

CARBONYLS IN OXIDIZING FAT  
 III. THE DISTRIBUTION OF VOLATILE AND NON-VOLATILE  
 CARBONYLS <sup>a</sup>

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The determination of carbonyls is useful in the study of flavor deterioration in fat containing foods. Reported methods (4, 6, 15, 22, 28, 29) have usually involved isolation by some type of volatilization procedure and estimation as 2,4-dinitrophenylhydrazones.<sup>b</sup> Steam volatile carbonyl 2,4-DNPH's have been separated into mono- and dicarbonyl fractions (15), and the monocarbonyls resolved into classes (14, 15) and individual compounds identified (13, 14, 16, 29). The carbonyls in the fat may be converted to 2,4-DNPH's, but complete separation from the fat has proven difficult. Procedures for the estimation of free monocarbonyls (30), and total determinable carbonyls and saturated and unsaturated components thereof (19), in the presence of the fat have been devised.

The carbonyl components in oxidized fat are extremely complex. The greater part of the determinable carbonyls are non-volatile (2, 8, 20, 22, 27) and are of unknown structure. Lea and Swobodi (22) have shown that aldehydes of C<sub>6</sub>-C<sub>14</sub> chain length can be recovered practically quantitatively from fats by vacuum distillation at 50° C (122° F). However, this procedure applied (22) to autoxidized fat yielded only 3% of the total carbonyls as determined by the method of Henick, Benca, and Mitchell (19). McKerrigan (27) and Chipault, Privett, Mizuno, Nickell, and Lundberg (8) have indicated that the volatile carbonyls may be mainly associated with "off" flavors. Holm, Ekblom, and Wode (20) and McKerrigan (27) have expressed the belief that the non-volatile carbonyls are of high molecular weight and are precursors of volatile odorous substances.

This report describes comparisons of various methods of isolating and estimating carbonyl compounds in autoxidized pork fat. The purpose of this work was to investigate the nature of the non-volatile carbonyls, and obtain information useful in the selection of procedures best suited to the study of relationships of carbonyls with rancid flavors.

The Girard T reagent (17) was used for the isolation of the non-volatile carbonyls from the fat. This reagent forms water soluble chlorotrimethylammonium acetylhydrazones with a wide variety of carbonyls. Its use has been recommended by several investigators (11, 33, 35) but little has been reported on its application to autoxidized fats. Hammond and Bird (18) have recently described its use in the study of autoxidized butterfat.

**Solvents, reagents, materials, and equipment.** These items were the same as used in similar operations described in earlier papers (10, 13, 14,

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<sup>b</sup> Hereafter referred to as 2,4-DNPH's.

15, 16) or in the particular method cited. Methanol and ethanol were purified by refluxing over sodium hydroxide and aluminum and distilling.

Vacuum distillation (22) of the fat was carried out in a 241.3 mm-long, flat bottomed tube of 41.3 mm inside diameter with a side-arm 177.8 mm from the bottom. The condensing surface, or cold finger, was a flat bottomed tube with 31.8 mm outside diameter. This was held in place at a depth of 206.4 mm in the larger tube (open end ground) by a bored large rubber stopper, which was placed in an inverted position on it. The end of the dry ice-cooled finger was 20.6 mm from the surface of a 10.0 g-sample of fat. A magnetic stirrer was used to agitate the fat.

#### EXPERIMENTAL

Fat used in this work was lard oxidized under ultraviolet light at 38° F (3.33° C) and 70° F (21.1° C) and in an oven at 60° C (140° F). As in previous studies (13, 15, 16), determinations were made on unheated fat and heated fat. The fat was heated at 165° C (329° F) for 15 min to approximate the effect of cooking.

Free carbonyls were determined by several procedures. Volatile carbonyls were determined by vacuum distillation of the fat at 50° C (122° F) for 1 hr at 1.0-0.5 mm pressure. Carbonyls condensed on the cold finger were reacted with 150 ml 2,4-dinitrophenylhydrazine reagent,<sup>o</sup> and the hydrazones isolated (15). Steam volatile carbonyls were isolated as 2,4-DNPH's as described by Gaddis, Ellis, and Currie (15). Free monocarbonyls in the fat were determined by the method of Pool and Klose (30). This method was extended to the approximation of free dicarbonyl and dicarbonyl-like compounds by thorough elution of the alumina column with ethanol. Total determinable carbonyls were estimated by the method of Henick, Benca, and Mitchell (19). Total carbonyls were isolated with the Girard T reagent (17). Ten grams fat were treated with 20 ml ethanol containing 5% Girard T reagent. Reactions were run by refluxing 1 hr with or without 0.2 g Dowex 50 cation resin (35).<sup>a</sup> They were also run by shaking at room temperature in an N<sub>2</sub> atmosphere for 3 hr. The Girard reaction mixtures were treated with 200 ml water and the fat recovered by extraction with petroleum ether. The water soluble Girard hydrazones were worked up by two different methods.

**Method 1.** The Girard hydrazones were treated with 220 ml 2,4-DNP reagent (15). After standing overnight at room temperature, the 2,4-DNPH's were extracted with CCl<sub>4</sub> (15). A second much smaller fraction was obtained by warming the extracted reaction mixture 1 hr on the steam bath and extracting again. CCl<sub>4</sub> extracts were combined.

**Method 2.** The aqueous solution of Girard hydrazones was made ½ N with concentrated HCl and steam distilled 22 min into 2,4-DNP reagent (15). This, after standing overnight, yielded the volatile carbonyl hydrazones. The residue from the steam distillation was treated with 50 ml 2,4-DNP reagent, and the next day the non-volatile 2,4-DNPH's were extracted with CCl<sub>4</sub>.

Fat hydroperoxides were reduced with sodium bisulfite by the method of Knight and Swern (21). Aqueous solutions of carbonyl bisulfites were converted to 2,4-DNPH's by addition of 4 ml concentrated HCl per gram of sodium bisulfite (32) and an excess of 2,4-DNP reagent.

Separations of total 2,4-DNPH's into mono- and dicarbonyls, and the monocarbonyls into classes, were made according to previously described methods (13, 14, 15, 16).

All determinations or reactions were run on 10 g fat except where the method specified smaller amounts. Quantitative figures were based on absorbance values at the wavelength of maximum absorption of the carbonyl hydrazones from 10 g fat in 100 ml of solution. Spectrophotometric readings were run in CCl<sub>4</sub> except in the Henick (19) method.

<sup>o</sup> Hereafter referred to as 2,4-DNP reagent.

<sup>a</sup> The mention of specific trade names throughout this paper does not constitute endorsement of the product used over comparable materials.

## RESULTS AND DISCUSSION

**Volatile and free carbonyls.** Differences between comparable methods were due to the degree of decomposition of hydroperoxides (9, 23) and/or complex secondary oxidation products (6, 7). A comparison of vacuum distillation, steam distillation, and Pool and Klose values is shown in Figure 1 for lard oxidized under ultraviolet light at 38° F (3.33° C) and 70° F

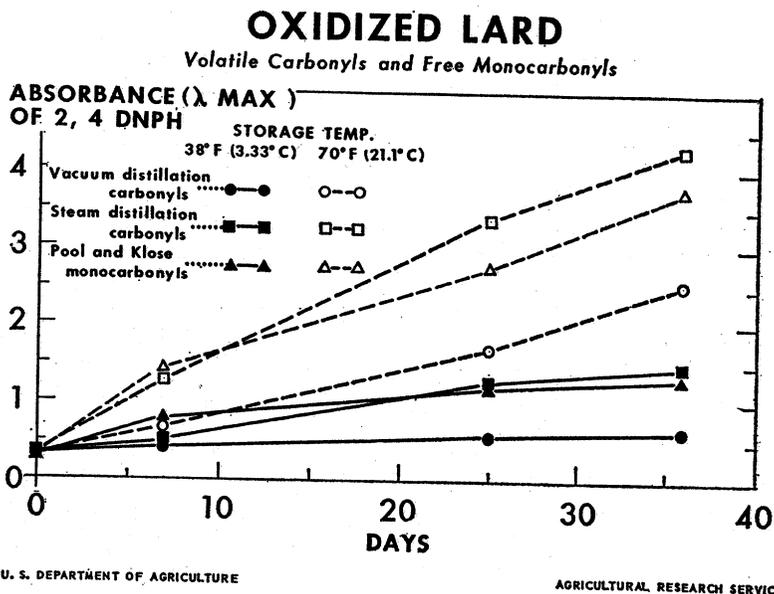


Figure 1. Oxidized lard. Volatile carbonyls and free monocarbonyls.

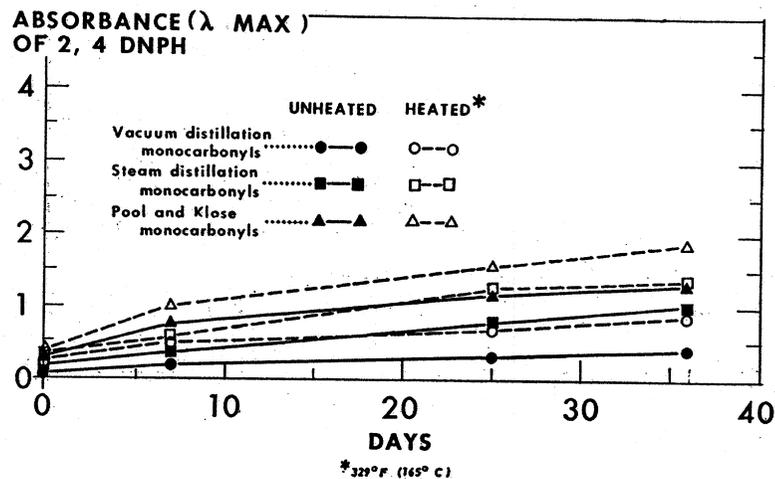
(21.1° C). Steam distillation values were higher than those of vacuum distillation. Some loss of C<sub>1</sub>-C<sub>5</sub> carbon chain monocarbonyls (22) could occur in the vacuum distillation, but this was probably of little significance since it has been shown (16) that very small proportions of such compounds are present in steam distillates. The differences between steam and vacuum distillation were evidently due to breakdown of peroxides or other precursors. Pool and Klose values were generally lower than the steam distillation values, but the former are free (volatile and non-volatile) monocarbonyls. Vacuum and steam distillation values averaged 2.5% and 4.0% of the Henick value for total carbonyls.

Volatile carbonyl 2,4-DNPH's were separated into mono- and dicarbonyls on 20% hydrated alumina. Proportions of the dicarbonyl fraction were approximately the same in the two methods. Figure 2 is a comparison of monocarbonyls found in unheated and heated lard oxidized under ultraviolet light at 38° F (3.33° C). Pool and Klose values were the highest. The difference between these and vacuum distillation monocarbonyls should represent free non-volatile monocarbonyls, provided that hydroperoxides and other precursors (3, 31) are not decomposed on the hydrated alumina. It is interesting that heating [165° C (329° F)] the fat did not decrease the differences between steam and vacuum distillation monocarbonyls. The

temperature of 176° C is a far more severe treatment than steam distillation and should decompose sensitive hydroperoxides rapidly (9, 23). The steam apparently exerted a special effect. Increase in steam volatile monocarbonyls produced by heating is a function of the degree of oxidation (15), and results in large increases in the proportion of unsaturated components (15, 16). The data indicated that the former statement was also true for vacuum distillation and Pool and Klose values. Furthermore, increases in the 3 values as the result of heating were approximately the same. The value for the non-volatile monocarbonyls was not significantly changed by heating. Heating an oxidized lard increased the steam volatile carbonyls 31.5%.

## OXIDIZED LARD, 38° F ( 3.33° C )

Volatile and Free Monocarboxyls



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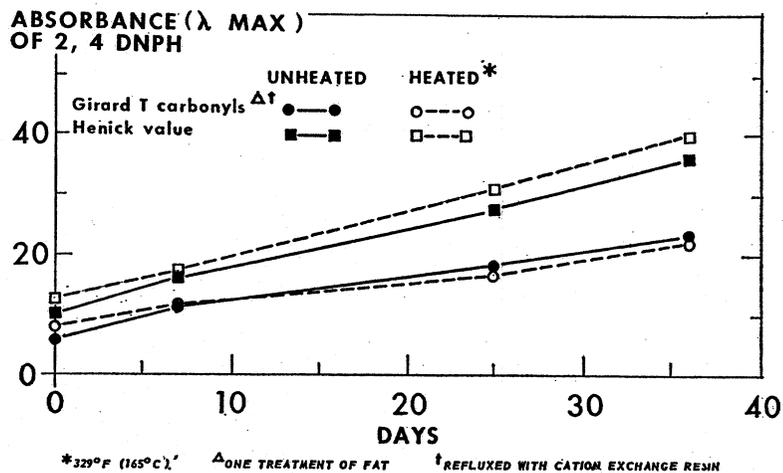
Figure 2. Oxidized lard 38° F (3.33° C). Volatile and free monocarbonyls.

However, steam distillation of the same unheated lard with trichloroacetic acid produced a 63.1% increase. The sum of values from vacuum distillation followed by steam distillation of recovered fat was the same as steam distillation of the whole fat. Pool and Klose, steam, and vacuum distillation monocarbonyls averaged 4.4%, 2.9%, and 1.4%, respectively, of the Henick value for total carbonyls. Shifts in the wave length of maximum absorption of the carbonyl fractions were similar to those described by Gaddis, Ellis, and Currie (15).

**Non-volatile and total determinable carbonyls.** The Henick method for total determinable carbonyls probably causes extensive decomposition of hydroperoxides as well as complex secondary oxidation products (20). The Girard T reagent offers a means of isolating non-volatile carbonyls. Teitelbaum (35) recently reported use of a cation exchange resin instead of acetic acid (17) in the reaction. This method was used in the following experiments, with refluxing in ethanol. Products were worked up by reaction with 2,4-DNP reagent and solvent extraction (Method 1). Figures 3 and 4

## OXIDIZED LARD, 38° F (3.33° C)

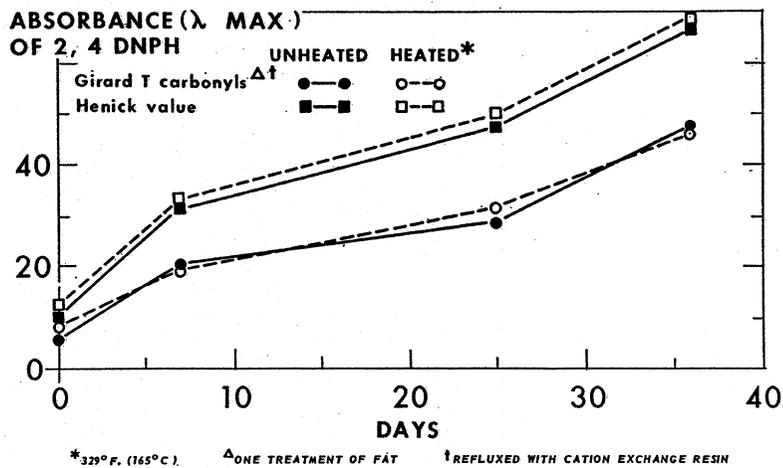
*Determinable and Isolated Carbonyls*



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**Figure 3. Oxidized lard 38° F (3.33° C). Determinable and isolated carbonyls.**

## OXIDIZED LARD, 70° F (21.1° C)

*Determinable and Isolated Carbonyls*



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**Figure 4. Oxidized lard 70° F (21.1° C). Determinable and isolated carbonyls.**

compare Girard and Henick values on unheated and heated lard oxidized at 38° F (3.33° C) and 70° F (21.1° C). Increases in the two values as the result of heating were small and not related to the degree of oxidation. The differences in the Girard values were not significant. This is in contrast to the effect of heating on volatile carbonyls. It indicated that both procedures broke down the same precursors that were decomposed by heat.

Girard T reagent, under these conditions, therefore, isolated both free and formerly bound carbonyls. Girard values averaged 64% of the Henick values. The partial isolation was probably due to the presence of more stable complexes. The Girard reaction removed all free monocarbonyls, as Pool and Klose determinations on recovered fat were negative. The sum of Girard values and Henick values on the recovered fat was approximately equal to Henick values on the whole fat.

**Distribution of carbonyls isolated by Girard T reagent.** Decomposition of Girard hydrazones by steam distillation in the presence of mineral acid, followed by conversion of the separated volatile and non-volatile carbonyls to 2,4-DNPH's (Method 2), gave an indication of the nature and distribution of the carbonyls isolated. Results are shown in Table 1. The lard used was oxidized at 60° C (140° F) to a peroxide value of 15. Girard derivatives were prepared by refluxing with Dowex 50 cation exchange resin. Results were similar for unheated and heated lard. The data show the presence of 41.1% volatile carbonyls, 33.6% monocarbonyl, 29.7% volatile monocarbonyl, and 3.9% non-volatile monocarbonyl based on total carbonyl isolated. The non-volatile monocarbonyl fraction was therefore of the order indicated by differences between Pool and Klose and vacuum distillation values. Volatile carbonyl fractions isolated by the Girard T reagent, although mostly coming from the breakdown of precursors, were at least superficially similar to the free volatile carbonyls present in the fat. The volatile monocarbonyls were separated by paper chromatography (14, 15) into the characteristic saturated aldehyde, 2-enal, and 2,4-dienal classes (15, 16). The non-volatile monocarbonyls separated into just one spot. The maximum of this material was 355-8 mmc, which may represent a new class (16). Dicarbonyls were 66.4% of the total carbonyls isolated. Volatile dicarbonyls were 11.4% of the total, had a maximum at 380-5 mmc (15), and were present in the proportions usually found in steam volatile carbonyls (15). Non-volatile dicarbonyls, or dicarbonyl-like compounds, were 55.0% of the total. These gave a red color with alcoholic alkali. An appreciable amount of these could not be removed from the 20% hydrated alumina with ethanol. The proportion of these compounds that are present as free non-volatile dicarbonyls is not known. Further examination of the fraction retained by the alumina in the Pool and Klose separation might provide a partial answer. However, contact with hydrated alumina may be attended by decomposition of oxidation products (3, 31). These non-volatile dicarbonyls are evidently of high molecular weight and could be precursors of the volatile carbonyls.

**The Girard reaction.** It is evident that much of the carbonyl was released in some manner by the Girard reagent and reaction conditions. The carbonyls may be present in bound forms or arise from oxidative polymers (6, 7) or partial decomposition of hydroperoxides. Production of carbonyls is probably influenced by the heat, the cation exchange resin and possibly by the Girard T reagent. It would be useful if the action of the Girard reagent could be limited to free carbonyls.

The literature indicates that cation exchange resins are frequently powerful catalysts. They are known to catalyze esterification, interesterification, and saponification (25, 34, 36) and form acetals (34). Cation ex-

**TABLE 1**  
The distribution of carbonyls isolated by the Girard T reagent<sup>1</sup>

	Carbonyl 2,4-dinitrophenylhydrazones									
	Absorbance ( $\lambda$ max)	Percent								
		Total			Volatile			Non-volatile		
		Mono-carbonyls	Di-carbonyls	Total	Carbonyls	Mono-carbonyls	Di-carbonyls	Carbonyls	Mono-carbonyls	Di-carbonyls
Lard oxidized at 60° C (140° F) to peroxide value of 15										
Unheated lard										
Method 1	29.7	66.7	66.7	41.9	31.2	10.7	58.1	3.7		54.4
Method 2	24.6	65.1	65.1							
Heated lard										
Method 1	30.3	69.6	69.6	40.3	28.2	12.1	59.7	4.1		55.6
Method 2	30.5	67.7	67.7							
Average values										
Method 1	31.9	68.1	68.1	41.1	29.7	11.4	58.9	3.9		55.0
Method 2	33.6	66.4	66.4							

<sup>1</sup> Refluxed with Dowex 50 cation exchange resin.

**TABLE 2**  
Effect of reaction conditions on yield of Girard carbonyls

	Total carbonyl 2,4-dinitrophenylhydrazones absorbance ( $\lambda$ max)									
	Repeated treatments of lard									
	Cation exchange resin					No cation exchange resin				
	No. 1	No. 2	No. 3	Total	No. 1	No. 2	No. 3	Total		
Lard oxidized at 70° F (21.1° C)										
Refluxed										
Unreduced lard										
Method 1	40.66	12.95	2.34	55.95	65.20	5.37	2.30	72.87		
Method 2	32.00	12.30	3.07	47.37	35.80	4.53	2.30	42.63		
Reduced lard										
Method 1	22.02	6.64	2.50	31.16	16.16	5.80	5.62	27.58		
Method 2	18.60	7.83	1.93	28.36	13.15	5.36	5.47	23.98		
Room temperature										
Unreduced lard										
Method 1	62.20	21.83	6.45	90.48	17.44	6.78	2.86	27.08		
Method 2	39.00	21.00	6.08	66.08	15.80	6.14	2.54	24.48		
Reduced lard										
Method 1	24.75	9.04	.....	33.79	15.72	6.04	6.08	27.84		
Method 2	23.60	8.98	.....	32.58	11.42	3.80	5.46	20.68		

change resins also are capable of catalyzing condensation and polymerization of aldehydes (1). Much of this potential catalytic action was probably inhibited by the two-phase system, fat and ethanol, in which the reaction was run. The ability of replacing mineral acids makes it seem likely that the chief action was depolymerization of oxidative polymers (6, 7) and decomposition of hydroperoxides.

Girard and Sandulesco (17) specified the use of acetic acid as catalyst in the reaction. However, Lederer and Nachmias (24) have stated that good yield was obtained in the Girard reaction without the use of acid. Hammond and Bird (18) reported that the peroxide value of oxidized butterfat was unchanged when the reaction was run without acid or catalyst at 40° C (104° F). The hydroperoxides can be reduced with sodium bisulfite (21). Such a step involves opposing side reactions (26). Carbonyls presumably free, are extracted as bisulfites. These may be accounted for by conversion to 2,4-DNPH's (32). Unsaturated carbonyls may be lost through the formation of sulfonic acid derivatives (32, 37). There may also be an increase in free carbonyls through the reduction of low molecular weight peroxides of the per acid type (12).

Data in Table 2 show the effect of cation exchange resin, heat, and reduction of hydroperoxides on the total carbonyls isolated. The lard used in the experiment was oxidized under ultraviolet light at 70° F (21.1° C) to a peroxide value of 32.0. The Girard reaction was repeated twice in succession on recovered lard. Reaction mixtures were worked up by Methods 1 and 2. Total carbonyls found by the various other methods were vacuum distillation 2.08, steam distillation 4.60, Pool and Klose 3.96, and Henick 70.67. Reduction with sodium bisulfite diminished the peroxide value to 0.4. The fat then had carbonyl values of vacuum distillation 1.65, steam distillation 5.16, Pool and Klose 3.80, and Henick 45.11. Carbonyl bisulfite extracted during the reduction was 5.83. Carbonyl 2,4-DNPH's isolated as bisulfites were 75.6% monocarbonyls. The maximum of this fraction indicated it was composed of saturated aldehydes. The 12.2% dicarbonyls could not be eluted from the hydrated alumina with ethanol. Since free carbonyl values were little changed, the reduction apparently resulted in an over-all increase in free carbonyl despite the potential loss of unsaturated free carbonyls. The Henick value (45.11) for total determinable carbonyls was considerably decreased by reduction. Girard isolated carbonyls were greatly diminished by reduction, except at room temperature without cation exchange resin. This indicated that hydroperoxide decomposition was produced by both cation exchange resin and heat. Results of Method 2 generally correlated with those of Method 1, but were much lower. Apparently steam distillation in the presence of HCl was destructive of carbonyl compounds. Oddly, this effect was greatest in the initial reaction. The combination of heat and cation exchange resin decreased the yield of carbonyls. However, at room temperature the presence of cation resin gave the highest total values. The lowest yield of carbonyl from unreduced lard was at room temperature without catalyst. Both heat and the cation exchange resin in the presence of Girard T reagent were able to break down hydroperoxides and other precursors. The cation exchange resin was probably more effective than heat in decomposing the latter.

**TABLE 3**  
Influence of reaction conditions on distribution of Girard isolated carbonyls<sup>1,2</sup>

	Carbonyl 2,4-dinitrophenylhydrazones										Percent		
	Absorbance ( $\lambda$ max)										Mono-carbonyl	Non-volatile dicarbonyl	Dicarbonyl retained by alumina
	Total	Steam volatile	Non-volatile	Total		Volatile		Non-volatile		Di			
				Mono	Di	Mono	Di	Mono	Di				
Cation exchange resin													
Refluxed	40.66											30.2	28.2
Method 1	32.00	17.68	14.32	12.30	16.92					6.72	1.18	45.3	27.1
Method 2				14.51	8.84								
Room temperature	62.20			15.76	24.00							25.3	36.1
Method 1	39.00	20.80	18.20	13.68	11.26					8.48	0.39	35.0	36.1
Method 2													
No cation resin													
Refluxed	65.2			29.30	21.40							44.9	22.2
Method 1	35.8	21.4	14.4	21.05	7.59					5.29	2.25	58.7	20.0
Method 2				4.57	8.34							26.2	26.0
Room temperature	17.44	6.26	9.54	5.40	6.20					5.25	0.77	34.2	26.6
Method 1	15.80												
Method 2													

<sup>1</sup> From first treatment of fat with Girard T.  
<sup>2</sup> Unheated unreduced lard.

**TABLE 4**  
Carbonyl distribution analysis of an oxidized lard<sup>1</sup>

Girard reaction <sup>2</sup>	Lard										Reduced lard		
	Absorbance ( $\lambda$ max)										Absorbance ( $\lambda$ max)		
	Total carbonyls	Mono-carbonyls	Di-carbonyls	Henickon recovered lard	Percent Mono-carbonyls	Total		Mono-carbonyls	Di-carbonyls	Percent Mono-carbonyls			
						Mono	Di						
Refluxed, cation resin	17.64	6.86	7.12	9.26	39.0	13.20	3.36	5.70	25.4				
Refluxed, no cation resin	20.00	6.98	9.39		34.6	11.52	2.48	5.48	21.5				
Room temperature, cation resin	25.08	7.52	11.40	11.70	30.0	16.88	4.11	6.76	24.3				
Room temperature, no cation resin	13.10	3.06	6.72		23.4	15.44	3.12	6.70	20.2				
Room temperature, no cation resin	35.74					28.65							
Henick value													
Pool and Klöse value	2.00	2.30	3.95		62.5	3.36	2.06	0.44	61.0				
Steam distillation	0.95	1.25	0.38		66.3								
Bisulfite extracted		0.63	0.19										

<sup>1</sup> Peroxide = 18.  
<sup>2</sup> Initial reaction with Girard A reagent.

The Girard reagent, even under the mildest of conditions, appeared capable of breaking down precursors. This was indicated by the isolation of more carbonyl on repeated treatment. The initial value for lard at room temperature without catalyst could approximate the free carbonyls present. The Pool and Klose monocarbonyl value for unreduced lard was 3.96. Elution of the 20% hydrated alumina column with ethanol gave a dicarbonyl 2,4-DNPH fraction of 7.53. This gave a total carbonyl of 11.49. However, ethanol did not completely remove the 2,4-DNPH's from the column. When the lard in benzene solution was applied to a 20% hydrated alumina column, and the column eluted with benzene and then ethanol, the resulting fractions, when tested with 2,4-DNP reagent, had values of 5.29 and 11.80. The total, 17.09, agree closely with the initial Girard value, 17.44. Thus it may be possible to isolate only free carbonyls with the Girard T reagent by the use of optimum conditions.

Table 3 is an extension of the data in Table 2, showing the distribution of carbonyls isolated in the initial Girard reaction on the unreduced lard. The loss of carbonyls by Method 2 is clearly shown to be in the dicarbonyl fraction. Amounts of the various fractions were influenced by the reaction conditions. Approximately 25-30% of the total carbonyls could not be removed from the hydrated alumina with ethanol. Further study of the Girard reaction with autoxidized fat is required. The complexity of the reaction is emphasized by the comprehensive data in Table 4 for a lard oxidized under UV light at 70° F (21.1° C) to a peroxide value of 18. This analysis generally supports the conclusions already stated. Yields of carbonyls were diminished in both reduced and unreduced lard by the combination of the cation exchange resin and heat, and heat alone. Most of this effect appeared to be on the dicarbonyl fraction. Production of carbonyl through hydroperoxide breakdown was an important source of differences. Apparently, hydroperoxide decomposition was minimized at room temperature without cation resin. Values obtained under mild conditions may then approach the actual free carbonyl content, which is much lower than the total carbonyl determinable.

#### SUMMARY

A study has been made of the distribution of volatile and non-volatile carbonyls in oxidized lard. Most of the carbonyls determinable were non-volatile and/or bound. Results indicated that a large part of the determinable carbonyls do not originally exist in the oxidized fat as carbonyls. They apparently are produced through breakdown of precursors by reaction conditions. Free monocarbonyls were 4.4%, volatile monocarbonyls 1.4%, and volatile dicarbonyls 1.1% of the total determinable carbonyls. Initial reaction with the Girard T reagent isolated an average of 64% of the total carbonyls determinable. In a moderately oxidized fat, carbonyls isolated by the Girard reagent were 33.6% monocarbonyl and 66.4% dicarbonyl, 41.1% volatile carbonyl and 58.9% non-volatile carbonyl, 29.7% volatile monocarbonyl and 11.4% volatile dicarbonyl, and 3.9% non-volatile monocarbonyl and 55.0% non-volatile dicarbonyl. The amount of carbonyls isolated by the Girard reagent was greatly influenced by reaction conditions. In general, results emphasize the need for a reliable method of isolating and determining the free total carbonyl in fat.

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