A Rapid Test for Alpha Dicarbonyls

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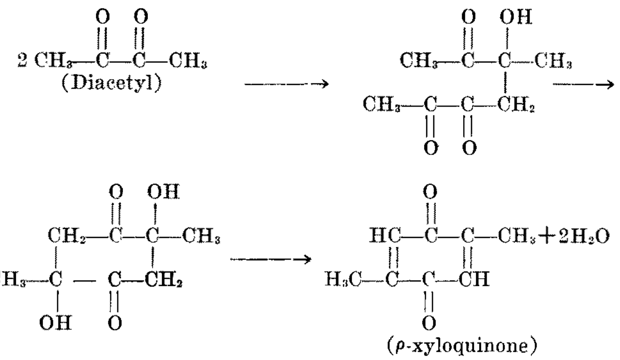
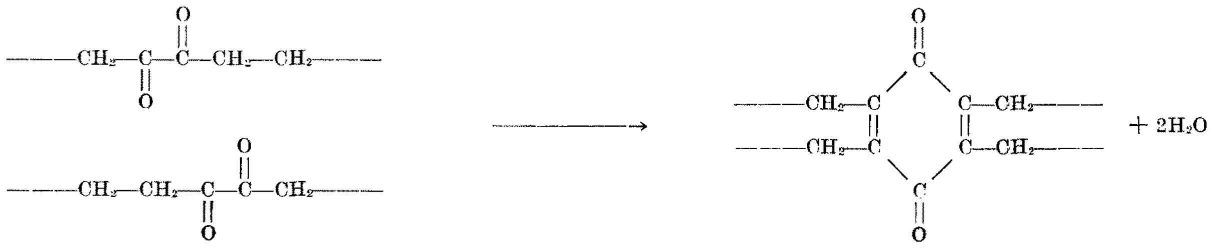
Recent discussions (3, 6) of the composition of auto-oxidation products of fats and oils indicate the probable presence of alpha-diketo structures and their precursors, alpha-dihydroxy compounds, among the complex reaction products. Prill (4) has described color tests for alpha-dicarbonyl compounds based upon oximation of the fatty material with hydroxylamine either in pyridine or in absloute ethanol in the presence of potassium acetate. The resulting dioximes are subsequently converted to Ni, Fe, or Cu derivatives which are soluble in benzene, yielding characteristically colored solutions. Prill clearly demonstrated the presence of alpha-dicarbonyls in several samples of oxidized fatty materials by the application of this test. Additionally, peroxides were destroyed by treatment with ferrous chloride without affecting the color test, thus definitely eliminating the possibility of side reactions between peroxides, hydroxylamine, and oxidized components other than alpha-dicarbonyls to form dioximes which might yield interfering colored metallic derivatives.

The simplest alpha-diketone exhibiting a structure similar to that existing in an alpha-dicarbonyl derived from the oxidation of an unsaturated fatty acid is diacetyl (2, 3 dioxy butane). Diacetyl is soluble in water yielding solutions which are bright yellow in high concentration and practically colorless when dilute. Aqueous solutions treated with caustic alkalies undergo successive aldol condensations eliminating two molecules of water from two molecules of diacetyl to form one molecule of para-xyloquinone. The reactions are as follows:



A deep red color is produced immediately upon adding caustic alkali to a solution of diacetyl, due to the formation of para-xyloquinone, the depth of color being proportional to the concentration of the diacetyl. Auto-oxidized fats and oils treated with alcoholic caustic potash likewise yield solutions which are more or less highly colored, depending upon the extent of auto-oxidation. The color is undoubtedly due to quinoid compounds formed by aldol condensation of alpha-diketones in a manner entirely analogous to the formation of para-xyloquinone from diacetyl. The molecular changes involved are probably as shown at bottom of page.



Procedure

The color developed by the alcoholic alkali treatment of fats, oils, or fatty acids is determined by warming a mixture of 50 grams of the fatty material, 50 ml. of formula 3A alcohol, and 20 ml. of a 50 per cent aqueous solution of potassium hydroxide to approximately 155°F. (68°C.) with continuous agitation. The fatty material rapidly saponifies and dissolves in the alcohol. The Lovibond color of a 5¼ inch column is read in a graduated color tube or in a standard 4 ounce oil sample bottle as soon as reaction is complete and the solution clears. The procedure yields approximately the required amount of solution to fill a 4 ounce bottle to the 5¼ inch level. Obviously reaction and color readings may be carried out with much smaller amounts if only a limited quantity of the material is available.

Fats and oils often are rather dark colored, although the colored components have no relation to the quinoid compounds formed by treatment with alkali. If the original material is highly colored, its color reading may be subtracted from the color obtained in the alcoholic alkali test to determine the actual color increment resulting from the conversion of alpha-dicarbonyls to quinoid compounds.

Relation Between Extent of Auto-oxidation and Color of Alcoholic Alkali Test

If alpha-dicarbonyls are formed in the auto-oxidation of fats and oils, a progressive increase in concentration and consequently a progressive increase in depth of color in the alcoholic alkali test should occur. Table I contains data comparing alcoholic alkali color and peroxide number of several fats and oils after varying storage periods under conditions favoring rapid oxidation. Refined and bleached fats and oils were exposed in thin layers in an oven at a temperature of 145°F. (63°C.), a condition frequently employed in accelerated ageing. The data clearly indi-

TABLE I

Effect of Oxidation on Alcoholic Alkali Color of Various Fats and Oils

Days' Storage at 145°F.	Tallow		Coconut Oil		Cottonseed Oil		Peanut Oil	
	Alcoholic Alkali Color, Lovibond Red	Peroxide Value*	Alcoholic Alkali Color, Lovibond Red	Peroxide Value*	Alcoholic Alkali Color, Lovibond Red	Peroxide Value	Alcoholic Alkali Color, Lovibond Red	Peroxide Value
0	5.0	0.3	3.5	0.4	12.0	0.3	2.5	6.4
1	20.0	7.4	3.4	0.8	13.0	6.1	2.5	6.4
2	32.0	15.0	5.0	3.3	16.0	6.4	3.2	8.0
5	45.0	34.3	20.0	12.0	32.0	24.5	3.5	12.3
8	54.0	45.0	26.0	15.0	65.0	30.0	30.0	42.0
11	62.0	68.0	32.0	22.0	100.0	42.0	80.0	60.0
Iodine Value of Original Stock	50.9		9.8		106.9		88.5	

* Millimoles of oxygen per 1,000 grams of fat.

cate that the alcoholic alkali color and the peroxide number increase progressively during auto-oxidation. All of the samples had a low initial peroxide number and alcoholic alkali color, hence the initial degree of oxidation was negligible. The fats and oils varied considerably in stability to oxygen. Coconut oil, which is commonly considered to be the most stable of the fatty materials tested, had the smallest increase in the alcoholic alkali color and peroxide number, while the largest changes occurred with refined cottonseed oil, which oxidizes readily even at room temperatures.

While the results reported in Table I were obtained on fats which were auto-oxidized at slightly elevated temperatures, a similar marked increase in alcoholic alkali color occurs in fatty materials subjected to prolonged storage at room temperatures under conditions permitting free access of air.

Stability of Alpha-Dicarbonyls

The peroxide number is frequently employed as an index of the degree of oxidation of a fat or oil. It is true that oxidative changes result in a substantial increase in the peroxide value, and it is also generally recognized that common processing methods such as bleaching and deodorizing materially reduce or entirely destroy peroxides. While a high peroxide number is evidence that a fat has been oxidized, it does not follow that fats having very low peroxide values are free from extensive oxidative changes.

Alpha-dicarbonyls are stable under ordinary processing conditions, and in contrast to the peroxide value, the alcoholic alkali color remains substantially unaffected. The advantages of the alkali color test for estimating the probable degree of oxidation of a given fat sample are therefore obvious.

Samples of refined and bleached tallow having a peroxide value of 26 were deodorized with steam in a small glass laboratory vacuum deodorizer at a temperature of 400°F. for 15 minutes, which reduced the peroxide value to zero. The alcoholic alkali color remained substantially the same as that of the undeodorized stock. A tallow having a peroxide number of 11.5 was bleached for five minutes at 105°C. with amounts of fuller's earth varying from 1 to 6%. The peroxide number was reduced to 0.3 or less in all instances, and the alcoholic alkali color was practically unaffected. These results are indicative of the marked stability of alpha-dicarbonyl compounds and demonstrate that the alcoholic alkali color test is more significant than the peroxide value for estimating the actual state of oxidation of a fatty material.

The Precursors of Alpha-Dicarbonyls

Considerations concerning the mechanism of the formation of peroxides, alpha-dicarbonyls and other oxidation products have usually been limited to a single point of unsaturation in the fatty acid molecule. It is obvious that one or all of the unsaturated fatty acids present in fatty material may yield alpha-dicarbonyl compounds upon oxidation. Oleic, linoleic, and linolenic are the commonly occurring unsaturated fatty acids, although small amounts of palmitoleic acid are present in tallow, and the presence of myristoleic acid has been reported in several fats and oils. Additionally, fish oils contain high molecular weight fatty acids with several double bonds.

Methyl esters of tallow fatty acids were fractionally distilled, and the C_{16} and C_{18} fractions were auto-oxidized in order to determine whether all fractions containing unsaturated acids developed alpha-dicarbonyls. The data in Table II indicate that the C_{18} fraction, consisting of a mixture of oleic, linoleic, and stearic esters, contained alpha-dicarbonyl compounds after oxidation. After 384 hours' storage the increase in the alcoholic alkali color of the C_{16} fraction was negligible as compared with the C_{18} fraction. The results suggest that palmitoleic acid does not oxidize to alpha-dicarbonyls, although the samples become strongly rancid, as judged by the odor. It therefore became of interest to determine whether pure oleic and linoleic esters auto-oxidize to yield alpha-dicarbonyls.

TABLE II

Effect of Oxidation on Alcoholic Alkali Color of Fractionated Methyl Esters Derived From Tallow Fatty Acids

Hours' Storage at 145°F.	Alcoholic Alkali Color—Lovibond Red	
	C_{16} Fraction	C_{18} Fraction
0	.2	.2
24	.2	7.0
120	.5	22.0
288	.9	50.0
384	1.2	84.0
Iodine Value of Original Ester	14.5	62.0

Methyl oleate was prepared by fractionating methyl esters derived from olive oil (7). The C_{18} esters consisting of a mixture of methyl oleate, methyl stearate, and a small quantity of methyl linoleate were then fractionally crystallized from acetone at —60°C., to substantially remove the soluble methyl linoleate. Subsequently the mixed oleic and stearic esters were dissolved in acetone and fractionally crystallized at —37°C., removing all of the methyl stearate and some of the methyl oleate in the solid phase, while the bulk of the methyl oleate remained in solution. The substantially pure methyl oleate was recovered from the acetone solution and then redissolved in acetone in the ratio of one part ester to fifteen parts of solvent. The solution was recrystallized at —60°C. to separate the last traces of methyl linoleate. The crystallized methyl oleate had an iodine value of 85.0, as compared with the theoretical of 85.7. The Kaufmann value agreed with the iodine value within the probable error of the determinations, hence the preparation was substantially free from linoleic acid.

Methyl linoleate was prepared (5, 1) by fractionally crystallizing 1,400 grams of fatty acids from cottonseed oil dissolved in 5 liters of acetone at a temperature of —15°C. to remove the saturated acids and some of the oleic acid. The unsaturated fatty acids in the supernatant liquor were recovered by evaporating the acetone, and subsequently dissolved in petroleum ether and completely brominated at 0°C. The insoluble tetra-bromo-stearic acid was separated and recrystallized twice at 0°C. from petroleum ether. The purified product was debrominated and methylated by treatment with zinc dust and sulphuric acid in methyl alcohol solution. The methyl linoleate was extracted with petroleum ether from an aqueous suspension of the reaction mixture, and the ether solution was washed repeatedly with water to remove the sulphuric acid. The purified compound had an iodine value of 170.9, as compared with the theoretical of 171.3, indicating that the product was substantially pure.

TABLE III

Alcoholic Alkali Color Changes in Methyl Oleate and Methyl Linoleate During Auto-Oxidation
(5-Gram Sample Used for Alcoholic Alkali Test)

Hours' Storage at 145°F.	Alcoholic Alkali Color—Lovibond Red	
	Methyl Oleate	Methyl Linoleate
0	8.0	0.4
24	8.0	30
72	8.7	60
96	9.0	*100
288	8.8	*600

* Colors estimated from readings of diluted solutions.

The data in Table III clearly demonstrate that the alcoholic alkali color derived from methyl oleate remained substantially constant during the entire period of auto-oxidation in the 145°F. oven, whereas the alcoholic alkali color of methyl linoleate increased tremendously. Both esters were extensively oxidized, since the methyl oleate had a peroxide number of 170 and the methyl linoleate 485 after 96 hours' storage. The methyl oleate developed and maintained a characteristic and overpoweringly rancid odor, while the methyl linoleate was practically odorless during the entire storage period. The evidence indicates that the unsaturated fatty acids containing a single double bond do not yield alpha-dicarbonyls, and apparently at least two double bonds in the fatty acid molecule are necessary for their formation.

There are a number of unsaturated fatty acids, such as linolenic, which contain more than two double bonds. While it has not been definitely demonstrated in this investigation that linolenic acid yields alpha-dicarbonyls, Morrell (2) reported that dark-colored products were formed when oxidized linolenic acid was treated with caustic soda. The probabilities are, therefore, that the tri-unsaturated acids upon oxidation also yield alpha-dicarbonyls.

The theoretical implications of these findings are not immediately obvious. It has been pointed out that organoleptic evidences of rancidity are due primarily to oleic rather than linoleic acid, presumably because oleic acid splits at the double bond to yield volatile higher aldehydes. Such a schism of the molecule would of course preclude the formation of alpha-dicarbonyls. The findings amply indicate the complexity of the oxidative changes, and the inadequacy of present theoretical knowledge.

All of the common fats and oils contain appreciable and sufficient quantities of linoleic acid to yield definite alcoholic alkali color changes upon auto-oxidation. It is calculated from the data for methyl linoleate in Table III that 50-gram samples of auto-oxidizing fat containing 1% of linoleic acid would ultimately develop a color of at least 60 R in the alcoholic alkali test.

Comparison of Alpha-Dicarbonyl Tests

Benzene solutions of the iron derivatives of the dioximes prepared from several oxidized fats by Prill's pyridine procedure increased in color with increasing degree of oxidation in a manner analogous to the alcoholic alkali color test. The preparation of the dioximes requires approximately two hours elapsed time, and while the depth of the color is roughly related to the degree of oxidation, the method has no apparent advantages over the simple, rapid, and convenient alcoholic alkali test.

Fatty acids isolated from highly colored alcoholic alkali solutions prepared from oxidized tallow did not yield colored metallic dioxime derivatives, although the original tallow was high in alpha-dicarbonyls by the Prill test. The negative Prill test on the fatty acids is confirmation that the alpha-dicarbonyls in the oxidized tallow were converted to quinoid derivatives by the action of the caustic potash. The quinoid condensation product would of course be stable in acid solution, and the possibility of dioxime formation is thus eliminated.

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