A Method for Determining the Carbonyl Value in Thermally Oxidized Fats[1,2]

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A simple method for the determination of the carbonyl value of thermally oxidized fats, using hydroxylamine hydrochloride, is described. Normal octyl alcohol containing pyridine was used as a solvent, and the reaction was allowed to proceed at room temperature for 24 hrs. The titrations were carried out with the aid of a pH meter. The method was standardized against aliphatic aldehydes and ketones. The results of analyses showed that the different pure compounds reacted to an extent of 98% or better at room temperature.

The method has been successfully applied for the determination of the carbonyl value of thermally oxidized fats. n-Valeric acid and the fatty acids with higher molecular weight were not found to interfere in the determination of the carbonyl value by the present method.

A NUMBER of analytical procedures for the determination of aldehydes and ketones, based on the use of hydroxylamine hydrochloride, have been reported. The amount of carbonyl oxygen present was quantitatively determined by estimating either the amount of unreacted hydroxylamine (1,2) or the amount of water formed during the reaction (3), or by estimating the hydrochloric acid liberated (4,5,6, 7,8). Lappin and Clark (9) have described a procedure which employs 2,4-dinitrophenylhydrazine for determining traces of carbonyls in aqueous or alcoholic solutions. This method with some modifications was employed by Henick *et al.* (10) for the determination of carbonyls in rancid fats and foods. These analytical procedures were not found to be reliable for the quantitative determination of carbonyl oxygen in thermally oxidized fats because of their limited solubility in all but a few suitable solvents. A method for the determination of the carbonyl content of thermally oxidized fats, using hydroxylamine hydrochloride as a reagent and n-octyl alcohol as a suitable solvent, is presented in this paper.

Experimental

Reagents

Hydroxylamine Hydrochloride Reagent. Thirty-five grams of hydroxylamine hydrochloride (reagent grade) were dissolved in 160 ml. of distilled water, and the resulting solution was diluted to 1 liter with 95% carbonyl-free ethyl alcohol.

Standard Sodium Hydroxide in Methanol. Twenty grams of sodium hydroxide (analytical reagent) were dissolved in a small amount of water and diluted to 1 liter with absolute methyl alcohol and standardized against standard hydrochloric acid (0.5 N).

Pyridine-octyl Alcohol Solvent. Five ml. of pyridine (analytical reagent) were diluted to 1 liter wth n-octyl alcohol.

Apparatus

A Beckman pH meter, Laboratory Model H2, equipped with standard glass electrode and standard calomel electrode, was used in this work.

Analytical Procedure

One gram of the sample to be analyzed was weighed into a 250-ml. glass-stoppered Erlenmeyer flask. Fifty ml. of pyridine-octyl alcohol solvent were pipetted into it, and, if necessary, the flask was heated on a hot plate until the sample was dissolved. Fifteen ml. of hydroxylamine hydrochloride reagent was then added with a pipette. The flask was stoppered, the contents were mixed well and left in the dark for 24 hrs. at room temperature. A blank determination was carried out simultaneously, following the same procedure, but without the fat sample.

At the end of the reaction time contents of the flask were quantitatively transferred to a 250-ml. beaker with 10 ml. of 95% carbonyl-free ethyl alcohol. The pH of the blank was recorded. The reaction mixture containing the fat sample was then potentiometrically titrated against standard 0.5 N sodium hydroxide solution in a 10-ml. micro burette. The alkali was added in increments of 0.1 ml. with shaking after each addition until the pH of the solution approached that of the blank. The titration was then completed by adding the alkali drop by drop until the pH of the titrated solution was the same as that of the blank.

Calculations

$$\text{Carbonyl value, meq/kilogram} = \frac{V \times N \times 1{,}000}{W}$$

where V = ml. of standard sodium hydroxide required for titration

N = normality of standard sodium hydroxide

W = weight of sample in grams used to determine carbonyl value

Results and Discussion

A series of aldehydes and ketones was obtained commercially and purified in the laboratory by distillation. These aldehydes and ketones were analyzed by the procedure of Bryant and Smith (4) and by the modified procedure described in this paper (Table I). The results obtained by the present method agreed closely with those obtained by the method of Bryant and Smith (4). Low values obtained by both the methods in some of the samples probably resulted from the impurities carried over during distillation. The results indicated that the modified procedure can be successfully used in the analysis of carbonyl compounds.

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TABLE I

Analysis of Carbonyl Compounds

Compound	% Found (Bryant and Smith method)	% Found (Present method)
n-Butanal	94.5	95.1
Crotonaldehyde	95.1	95.3
n-Hexanal	96.1	95.8
n-Heptanal	95.2	96.3
n-Octanal	97.6	99.1
n-Nonanal	96.5	97.2
n-Decanal	98.2	98.8
n-Dodecanal	98.6	98.4
2-Heptanone	98.4	98.3
5-Hexene-2-one	98.7	98.5
5-Nonanone	97.7	98.1
Di-iso-propyl-ketone	98.2	99.0
Benzaldehyde	98.5	98.6
Anisaldehyde	94.6	95.0
2-Octanone	94.1	94.2

It has been shown that, during thermal oxidation of a fat, there is an increase in the carbonyl value (11). The attack of oxygen produces carbonyl groups in the glyceride molecules. Part of this attack takes place at the double bond, and some of the changes are also known to occur at the methylene group since the presence of α,β-unsaturated carbonyl group has been demonstrated (12). In order to find out if the carbonyl group present in the triglyceride molecules could be determined, triglycerides containing one or two moles of keto acids (12-oxo-9-*cis*-octadecenoic acid) were prepared in the laboratory according to the method of Hartman (13). These triglycerides and the keto acid were analyzed for the carbonyl content according to the modified procedure, and the results are presented in Table II. The results indicated that hydroxylamine hydrochloride reacted with the carbonyl groups in the glyceride molecules and gave 97–98% reaction. This method can therefore be used not only for free aldehydes and ketones but also for the carbonyl groups present in the triglyceride molecules.

TABLE II

Analysis of Triglycerides Containing Keto Acid [a]

Compound	% Carbonyl found
12-Oxo-9-*cis*-octadecenoic acid	98.2
α,α'-Palmito, β-ketoyl triglyceride	97.2
α-Palmito. $\alpha'\beta$-ketoyl triglyceride	98.1

[a] Keto acid = 12-oxo-9-*cis*-octadecenoic acid.

TABLE III

Effect of Fatty Acid on Carbonyl Value

Mixture	% acid added	Carbonyl value,[a] meq/1,000 g.
Fresh corn oil containing 5.13% of n-hexanal, valeric acid	0	486.0
	3	491.5
	6	482.3
Fresh corn oil containing 4.96% of n-octanal, valeric acid	0	382.4
	3	378.7
	6	380.2
Thermally oxidized corn oil, valeric acid	0	321.6
	1	319.8
	3	320.3
	6	320.8
Thermally oxidized corn oil, caproic acid	0	323.1
	1	324.2
	3	321.7
	6	322.6
Thermally oxidized corn oil, palmitic acid	0	324.6
	1	324.3
	2	325.0
	4	322.7

[a] Calculation of the carbonyl value is based on the weight of fat used for the experiment.

TABLE IV

Effect of Reaction Time on Carbonyl Value of Thermally Oxidized Corn Oil

Time of reaction, hours	Carbonyl value, meq/1,000 g. fat
4	190
8	270
12	297
24	319
48	320

TABLE V

Recovery of Carbonyl Compounds from Synthetic Mixtures

Mixture	% Carbonyl added	Carbonyl value calculated	Experimental,[a] meq/1,000 g.
Fresh corn oil and n-hexanal [b]	0.0	0.0	0.0
	0.71	68.0	68.5
	1.32	126.5	128.2
	2.50	239.5	242.3
	3.60	344.9	341.3
	5.13	491.5	486.0
	9.07	869.0	861.6
Fresh corn oil and 2-heptanone [c]	0.86	74.3	75.8
	1.44	121.6	124.0
	3.34	286.7	292.1
	5.26	452.0	457.8
	8.38	719.3	707.0

[a] Average of three determinations.
[b] n-Hexanal 95.8% purity.
[c] 2-Heptanone 98.3% purity.

Small amounts of carboxylic acids are usually formed during thermal oxidation of a fat (11). A number of mixtures of thermally oxidized corn oil and free fatty acids were therefore prepared and analyzed for carbonyl value to determine the effect of free fatty acids (Table III). The results indicated that the addition of free fatty acids up to 6% to the thermally oxidized corn oil did not affect the carbonyl value. Addition of valeric acid to fresh corn oil containing 5.13% n-hexanol or 4.98% n-octanal was also not found to interfere in the carbonyl value determination (Table III). It was further observed that addition of 75 mg. of valeric, caproic, or palmitic acid to the blank solution used in the experimental procedure did not lower the pH of the solution. Free fatty acids were thus not found to interfere in the determination of carbonyl value when the titration of hydrochloric acid liberated from hydroxylamine hydrochloride was carried out up to the pH of the blank solution in the experimental procedure.

The effect of varying the time of reaction on the carbonyl value of thermally oxidized fat was carried out to determine the optimum time for completion of reaction (Table IV). Corn oil subjected to thermal oxidation for 24 hrs. at 200°C. required about 24 hrs. for the completion of the reaction at room temperature. There was no significant increase in carbonyl value when the reaction mixture was allowed to stand for 48 hrs.

Fresh corn oil was mixed with n-hexanal or 2-heptanone in varying proportions, and these were analyzed to study the recovery of carbonyl compounds (Table V). The experimental values agreed closely with the theoretical values; recovery was found to be between 98.5 and 102%. Reproducibility of the method was studied by carrying out triplicate analyses on four successive days, using thermally oxidized corn oil. Averages of triplicates were found to be in good agreement and there was no significant variation in the results when the analyses were carried out on different days (Table VI).

The method was applied to the estimation of the carbonyl value of heated fats. Fresh corn oil, cot-

TABLE VI

Reproducibility of Carbonyl Value

Day	Carbonyl value of T.O. corn oil,[a] meq/1,000 g.			Average
	A	B	C	
1	319.2	321.7	317.8	319.6
2	319.3	319.6	322.1	320.3
3	320.7	320.3	316.4	319.1
4	321.7	318.2	319.2	319.7

Average 319.7 ± 1.71.
[a] Corn oil heated for 24 hrs. at 200°C.

TABLE VII

Effect of Thermal Oxidation[a] on Carbonyl Value of Fats

Hours of heating	Corn oil		Carbonyl value as meq/1,000 g. cotton seed oil		Coconut oil	
	A	B	A	B	A	B
0	0	0	0	0	0	0
4	86	0	77	0	132	0
8	174	124	179	143	160	119
12	238	167	234	171	184	131
24	320	219	296	233	244	175

[a] Thermal oxidation carried out at 200°C.
A=Carbonyl value determined according to the present method.
B=Carbonyl value determined according to the method of Bryant and Smith (4).

tonseed oil, and coconut oil were subjected to thermal oxidation at 200°C. Samples were drawn at various intervals, and carbonyl values were determined (Table VII). It was observed that the carbonyl value of the fat increased with an increase in the time of heating. It was further observed that the method of Bryant and Smith (4) gave lower results for the carbonyl values of heated fats.

Thermally oxidized fats exhibit a dark brown color when they are dissolved in a solvent. This dark color was found to interfere with the detection of the end-point according to the method of Bryant and Smith (4), in which bromophenol blue is used as an indicator. Furthermore various solvents, namely, ethyl alcohol (4), isopropyl alcohol (14), and benzene (10) were not found to be suitable for the determination of carbonyl values of thermally oxidized fats because the reactants did not remain in solution during the reaction with hydroxylamine hydrochloride, resulting in an incomplete reaction. If the thermally oxidized fat is converted to methyl esters either by saponification, followed by methanolysis or by direct transmethylation, using sodium methoxide as a catalyst, it is possible to keep the various reactants in solution during reaction with hydroxylamine hydrochloride. However it was observed that there was a reduction in the carbonyl value of about 40% as compared with the results obtained by the present method (Table VIII).

In order to get an accurate estimation of the carbonyl value of a fat it is therefore desirable to maintain solution of the sample during reaction and to retain the glyceride structure of the fat. Among the various solvents investigated, n-octyl alcohol was found to be most effective in maintaining the fat in solution during the reaction with hydroxylamine hydrochloride. The difficulty of judging the end-point of the dark-colored solutions was overcome by using a pH meter for titrations.

TABLE VIII

Effect of Saponification or Transmethylation on Carbonyl Value

Sample	Carbonyl value meq/1,000 g.
T.O. corn oil[a]	433
Methyl esters of T.O. corn oil[b]	253
Methyl esters of T.O. corn oil[c]	240

[a] Corn oil heated for 48 hrs. at 200°C.
[b] By saponification and methanolysis.
[c] By transmethylation using sodium methoxide.

In the determination of carbonyl value by the present method there is a decrease in pH because of the release of hydrochloric acid, which is subsequently titrated against standard sodium hydroxide solution. Roe and Mitchell (6) have shown that inorganic and organic acids interfered with the determination of carbonyl compounds by the differential pH method, causing high results. These authors have however used only acetic acid to show the effect of free fatty acids. During thermal oxidation a fat is exposed to very high temperatures, namely, 180°C. or above. At this high temperature the fat is not expected to contain free acetic, propionic, or butyric acid. These fatty acids, if formed during thermal oxidation, will be removed from the oxidizing sample because of their low boiling point. In recent experiments carried out in our laboratory on the thermal oxidation of tripalmitin, monolauro-dipalmitin, and monoöleo-dipalmitin, free fatty acids were isolated from the thermally oxidized fats and analyzed by gas-liquid chromatography. None of the samples were found to contain low-molecular-weight fatty acids up to and including capric acid. Furthermore it is evident from the data presented in Table III that valeric acid or fatty acid with higher molecular weight, which are most likely to be present in thermally oxidized fat, do not interfere in the determination of carbonyl value by this method.

Although peroxides are known to interfere with the determination of carbonyl compounds (15), this problem does not arise in the case of thermally oxidized fats. The peroxides formed during oxidation are decomposed at the high temperature, and almost none is present in the final product. Therefore no attempt has been made in this paper to study the interference of peroxides in the determination of carbonyl value.

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