Interference of Peroxides with the Determination of Total Carbonyls in Autoxidized Fats[1]

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Abstract

The contribution of hydroperoxides to the carbonyl content of autoxidized fats measured by a colorimetric 2,4-dinitrophenylhydrazone procedure has been studied. Carbonyls were determined in radiation oxidized methyl myristate, in autoxidized methyl esters of oleic, linoleic and linolenic acids and in autoxidized oils, before and after reduction of hydroperoxides to hydroxyl groups.

The results indicate that hydroperoxides decompose to carbonyl compounds during the carbonyl determination and give carbonyl contents that are too high. The extent of the interference depends on the nature of the peroxides and, therefore, on the fatty acid composition of the material and on other factors probably associated with the conditions during autoxidation and subsequent storage. For these reasons it is not possible to apply a correction for peroxide interference based on the determined peroxide value.

Carbonyl determinations on autoxidized lipids should be preceded by reduction of the peroxides to non-carbonyl compounds, and care should be taken to prevent losses of low molecular weight carbonyls during this procedure.

Introduction

THE WELL-KNOWN but variable relationship between organoleptic rancidity and peroxide value is indirect and empirical because peroxides are odorless and tasteless. Carbonyl compounds with pungent odors and unpleasant flavors are also present in autoxidizing fats, and efforts have been made to correlate the organoleptic deterioration of fats with their carbonyl content. The measurement of carbonyls in fats, however, is not simple and the several methods described in the literature usually give different results (1).

A very sensitive method based on the colorimetric measurement of 2,4-dinitrophenylhydrazones in alkaline solutions was devised by Lappin and Clark (2) to determine traces of carbonyl in various media. Henick, Benca and Mitchell (3) modified the procedure and used it to measure carbonyl compounds in autoxidized fats. The method is rapid, direct and simple, and has been employed frequently. Because the results are usually higher than those of other carbonyl procedures, it has often been assumed that it gave a more reliable indication of the total carbonyls present in the fats.

However, several workers (1,4,5) have suggested that, under the conditions specified by Henick et al. (3), hydroperoxides are decomposed to carbonyls, thus giving results much higher than the true carbonyl content of the samples. Horikx (6) has shown that the hydroperoxides of oxidized methyl oleate give good yields of aldehyde 2,4-dinitrophenylhydrazones on a column of celite impregnated with dinitrophenylhydrazine-hydochloric acid. On the other hand, it has been reported that hydroperoxides decompose to hydroxyl groups under acidic conditions (7,8), and Schwartz (9) could find no carbonyl hydrazones when methyl linoleate hydroperoxides were passed over a column coated with 2,4-dinitrophenylhydrazine and phosphoric acid, although all the peroxides were destroyed.

In this study, information on the interference of peroxides with the measurement of carbonyls has been obtained by comparing the total carbonyl contents of several autoxidized lipids determined before and after reduction of the peroxides to non-carbonyl compounds.

Experimental

The quantitative reduction of hydroperoxides to hydroxyl groups is common practice with many investigators of lipid autoxidation, and it has been noted by us and by others (10,11) that fats recovered from reduction mixtures had decreased carbonyl contents. However, the following experiment showed that carbonyls could be easily lost during evaporation of solvents from the recovered reduced fat: A sample of methyl linoleate (peroxide value 202 mmole/kg) was reduced with stannous chloride as will be described later. Ether was used to extract the reduced fat from half the reduction mixture and the other half was extracted with benzene. After identical washing procedures the solvent was removed from portions of each extract as described in Table I, and the carbonyl content of the fractions was determined. The results in Table I show that recovery of reduced fat by solvent removal resulted in serious losses of carbonyls. To avoid these difficulties in subsequent work, the reduced lipids were extracted with benzene and the carbonyls were determined directly on the benzene solution without removing any solvent.

Preparation of Autoxidized Samples

All the methyl esters used were more than 99% pure. Methyl myristate was autoxidized at approximately 35C for 8 hr while exposed to the gamma rays of a cesium-137 source. The total dose received was approximately 10 megarads and a stream of finely dispersed oxygen was bubbled through the ester during the entire irradiation period.

The unsaturated methyl esters were autoxidized in layers 2 to 3 mm thick exposed to air at room temperature. The vegetable oils were commercial samples that had been stored for some time at room temperature. The corn, safflower and linseed oils had peroxide values of 92, 36 and 40, respectively, and

TABLE I

Losses of Carbonyls During Removal of Solvent from Reduced Autoxidized Methyl Linoleate

Material analyzed and treatment	Carbonyl content
	mmole/kg
Benezene extract — No solvent removed	74
Benezene extract — Solvent removed in stream of N_2 at 60C	30
Ether extract — Solvent removed in stream of N_2 at room temperature	39
Ether extract — Solvent removed under vacuum in rotating evaporator at 60C	32

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TABLE II

Effect of Reduction and Mock Reduction on Carbonyl Content

Sample	Carbonyl content [a]		
	Original	Mock reduced	Reduced
	mmole/kg	*mmole/kg*	*mmole/kg*
n-Heptaldehyde in fresh methyl oleate	35.4	37.3	37.0
Autoxidized methyl oleate	51.4	51.8	11.5
Autoxidized methyl oleate	39.6	38.9	11.2
Autoxidized methyl oleate	41.8	40.6	8.8
Autoxidized methyl	41.8	40.6	8.8

[a] Average of two determinations, each value deviating from the average by not more than 1.7%.

were examined without further treatment. The olive oil with a peroxide value of 4 was further oxidized in bulk by exposing it to ultraviolet light while a slow stream of oxygen was bubbled through it until a peroxide value of 56 had been reached.

All solvents were made carbonyl-free as recommended by Henick et al. (3). The solvents and reagent solutions were air-free and kept in an atmosphere of nitrogen, and all operations were performed under nitrogen.

Reduction and Carbonyl Determination

A 125 ml separatory funnel was flushed with a stream of purified nitrogen, and 100 mg of stannous chloride, 5.0 ml of methanol and 5.0 ml of benzene were added to the funnel. When the stannous chloride was completely dissolved, 1.0 ml of the peroxidized fat was introduced, the nitrogen inlet tube was removed and the funnel was tightly stoppered and allowed to stand at room temperature for 2 hr with occasional gentle shaking. The mixture was then diluted with 40 ml of a 20% KCl solution in 1.2 N aqueous HCl, extracted three times with 25 ml portions of benzene and the combined benzene extracts were washed four times with 25 ml portions of 30% KCl. (The use of KCl solutions minimized the formation of stubborn emulsions which occurred when water was used. In cases where slight emulsions did form, they were readily broken by adding small amounts of solid KCl.) The benzene solution was dried over anhydrous sodium sulfate and the solvent was completely removed from 10 ml portions for peroxide measurements (12) and to determine, gravimetrically, the concentration of fat in the solution.

Carbonyls were determined directly on 5 ml aliquots of the benzene solution by a slight modification (13) of the procedure described by Henick et al. (3).

Results and Discussion

If a decreased carbonyl content after peroxide reduction is to indicate that peroxides contribute to the Henick carbonyl value, then it must be established that the reduction procedure does not, in any way, change carbonyls that are already present in the peroxidized fat. It has been shown that hydroperoxides can be reduced quantitatively to hydroxyls by several procedures (14—16). The stannous chloride reagent was preferred by Privett et al. (15) because of its solubility in methanol and because the mild conditions of the reduction gave no evidence of side reactions. Consequently, no new carbonyl compound should be formed from the reduction of peroxides with stannous chloride. Barnard and Hargraves (17) found that ketones were unaffected and that aldehydes were reduced only very slightly by the dilute aqueous stannous chloride at room temperature. This was confirmed in an experiment in which a solution of *n*-heptaldehyde in pure unoxidized methyl oleate was subjected to the stannous chloride reduction procedure. As shown in Table II, no loss of carbonyl occurred as a result of reduction.

A decrease in carbonyl content after reduction could result also from losses of pre-formed carbonyls during extraction, washing and other manipulations necessary to carry out the reduction procedure. To test this possibility, carbonyl compounds were determined in oxidized methyl oleate before and after a mock reduction in which the samples were treated exactly as if they were reduced, except that no stannous chloride was used. As shown in Table II also, this mock reduction resulted in no change in the carbonyl content of the samples, while actual reduction of the peroxides gave a sharp decrease. Therefore, it can be concluded with confidence that a decrease in the carbonyl content of an autoxidized lipid, after stannous chloride reduction, must result directly from destruction of the peroxides and is not due to side reactions or losses caused by treatments and manipulations incidental to the reduction.

Table III shows the results obtained with oxidized samples of methyl esters of pure fatty acids and several oils. The last column indicates the portion of the peroxides estimated to yield carbonyl compounds when they are subjected to the conditions of the carbonyl determination of Henick et al. (3). Methyl myristate had a low peroxide value and the difference between the carbonyl contents of the original and reduced samples is only slightly larger than the estimated experimental error of the carbonyl determination. Furthermore, oxidation of the sample was promoted by high energy radiations and the nature of the oxidation products obtained under these conditions is uncertain. The high ratio of carbonyls to peroxides in this sample suggests that either carbonyls were formed directly or that, if peroxides were first produced, most of them were decomposed to carbonyls during irradiation. For these reasons, the results obtained with irradiation-oxidized methyl myristate will not be discussed further.

The data for the unsaturated methyl esters show that 72% of the oleate peroxides are converted to carbonyl compounds during the carbonyl determination. This conversion is much lower for linoleate and practically nil for linolenate. At first evaluation these figures might be interpreted to mean that methyl oleate peroxides are very easily decomposed while the peroxides from the more unsaturated esters are more stable under these experimental conditions. This point of view, however, is in conflict with the generally accepted belief that the stability of peroxides decreases with increasing unsaturation.

TABLE III

Effect of Peroxide Reduction on Carbonyl Values of Autoxidized Fats

Sample	Peroxide value		Carbonyl content		Peroxides converted to carbonyls [a]
	Original	Reduced	Original	Reduced	
	mmole/kg	*mmole/kg*	*mmole/kg*	*mmole/kg*	%
Methyl myristate	7.9	0	23.7	22.1	20.2
Methyl oleate	52.0	0	44.3	6.8	72.1
Methyl linoleate	203.0	3.6	59.3	34.4	12.5
Methyl linolenate	78.0	4.1	29.7	28.7	1.3
Olive oil	56.0	0	48.1	20.2	50.0
Corn oil	92.0	0	39.4	22.6	19.0
Safflower oil	36.0	0.9	30.4	26.5	11.0
Linseed oil	40.0	3.3	122.3	114.1	22.0

[a] Percent of total peroxides converted to carbonyls during the carbonyl analysis of the original material. Calculated from difference in carbonyl content before and after reduction assuming that 1 mole of peroxide yields 1 mole of carbonyl.

Another explanation may be based on the difference in stability of various hydroperoxides. In addition to the effect of unsaturation on stability, much evidence has been presented to indicate that autoxidation of any single unsaturated fatty acid methyl ester yields more than one peroxide and that the stability of these peroxides varies widely (18–22). It is possible, therefore, that at the time the autoxidized methyl esters were reduced and analyzed, essentially all the unstable peroxides of linolenate had already decomposed leaving only the more stable peroxides which, indeed, proved to be stable even to the conditions of the carbonyl determination. On the other hand, the "unstable" oleate peroxides which are considerably more resistant to decomposition under normal conditions than the linolenate peroxides, remained virtually unchanged before analysis but were decomposed to carbonyl compounds during the determination. The linoleate peroxides occupy an intermediate position with regard to their stability and their decomposition during the carbonyl determination. In support of this explanation may be cited the low ratios of peroxide value to true carbonyl content (determined after reduction) for linolenate (2.7), compared to linoleate (5.9) and oleate (7.6).

In general, the results obtained with the four vegetable oils agree with those from the pure methyl esters. Olive oil, the unsaturated component of which is almost exclusively oleic acid, behaves as methyl oleate and shows a high conversion of peroxides to carbonyls during the carbonyl determination. The more unsaturated oils containing linoleic and linolenic acids show less peroxide interference. In this study, clear-cut differences between corn, safflower and linseed oils should not be expected because the rate of autoxidation of individual unsaturated fatty acid in mixtures is much different from that of the pure compounds and also because these oils had been oxidizing under uncontrolled conditions for different periods of time.

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Preparation of 2-Hydroxytridecanenitrile from Petroselinic Acid

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Abstract

The cyanohydrin of dodecanal has been isolated in 90% crude yield from reaction of hydrogen cyanide formed in situ with the mixed aldehydes resulting from reductive ozonization of petroselinic acid. Attempts to isolate the cyanohydrin of the other fragment, adipaldehydic acid, were unsuccessful.

Introduction

IN THE SEARCH for new oilseed crops in the New Crops program of the U.S. Department of Agriculture, one of the families of plants selected for study has been the *Umbelliferae* (carrots, fennel, parsley, etc.). The seed oils of these plants contain 30–75% petroselinic (*cis*-6-octadecenoic) acid, an isomer of oleic acid found with very few exceptions only in this family of plants. Any industrial utilization of the oils would depend primarily on the petroselinic acid or its derivatives. Consequently, the utilization research on these oils has been concentrated on the chemistry of petroselinic acid and various derivatives of the acid.

Reductive ozonization of petroselinic acid gives a mixture of dodecanal and adipaldehydic acid (1). This paper reports the reaction of such a mixture with hydrogen cyanide and work done to isolate the products of the reaction. The reactions of these aldehydes with sodium cyanide and hydrochloric acid (the latter reagents yielding hydrogen cyanide in situ) are as follows:

$$CH_3(CH_2)_{10}CHO + NaCN + HCl \longrightarrow CH_3(CH_2)_{10}\underset{\displaystyle OH}{\underset{|}{CH}}—CN$$

$$HOOC(CH_2)_4CHO + NaCN + HCl \longrightarrow HOOC(CH_2)_4\underset{\displaystyle OH}{\underset{|}{CH}}—CN$$

These cyanohydrins, which do not appear to have been reported in the literature, would be versatile intermediates for preparing many useful products. They could be hydrolyzed to alpha-hydroxy acids, for example, or reduced to alkyl-substituted ethanolamines (alkylolamines). Alpha-hydroxy acids have found utility in preparing resinous products (2), improved lubricating greases (3), and stabilizers for vinyl

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