A Simple Method for Estimating Carbonyl Content in Peroxide-Containing Oils

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A simple method is described for the estimation of carbonyl content in peroxide-containing oils. In this method, peroxides, which interfere with carbonyl determination, are reduced with triphenyl phosphine prior to carbonyl determination. Carbonyl content is measured by a colorimetric 2,4-dinitrophenyl hydrazone procedure. Neither triphenyl phosphine nor triphenyl phosphine oxide, oxidation products of triphenyl phosphine by reaction with peroxides, interfere with the measurement of carbonyl content. The method is applicable to several kinds of oxidized oils in any oxidation stage.

The estimation of carbonyl compound content is a useful approach for the determination of the extent of lipid oxidation, because carbonyl compounds formed by the degradation of hydroperoxides induce rancid and unpleasant flavors more than other secondary oxidation products (1-3).

The most reliable and widely used method for the estimation of total carbonyl compound content in oil is the colorimetric measurement of 2,4-dinitrophenl hydrazones in alkali solution, outlined by Henick et al. (4). This method is rapid, simple, and sensitive, but has been criticized for use in the estimation of carbonyl content in peroxide-containing oils because hydroperoxides decompose to carbonyl compounds under the conditions for the carbonyl determination, yielding results much higher than the true carbonyl content of a sample oil (5-10).

Mizuno and Chipault (10) proposed a modified procedure which included reduction of hydroperoxides with stannous chloride to noncarbonyl compounds in benzene-methanol solution prior to carbonyl determination. Although neither side reactions nor loss of carbonyl compounds were reported during the reduction procedure, it was rather tedious because carbonyl compounds must be extracted with benzene from the reduction mixture. Fioriti (9) found that the decomposition of hydroperoxides was dependent on the reaction temperature in carbonyl determination, and that the interference from hydroperoxides could be reduced at 5°C, but under these mild conditions the reaction to form hydrazones must be carried out for 20 hr. These modified procedures are applicable to the estimation of carbonl content in peroxide-containing oils, but they have not been commonly used because they are often time-consuming and/or troublesome.

Thus, in this paper we propose a simple procedure involving the reduction of hydroperoxides with triphenyl phosphine (TP) prior to carbonyl determination. By using TP as a reducing reagent, the reduction time can be shortened by about 30 min over Mizuno's procedure of 2 hr (10), and the reduced mixture is directly applicable to the subsequent procedure. TP is a common reducing

reagent for organic hydroperoxides and is oxidized to triphenyl phosphine oxide (TPO) in the following reaction:

$$(C_6H_5)_3P + LOOH \rightarrow (C_6H_5)_3PO + LOH$$
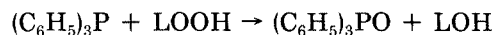
Simultaneously, lipid hydroperoxides (LOOH) in oxidized oils are reduced to hydroxy compounds (LOH), which have no influence on carbonyl determination. TP has been used as a reducing agent for analyzing the composition of lipid hydroperoxides and the positions of hydroperoxy groups in a fatty acid chain (11-14). A derivative of TP was recently used for fluorometrical determination of hydroperoxides in autoxidized oils (15,16).

EXPERIMENTAL PROCEDURES

Materials. All solvents were purified to a carbonyl-free state as described by Henick et al. (4). Triphenyl phosphine (Wako Pure Chem., Osaka, Japan) and triphenyl phosphine oxide (Aldrich, Milwaukee, WI) were used without further purification. All reagents were of either special purity or analytical grade. Soybean oil, safflower oil and corn oil were obtained from commercial suppliers and had peroxide values of less than 1.0 meq/kg before oxidation. Methyl linoleate (purity >99%, peroxide value <0.5 meq/kg) was prepared from methyl esters of safflower oil as previously described (17).

Autoxidation procedure. Oils were autoxidized in bulk at 46°C either under irradiation with 20 W fluorescent lamps or in the dark. Peroxide values of oxidized oils were determined by the AOCS official method (18).

Estimation of total carbonyl content. Weighed samples to be analyzed were dissolved in 5 ml of benzene solution containing TP (2.1 mg/5 ml), unless otherwise noted. The peroxide content in the mixture should be less than 8.0×10^{-6} mol. If the peroxide content in the mixture is higher than this level, a higher concentration of TP is required. The reaction mixtures stood for 30 min in the dark at room temperature. After reduction, carbonyl contents were directly estimated by a colorimetric method according to either of two procedures, Henick's (4) or Kumazawa's procedure (7,19). The total carbonyl content of one sample was also determined by a conventional procedure in which the weighed sample was dissolved in 5 ml of benzene and immediately measured for carbonyl content by both of the procedures. In both procedures, absorbances were measured against distilled water and were corrected for by subtracting the absorbance obtained from a blank solution which was prepared similarly, except it contained no sample. Although Kumazawa's procedure, which is a modified procedure of Henick's procedure, was unfamiliar outside of Japan, it has been employed in Japan as the standard method by the Japan Oil Chemists' Society (20), and has been used commonly ever since.

RESULTS AND DISCUSSION

Reactions of TP with peroxides in autoxidized oils. When TP-benzene solution was added to oxidized oils, peroxide

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TABLE 1

Effect of Triphenyl Phosphine Reduction on Peroxide Contents of Autoxidized Oils

| Autoxidized oil (Peroxide value: meq/kg) | Reduction time (min) | Peroxide content | | Amounts of reduced peroxides ($\times 10^{-5}$ mol) |
		Original ($\times 10^{-5}$ mol)	After reduction ($\times 10^{-5}$ mol)	
Methyl linoleate (265.7)	10	2.77	1.20	1.57
	30	3.04	1.44	1.60
	60	3.23	1.59	1.64
Corn oil (313.4)	30	5.98	3.98	2.02
Safflower oil (408.4)	30	9.26	7.24	2.00
Methyl linoleate (272.8)	30	1.33	nil	1.33
Safflower oil (408.4)	30	1.12	nil	1.12

Weighed samples and 10 ml of TP benzene solution (1.61×10^{-6} mol/ml) were mixed in an Erlenmeyer flask, and reacted for a definite time in the dark at room temperature. Then peroxide values of the reaction mixtures were directly measured. Peroxide content was calculated from peroxide values as molar amount of $-OOH$. Nil shows that no peroxide value can be detected by the iodometric method.

values of the oils decreased rapidly and became nearly constant within 30 min (Table 1). The molar quantity of the reduced peroxides with TP were nearly equivalent to the amount of TP added, and peroxides in oxidized oils could be completely eliminated by adding a greater amount of TP. Accordingly, a reaction time of 30 min was found to be sufficient for reducing peroxides in oxidized oils prior to carbonyl determination.

Influence of TP and TPO on carbonyl determination. Benzene solutions containing either TP or TPO were subjected to Kumazawa's procedure to examine whether either TP or TPO reacts with some reagents under conditions for carbonyl determination to give some additional absorbance. The relationships between the absorbances at 440 nm measured by the procedure and with the added amounts of TP or TPO are shown in Figure 1. The presence of TP brought about a slight increase of absorbance which rose linearly with the amount of TP added. On the other hand, with the addition of TPO, which was formed from TP by the reaction with hydroperoxides, the absorbances measured from the TPO-benzene solutions
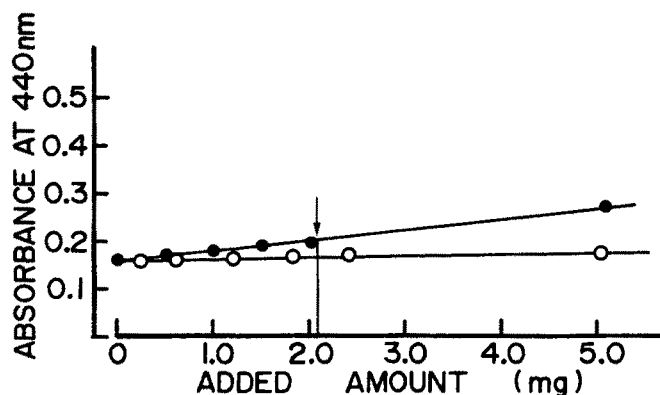

FIG. 1. Effect of added amount of triphenyl phosphine and triphenyl phosphine oxide on absorbance at 440 nm in carbonyl determination. Absorbance at 440 nm was measured by Kumazawa's procedure against distilled water. Five ml of benzene solution containing either triphenyl phosphine (●) or triphenyl phosphine oxide (○) (0.0–20.0 mg/5 ml) was used for a determination. The arrow indicates the level of TP in the standard procedure.

were nearly equal to those of the blank solution containing no TPO. Although the increase of absorbances by the addition of TP is undesirable for the estimation of carbonyl content, this could be compensated for by the subtraction of absorbances from the blank solution which was prepared in a similar manner, except that it did not contain a sample. In case of oils containing high levels of hydroperoxides, reducing the background absorbance caused by TP may be assumed to give low carbonyl content. However, these effects are not significant in autoxidized vegetable oils as shown in the next term. Figure 2 shows the absorption spectra of the reaction mixtures 15 min after the color development by alkali in Henick's procedure, authentic aldehydes being used as samples. Absorption spectra in the range of 400–500 nm derived from aldehyde-benzene solutions with and without TP overlay each other almost completely. Similar results were obtained from the determination by Kumazawa's procedure.

TP-reduction procedure did not impair the determination of the carbonyl content in benzene solutions containing an authentic aldehyde and the values obtained were reproducible (Table 2). Furthermore, a plot of the absorbances at 440 nm measured by the conventional procedure (x), against those by the TP-reduction procedure (y), with the variation in sample size of tr. tr. 2,4-decadienal gave a single straight line ($y = 0.987x + 0.011$) with a correlation coefficient, 0.999. These results suggest that TP does not affect the formation of 2,4-dinitrophenyl hydrazones and subsequent color development by alkali.

Determination of carbonyl content in oxidized oils. Table 3 shows the results of the application of the new procedure on oxidized oils. In these measurements 2.1 mg of TP, which is capable of reducing 8.0×10^{-6} mol hydroperoxides, was used on a test run. This amount is enough to reduce hydroperoxides in an aliquot of oxidized oils weighed for carbonyl determination. To assess the effect of hydroperoxides on the background absorption caused by TP, the carbonyl content was also calculated by taking account of the decrease of TP by the reaction with hydroperoxides. For comparison with another procedure proposed for carbonyl determination of peroxide-containing oils, we measured carbonyl content by Mizuno and Chipault's procedure (10) involving $SnCl_2$-reduction.
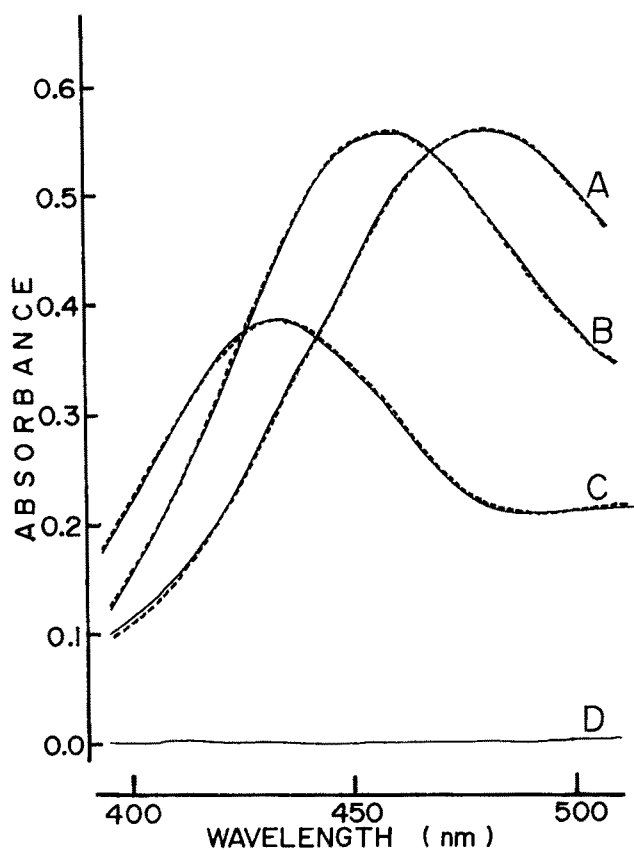
FIG. 2. Absorption spectra of reaction mixtures 15 min after addition of 4% KOH-ethanol in Henick's procedure with (——) and without (———) TP. These spectra were measured against a blank solution which was prepared in a similar manner, except that it contained no sample. Samples are A, *trans,trans*-2,4-decadienal (0.1128 mg); B, *trans*-2-hexenal (0.0984 mg); C, hexanal (0.1008 mg); and D, TP (9.64 mg).

TABLE 2

Effect of Addition of Triphenyl Phosphine on Absorbance at 440 nm in Carbonyl Determination

Carbonyl compound ($\times 10^{-2}$ mg)	Absorbance at 440 nm	
	Conventional	TP reduction
Hexanal (4.16)	0.319 ± 0.005	0.317 ± 0.003
Nonanal (8.30)	0.503 ± 0.001	0.509 ± 0.002
trans-2-Hexenal (6.40)	0.379 ± 0.003	0.378 ± 0.003
trans-2-Nonenal (4.68)	0.360 ± 0.001	0.357 ± 0.001
trans,trans-2,4-Heptadienal (4.96)	0.525 ± 0.001	0.526 ± 0.004

Absorbance at 440 nm was measured by Kumazawa's procedure against a blank solution which was prepared similarly except for removing a sample carbonyl. In TP-reduction procedure, 5 ml of carbonyl-benzene solution containing TP (2.1 mg/5 ml) was stood for 30 min in the dark at room temperature; then the mixture was subjected to Kumazawa's procedure. Values are mean ± S.D. of four determinations.

Carbonyl content of all oxidized oils considerably decreased after reduction, especially in oxidized oils with higher peroxide values (Table 3). Although the carbonyl content in autoxidized oils with high peroxide values was increased by the correction, the differences were less than 5%. Therefore, in most cases, any special background correction will be unnecessary. The values obtained from the TP-reduction method are comparable to those of the SnCl$_2$-reduction method. In some of the samples the values obtained from TP-reduction are lower than those from SnCl$_2$-reduction. When measured after reduction, the peroxide values of the sample oils were zero after TP-reduction, but not after SnCl$_2$-reduction. The remaining hydroperoxides in the SnCl$_2$-reduced oil is apparently

TABLE 3

Effect of Peroxide Reduction on Total Carbonyl Contents of Autoxidized Oils

Autoxidized oil	Carbonyl content (mmol as hexanal/kg)				Peroxide value (meq/kg)		
	Conventional	Reduced			Original	Reduced	
		TP	TP-corrected by peroxide value[a]	SnCl$_2$[b]		TP	SnCl$_2$[b]
Methyl linoleate	39.5	8.8 (25.7)	9.0	10.3 (24.4)	119.6	nil	2.9
Soybean oil	28.0	9.6 (38.0)	9.7	9.6 (38.1)	48.3	nil	nil
Soybean oil	30.7	9.5 (35.3)	9.7	14.2 (27.5)	60.0	nil	—[c]
Safflower oil	25.7	16.1 (27.7)	16.1	16.4 (26.8)	34.6	nil	nil
Safflower oil	42.5	14.4 (20.0)	14.8	20.2 (15.9)	140.4	nil	5.3
Safflower oil	88.0	24.3 (18.6)	25.4	41.8 (13.5)	343.0	nil	10.6

Carbonyl content was measured by Kumazawa's procedure. The value in parentheses shows percent of original peroxides converted to carbonyl during carbonyl determination of the conventional procedure and is calculated from difference in carbonyl content before and after reduction, assuming that 1 mol of peroxide yields 1 mol of carbonyl. Nil shows that no peroxide value can be detected by the iodometric method.

[a]The background absorbance by TP was reduced by taking account of the reaction with hydroperoxides.

[b]The procedure by reduction with SnCl$_2$ was according to Mizuno and Chipault (10).
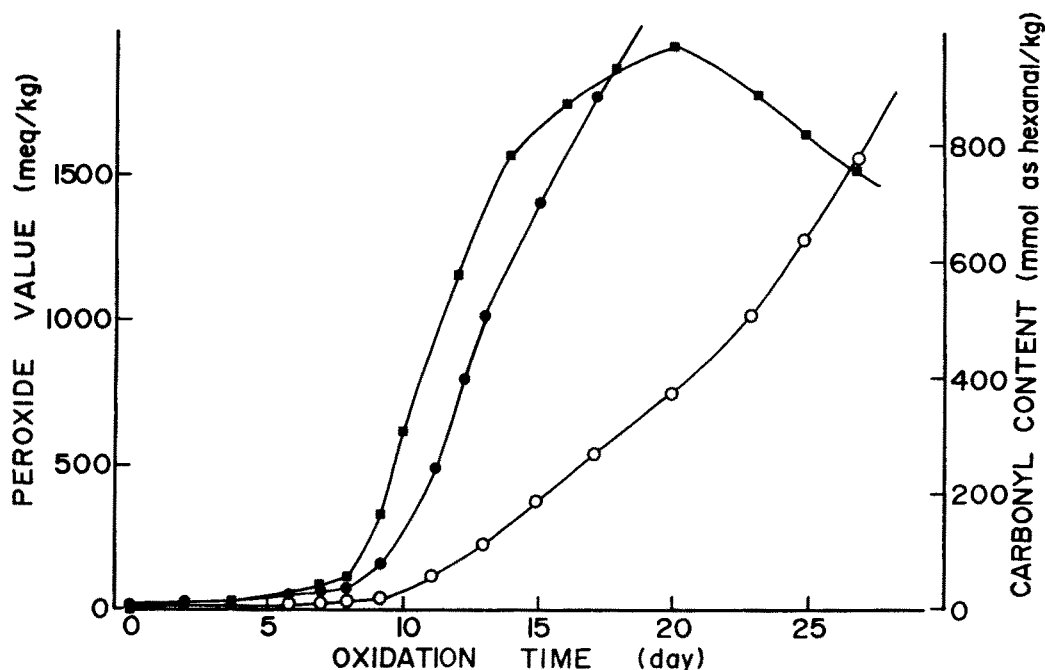
[c]Not measured.

FIG. 3. Changes of carbonyl content and peroxide value with oxidation time during autoxidation of safflower oil. Carbonyl content was measured by Kumazawa's procedure. Safflower oil was oxidized at 46°C under irradiation with a 20 W fluorescent lam. ●, Carbonyl content obtained from the conventional procedure; ○, carbonyl content obtained from the TP-reduction procedure; ■, peroxide value.

responsible for the higher carbonyl content in this method as compared to the TP-reduction method.

The time courses of carbonyl content and peroxide values during the autoxidation of safflower oil are illustrated in Figure 3. Carbonyl content determined by the conventional procedure changed with oxidation time as did the peroxide values, because they are strongly influenced by peroxide content (5-10), as shown in Table 3. However, the time course pattern of carbonyl content in the TP-reduction procedure was quite different. The carbonyl content in the TP-reduction procedure increased at one-third the rate of that in the conventional procedure after the induction period of eight days. The rate rose gradually to about two times higher after the peroxide value reached a maximum. This pattern is fairly consistent with the carbonyl-formation mechanism in lipid autoxidation (21,22).

The ratio of hydroperoxide-decomposition to carbonyls during the carbonyl determination by the conventional method was calculated assuming that one mole of hydroperoxide yields one mole of carbonyl compound. The values change with oxidation time; that is, in the beginning of oxidation, 18-30% of hydroperoxides were decomposed, while 35-40% were decomposed after the peroxide values reached a maximum. The lability of hydroperoxides in extensively oxidized oils under the conditions of carbonyl determination is thought to be attributable to the increase in hydroperoxide-bearing secondary products, such as dihydroperoxides and dimers (1,23).

Carbonyl content was also followed by the method of $SnCl_2$-reduction (10,19,20) during the autoxidation of safflower oil (data not shown), and showed a similar profile to that of the TP-reduction procedure in the early

stage of autoxidation. However, with the progress of oxidation the $SnCl_2$-method gave much larger values than those of the TP-method, and the recovery of sample oils decreased in the $SnCl_2$-method in which oils were extracted with benzene. Therefore, it is supposed that the TP-method is more suitable for highly oxidized oils than the $SnCl_2$-method.

The present study developed an improved method for total carbonyl content estimation in peroxide-containing oils. In this method peroxides are reduced with TP prior to carbonyl determination. This method is applicable to several kinds of oxidized oils in any oxidation stage.

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