A Highly Sensitive Method for the Micro-determination of Lipid Hydroperoxides by Potentiometry

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A modified method for peroxide value (POV) determination of lipids was developed through the application of potentiometry to conventional POV tests such as the official method of the Japan Oil Chemists' Society (JOCS). The new method permits a simple and reliable determination of low hydroperoxide levels in the initial stages of lipid autoxidation when only very small amounts of sample are available, even when those levels are measured on less than 10 mg of lipid. Using the present method, hydroperoxide levels as low as 20 nanoequivalents (neq) were determined with reasonable precision. This method is applicable to all lipids tested including oils and fats, free fatty acids, phospholipids, glycolipids and cholesterol esters.

A majority of the interest in the field of lipid chemistry currently lies in the influence of lipid peroxides in vivo, particularly in the area of geriatric diseases and aging. However, this biochemical field is limited to some degree, because there is no simple, highly sensitive and reliable method which can directly determine very small amounts of lipid hydroperoxides. Although some polyunsaturated fatty acids such as γ-linolenic, arachidonic, icosapentaenoic and docosahexaenoic acids are commercially available as precursors of prostaglandins and leukotrienes, it is very difficult to maintain the quality of these acids because they autoxidize easily.

Currently, there are many methods which determine small amounts of lipid peroxides including thiobarbituric acid (TBA) (1,2), chemiluminescence (3), enzymatic (4,5), coulometric (6), amperometric (7), Stamm (8) and GLC (9) procedures, but all have negative aspects which counter their merits. For example, one method may be simple to apply but unreliable or expensive while another may be highly sensitive but difficult to control. Therefore, we have modified the JOCS official POV method (10), which is a frequently used iodometric titration method and is based on the stoichiometric reaction between lipid hydroperoxide and potassium iodide. The JOCS official method (10) is not accurate for low POV because of difficulties in determining the titration end-point by the iodine-starch color reaction. Hence, we modified the final step of titration by replacing it with potentiometric determination. As the main reaction procedure was not changed, comparable results were obtained.

EXPERIMENTAL PROCEDURES

Principle. All substances that oxidize potassium iodide stoichiometrically under the conditions of the test are determined titrimetrically in terms of milliequivalents (meq) of peroxide per one kg of sample as shown below. Those substances are assumed mainly to be hydroperoxides, which are primary products of lipid autoxidation.

$$-CH_2-\underset{\displaystyle OOH}{\underset{|}{CH}}-CH=CH-CH_2- + KI \rightarrow$$

$$-CH_2-\underset{\displaystyle OH}{\underset{|}{CH}}-CH=CH-CH_2- + I_2 + K_2O$$

$$I_2 + Na_2S_2O_3 \rightarrow NaI + Na_2S_4O_6$$

Equipment. For convenience, an automatic titrator (Mitsubishi Chemical Industries Ltd., Tokyo, Japan, Model GT-05) was used, equipped with a platinum combination electrode and a burette which is able to provide a minimum droplet of four μl. Also used was a potentiometer (Corning Glass Works, Medifield, Massachusetts, Model-130 pH Meter) equipped with a platinum and reference electrodes, and a repeating microsyringe as the burette.

Reagent. All chemicals used were of commercial analytical grades. Distilled water for dissolving reagents was used after purging with nitrogen. Saturated potassium iodide solution was prepared by dissolving potassium iodide into freshly distilled boiled water and kept in a brown bottle covered with aluminum foil to protect the solution from light. That the solution remains saturated was indicated by the presence of undissolved crystals. N/1000 sodium thiosulfate solution was prepared as follows: 0.25 g of sodium thiosulfate was dissolved in freshly distilled water containing no carbon dioxide and brought to 1000 ml, followed by potentiometric standardization using authentic potassium dichromate solution. It is necessary to check the titer of this solution prior to use because of its instability. When the peroxide level of a sample was high, either smaller amounts of the sample were taken for determination or a higher concentration of sodium thiosulfate solution was used.

TABLE 1

Effect of Sample Amounts on POV Determination by JOCS Official Method (10)

Sample (g)	POV (meq/kg)		
	Sample(1)[a]	Sample(2)[a]	Sample(3)[b]
5.00	32.79 ± 0.21	25.95 ± 0.76	1.91 ± 0.02
1.00	32.83 ± 0.18	25.98 ± 0.86	1.91 ± 0.01
0.50	33.07 ± 2.42	26.33 ± 1.04	2.00 ± 0.06
0.20	—	—	1.92 ± 0.08
0.10	46.27 ± 9.53	19.66 ± 2.46	1.91 ± 0.24
0.07	—	—	1.57 ± 0.11
0.05	50.19 ± 5.64	19.60 ± 8.23	—
0.01	—[c]	—[c]	—[c]

[a]Used with N/100 $Na_2S_2O_3$.
[b]Used with N/1000 $Na_2S_2O_3$.
[c]Undetectable.

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TABLE 2

Comparison Between JOCS Official Method (10) and Potentiometry for POV Determination of Fresh and Autoxidized Salad Oils

Sample[a]	POV (meq/kg)[b]	
	JOCS official method	Potentiometry
1	0.49 ± 0.05	0.54 ± 0.01
2	1.58 ± 0.07	1.60 ± 0.05
3	25.72 ± 0.95	26.50 ± 0.42
4	32.83 ± 0.18	34.10 ± 0.73

[a]Sample volume used, 1 g.
[b]n,10.

Procedure. The determination was conducted as follows: (i) a few mg of sample were weighed in a 250-ml glass-stoppered wide mouth bottle; (ii) the sample was completely dissolved in 10 ml of chloroform, then 15 ml of glacial acetic acid was added and swirled in the bottle; (iii) after air was substituted in the bottle with a stream of either pure nitrogen or carbon dioxide gas (11), exactly 0.3 ml of saturated potassium iodide solution was added and swirled in the bottle for one min, followed by cooling in an ice bath for five min in a dark area; (iv) 100 ml of ice-cold distilled water was added and shaken vigorously, then potentiometric titration was conducted using N/1000 sodium thiosulfate with the bottle kept in an ice bath. The end-point of titration was calculated by the second differential method. The amount of N/1000 sodium thiosulfate solution used for a blank test must not exceed 0.15 ml. If the blank run had volumes more than 0.15 ml of N/1000 sodium thiosulfate solution, the saturated potassium iodide solution must be reprepared for POV measurement.

Calculation. The peroxide value was calculated as milli-equivalents (meq) of peroxide/kg of sample:

$$\text{POV (meq/kg)} = (A-B) \times F \times N \times 1000/C$$

where A is the number of ml of N/1000 sodium thiosulfate solution used in the main experiment; B is the number of ml of N/1000 sodium thiosulfate solution used in the blank run; C is the weight of sample (g); F is the titer of N/1000 sodium thiosulfate solution and N is the normality of sodium thiosulfate solution.

TABLE 3

Effect of Sample Amounts on Low POV Determination by Potentiometry

Sample (g)	POV (meq/kg)	
	Sample(1)[a]	Sample(2)[a]
1.00	2.43 ± 0.14	0.74 ± 0.04
0.20	2.35 ± 0.09	0.70 ± 0.01
0.10	2.40 ± 0.24	0.71 ± 0.03
0.08	2.43 ± 0.09	0.72 ± 0.42
0.06	2.49 ± 0.08	0.59 ± 0.90
0.04	2.45 ± 0.02	—
0.02	2.49 ± 0.06	—
0.01	2.83 ± 0.19	—

Used with N/1000 $Na_2S_2O_3$.
[a]n,10.

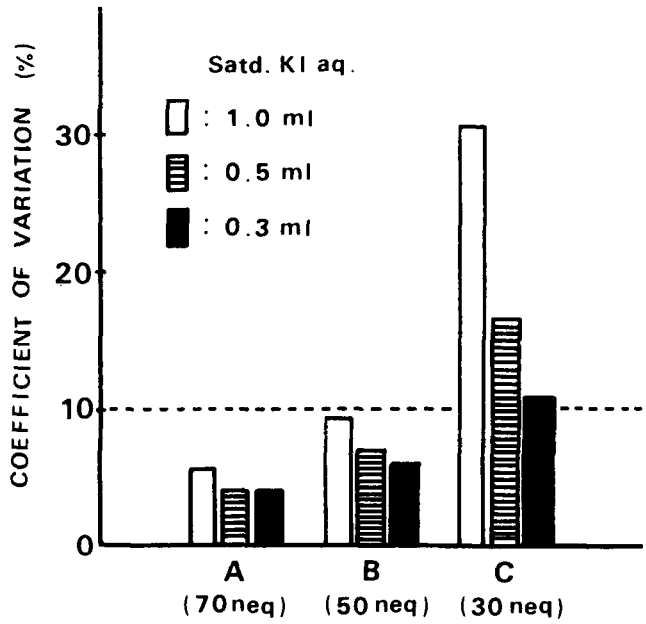

FIG. 1. Effect of saturated aq. KI amounts on peroxide determination by potentiometry.

RESULTS AND DISCUSSION

Prior to the development of this new method, we investigated how POVs are affected by sample amounts in conventional methods. Table 1 shows the POV of plant oil determined by the JOCS official method (10). Using N/100 sodium thiosulfate solution, this method required at least 0.5 g of sample when the POV was roughly 30 meq/kg. In the case of titration with N/1000 sodium thiosulfate solution, the POV of 0.1 g sample was determinable when the POV was roughly two meq/kg. However, the violet coloration accompanying the 1% starch solution end-point was too pale for the low level of peroxides to be ascertainable. Table 2 shows the results of the POV measurements of autoxidized plant oils by the JOCS official method (10) and potentiometry. The use of potentiometry gave higher values compared to the JOCS official method (10) because of the shifting of the end-point, but it proved to be useful for POV determination. Table 3 shows the detection limits of low levels of the POV by potentiometry. The mean values and their standard deviations calculated from 10 measurements of each sample are shown in the table.

TABLE 4

Potentiometric Determination of Peroxides at Room Temperature and Ice-cold Conditions

Sample	Room temperature		Ice-cold condition	
	Peroxide (neq)	CV (%)[a]	Peroxide (neq)	CV (%)[a]
1	61.7	6.0	61.6	5.8
2	38.0	11.3	38.3	6.8
3	25.1	16.8	24.9	8.9

[a]Coefficient of variation (n,5).

TABLE 5

POV Determination of Different Lipids Containing Low Levels of Peroxides by Potentiometry

Sample	Sample weight (mg)	POV (meq/kg)	CV[a] (%)	Peroxides (neq)
Soybean oil	36.4	0.68 ± 0.03	4.4	24.8
Linoleic acid	11.2	4.22 ± 0.26	6.2	47.3
Cholesterol	20.0	3.03 ± 0.28	9.3	60.6
Soybean phospholipid	25.0	2.40 ± 0.09	3.8	60.0

[a]Coefficient of variation (n,10).

Judging from these results, it is possible to use only 20 mg of lipids when the POV is higher than 1 meq/kg, and 100 mg of lipids when it is lower than one meq/kg. The present method is 100 times more sensitive than the JOCS official method (10). Because the one ml of saturated potassium iodide solution used in the official method (10) was considered too high for determination of a low POV (less than 100 meq/kg) sample, we adjusted the amount of the solution for potentiometry to achieve much higher reliability, as shown in Figure 1. It is well known that saturated potassium iodide solution is easily oxidized with atmospheric oxygen to produce iodine and is difficult to keep fresh. Therefore, iodometry with sodium thiosulfate should be performed on the blank run, and less saturated potassium iodide solution should be used in order to avoid titration error. In the present experiment, the free iodine contained in the saturated potassium iodide solution was monitored potentiometrically as a blank run, and the amount of saturated potassium iodide solution was reduced from one ml, as in the JOCS official method (10), to 0.5 ml and 0.3 ml. The determination was conducted seven times each on samples that contained 70, 50 and 30 neq of peroxides. It was apparent from Figure 1 that the coefficient of variation of the POV became much smaller with a decrease in the amount of potassium iodide. We concluded that such changes in the coefficient of variation were caused by a very small amount of free iodine in the saturated potassium iodide solution, because lower amounts of the solution or higher levels of hydroperoxides gave smaller coefficients of variation. Moreover, the time for titration was shorter, and the endpoint of titration clearer, with such a decrease, giving more reliable results. As these results indicate, this modification has made it possible to detect about 40 neq hydroperoxides with high accuracy and rapidity.

We were able to obtain better results by cooling the system throughout the entire reaction and titration periods. It was necessary to keep a constant reaction temperature due to the fact that the solubility of potassium iodide in water is influenced by the atmospheric temperature and potassium iodide easily reacts with ambient oxygen to produce free iodine at higher temperatures in which lipids are also easily peroxidized.

It was obvious from Table 4 that POVs obtained from five min standing with occasional shaking under cool conditions showed smaller coefficient of variations compared to the results under room temperature, although the iodometric reaction is conducted at room temperature for five min for the JOCS (10) and one min for the AOCS (12) official methods.

Results from the potentiometric determination of POV of various lipid samples are shown in Table 5, where the sample weight was determined as follows: a few hundred mg of each lipid was weighed exactly into a 200-ml volumetric flask and dissolved with chloroform; then, 10 ml of each was pipetted into a 250-ml glass-stoppered wide mouth bottle.

Application of this highly sensitive and reliable method for POV determination in biological studies, quality control of polyunsaturated fatty acids and phospholipids indicates that lipid hydroperoxides can be determined at very low concentrations (around 20 neq).

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