Adaptation of the AOCS Official Method for Measuring Hydroperoxides from Small-Scale Oil Samples

Tammy D. Crowe and Pamela J. White*

Food Science and Human Nutrition Department and Center for Crops Utilization Research, Iowa State University, Ames, Iowa 50011

ABSTRACT: An adaptation of the American Oil Chemists' Society Official Method Cd 8-53 for determining peroxides in fats and oils using a 0.5-g sample is described. Comparisons of the Official Method and the small-scale method were performed by analyzing soybean oil samples spiked with *t*-butyl hydroperoxide and autoxidized soybean oil samples. A linear relationship between the Official Method and the small-scale method was obtained with an R^2 of 0.998. The small-scale method is sensitive, precise, and suitable for small sample sizes and uses only about 10% of the chemicals necessary for the Official Method.

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KEY WORDS: AOCS Official Method, hydroperoxides, method for small oil sample, micro-peroxide value method, oil analyses, peroxide value.

The American Oil Chemists' Society (AOCS) Official Method Cd 8-53 for determining peroxide value (PV) is an effective, easy-to-use method for which the equipment and glassware required are readily available in most laboratories. One negative aspect of this method is the need for a relatively large sample size of ~5.0 g (1). In some research studies, oil quantities may be limited, either by physical space during storage studies or by sample availability, as in the case of experimental or exotic oilseed crops. Thus, the AOCS procedure is not a viable method in these situations. Several methods suitable for use with small sample sizes have been proposed (2–5); however, these methods are often too complex for evaluation of small numbers of samples and can require special reagents or equipment. The use of smaller sample sizes with the AOCS Official Method would result in less chemical waste and reduced exposure to potentially toxic solvents such as chloroform. The objective of this study was to modify and evaluate the AOCS Official Method Cd 8-53 of PV determination for use with 0.5-g oil samples, a sample size of about 10% of the weight recommended in the AOCS Official Method.

EXPERIMENTAL PROCEDURES

Refined, bleached, and deodorized (RBD) soybean oil was obtained from Archer Daniels Midland Company (Decatur, IL). Three sets of samples were evaluated: stored soybean oil samples (Oil Set I), standard soybean oil samples spiked with different amounts of *t*-butyl hydroperoxide (TBHP; Oil Set II), and a variety of oils from several sources oxidized to various levels (Oil Set III). The PV analyses by the official and small-scale methods were performed in triplicate. The entire experiment was replicated three times for Oil Sets I and II.

Stored soybean oil (Oil Set I). To simulate oxidative stability testing conditions, RBD soybean oil was subjected to accelerated storage conditions. The oil was stored in a 60°C oven in the dark for 14 d in 100-mL beakers containing 50 mL of oil [surface area-to-volume ratio 0.03 (mm^2/mL)] and loosely covered with plastic wrap. The PV were measured every 2 d during storage.

Standard RBD soybean oil spiked with TBHP (Oil Set II). A 5.5-M solution of TBHP (Aldrich Chemicals, Milwaukee, WI) in isooctane was prepared in the laboratory. Various amounts of the TBHP solution were added to the RBD soybean oil, which had been sparged at 50°C with helium for 16 h to destroy any existing hydroperoxides, to produce PV ranging from 0 to 100 meq/kg oil.

Oil from various sources (Oil Set III). To evaluate the small-scale PV method in a variety of samples, several oils purchased at a local grocery store (soybean, corn, olive, and sunflower oils) or extracted in our laboratory using supercritical carbon dioxide extraction (walnut oils 1 and 2) were analyzed for PV in triplicate. These oils were allowed to oxidize at room temperature for periods of between 2 wk and 2 yr.

AOCS Official Method Cd 8-53. The AOCS iodometric procedure Cd 8-53 (1) was performed without modification using oil samples of approximately 5.0 g. A 0.1 N solution of sodium thiosulfate ($Na_2S_2O_3$) was prepared and standardized according to the Association of Official Analytical Chemists (AOAC) Method #942.27 (6).

Small-scale method. A 0.001 N sodium thiosulfate solution was prepared by diluting the 0.1 N solution 100 times with boiled Milli-Q water. The 0.001 N solution was standardized according to the AOAC Method #942.27 (6). The AOCS iodometric procedure Cd 8-53 (1) was performed using a 0.5-g sample, with all reagents at 10% of the amounts recommended for the standard procedure. Oil samples were titrated with a 0.001 N sodium thiosulfate solution into 50-mL beakers.

Statistical analysis. Data were analyzed by analysis of variance (ANOVA) using the General Linear Model procedure of SAS (7). Coefficients of determination (R^2) and coefficients of variation were determined to evaluate the suitability of the method. Significance was established at $P < 0.05$.

*To whom correspondence should be addressed at Food Science and Human Nutrition Department, Iowa State University, 2312 Food Sciences Building, Ames, IA 50011-1060. E-mail: pjwhite@iastate.edu

TABLE 1
Peroxide Values (PV) of Oxidized Soybean Oils (Oil Set I) Determined by American Oil Chemists' Society (AOCS) and Small-Scale Methods

Method	PV (meq/kg) at specified days								Overall mean (%)	CV[a] (%)
	0	2	4	6	8	10	12	14		
AOCS Official	0.4	15.7	41.8	49.9	66.3	78.7	91.6	100.7	55.6	1.3
Small-scale	0.5	15.7	43.8	49.3	64.9	76.2	91.8	100.8	55.4	2.7

[a]CV, coefficient of variation.

RESULTS AND DISCUSSION

Oil Set I. Table 1 shows the PV determined by the AOCS Official Method and by the small-scale method. There was no significant ($P < 0.05$) difference in the overall means for the PV determinations by the two methods in samples oxidized at 60°C in the dark with PV levels of 0 to 100 meq/kg of oil. The percentage difference between the two methods was greatest at the lowest PV level. Subjective determination of the endpoint in this procedure was difficult, particularly in samples with low PV, so a 50-mL beaker was used for the small-scale procedure in place of the Erlenmeyer flask recommended in the Official Method. The greater depth of field of the beaker allowed easier visualization of the colorimetric endpoint; however, visualization at low PV was still difficult. The greater differences at these low PV was likely a result of human error in judgment of the endpoint. Figure 1 shows a plot of the relationship between the PV obtained by the Official Method and those obtained using the small-scale method. The correlation coefficient (R^2) between the two methods was 0.998.

Oil Set II. Similarly, a comparison of the methods using samples prepared from sparged soybean oil with various levels of added TBHP to produce a PV range of 0 to 100 resulted in an R^2 value of 0.994 (Fig. 2). The PV obtained from both the AOCS Official Method and the small-scale method were linearly related to each other within the 0–100 PV range. This range is expansive enough to encompass PV of most fats and oils during normal and accelerated storage conditions.

Oil Set III. Because the iodometric titration method is highly empirical, the results and accuracy of the test are strongly dependent on experimental conditions, including sample type. The results from PV determinations of various oil samples using both the AOCS Official Method and the small-scale method are shown in Table 2. Both methods resulted in similar PV for each of the different oils, with relatively small standard deviations.

The two principal sources of error in the iodometric methods for the determination of peroxides are (i) the absorption of iodine at unsaturated bonds of the fatty material and (ii) the liberation of iodine from potassium iodide by oxygen present in the solution being titrated (8). Oxygen in the solution

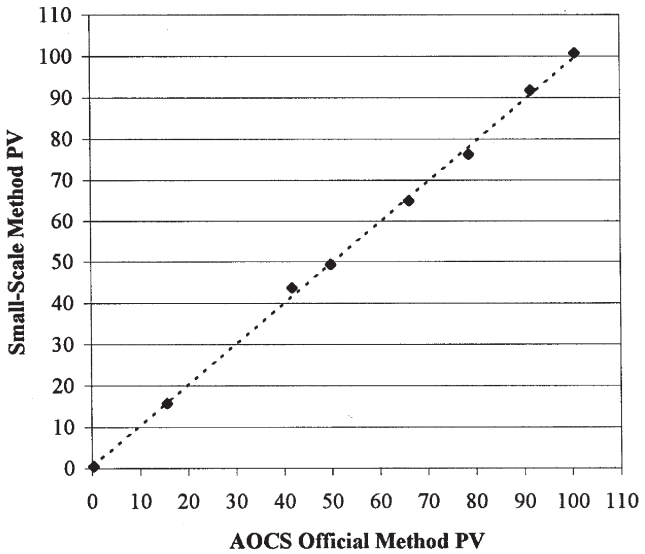

FIG. 1. Relationship between the AOCS Official Method and the small-scale method for determination of peroxide value (PV; meq/kg) in autoxidized soybean oil (Oil Set I). The correlation coefficient (R^2 value) between the two methods was 0.998.

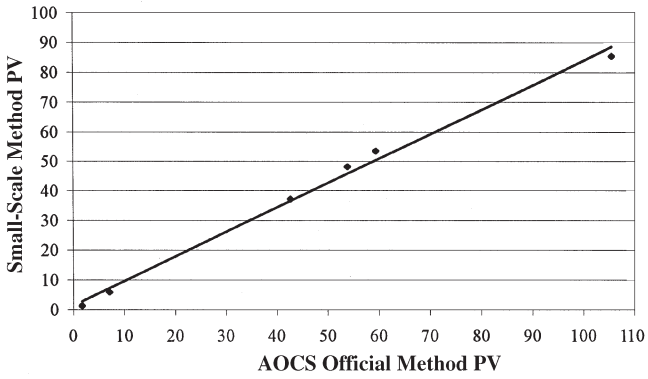

FIG. 2. Relationship between the AOCS Official Method and the small-scale method for determination of PV (meq/kg) in soybean oil spiked with *t*-butyl hydroperoxide (Oil Set II). The correlation coefficient (R^2 value) between the two methods was 0.994. See Figure 1 for abbreviation.

TABLE 2
PV of Autoxidized Oils (Oil Set III) Determined by the AOCS and Small-Scale Methods

Oil	PV[a] (meq/kg)	
	AOCS Official Method	Small-scale method
Soybean	5.3 ± 0.15	5.1 ± 0.15
Corn	12.3 ± 0.22	12.6 ± 0.26
Olive	8.9 ± 0.28	9.3 ± 0.21
Sunflower	26.7 ± 0.75	28.0 ± 0.92
Walnut #1	1.2 ± 0.15	1.4 ± 0.20
Walnut #2	9.8 ± 0.25	9.5 ± 0.30

[a]Mean of three replications ± standard deviation. For abbreviations see Table 1.

of a sample causes the liberation of iodine from potassium iodide by the following reaction:

$$4\ I^- + O_2\ (air) + 4\ H^+ \rightarrow 2\ I_2 + 2\ H_2O \quad [1]$$

This reaction, which is accelerated in the presence of light and peroxides, is sometimes referred to as the oxygen error and leads to high results in peroxide determination. The assumption that subtracting a blank determination negates this error may be incorrect, because the effect of oxygen is more pronounced in the presence of peroxides.

Because of the large surface area-to-volume ratio of the samples used for the small-scale method, there was concern that oxygen would be rapidly absorbed into the sample, resulting in elevated PV measurements. To attenuate the risk of oxygen error, each sample was weighed and immediately analyzed before the next sample was weighed. Preliminary determinations of PV in RBD soybean oil samples indicated that this precaution reduced coefficients of variation, particularly in samples with high levels of peroxides. For the small-scale method, standardizing the 0.001 N sodium thiosulfate, which was prepared by diluting the 0.1 N solution, also reduced error, particularly at very low (0–2 PV) peroxide levels.

In addition, variation in weight of sample, variation in reaction conditions, such as time and temperature, the type and grade of solvent used, and the types and reactivity of the peroxides being titrated can significantly influence measured PV. In an effort to test this method under the most lax conditions, thus maximizing the ease and convenience of the small-scale method, no efforts to control temperature, such as utilization of ice baths, were made. All samples were analyzed under ambient conditions with both Official Method and small-scale method determinations for individual samples being performed on the same day.

The type of peroxide present in a sample also can influence the liberation of iodine. For example, dialkyl peroxides, which may be formed during the termination reaction of fat oxidation, are much less easily reduced than are hydroperoxides (9). For this reason, it is important that validation of tests designed to measure PV include side-by-side methodological comparisons of samples with known storage histories. Simply adding known quantities of peroxides may not be indicative of how a test will perform under real conditions, because as oxidation progresses, several stages of oxidation occur simultaneously. Thus, several different species of peroxides, with varying degrees of reducibility, may exist concurrently in a given sample.

Results from the small-scale method agree closely with those obtained from the AOCS Official Method over a wide PV range with soybean oil as well as plant oils from other sources. The small-scale method is advantageous because its use greatly reduces the use and disposal of organic solvents and is effective for evaluation of small (0.5 g) oil samples.

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