Comparison of Four Analytical Methods for the Determination of Peroxide Value in Oxidized Soybean Oils

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ABSTRACT: Previous work in our laboratory demonstrated that soybean oil oxidation, expressed as PV, can be determined using NIR transmission spectroscopy as an alternative to the official AOCS iodometric titration method. In the present study, a comparison of four peroxide analytical methods was conducted using oxidized soybean oil. The methods included the official AOCS iodometric titration, the newly developed NIR method, the PeroxySafe™ kit, and a ferrous xylenol orange (FOX) method, the latter two being colorimetric methods based on oxidation of iron. Five different commercially available soybean oils were exposed to fluorescent light to obtain PV levels of 0–20 meq/kg; periodic sampling was done to ensure having representative samples throughout the designated range. A total of 46 oil samples were analyzed. Statistical analysis of the data showed that the correlation coefficient (*r*) and standard deviation of differences (SDD) between the standard titration and NIR methods were $r = 0.991$, SDD = 0.72 meq/kg; between titration and the PeroxySafe™ kit were $r = 0.993$, SDD = 0.56 meq/kg; and between the standard titration and FOX method were $r = 0.975$, SDD = 2.3 meq/kg. The high correlations between the titration, NIR, and PeroxySafe™ kit data indicated that these methods were equivalent.

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KEY WORDS: Edible oil, lipid oxidation, method comparison, near-infrared spectroscopy, peroxide value, PeroxySafe™ kit, soybean oil, xylenol orange.

PV is most commonly used as an indicator of the early stages of oxidation in fats and oils. Warner *et al*. (1) reported ranges of PV for oxidized vegetable oils, including soybean, sunflower, and canola, to be 3–5 for low oxidation, 10–12 for moderate oxidation, and 16–18 for high oxidation. Existing analytical procedures for measuring the oxidation level in vegetable oils are simple but also time-consuming, destructive to the sample, costly, and require large amounts of potentially hazardous reagents. An advantage of the PV determination is that it directly measures the lipid peroxides, which are the primary lipid oxidation products. On the other hand, a major disadvantage is susceptibility to interference by molecular oxygen, as well as the reaction of liberated iodine with other components in the system (2).

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Over the past few years, numerous new methods have been developed for measuring PV. These studies have described rapid methods for the quantitative determination of PV of vegetable oils and different foods by FTIR transmission spectroscopy (3–5); by coordination ion spray mass spectrometry (CIS/MS) (6); by GC–MS (7); by HPLC (8–14); by IR and NMR spectroscopic techniques (15); by conventional, difference, or difference-derivative UV spectrophotometry (16); by thermogravimetry (17); by measuring thermoluminescence (18); and by using fluorescence and autofluorescence spectroscopy (19,20). A simple and sensitive spectrophotometric lipid hydroperoxide measurement using oxidation of ferrous (Fe^{2+}) to ferric ions (Fe^{3+}) by hydroperoxides has already been developed. Under acidic conditions and in the presence of a ferric ion indicator, xylenol orange (XO), this ferrous xylenol orange (FOX) method has been successfully applied to edible vegetable oils and fats, to the estimation of dietary hydroperoxide intakes (21,22), and to biological samples (23–26). In addition to edible oils, the FOX method has been successfully applied to determine the PV of beef, chicken, butter, fish, vegetable products (27), and raw and cooked dark chicken meat (28). The FOX method also has been reported to have high sensitivity, being comparable to or even better than the iodometric method (21–23), and is the official method of the International Dairy Federation (27,29).

Additionally, a PeroxySafe™ kit has recently been made available (Diamed AG, Cressier sur Morat, Switzerland; marketed in North America by Safety Associates, Inc., Tustin, CA). This is also a colorimetric method that measures lipid peroxide concentration in oil samples based on the hydroperoxide-mediated oxidation of acidified iron. The results of the method are expressed in milliequivalents (meq) of peroxide per kilogram (kg) of oil.

Our previous study (30) demonstrated that soybean oil (SBO) PV was successfully determined by NIR spectroscopy, giving results comparable to the AOCS standard titration method (31). This new method is simple, fast, and safe, and by applying it, the amount of hazardous solvents as well as the cost of labor can be reduced dramatically.

The objective of the present research was to compare and evaluate PV determination in oxidized SBO using four analytical methods. The AOCS standard titration method (Cd 8-53; Ref. 31) was compared with other available alternative analytical methods including the NIR spectroscopic method

previously developed in our laboratory, the PeroxySafe kit, and a FOX method based on the procedure of Nourooz-Zadeh *et al.* (22).

EXPERIMENTAL PROCEDURES

Samples. Five SBO samples were purchased from local supermarkets in Lincoln, Nebraska. To achieve variation in the oils, samples were selected from different commercial brands of nonhydrogenated, fully refined, and bleached oils. Oils had been winterized and also contained added citric acid. Care was taken to avoid duplication of samples. This was done by tracking "sell by" dates and batch codes, if available, from packaging. It was assumed that containers from the same batch would not have different "sell by" dates.

Oxidation of SBO. A light-catalyzed system was used to generate different levels of oxidation. Subsamples of 100 g from each of the five commercial samples were placed under a fluorescent light source (4200 lux) as described by Hall (32). The oils were exposed to fluorescent light to obtain PV levels of 0–20 meq/kg; periodic sampling was done to ensure having representative samples throughout the designated range. At each sampling, the AOCS iodometric titration method and the FOX method were conducted. The samples were then placed under nitrogen gas in brown glass bottles with minimal headspace and kept in a freezer (–100°C) until analyzed using the PeroxySafe kit and NIR. A total of 46 oil samples were analyzed.

Standard titration method. The oxidation level of each oxidized SBO sample was analyzed under the same conditions according to AOCS Official Method Cd 8-53 for PV (31). Means were calculated from duplicate analyses. All solvents and reagents used were of analytical grade.

PeroxySafe™ kit assay. This assay is based on two different kits: one intended for oil samples with peroxide levels >2 meq/kg (2.0 to 20 meq/kg, PeroxySafe kit for pressed and nutritional oils) and the other for peroxide levels <2 meq/kg (0.01 to 2.0 meq/kg, PeroxySafe kit for refined oils). For this study, we used both kits since we were monitoring PV from 0 to 20 meq/kg. After oil samples had reached room temperature, they were analyzed using kit instructions. Samples (50 μL or less) were solubilized in the proprietary preparation reagent, held 15 min, then measured at 570 nm.

FOX method. A colorimetric FOX method was used for determination of lipid hydroperoxides in edible oils as described by Nourooz-Zadeh *et al.* (22). The 30-min incubation time at room temperature was adhered to strictly, because the intensity of color changed with time. Also, the reagent containing methanol, sulfuric acid, BHT, XO, and ammonium iron (II) sulfate was prepared fresh each day. A daily calibration was done using hydrogen peroxide as described by Wolff (29), and absorbance measurements for a colored (blue-purple) complex were measured at 560 nm, the absorbance maximum, using a Beckman DU 640 spectrophotometer.

Collection of NIR spectral data. Spectroscopic data from individual vegetable oils were collected as described by Yildiz *et al.* (30) using a Foss NIRSystems Model 6500 scanning spectrophotometer. The single-beam instrument was configured for direct transmission measurements with a standard 2-mm quartz cuvette. Transmittance spectra were recorded in log 1/T format at 2-nm intervals from 400–2500 nm, using ambient air as the reference. All samples were allowed to reach room temperature (25 ± 2°C) prior to collection of spectra. The sample transport temperature control unit was set at 26°C. Each spectrum was then obtained by collecting and averaging 32 individual spectral scans. An equation based on partial least squares (PLS) regression of first derivative spectra was then applied to the spectral data from each sample to predict the PV.

Statistical analysis/data analysis. Data were analyzed by analysis of standard deviation of differences (SDD) and linear regression between the reference titration method and each alternative method. An *F*-test was performed using a two-tailed *F*-test at α value = 0.05 to evaluate the statistical significance of differences between methods (33). Significance between SDD was established when the α value was <0.05.

RESULTS AND DISCUSSION

Sample distribution. The five original oils had titration PV that ranged from 0.70 to 2.5 meq/kg (mean 1.4 meq/kg). The oils were exposed to fluorescent light to obtain PV levels of 0–20 meq/kg. Periodic sampling was done over times ranging from 0 to 216 h.

Method comparisons. (i) NIR and PV. Strong correlations between NIR-predicted values and standard titration data for PV of the oxidized SBO were obtained. With the best PLS calibration from the previous work (30), statistical analysis of the data showed that *r* and SDD between the official titration method and the NIR were $r = 0.991$, SDD = 0.72 meq/kg. The plot of NIR predicted vs. standard titration data was linear and had a slope close to unity (Fig. 1).

(ii) PeroxySafe kit and PV. Statistical analysis of the PeroxySafe data and reference method data for PV showed that the *r* and SDD between titration and the PeroxySafe kit were $r = 0.993$, SDD = 0.56 meq/kg. The plot of PeroxySafe vs.

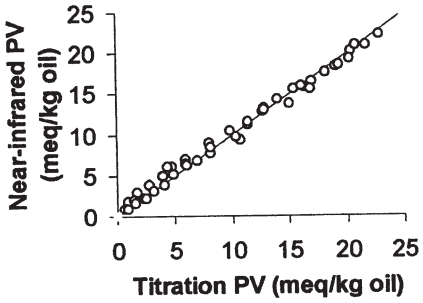

FIG. 1. Scatter plot of data for the official AOCS iodometric titration method Cd 8-53 (31) vs. the NIR spectroscopic method, compared to a 45° line (—) representing the theoretical perfect fit. Abscissa values are PV as determined by the reference procedure.

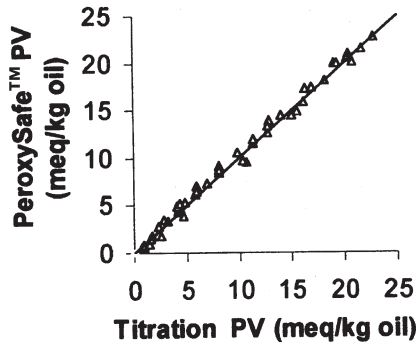

FIG. 2. Scatter plot of data for the official AOCS iodometric titration method Cd 8-53 (31) vs. the PeroxySafe™ method (Safety Associates, Inc., Tustin, CA), compared to a 45° line (—) representing the theoretical perfect fit. Abscissa values are PV as determined by the reference procedure.

standard titration data was linear and had a slope close to unity (Fig. 2).

The SDD between the NIR and titration methods was compared to the SDD between the PeroxySafe and titration methods to determine whether there was a statistically significant difference in performance between the two alternative methods. Results of an *F*-test (α value = 0.05) found that there was no statistically significant difference between the SDD of the two alternative methods (Table 1). This indicates that the NIR and PeroxySafe methods emulate the titration procedure equally well.

(iii) FOX and PV. Work was also done to compare the official AOCS PV method with a published FOX method (22) for determining PV in vegetable oils. The FOX method described in the literature is being considered by some researchers as a replacement for the official method because it uses less sample and a less toxic solvent than the official method. In a preliminary study, a total of 30 oil samples from three different commercial brands were analyzed. Statistical analysis showed that the results from the two analytical methods were highly correlated ($r \geq 0.95$), and these two methods appeared to be equivalent (data not shown). It was concluded that more work was needed to verify this equivalency, so in the present study a total of 46 oil samples were analyzed. Statistical analysis of the raw data showed that the *r* and SDD between titration and the FOX method were $r = 0.975$, SDD = 2.3 meq/kg (Fig. 3).

TABLE 1
Summary of Statistical Results Obtained for the PV Methods

Modeling	*n*	*r*	SDD[a]
Titration vs. NIR	46	0.991	0.72 a
Titration vs. PeroxySafe™[b]	46	0.993	0.56 a
Titration vs. $FOX_{Author\text{-}corrected}$	46	0.975	1.15 b
Titration vs. $FOX_{Literature\text{-}corrected}$			7.18 d
Titration vs. $FOX_{Initial}$			2.36 c

[a]The same letter within a column indicates the values are not significantly different at a 5% level. FOX, ferrous xylenol orange method; SDD, standard deviation of differences.
[b]Source: Safety Associates, Inc., Tustin, CA.

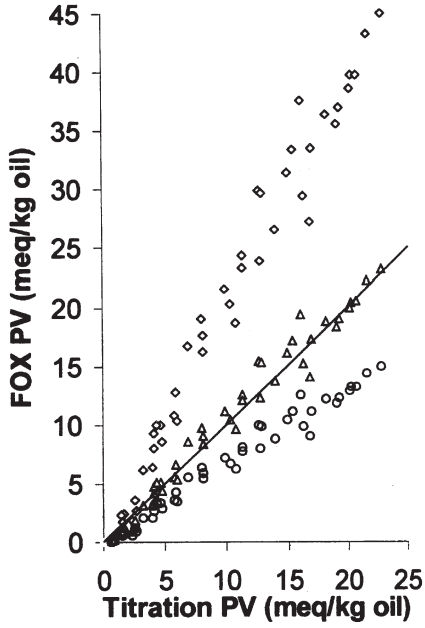

FIG. 3. Scatter plot of data for the official AOCS iodometric titration method Cd 8-53 (31) vs. the ferrous xylenol orange (FOX) method, compared to a 45° line (—) representing the theoretical perfect fit. Data from the FOX method are presented without correction (○), corrected based on Reference 21 (◇), and corrected with an optimal slope correction factor (△). Abscissa values are PV as determined by the reference procedure.

This method consists of the peroxide-mediated oxidation of ferrous ions in an acidic medium containing the dye XO, which binds the resulting ferric ions to produce a blue-purple complex with a maximum absorbance between 550 and 600 nm. Although a relatively strong correlation was found between the FOX and titration methods, initially there were problems with the level of PV measured with the FOX method (Fig. 3). These initial values, without any correction, consistently underestimated the PV level when they were plotted against standard titration data. When the data were corrected as described by Jiang *et al.* (21), who made the assumption/calculation that 3 moles of ferric ion were produced for each mole of hydroperoxide, the PV levels were consistently overestimated. Figure 3 shows the scatter plots of data for the official AOCS standard titration method vs. the FOX method, compared to a 45° line representing the theoretical perfect fit. For the theoretical perfect fit, the slope value is one and the correlation coefficient is one. Given the overestimated and underestimated results, we made a slope correction to yield a slope close to unity, which required a correction factor of 0.515. When this factor was applied to the data, as calculated by the method of Jiang *et al.* (21), we found the best fit with the 45° theoretical line (Fig. 3). This is equivalent to a stoichiometry of 3 moles of ferric ion produced from 2 moles of hydroperoxide. According to Přibil (34), it is generally considered that 1:1 complexes are formed in the presence of excess of XO, and the composition of the complexes is strongly dependent on pH. However, stable 2:1 complexes are also reported to exist. If XO reacts with two Fe^{3+} ions instead

TABLE 2
Summary of Requirements for the Four Methods Used for Determination of PV[a]

	AOCS titration	NIR	PeroxySafe™	$FOX_{corrected}$
Time	1–6 samples/20–40 min	1–6 samples/3–15 min	6 samples/25–30 min	1–6 samples/40–60 min
Reagent volume	50–150 mL	Zero	2.5 mL	1 mL
End point	Subjective	—	Objective	Objective
Sample volume	1–5 g	3–5 g	50 µL or less	0.01–0.1 g
Reagent toxicity	Toxic	None	Low toxicity	Low toxicity
Destruction of sample	Destructive	Nondestructive	Destructive	Destructive
Possibility of automation	No	Yes	Partial	Partial
On-line measurement	No	Yes	No	No
Initial equipment costs	Very low	Very high	High	Low

[a]See Table 1 for abbreviations and company source.

of just one, the stoichiometry of $2HO_2$ for $3XOFe_2$ could be explained (Eq. 1):

$$6H^+ + 2HO_2 + 6e^- \rightarrow 4H_2O$$
$$6Fe^{2+} \rightarrow 6Fe^{3+} + 6e^-$$
$$6Fe^{3+} + 3XO \rightarrow 3XOFe_2$$
$$\overline{6H^+ + 2HO_2 + 6Fe^{2+} + 3XO \rightarrow 3XOFe_2 + 4H_2O} \quad [1]$$

This would give 3 moles of ferric XO per 2 moles of hydroperoxide, consistent with our fitted stoichiometry.

The SDD (i) between the titration method and the initial FOX data, (ii) between the titration method and the literature-corrected FOX data, and (iii) between the titration method and the author-corrected FOX data were compared to the SDD between the titration and NIR methods, as well as between the titration and PeroxySafe kit. *F*-test results showed that the SDD of all three FOX procedures, as compared to the SDD achieved by the NIR or PeroxySafe methods, were significantly greater (Table 1). This indicates that the FOX method that we evaluated does not emulate the titration method as well as do the NIR and PeroxySafe techniques, especially for samples with a PV <2.0.

In summary, all four analytical methods used could detect oxidation of the SBO samples, and the three available alternative methods strongly correlated to the standard titration method. All three of the alternative analytical methods have some advantages over the standard titration method (Table 2). NIR spectroscopy offers several advantages over the standard titration method, including high speed and nondestruction of the sample. NIR analysis is also economical and environmentally friendly because reagents are not needed, labor requirements are low owing to minimal sample preparation, and no chemical wastes are produced. Therefore, the NIR method is a clean analytical method. Moreover, the NIR method is easy to use and applicable for on-line measurement systems. On the other hand, the initial equipment cost of this method is quite high, and before use, it needs to be calibrated.

The newly available PeroxySafe kit also has numerous advantages over the standard titration method and the other colorimetric method based on oxidation of ferrous iron (Table 2) for measuring PV of oxidized vegetable oils. This method requires a very small sample size and also is rapid and straightforward. Other highlights of the PeroxySafe method are good method-to-method agreement with the traditional titration and reduced toxicity.

The high correlation between the titration, NIR, and PeroxySafe kit methods showed that these methods are equivalent. Although the FOX method had a reasonably high correlation with the titration procedure, there were accuracy problems with samples that had a PV <2.0, and the uncorrected PV were consistently lower (underpredicted) than those found with the other three methods. Moreover, in the literature there is a question on the calculation of the FOX method results that needs more investigation.

The wet chemical titration method has disadvantages in meeting an increasing demand for rapid, clean, and cost-effective PV measurement in the food industry. Results of this study have shown that there are alternative analytical methods capable of replacing the standard titration procedure currently in use for determining PV in commercial vegetable oils.

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REFERENCES

1. Warner, K., E.N. Frankel, and T.L. Mounts, Flavor and Oxidative Stability of Soybean, Sunflower, and Low Erucic Acid Rapeseed Oils, *J. Am. Oil Chem. Soc. 66*:558–564 (1989).
2. Løvaas, E., A Sensitive Spectrophotometric Method for Lipid Hydroperoxide Determination, *Ibid. 69*:777–783 (1992).
3. van de Voort, F.R., A.A. Ismail, J. Sedman, J. Dubois, and T. Nicodemo, The Determination of Peroxide Value by Fourier Transform Infrared Spectroscopy, *Ibid. 71*:921–926 (1994).
4. Ma, K., F.R. Van De Voort, J. Sedman, and A.A. Ismail, Stoichiometric Determination of Hydroperoxides in Fats and Oils by Fourier Transform Infrared Spectroscopy, *Ibid. 74*:897–906 (1997).
5. Guillèn, M.D., and N. Cabo, Usefulness of the Frequency Data of the Fourier Transform Infrared Spectra to Evaluate the Degree of Oxidation of Edible Oils, *J. Agric. Food Chem. 47*:709–719 (1999).
6. Porter, N.A., Mechanisms of Free Radical Oxidation: New Methods for Lipid Peroxidation Analysis, Paper No. ORGN-

307, Abstracts from the 220th American Chemical Society National Meeting, Washington, DC, August 20–24, 2000.
7. Frankel, E.N., W.E. Neff, and T.R. Bessler, Analysis of Autoxidized Fats by Gas Chromatography–Mass Spectrometry: V. Photosensitized Oxidation. *Lipids 14*:961–967 (1979).
8. Grau, A., R. Codony, M. Rafecas, A.C. Barroeta, and F. Guardiola, Lipid Hydroperoxide Determination in Dark Chicken Meat Through a Ferrous Oxidation–Xylenol Orange Method, *J. Agric. Food Chem. 48*:4136–4143 (2000).
9. Sugino, K., Simultaneous Determination of Different Classes of Lipid Hydroperoxides by High-Performance Liquid Chromatography with Post-column Detection by a Ferrous/Xylenol Orange Reagent, *Biosci. Biotechnol. Biochem. 63*:773–775 (1999).
10. Christensen, T.C., and G. Hoelmer, Lipid Oxidation Determination in Butter and Dairy Spreads by HPLC, *J. Food Sci. 61*:486–489 (1996).
11. Sjovall, O., A. Kuksis, L. Marai, and J.J. Myher, Elution Factors of Synthetic Oxotriacylglycerols as an Aid in Identification of Peroxidized Natural Triacylglycerols by Reversed-Phase High-Performance Liquid Chromatography with Electrospray Mass Spectrometry, *Lipids 32*:1211–1218 (1997).
12. Korytowski, W., P.G. Geiger, and A.W. Girotti, Lipid Hydroperoxide Analysis by High-Performance Liquid Chromatography with Mercury Cathode Electrochemical Detection, *Methods Enzymol. 300*:23–33 (1999).
13. Hui, S.-P., T. Yoshimura, T. Murai, H. Chiba, and T. Kurosawa, Determination of Regioisomeric Hydroperoxides of Fatty Acid Cholesterol Esters Produced by Photosensitized Peroxidation Using HPLC, *Anal. Sci. 16*:1023–1028 (2000).
14. Bauer-Plank, C., and L. Steenhorst-Slikkerveer, Analysis of Triacylglyceride Hydroperoxides in Vegetable Oils by Nonaqueous Reversed-Phase High-Performance Liquid Chromatography with Ultraviolet Detection, *J. Am. Oil Chem. Soc. 77*:477–482 (2000).
15. Khatoon, S., and A.G.G. Krishna, Assessment of Oxidation in Heated Safflower Oil by Physical, Chemical, and Spectroscopic Methods, *J. Food Lipids 5*:247–267 (1998).
16. Lezerovich, A., Determination of Peroxide Value by Conventional Difference and Difference-Derivative Spectrophotometry, *J. Am. Oil Chem. Soc. 62*:1495–1500 (1985).
17. Felsner, M.L., and J.R. Matos, Analysis of the Thermal Stability and Oxidation Temperature of Commercial Edible Oils by Thermogravimetry, *An. Assoc. Bras. Quim. 47*: 308–312 (1998).
18. Miyazawa, T., K. Fujimoto, M. Kinoshita, and R. Usuki, Rapid Estimation of Peroxide Content of Soybean Oil by Measuring Thermoluminescence, *J. Am. Oil Chem. Soc. 71*:343–344 (1994).
19. Aubourg, S.P., Recent Advances in Assessment of Marine Lipid Oxidation by Using Fluorescence, *Ibid. 76*:409–419 (1999).
20. Wold, J.P., and M. Mielnik, Nondestructive Assessment of Lipid Oxidation in Minced Poultry Meat by Autofluorescence Spectroscopy, *J. Food Sci. 65*:87–95 (2000).
21. Jiang, Z.Y., A.C.S. Woollard, and S.P. Wolff, Lipid Hydroperoxide Measurement by Oxidation of Fe^{2+} in the Presence of Xylenol Orange: Comparison with the TBA Assay and an Iodometric Method, *Lipids 26*:853–856 (1992).
22. Nourooz-Zadeh, J., J. Tajaddini-Sarmadi, and S.P. Wolff, Measurement of Hydroperoxides in Edible Oils Using the Ferrous Oxidation in Xylenol Orange Assay, *J. Agric. Food Chem. 43*:17–21 (1995).
23. Jiang, Z.Y., J.V. Hunt, and S.P. Wolff, Ferrous Ion Oxidation in the Presence of Xylenol Orange for Detection of Lipid Hydroperoxides in Low Density Lipoproteins, *Anal. Biochem. 202*:384–389 (1991).
24. Nourooz-Zadeh, J., J. Tajaddini-Sarmadi, and S.P. Wolff, Measurement of Plasma Hydroperoxide Concentrations by the Ferrous Oxidation–Xylenol Orange Assay in Conjunction with Triphenylphosphine, *Ibid. 220*:403–409 (1994).
25. Sodergren, E., J. Nourooz-Zadeh, L. Berglund, and B. Vessby, Re-evaluation of the Ferrous Oxidation in Xylenol Orange Assay for the Measurement of Plasma Lipid Hydroperoxides, *J. Biochem. Biophys. Methods 37*:137–146 (1998).
26. Nourooz-Zadeh, J., Measurement of Hydroperoxides in Edible Oils and Fats Using the Ferrous-Oxidation in Xylenol Orange (FOX) Assay and Application of the Method to Estimation of Dietary Hydroperoxide Intakes in the United Kingdom, *Recent Res. Dev. Oil Chem. 2*:53–61 (1998).
27. Shantha, N.C., and E.A. Decker, Rapid, Sensitive, Iron-Based Spectrophotometric Methods for Determination of Peroxide Values of Food Lipids, *J. AOAC Int. 77*:421–424 (1994).
28. Grau, A., F. Guardiola, J. Boatella, M.D. Baucells, and R. Codony, Evaluation of Lipid Ultraviolet Absorption as a Parameter to Measure Lipid Oxidation in Dark Chicken Meat, *J. Agric. Food Chem. 48*:4128–4135 (2000).
29. Wolff, S.P., Ferrous Ion Oxidation in Presence of Ferric Ion Indicator Xylenol Orange for Measurement of Hydroperoxides, in *Methods in Enzymology*, edited by L. Packer, Academic Press, New York, 1994, Vol. 233, pp. 182–189.
30. Yildiz, G., R. Wehling, and S. Cuppett, Method for Determining Oxidation of Vegetable Oils by Near-Infrared Spectroscopy, *J. Am. Oil Chem. Soc. 78*:495–502 (2001).
31. AOCS, *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 4th edn., American Oil Chemists' Society, Champaign, 1990.
32. Hall, C., III, The Effect of *Rosmarinus officinalis* (rosemary) on the Photooxidation of Soybean Oil. A Study Involving the Use of a Commercial Rosemary Oleoresin and a Synthesized Rosemary Antioxidant, Rosmariquinone, M.S. Thesis, University of Nebraska–Lincoln, Lincoln, 1991, pp. 70–71.
33. Snedecor, W.G., and W.G. Cochran, *Statistical Methods*, 6th edn., The Iowa State University Press, Ames, 1967, pp. 117.
34. Přibil, R., *Applied Complexometry, Pergamon Series in Analytical Chemistry, Vol. 5,* Pergamon Press, Oxford, 1982, pp. 33–37.

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