Evaluation of SafTest™ Methods for Monitoring Frying Oil Quality

Swee Yee Foo, Susan Cuppett, and Vicki Schlegel*

Department of Food Science and Technology, University of Nebraska, Lincoln, Nebraska 68583-0919

ABSTRACT: The feasibility of applying methods developed by Safety Associates, Inc., to monitor oil degradation products, including malondialdehydes (AldeSafe™), FFA (FASafe™), and peroxides (PeroxySafe™), in fresh and heat-abused deep-fat frying oil was evaluated. Based on performance qualification studies, the AldeSafe method was the most suitable SafTest™ assay for monitoring the quality of frying oil because of its high accuracy, precision, linearity, and reproducibility, and low detection/quantitation limits. A strong correlation ($r = 0.924$) between the AldeSafe method and its counterpart, AOCS Official Method Cd 19-90, also supported the suitability of the SafTest method for monitoring oil quality. Moreover, the FASafe method had a moderately strong relationship with AOCS Official Method Ca 5a-40 ($r = 0.761$). Our studies suggest that this test can be applied for monitoring frying oil; however, certain method performance limitations must be considered for routine analysis purposes. In contrast, the PeroxySafe method probably should not be used to monitor heat-abused oil without further development because of high variability, low accuracy, and low correlation ($r = 0.062$) with the AOCS Official Method Cd 8-53 assay.

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KEY WORDS: Assay correlation, deep frying, free fatty acids, method qualification, malondialdehyde, oil degradation products, oil quality indicators, peroxides.

Controlling deep-fat frying processes is crucial in the food industry, as oil degradation products can compromise the quality of the oil over time and may be harmful to human health (1–6). Peroxides and malondialdehyde (MDA) are applicable indicators of oil quality, as these oxidation by-products are produced from several frying parameters, i.e., light, elevated temperatures, oxygen, water in the food, and the like (7,8). FFA are another oil quality indicator because these molecules are cleaved from TAG when steam reacts with oil at the elevated temperatures typically used in a deep-fat frying process (2). Although AOCS official methods are available for monitoring the cited indicators of degraded oil, several disadvantages exist in their application (9–11). For example, hazardous chemicals are needed for some of the tests; therefore, appropriate safety equipment and additional waste disposal must be considered. Bias and manual errors can also occur when performing the titration-based methods for FFA and peroxides. To complete the MDA-AOCS method, 2-thiobarbituric acid (TBA) is combined with the test sample and a red pigment is formed in the presence of MDA, which can then be monitored at a wavelength of 530 nm (8,12). However, TBA reacts with other molecules, including ketones, ketosteroids, acids, esters, sugars, imides and amides, amino acids, oxidized proteins, pyridines, and pyrimidines (13). In addition, solution and/or standard preparation requirements increase the time required to complete the AOCS assays.

Methods developed by Safety Associates, Inc., (Tempe, AZ) under the brand name of SafTest™ may be promising alternatives to the official AOCS assays for monitoring frying oil quality because peroxides, MDA, and FFA can easily be detected (14). Samples are prepared for this system by solubilizing the food matrix with a proprietary preparation solution, separating the quality indicator from other food components when necessary, and reacting the ensuing extract with proprietary reagents. The quality indicators are detected with an optical reader programmed to quantitate the molecule of interest against a previously established calibration curve. All the test reagents, calibration standards, controls, membrane packs, and so on are available in kits specific to the quality indicator of interest. As a result, the SafTest system generates less waste, does not require timely standardized solution preparations, and is capable of higher sample analyses than the AOCS methods.

Despite internal studies conducted by the manufacturer showing the successful application of the SafTest methods to various food systems, these assays have not been used extensively in the food industry. The purpose of these studies was to determine the feasibility of using the SafTest system to monitor the quality of frying oil heated for extended periods of time. Assay qualification studies were initially performed to evaluate the basic performance characteristics of the SafTest assays. These methods were then compared with their official AOCS counterparts.

MATERIALS AND METHODS

Reagents and solutions. AldeSafe™, FASafe™, and PeroxySafe™ kits were purchased from Safety Associates, Inc. Each kit contained the following items: preparation solution, analyte reaction reagents, positive controls (low, high, and medium), and 3 to 5 standards of known concentrations. The reagents purchased to complete the AOCS test consisted of acetic acid, 1 N sodium hydroxide, glacial acetic acid (J.T.Baker,

*To whom correspondence should be addressed at Department of Food Science and Technology, 143 Filley Hall, University of Nebraska, Lincoln, NE 68583-0919. E-mail: vlschleg@unlnotes.unl.edu

Phillipsburg, NJ), chloroform (Mallinckrodt Baker, Paris, KY), 1,1,3,3-tetraethoxypropane, TBA, phenolphthalein (Sigma, St. Louis, MO), potassium iodide (VWR, West Chester, PA), starch indicator, sodium thiosulfate, and 2-propanol (Fisher Scientific, Fair Lawn, NJ).

Assays for MDA levels (nmol/mL). MDA levels were detected with the AldeSafe method by diluting samples with the proprietary preparation reagent to a suitable dilution that could be monitored by the SafTest optical reader. After incubation at 55°C for 15 min, 150 μL of a test sample was combined with AldeSafe reagent A (1.9 mL) and reagent B (0.7 mL). The mixtures were vortexed and placed on a tube rocker. At intervals ranging between 5 and 10 min, the color of the sample was compared with the color of the highest standard (50.00 nmol/mL). Additional dilutions were performed when the color of the sample was darker than the standard. Calibration curves were prepared with 0, 5, 10, 25, and 50 nmol/mL standards under the conditions of the test. The results were adjusted by the dilution factor to prepare a sample, if needed.

AOCS Method Cd 19-90 (10) was modified slightly as described by Guillen-Sans and Guzman-Chozas (7) and Younathan *et al*. (15) (MDA-AOCS test). The modified method consisted of diluting 1 mL of an oil sample with 9 mL of an 1:1 acetic acid–isopropanol solution. The samples were incubated at 55–60°C for 15 min, and 1 mL of this sample solution was combined with 1 mL of 0.02 M TBA. This solution was mixed and incubated in a 95°C oven for 90 min. The absorbance of the reaction solution was measured at a wavelength of 530 nm with a UV-vis spectrophotometer. A calibration curve was prepared by diluting a stock solution of 10^{-2} M of 1,1,3,3-tetraethoxypropane to concentrations ranging between 0 and 87.5 nmol/mL of MDA.

Assays for FFA (percentage of oleic acid). FFA levels were determined with the FASafe method by dissolving the oil samples in an appropriate amount of the preparation reagent. The diluted samples were vortexed and incubated at 55°C for 15 min. The test sample (100 μL) was combined with FASafe reagent A (2.35 mL) and reagent B (0.25 mL), vortexed for 30 s, and heated at 45°C for 10 min. The results were corrected by multiplying by the appropriate dilution factor. A calibration curve was constructed by analyzing five calibration standards (0.04, 0.26, 0.62, 1.02, and 2.01% of oleic acid) as cited for the FASafe test. AOCS Official Method Ca 5a-40 (FAA-AOCS) was performed by combining the oil sample with 5 mL of isopropanol and 5 to 6 drops of phenolphthalein indicator (11). FFA levels were determined by alkaline titration (0.01 N NaOH) and the results were reported as the percentage of oleic acid.

Peroxide levels (meq/kg). Peroxides were measured by the PeroxySafe method by first diluting each sample with the preparation reagent to a dilution factor that could be detected by the optical reader. The sample solutions were incubated at 55°C for 15 min or until the sample solutions became clear. Test samples (50 μL) were mixed with PeroxySafe reagent A (2.1 mL), reagent B (0.1 mL), and reagent C (0.3 mL). The samples were then mixed and placed on a tube rocker for an additional 15 min. If there was any indication of cloudiness during the mixing period, the samples were reheated to 55°C for 5 to 6 min. A calibration curve was constructed with the three standards (0.05, 0.2, and 0.5 meq/kg) supplied with the kit. AOCS Official Method Cd 8-53 (PV-AOCS) was performed by dissolving 5 g of an oil sample in 30 mL of a 3:2 acetic acid–chloroform solution and 0.5 mL of a saturated potassium iodide solution (9). After mixing the solution for 1 min, 30 mL of water and 2 mL of starch indicator were added to the sample solution and titrated with 0.001 M of sodium thiosulfate until the blue violet color disappeared.

Qualification of SafTest methods. Method linearity was evaluated by measuring the standards supplied with a kit at least 5 to 7 times and constructing calibration curves from these measurements. The calibration curves were statistically analyzed to ensure reproducibility of the reagents and other test supplies. To assess kit-to-kit variability, a single calibration curve was prepared from each of 5 to 10 kits, and the results were compared. Other method performance characteristics were performed according to the guidelines established by the *United States Pharmacopoeia* (16) and included percentage recovery, accuracy/specificity, precision, limit of detection, and limit of quantitation.

Experimental design for the frying process. Partially hydrogenated soybean oil (FryMax®, Memphis, TN) was heated in two 6-quart fryers (Options™; Presto®, Eau Claire, WI) for 8 h per day at approximately 190°C for a 7-d period. To facilitate the production of FFA from hydrolytic reactions, water was added to each fryer with a Beckman® Accu-Flo pump at 2% by weight of the oil per hour. Two to five samples were collected for each assay every 4 and 8 h on a daily basis. A total of four replicate trials were completed to compare the MDA assays, whereas the peroxide and FFA methods were applied to eight replicate trials. The oil was sealed under nitrogen gas (Linweld, Lincoln, NE) when a process was stopped at the end of each 8-h day.

Mathematical and statistic analyses. Correlation and regression analyses was used to compare the SafTest methods with the AOCS methods. ANOVA tests were performed to verify whether calibration data were significantly different at the 95% confidence interval ($P < 0.05$). Potential outliers were assessed with the Grubbs test at a 5% risk for rejection. Calculations for percentage recovery, detection limit, quantitation limit, and precision were determined according to *U.S. Pharmacopoeia* guidelines (16). The statistical tests were performed with StatGraphics Plus software, version 4 (Rockville, MD). Regression curves were fitted using Microsoft Excel (Redmond, WA).

RESULTS AND DISCUSSION

Qualification of any analytical method ensures that the test performs suitably for its intended purpose. In essence, qualifying an analytical method after the development stage produces well-characterized techniques. Various performance characteristics, including accuracy, precision, linearity, detection limit,

and quantitation limit, were thereby evaluated for each SafTest method. The tests were then applied to the analysis of samples collected from deep-fat frying oil that was exposed to elevated temperatures over a 7-d period. The ensuing data from each SafTest method was compared with that obtained from its AOCS counterpart to determine the degree of correlation between the two tests.

Qualification of the AldeSafe method. Linearity was determined for a single AldeSafe kit by analyzing replicate standards (n = 7) ranging in concentrations from 0.00 to 50 nmol/mL. From these results, a linear equation was generated with a correlation coefficient (r) of 0.9991 (Table 1). To verify whether linearity varied from kit to kit, the standards and reagents supplied with each of 5 kits were used to produce a single calibration curve. A linear relationship again was found, resulting in an r value of 0.9991. P values >0.05 were further calculated when the data obtained from each of the different kits were statistically analyzed as different treatments. These results suggest that consistent and highly linear curves could be prepared with different lots of AldeSafe kits. Method precision also increased with higher analyte concentrations, as evidenced by the relative standard deviations (RSD) produced from the replicate analyses of standards supplied with different kits (Table 1) and from a single kit (data not shown). The evaluation of heat-abused oil also confirmed a high degree of method precision, as the RSD for 5 replicate samples collected from 20 different time points were less than 5% (Table 1).

The calibration curve prepared from reagents supplied with 5 different kits was subsequently used for the remaining experiments. As a result, a detection limit of 1.9 nmol/mL and a quantitation limit of 6.9 nmol/mL was calculated from the mean blank response + 2 (SD) and the mean blank response + 10 (SD), respectively (16). Considering that the calibration curve range extended to 50 nmol/mL, both the detection and quantitation limits were acceptably low.

To assess the accuracy of the AldeSafe method, standards (0.00, 10.0, and 50.0 nmol/mL) were added to fresh oil at a ratio that allowed the analyte to be measured at concentrations at or above that expected in the sample. The mean recovery of the 0.00 nmol/mL standard spiked into fresh oil was below the detection limit of the assay, whereas standards 10.0 and 50.0 nmol/mL were recovered from fresh oil samples at 98 and 95%, respectively. The accuracy of the test when applied to the heated oil was determined by adding standards to samples collected from four different time points. All of the spikes were recovered from the oil matrices at percentages greater than 90%, with a grand mean percentage of 95% (Table 1). In addition, every negative control analyzed under the conditions of the test was below the detection limit of the assay. Coupled with the percentage recovery data, these results indicated that fresh oil or heat-abused oil matrices did not affect the test by either enhancing or inhibiting the assay.

Qualification of the FASafe method. Calibration curves constructed with standards supplied with the FASafe kit generated a negatively curved regression line. The curve was thus analyzed over the lower quantitation region (0.04 to 0.62% oleic acid) and over the entire region (0.04 to 2.01% oleic acid) (Table 2). For a single kit, the lower quantitative region of the curve was slightly more linear (r = 0.9753) compared with the entire regression line (r = 0.9552). In addition, the slope of the line for the lower quantitation region was greater than that for the entire region, indicating that the test was more sensitive to changes in analyte levels within this concentration range. For multiple kits, the lower quantitative region was again more linear (r = 0.9091) than the entire curve (r = 0.8752) (Table 3). Still, the correlation coefficients were higher in both cited regions when the curves were constructed from the reagents supplied with a single kit. Compared with multiple kits, the precision of a single kit was also higher, as the RSD calculated from replicate analyses of standards and the heat-abused oil samples were below 5% except for the lowest standard, which was only slightly higher (Table 2). Conversely, method precision ranged from 10 to 15% for each standard concentration originating from different kits (data not shown). Although there was no statistically significant difference for the "between-kit" calibration curves ($P > 0.05$), the precision data suggest that a well-constructed calibration curve should be prepared with each new kit. As a result, the ensuing studies were completed by constructing a FFA calibration curve with the same kit reagents used to analyze a given set of test samples.

TABLE 1
Performance Characteristics of the AldeSafe™ Method

Performance characteristics	Results
Linearity (regression analysis)	
Within kit (n = 7)	$Y = 0.016 + 0.005x$, $r = 0.9991$
Between kits (n = 5)	$Y = 0.017 + 0.005x$, $r = 0.9991$
Precision (RSD %)[a]	
Standard: 0 nmol/mL (n = 5)	15
Standard: 5 nmol/mL (n = 5)	8.5
Standard: 10 nmol/mL (n = 5)	4.9
Standard: 25 nmol/mL (n = 5)	3.1
Standard: 50 nmol/mL (n = 5)	2.0
Heat-abused oil[b] (n = 3)	Less than 5
Detection limit (nmol/mL)	
Blanks (n = 6)	1.9
Quantitation limit (nmol/mL)	
Blanks (n = 6)	6.9
Accuracy/specificity (% recovery)	
Fresh oil: 0.00 nmol/mL[c] (n = 5)	BDL[c]
Fresh oil:10.00 nmol/mL (n = 5)	98 ± 3.1
Fresh oil: 50.00 nmol/mL (n = 5)	95 ± 4.2
R4-D3-H4-A[d] (n = 5)	100 ± 1.3
R4-D3-H4-B (n = 5)	92 ± 3.5
R4-D6-H4-B (n = 5)	93 ± 1.5
R4-D6-H4-A (n = 5)	94 ± 3.4
Negative controls (nmol/mL)	
Fresh oil (n = 5)	BDL[e]
Heat-abused oil (n = 10)	BDL

[a]RSD, relative standard deviation.
[b]For three replicate samples obtained at 20 different time points during a single process.
[c]Fresh oil spiked with indicated standard concentration.
[d]BDL, below detection limit.
[e]R, replicate trial; D, day; H, hour; A or B, fryer A or B.

The accuracy/specificity studies supplied additional evidence for using only the lower region of the FASafe calibration curve. A recovery of 110% resulted when the 0.62% standard was spiked into fresh oil but was only 80.3% when the 2.01% standard was used. The signal from the former sample was analyzed *via* the lower quantitative region of the calibration curve (0.04 to 0.62% oleic acid), whereas the 2.01% standard spike was evaluated with the upper quantitative region (0.62 to 2.01% oleic acid). When the 0.62% standard was added to the heat-abused oil, the responses could be quantified within the lower quantitative region of the calibration curve and resulted in a grand mean percentage recovery of 96%. Last, the response of replicate tests of negative controls fell outside the highest range of the entire calibration curve (Table 2). Yet when kit reagents were added to the negative controls, the signal was below the detection method of the assay. These combined results show that the continually changing oil matrix did not significantly contribute to the background signal of the FASafe assay, nor did it affect the assay at levels below 0.62% oleic acid.

A detection limit of 0.12% and a quantitation limit of 0.26% were calculated by using the regression equation determined for the lower quantitative region of the calibration curve of a single kit. Because the quantitation range was 0.26 to 0.62%, more sample handling is needed to ensure that materials containing high FFA levels are properly diluted to quantify within the lower and rather narrow quantitative range of the calibration curve. Detection/quantitation limits may also change with each new kit, which will then affect sample handling dilution conditions.

Qualification of the PeroxySafe method. When the calibration curves were statistically analyzed, a P value of >0.05 was obtained for both the within-kit and between-kit" data (Table 3). Moreover, the calibration curves constructed from a single PeroxySafe kit and from multiple kits produced linear equations with r values of 0.9895 and 0.9907, respectively (Table 3). These results indicate that PeroxySafe kits were not significantly different on a lot-to-lot basis, although the linearity and sensitivity (the slope of the regression line) were less than that of the AldeSafe, i.e., the other SafTest assay based on an oxidation reaction product. A detection limit of 0.00 meq/kg and a quantitation limit of 0.03 meq/kg were subsequently calculated by using the between-kit linear regression curve. Although the limits of detection and quantitation were low, sample analysis was limited given that analyte concentrations had to be between 0.03 and 0.5 meq/kg. When peroxide concentrations were above the upper range of the calibration curve, the sample had to be diluted appropriately to fall within this region. Considering that variability increased with lower peroxide concentrations (Table 3), higher precision could probably be achieved by extending the curve while increasing the quantitation region of the method, but standards greater than 0.50 meq/kg were not provided with this kit.

The accuracy of this assay was established by testing replicate samples of 0.02 and 0.5 meq/kg standards spiked into fresh oil and heated oil. As shown in Table 3, the analyte was consistently recovered from fresh oil at a grand mean average of 108%, whereas the mean percentage recovery was 78% when heat-abused oil samples were used as the matrix. These results indicate that the heat-abused oil negatively affected the quantitation of peroxides. Nonetheless, the oil matrix did not bias the test, as negative controls prepared with fresh and heat-abused oil were below the detection limit of the assay.

TABLE 2
Performance Characteristics of the FASafe™ Method

Performance characteristics	Results
Linearity (regression analysis)	
Within kit: 0.04 – 0.62% (n = 7)	Y = 1.28 – 0.719x, r = 0.9753
Within kit: 0.04 – 2.01%	Y = 1.17 – 0.379x, r = 0.9552
Between kit: 0.04 – 0.62% (n = 8)	Y = 1.33 – 0.929x, r = 0.9091
Between kit: 0.04 – 2.01%	Y = 1.18 – 0.414x, r = 0.8752
Precision (RSD %)[a]	
Standard: 0.04% (n = 7)	5.3
Standard: 0.26% (n = 7)	2.7
Standard: 0.62% (n = 7)	2.6
Standard: 1.02% (n = 7)	2.5
Standard: 2.01% (n = 7)	2.9
Heat-abused oil[b] (n = 5)	Less than 5
Detection limit (%)	
Blanks (n = 5)	0.12
Quantitation limit (%)	
Blanks (n = 5)	0.26
Accuracy/specificity (% recovery)	
Fresh oil: 0.62%[c] (n = 3)	110 ± 11
Fresh oil: 2.01% (n = 3)	80 ± 2.1
R4-D3-H4-A[d] (n = 5)	95 ± 0.90
R4-D6-H4-B (n = 5)	97 ± 0.84
Negative controls (%)	
Fresh oil (n = 5)	BDL[e]
Heat-abused oil (n = 10)	BDL

[a]RSD, relative standard deviation.
[b]For five replicate samples obtained at 20 different time points.
[c]Fresh oil spiked with the indicated standard concentration.
[d]R, replicate trial; D, day; H, hour; A or B, fryer A or B.
[e]BDL, below the detection limit.

Comparison of SafTest methods with AOCS methods. Based on the qualification studies, the AldeSafe, PeroxySafe, and FASafe methods were used to monitor the quality of oil exposed to high temperatures over a 7-d period, and these results were correlated with those obtained from the AOCS methods. In general, MDA levels increased steadily with frying time, as monitored by both methods (Fig. 1). However, the MDA-AOCS method detected higher analyte levels compared with the AldeSafe method. Detection of higher MDA concentrations by the former method probably occurred because TBA reacts with other types of aldehydes and TBARS, whereas the proprietary reagents supplied with the AldeSafe kit were selective only for MDA (15). This high selectivity was confirmed by the method qualification studies of the AldeSafe assay, as analysis of fresh oil samples resulted in responses below the quantification limit of the assay, as expected. However, when fresh oil was tested *via* the AOCS method, a mean of 610 nmol/mL was obtained. Despite this bias, comparison of the two methods supported the suitability of the AldeSafe method for monitoring oil quality, as this method correlated

TABLE 3
Performance Characteristics of the PeroxySafe™ Method

Performance characteristics	Results
Linearity (regression analysis)	
Within kit (n = 7)	Y = –0.006 + 0.746x, r = 0.9895
Between kits (n = 10)	Y = –0.002 + 0.680x, r = 0.9907
Precision (RSD %)[a]	
Standard: 0.05 meq/kg (n = 10)	14
Standard: 0.2 meq/kg (n = 10)	7.9
Standard: 0.5 meq/kg (n = 10)	5.8
Heat-abused oil[b] (n = 3)	Less than 5
Detection limit (meq/kg)	
Blanks (n = 5)	0.0
Quantitation limit (meq/kg)	
Blanks (n = 5)	0.03
Accuracy/specificity (% recovery)	
Fresh oil: 0.05 meq/kg[c] (n = 3)	107 ± 0.24
Fresh oil: 0.2 meq/kg (n = 3)	107 ± 3.4
Fresh oil: 0.50 meq/kg (n = 3)	111 ± 4.4
R4-D3-H4-A[d] (n = 5)	81 ± 3.0
R4-D3-H4-B (n = 5)	66 ± 2.3
R4-D6-H4-B (n = 5)	74 ± 2.0
R4-D6-H4-A (n = 5)	91 ± 4.5
Negative controls (meq/kg)	
Fresh oil (n = 5)	BDL[e]
Heat-abused oil (n = 6)	BDL

[a]RSD, relative standard deviation.
[b]For five replicate samples obtained at 20 different time points.
[c]Fresh oil spiked with indicated standard concentration.
[d]R, replicate trial; D, day; H, hour; A or B, fryer A or B.
[e]BDL, below the detection limit.

strongly with the modified MDA-AOCS method (r = 0.924) (Fig. 1).

Analysis of FFA levels in samples collected from heat-abused oil indicated that this oil analyte increased linearly over time, as detected by both methods (data not shown), but the FASafe method correlated only moderately with the FFA-AOCS method (r = 0.761) (Fig. 2). These results may be due to the quantification and linearity constraints associated with the FASafe method, as was determined from the method performance studies. As an outcome, the FASafe method could be used to monitor frying oil, but performance limitations must be considered to adapt the test appropriately within a quality control setting. For example, a well-constructed calibration curve must be prepared for every kit, and samples may require additional handling steps to quantify them in the lower region of the calibration curve.

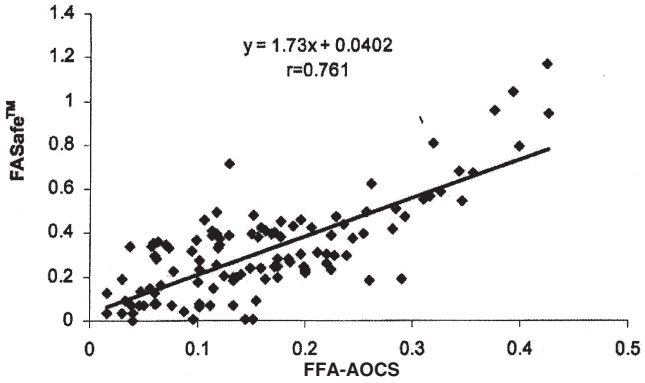

FIG. 2. Comparison of FASafeTM and the FFA-AOCS assay (in percentage oleic acid).

Regression analysis of the peroxide data obtained from eight replicate trials resulted in an exponential relationship between heat time and concentration as measured by the PeroxySafe method, but a square root x model occurred when the replicates were monitored by the PV-AOCS method (data not shown). These results confirm that the two methods differed when used to measure peroxides in heat-abused oil, as was supported by a direct comparison of the two methods (Fig. 3). Data points are randomly scattered around a regression line with an r value of 0.062. Although peroxide levels were inconsistent when monitored by either assay, the PeroxySafe method typically detected higher concentrations. This observation may be attributed to higher sensitivity or selectivity of the PeroxySafe test compared with the PV-AOCS method for monitoring a complex oil matrix. It must be noted, however, that most of the heat-abused samples were diluted in the concentration region close to the quantitation limit of the calibration curve, where precision decreased substantially. Pending elimination of the precision and accuracy problems, which may be achieved by simply using

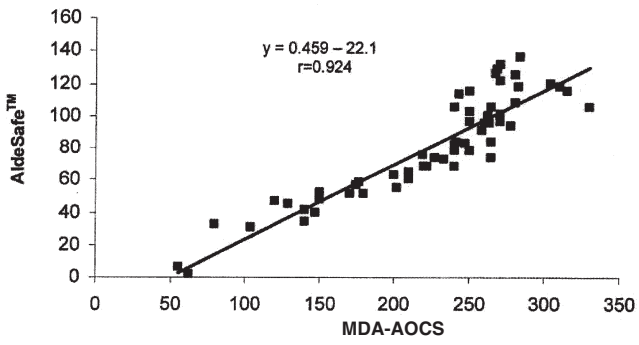

FIG. 1. Comparison of AldeSafe™ and the malondialdehyde (MDA) AOCS assay (in nmol/mL).

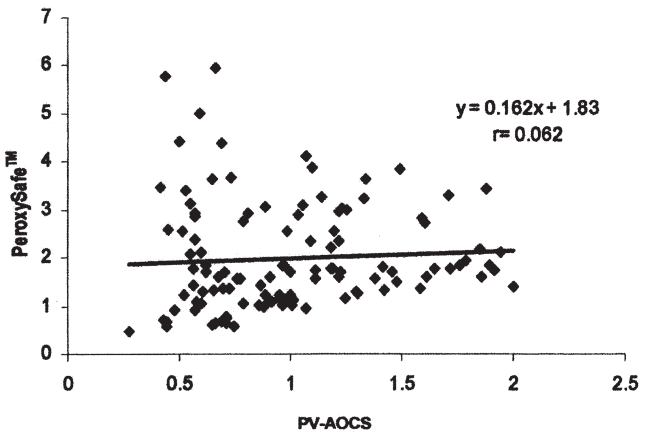

FIG. 3. Comparison of PeroxySafe™ and the PV-AOCS assay (in meq/kg).

more highly concentrated standards to extend the calibration curve, the PeroxySafe test should not be used to monitor the quality of heat-abused oil.

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