J Am Oil Chem Soc (2007) 84:893–897
DOI 10.1007/s11746-007-1124-7

ORIGINAL PAPER

The Use of Fast Methodologies (Kits) in Evaluating Deep-Frying Oils

Cibele Cristina Osawa · Lireny Aparecida Guaraldo Gonçalves · Sidnei Ragazzi

Received: 31 January 2006 / Revised: 3 April 2007 / Accepted: 10 August 2007 / Published online: 9 September 2007

Abstract Fried foods are frequently served by fast food establishments but the evaluation of the oil used is quite laborious, expensive, and requires a well-structured laboratory with sophisticated equipment. Moreover, *p*-anisidine, used as the reagent in the traditional test for monitoring the alkenal concentration of frying oils, is carcinogenic. The DiaMed F.A.T.S. kits for the determination of alkenal (AlkalSafe™ STD) and malonaldehyde (AldelSafe™ STD), equivalent to the *p*-anisidine and TBA tests, respectively, are safe, fast and accurate, using compact equipment and generating fewer residues than the official methods. The results obtained using these kits were compared with those obtained using the official methods for determining alkenal (AOCS Cd 18b-90) and malonaldehyde (AOCS Cd 19b-90), in 20 samples collected from an institutional restaurant. Based on the least squares regression analysis, the AlkalSafe kit results were highly correlated with the *p*-anisidine values ($r = 0.74$), but there was a lack of correlation between the results of the AldeSafe™ kit and the TBA test. Both kits were significantly more sensitive than the official methods, as revealed by the results of the Tukey test. Although the TBA values for the samples investigated were minimal, suggesting the inadequacy of the test for monitoring frying oils, the greater sensitivity of the kit makes it a relatively feasible option.

C. C. Osawa (✉) · L. A. G. Gonçalves
Laboratory of Oils and Fats, Department of Food Technology, Faculty of Food Engineering, State University of Campinas, P.O. box 6091, 13083-970 Campinas, SP, Brazil
e-mail: cibele_osawa@yahoo.com.br

S. Ragazzi
Department of Statistics, State University of Campinas, P.O. box 6065, 13083-970 Campinas, SP, Brazil

Keywords Correlation · Deep-frying · DiaMed F.A.T.S. · Fast methodologies · Frying oils · Kits · Official methods · *p*-Anisidine test · TBA test

Introduction

With the production of more than 20 million tons of frying oil per year, the deep fat frying of foods is considered the most common unit operation in food preparation worldwide [1].

In deep fat frying, the food is submerged in oil heated in the presence of water. The oil is exposed to the action of four agents that cause drastic changes in its structure: moisture from the food, giving rise to hydrolytic alteration; atmospheric oxygen entering the oil from the surface of the container, giving rise to oxidative alterations; the high temperature at which the operation takes place, which results in thermal alterations; and contamination by food ingredients [2].

Frying oils have to be discarded after a certain interval of use because of the harmful effects of the degradation products that form and accumulate in the oil. This has raised the need for a quantitative method to evaluate oil degradation [3].

The peroxides presented in oxidized oils are transitory intermediates, which decompose to several carbonylic compounds amongst others, especially 2,4-dienals and 2-alkenals, in the presence of *p*-anisidine in an acidic media. The *p*-anisidine test detects mainly 2-alkenals, secondary products of lipid oxidation [4].

The *p*-anisidine test can be used to monitor oil deterioration during deep-frying [5], since the anisidine value tends to increase linearly with the increasing time of frying/heating [6] and correlate with the frying time [7]. It also

correlates with the total polar compound determination, conjugated dienes [5], sensory analysis, polymers and volatile compounds, such as hexanal, heptanal, t-2-hexenal, t-2-heptenal, t-2-octenal and t,t-2,4-decadienal [6].

The TBA test is another empirical method frequently used for the detection of lipid oxidation. It relates to the level of aldehydes present in the oil in a similar way to the anisidine test [8].

Aldehydes correspond to more than 50% of the volatiles produced during lipid oxidation and many of those that have significant influence on flavor, are produced from the oxidation of soybean oil [9].

The TBA value is measured as milligrams of malonaldehyde per kilogram of sample and is based on the color reaction between TBA and fatty acid oxidation products. Almost all types of food have been reported to contain malonaldehyde, but those of greater concern are dehydrated foods, cooked meats, stored meats and fats from animal or vegetable sources used in industrial frying [10]. Further information about the TBA test can be found in the studies of Osawa et al. [11].

The AlkalSafe™ STD and AldeSafe™ STD kits of the DiaMed F.A.T.S. are alternative methods to the official *p*-anisidine and TBA tests, respectively. The quantifications of alkenals or malonaldehyde, expressed as nmol/kg or nmol/mL, are based on a colorimetric complex formed between aldehydes and indole, which are read spectrophotometrically at a wavelength of 550 nm.

This study intended to evaluate frying oil samples collected from the institutional restaurant of the State University of Campinas (Unicamp), using the AlkalSafe™ STD and AldeSafe™ STD kits and the official methods for determining malonaldehyde and alkenal.

Experimental Procedures

Materials

1. Twenty samples of frying oil were collected from the electric fryer (capacity 200 L, operating in a saline solution) of the institutional restaurant of Unicamp during approximately 3 months of discontinuous frying. The samples were treated with 2 g of anhydrous sodium sulfate per 10 g of sample, filtered and kept in a dark flask at −18 °C until analyzed.
2. For the kit determinations, dispensers, pipettes and tips, glass tubes (12 × 75 mm) and caps, a vortex mixer (DiaMed, Switzerland) and colorimeter (MicroChem™ Analyser, Source Scientific, USA) were used.
3. *Chemicals*. 2-Thiobarbituric acid 11496-000 (ACROS Organics); *p*-anisidine 99% A88255 (Aldrich); reagent grade 1-butanol, isooctane and acetic acid; Karl Fischer reagent 9258 (1 mL = minimum 5 mg of water, Merck, Germany) containing methoxyethanol and no pyridine in a single solution; reagents of the AlkalSafe™ STD and AldeSafe™ STD kits (Diamed, Switzerland).

Purification of the Reagents Before Use

1. *Isooctane*. The isooctane used was treated with concentrated sulphuric acid for 1 day, with occasional agitation and exchange of the acid four-times. A 0.1 M aqueous solution of potassium permanganate was then added. After 24 h, the permanganate solution was separated and removed, and the isooctane washed to a neutral pH. Finally the solution was dried and distilled, discarding the head and tail fractions, and maintained with anhydrous sodium sulfate until the day of the analyses, when it was filtered for use.
2. *Glacial acetic acid*. The glacial acetic acid used was treated with potassium permanganate in a ratio of 5 g per 1 L of acetic acid, maintaining the contact for 24 h. Similar to the procedure for isooctane, the solution was then distilled and maintained in contact with anhydrous sodium sulfate until use.
3. *p-Anisidine*. Four grams of *p*-anisidine was weighed into a 250 mL Erlenmeyer flask, and while maintained within collective protection equipment, water at approximately 80°C was added, sufficient to dissolve the crystals. The crystals were filtered while heating with a Bunsen burner to keep the temperature constant and increase the filtration yield. After filtration, the solution was immediately cooled in a cooled bath with agitation and maintained at 0 °C for at least 4 h, when the crystals were filtered in a vacuum system using cooled water to transfer them. The filter paper with the purified crystals was conditioned in an aluminum moisture dish, and then transferred to a vacuum desiccator operating at room temperature. The crystals were dehydrated for 20.5 h at room temperature, protected from the light [12], and then maintained under refrigeration in a dark flask with no tap, inside a plastic container sealed with tape and containing activated silica and zeolites to protect against moisture gain.

Methods

1. *Moisture by Karl Fischer*. In the *p*-anisidine test, the moisture content must be below 0.1% for the reaction to occur without interference by the water. In the TBA

test, the moisture content of the 1-butanol must be below 0.5% [4]. The moisture contents of the ten samples and all the reagents were determined in triplicate, according to the AOCS method Ca 2e-84 [4]. The Titroline alpha (Schott, Germany) titrator was used, with a 10-mL burette and a TM 125 magnetic stirrer (Schott, Germany). The initial agitation was adjusted to 30 s and the titration end point to 30 μA, when the equipment automatically stopped the transference of the titrant solution. In the water equivalence determination, 20 mg of water were injected into the titration vessel with a 1-mL syringe (Microstat Tuberculin, USA). For the moisture content determinations of the oils and reagents, 200 mg of *p*-anisidine crystals and the equivalent of 30 drops (200–500 mg) from a Pasteur pipette of the samples and reagents, were used.

2. *p-Anisidine test*. The anisidine values were determined in triplicate according to the AOCS method Cd 18b-90 [4], using 0.3–0.5 g of samples and a quartz cuvette. A blank tube was prepared for each sample and the samples read against visible and ultraviolet lights in a UV/VIS Lambda 20 spectrophotometer (Perkin Elmer, Germany).
3. *TBA test*. The TBA values of the samples were quantified in triplicate following the AOCS method Cd 19b-90 [4], using 0.20–0.36 g of samples and a 10-mL volumetric flask to dissolve the samples in 1-butanol. The absorbance was read in the same equipment cited above (2).
4. *DiaMed F.A.T.S. kits*. The malonaldehyde and alkenal determinations using the AldeSafe™ STD and AlkalSafe™ STD kits (DiaMed, Switzerland), respectively, were carried out the day after the official test determinations. The procedures adopted followed the manufacturer's recommendations.

Statistical Analysis

1. *Linear regression*. The linear regression model used to correlate the results of the kits employed in this study with those of the official methods was the least square method, using the software MiniTab for Windows version 12.1 at 95% of confidence and 95% of prediction. The analysis of variance (ANOVA) was used to test the linear models using the software SAS for Windows version 8.2.
2. *Comparison of means*. The ANOVA and Tukey tests were applied to prove the existence of significantly different samples at a significance level of 5%, evaluated by the same method. The software SAS for Windows version 8.2 was used again.

Results and Discussion

The moisture contents of the samples ranged from 0.101 ± 0.013 to 0.181 ± 0.006%, and the reagents presented moisture values of: 0.29 ± 0.02% for glacial acetic acid; 0.05 ± 0.01% for isooctane; and 1.2 ± 0.1% for the *p*-anisidine crystals. These values, except for that of isooctane, exceeded the limit for the *p*-anisidine test, even if the reagents were purified and the samples treated to eliminate moisture. The 1-butanol moisture content was 0.28 ± 0.04%, not interfering in the TBA test. No other study was found in the literature reporting carrying out the Karl Fischer moisture determination of the samples and reagents before the TBA and *p*-anisidine tests.

The *p*-anisidine values of the frying samples were 8.5 ± 1.5 for fresh oil and varied from 21.8 ± 1.3 to 64.4 ± 3.7 for the frying oils. The literature reports *p*-anisidine values of <0.1 [13]; 1.4 [14]; 1.81 [15], 2.0 [16]; 2.05 ± 0.11 [17]; 0.53–4.83 [18] and 6.3 [7] for fresh soybean oils and 1.51 ± 0.10; 1.56 ± 0.15 [19]; and 4.30 ± 0.49 [20] for fresh palm oils. Considering the wide ranges of values for the same kind of product, one can affirm that the *p*-anisidine value for soybean oil is similar to that of palm oil. Higher values for soybean oil would be expected if its higher percentage of linoleic acid were taken into account, as compared to palm oil. The latter is characterized by oleic acid amongst the unsaturated fatty acids, which is less prone to oxidation than linoleic acid. These similarities in the values were explained by the interference of water in the *p*-anisidine test [4]. The value obtained for the fresh soybean oil was much higher than the values found in the literature, leading to the conclusion that the conditions adopted here were the best possible ones.

The results obtained for the alkenal concentrations using the AlkalSafe™ STD kit ranged from 14 ± 1 to 649 ± 21 nmol/mL. These correlated well with the results of the AOCS official method Cd 18b-90. The linear correlation model was validated using the ANOVA test ($F_{Calc} >> F_{Tab}$) (Table 1) and the equation obtained was $y = 9.07 \times + 80.94$, explaining 55% of the variation in the alkenal concentrations, with a coefficient of correlation

Table 1 ANOVA test for linear regression of the correlation between the AlkalSafe·™ STD kit and the *p*-anisidine test

Source of variation	SS	DF	MSS	F_{Calc}	F_{Tab}	P
Regression	791385	1	791385	70.43	≅4.00[a]	0.000
Residual error	651692	58	11236			
Total	1443077	59				

SS sum of squares, *DF* degrees of freedom, *MSS* mean of sum of squares

[a] at the 5% significance level

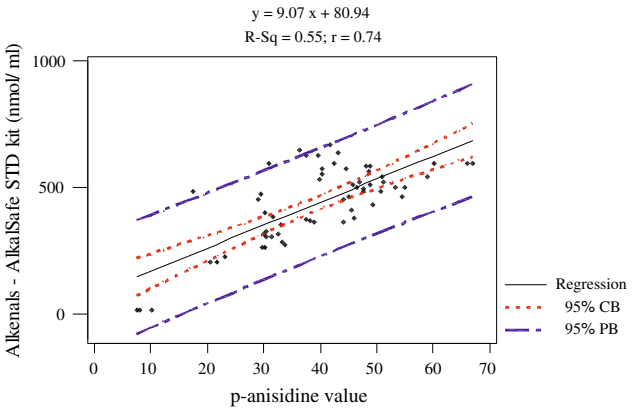

Fig. 1 Graph of the correlation between the results with the AlkalSafe™ STD kit and those of the AOCS official method Cd 18–90 for frying oil samples, with bands of 95% of confidence and (CB) and 95% of prediction (PB)

value r of 0.74 (Fig. 1). Consequently, the AlkalSafe™ STD kit can be used in substitution for the *p*-anisidine test, with improvements in the toxicity of the reagents. It is well-known that the *p*-anisidine reagent is irritant, toxic and possibly carcinogenic [4].

Applying the ANOVA and Tukey tests to verify statistical differences amongst frying oil samples ($\alpha = 0.05$) evaluated by the same method, it was possible to observe that the results obtained with the AlkalSafe™ STD were more precise than those of the *p*-anisidine test (Table 2), since it discriminated a larger number of samples.

The malonaldehyde concentrations in the frying oil samples were –1.3 ± 0.9 to 37.3 ± 1.0 nmol/mL and the TBA values were 0.018 ± 0.002–0.040 ± 0.002. The negative values obtained with the AldeSafe™ STD kit were possible due to experimental error and the fact that the concentrations of malonaldehyde were very small. No correlation between the kit and the official method results was found ($r = 0.25$). One explanation for this was the small range of TBA values and the lack of sensitivity of the test [11]. In the present experiment, it was only possible to group the results into three groups of significantly different samples using the TBA test. In comparison, the AldeSafe™ STD kit provided visibly more accurate results, differentiating a greater number of samples at the 5% level of significance (Table 3), as occurred with the AlkalSafe™ STD kit when compared with the *p*-anisidine test (Table 2). The TBA values of the frying oils were below unity and could be associated with the heat employed in the test, accelerating the oxidative process and resulting in a higher TBA value [11]. It must also be considered that the fresh

Table 2 Alkenal concentrations (AC), in nmol/mL, and anisidine values of the frying oil samples, organized in decreasing order for the same method

Samples	AC (nmol/mL)	Samples	Anisidine values
J	649 ± 21^{a}	I	64.3 ± 2.9^{a}
I	595 ± 0^{ab}	T	54.9 ± 4.0^{ab}
H	589 ± 33^{ab}	Q	52.2 ± 3.0^{bc}
G	585 ± 11^{b}	K	48.6 ± 1.7^{bcd}
O	572 ± 48^{bc}	P	47.9 ± 0.9^{bcd}
P	559 ± 31^{bc}	S	47.7 ± 3.0^{bcd}
T	520 ± 24^{cd}	N	46.1 ± 2.7^{bcd}
S	514 ± 10^{cde}	H	43.4 ± 1.1^{cd}
Q	480 ± 27^{def}	F	43.2 ± 4.3^{cd}
K	473 ± 6^{def}	L	40.2 ± 3.4^{de}
R	453 ± 16^{ef}	G	39.9 ± 9.0^{de}
N	443 ± 16^{fg}	O	39.9 ± 0.5^{de}
F	387 ± 22^{gh}	J	38.5 ± 2.9^{de}
M	366 ± 22^{hi}	C	31.6 ± 1.4^{ef}
L	357 ± 6^{hij}	M	31.6 ± 1.4^{ef}
D	319 ± 6^{ijk}	E	31.1 ± 2.2^{ef}
C	297 ± 12^{jk}	D	30.9 ± 1.3^{ef}
E	265 ± 6 k^{l}	R	25.4 ± 6.8^{f}
B	215 ± 12^{l}	B	21.8 ± 1.3^{f}
A	14 ± 1^{m}	A	8.5 ± 1.5^{g}

Samples with the same letter in the same column represent the absence of significant difference ($\alpha = 0.05$)

Table 3 Malonaldehyde concentrations (MC) in nmol/mL, obtained using the AldeSafe STD™ kit, and TBA values (decreasing order), for frying oils

Samples	MC (nmol/mL)	Samples	TBA values
S	37.3 ± 1.0^{a}	L	0.040 ± 0.002^{a}
J	35.2 ± 2.0^{ab}	E	0.039 ± 0.000^{ab}
P	32.7 ± 0.2^{bc}	I	0.038 ± 0.007^{ab}
O	32.6 ± 1.4^{bc}	Q	0.036 ± 0.001^{abc}
I	31.1 ± 2.0^{cd}	B	0.034 ± 0.004^{abc}
N	29.5 ± 0.4^{cd}	H	0.033 ± 0.010^{abc}
T	28.3 ± 3.2^{de}	J	0.033 ± 0.007^{abc}
G	25.6 ± 0.6^{e}	G	0.033 ± 0.004^{abc}
F	24.9 ± 0.3^{ef}	R	0.032 ± 0.001^{abc}
M	21.5 ± 0.5^{fg}	K	0.032 ± 0.002^{abc}
K	21.4 ± 0.3^{fg}	P	0.030 ± 0.006^{abc}
Q	19.4 ± 0.5^{gh}	C	0.030 ± 0.001^{abc}
D	16.4 ± 0.0^{hi}	A	0.028 ± 0.009^{abc}
E	15.7 ± 0.4^{ij}	T	0.027 ± 0.012^{abc}
L	15.2 ± 0.6^{ij}	D	0.027 ± 0.009^{abc}
C	14.8 ± 0.5^{ij}	M	0.025 ± 0.005^{abc}
H	12.2 ± 0.3^{jk}	O	0.024 ± 0.007^{abc}
B	9.4 ± 0.9^{k}	F	0.023 ± 0.007^{abc}
R	5.0 ± 0.0^{l}	S	0.021 ± 0.006^{bc}
A	–1.2 ± 0.9^{m}	N	0.018 ± 0.002^{c}

Samples with the same letter in the same column represent the absence of significant difference ($\alpha = 0.05$)

oil sample (sample A) was not the one with the smallest TBA value as one would expect, since this sample had the smallest amount of malonaldehyde (Table 3), and the smallest anisidine value and alkenal content (Table 2).

The results of this study lead to the conclusions that the AlkalSafe™ kit could be used satisfactorily to monitor frying oils and that the TBA test was not appropriate for this function.

In addition, both the AldeSafe™ and AlkalSafe™ kits were more sensitive than the official methods and could be feasible options, offering advantages related to good working conditions, safety, little generation of residues and sample sizes.

Acknowledgments The authors are grateful to Capes and DiaMed AG (Switzerland) for their financial support, Mr. Laércio de Melo for his active participation, Mrs. Evelyne de Groote for technical assistance and Mr. José Togashi who gave permission to collect the frying oil from the Institutional Restaurant of Unicamp.

References

1. Gertz C (2000) Deep frying remains an art. Eur J Lipid Sci Technol 102:529–538
2. Dobarganes MC, Márquez-Ruiz G (1998) Regulation of used frying fats and validity of quick tests for discarding the fats. Grasas y Aceites 48:331–335
3. Paul S, Mittal GS (1997) Regulating the use of degraded oil/fat in deep-fat/oil food frying. Crit Rev Food Sci Nutr 37:635–662
4. AOCS (2004) Official methods and recommended practices of the American Oil Chemists' Society. AOCS, Champaign
5. Houhoula DP, Oreopoulou V, Tzia C (2002) A kinetic study of oil deterioration during frying and a comparison with heating. J Am Oil Chem Soc 79:133–137
6. Tompkins C, Perkins EG (1999) The evaluation of frying oils with the *p*-anisidine value. J Am Oil Chem Soc 76:945–947
7. Lima JR, Gonçalves LAG (1994) Parâmetros de Avaliação da Qualidade de Óleo de Soja Utilizado para Fritura. Quím Nova 17:392–396
8. Rossell JB (1994) Measurement of rancidity, In: Allen JC, Hamilton RJ (eds) Rancidity in Foods, Chapman and Hall, London, pp 22–53
9. Przybylski R, Eskin NAM (1999) Measurement and significance of volatile compounds, In: Warner K, and Eskin NAM (eds) Methods to assess quality and stability of oils and fat-containing foods, AOCS, Champaign, 1995, pp 107–133 *apud* Tompkins C, Perkins EG (1999) The evaluation of frying oils with the *p*-anisidine value. J Am Oil Chem Soc 76:945–947
10. Addis PB (1986) Occurrence of lipid oxidation products in foods. Food Chem Toxicol 24:1021–1030
11. Osawa CC, de Felicio PE, Gonçalves LAG (2005) Teste de TBA aplicado a carnes e derivados: métodos tradicionais, modificados e alternatives. Quím Nova 28:655–663
12. DGF (1984) Deutsche Einheitsmethoden zur Untersuchung von Fetten, Fettprodukten und verwandten Stoffen. Wis-senschaftliche Verlagsgesellschaft mbH, Münster
13. Naz S, Siddiqi R, Sheikh H, Sayeed SA (2005) Deterioration of olive, corn and soybean oils due to air, light, heat and deep-frying. Food Res Int 38:127–134
14. Chu YH (1991) A comparative study of analytical methods for evaluation of soybean oil quality. J Am Oil Chem Soc 68:379–384
15. Man YBC, Liu JL, Jamilah B, Rahman RA (1999) Quality changes of refined-bleached-deodorized (RBD) palm olein, soybean oil and their blends during deep-fat frying. J Food Lipids 6:181–193
16. List GR, Evans CD, Kwolek WF, Warner K, Boundy BK, Cowan JC (1974) Oxidation and quality of soybean oil: a preliminary study of the anisidine test. J Am Oil Chem Soc 51:18–21
17. Tan CP, Che-Man YB, Selamat J, Yusoff MSA (2002) Comparative studies of oxidative stability of edible oils by differential scanning calorimetry and oxidative stability index methods. Food Chem 76:385–389
18. Medina-Juarez LA, Gonzalez-Diaz P, Gamez-Meza N, Ortega-Garcia J, Moreno-Varela AB, Bringas-Alvarado L, Angulo-Guerrero O (1998) Effects of processing on the oxidative stability of soybean oil produced in México. J Am Oil Chem Soc 75:1729–1733
19. Gil B, Cho YJ, Yoon SH (2004) Rapid determination of polar compounds in frying fats and oils using image analysis. Lebensm Wiss Technol 37:657–661
20. Al-Kahtani H (1991) Survey of quality of used frying oils from restaurants. J Am Oil Chem Soc 68:857–862