Analysis of FFA in Edible Oils by Catalyzed End-Point Thermometric Titrimetry (CETT)

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ABSTRACT: A novel titrimetric procedure has been developed for the determination of FFA in edible oils using an automated thermometric titration with KOH in isopropanol as titrant. The end point is indicated by the strongly exothermic base-catalyzed reaction between acetone and chloroform. The procedure is fast, easy to use, accurate, and highly reproducible. Analytical precisions (as one SD) in the range 0.001–0.003% w/w (as oleic acid) for new and used cooking oils were obtained. It is very suitable for the routine process and quality control of new and used oils in commercial frying operations, among other applications.

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KEY WORDS: Automated titration, fats, FFA, free fatty acids, oils, thermometric titration, thermometric titrimetry.

The FFA content of edible fats and oils is an important quality control parameter. The official titrimetric method for the determination of FFA involves titration with ethanolic KOH to a visual end point based on the color change of phenolphthalein indicator (AOCS Official Method Ca 5a-40; 1). In general, accuracy of titrations involving visual indicators is strongly influenced by the skill and color perception of the analyst, and variations between analysts can be considerable, particularly if the sample solution itself is colored. Potentiometric titration procedures employing a glass membrane pH electrode to indicate the end point have also been developed. Potentiometric titrations in nonaqueous media can be unreliable if the electrode membrane is not frequently rehydrated. To cope with the demands of modern commercial operations making or using edible oils, there is a need for a robust, simple, fast, accurate, and precise automated titrimetric procedure that is substantially independent of the analyst's skill and that is suitable for routine process and quality control.

Modern thermometric titrimetry employs a thermistor as the sensing element. Thermistors are solid-state devices that exhibit large changes in their electrical resistance for small changes in temperature. The end points in thermometric titrations are indicated by rates of change in the temperature of the solution. When small amounts of weakly acidic species are titrated in nonaqueous media with a titrant of relatively low strength, the heat evolved by the neutralization reactions may be quite small and easily swamped by other thermal effects such as solvent evaporation and the heat of mixing of the titrant with the sample solution. Vaughan and Swithenbank (2) found that when a sample containing a weak acid was dissolved in acetone, the first excess of base catalyzed a strong exothermic reaction resulting from the condensation of acetone to diacetone alcohol. The generic term of Catalyzed End-Point Thermometric Titrimetry (CETT) may be applied to the technique of using an external indicator system with a considerable reaction enthalpy to locate an end point. Vaughan (3) claimed that KOH in isopropanol gave a slightly higher rate of heat evolution than ethanolic or methanolic KOH and was therefore to be recommended. The method has been successfully applied to the determination of a range of acidic substances, including carboxylic acids, hydroxy acids, phenols, phenolic acids, keto-enols, imides, and some aromatic nitro-compounds. However, practical experience has demonstrated that in some sample matrices the inflection at the end point showed excessive rounding, with consequent loss of precision and accuracy. The base-catalyzed strongly exothermic polymerization of such monomers as acrylonitrile, methyl acrylate, and dimethyl itaconate has also been used for the determination of acids (4). However, the high toxicity of these reagents coupled with the fact that they were used in large quantities as the primary solvent would appear to preclude their use in routine process and quality control applications. Another base-catalyzed reaction has been considered as a candidate to indicate end points in the determination of weak acids. Acetone and chloroform react exothermically (and under certain conditions, violently) in the presence of base. The following reaction mechanism appears likely:

$$\mathrm{CHCl_3 + OH^- \rightarrow CCl_3^- + H_2O} \quad [1]$$

$$\mathrm{CCl_3^- + (CH_3)_2C{=}O + H_2O \rightarrow (CCl_3)(CH_3)_2C{-}OH + OH^-} \quad [2]$$

Preliminary experiments showed that the reaction in the titration vessel behaved in a safe and predictable manner without any sign of uncontrolled temperature rise, and considerable promise for further investigation was indicated. In samples where a range of acidic species may be anticipated, a thermometric procedure that uses a base-catalyzed indicator reaction may be preferred to a potentiometric procedure where the end point inflection can be influenced by the relative amounts of each of the acidic species and their respective p*K* values. In the thermometric procedure, the end point is seen only when all acidic species capable of reacting with KOH have been

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neutralized. Thermometric sensors based on thermistors are also ideally suited for routine process and quality control, requiring no maintenance other than rinsing between titrations.

EXPERIMENTAL PROCEDURES

Titrations were carried out using an automated titration system employing thermometric sensing (Multitrator Pty. Ltd., Brisbane, Australia). Titration software was Multitrate version 1.2.0. The titrant was 0.1 mol/L KOH in isopropanol, which had been standardized against 99.8% pure benzoic acid dissolved in acetone. The titrant was delivered at a constant rate of 1 mL/min from a stepper motor-driven buret under the control of a dedicated titration software program. The output of the thermistor was sampled at a rate of 10 times per second. To obtain the end point, the raw temperature data were subjected to smoothing by a signal conditioning algorithm designed to impart minimum shift to the end point as a function of the degree of smoothing employed. End points were determined using a peak-picking algorithm applied to the second derivative curve computed from the smoothed temperature data. A first-derivative curve was also computed to monitor reaction rates and to permit activation of the automatic cutoff feature that stops the titration automatically after the end point has been reached. At the end of each titration, sample and titration data were automatically sent for identification and computation, respectively, to a dedicated, user-defined spreadsheet prepared in Microsoft Excel.

All reagents were of analytical reagent (A.R.) quality. The grades of isopropanol and acetone were chosen for their minimal water contents. Grades of these solvents manufactured for HPLC analyses were certified to have very low water contents and were of a reasonable price.

Preparation and standardization of 0.1 mol/L KOH titrant. KOH (5.6 g) was dissolved in isopropanol in a 1000-mL volumetric flask. Approximately 0.32 g benzoic acid was weighed accurately into a 200-mL volumetric flask, dissolved, and made to volume with acetone. A calibration curve was prepared after titrating solutions made according to volumes listed in Table 1. Volumes of benzoic acid solution were delivered into the titration vessels using volumetric pipets. Each titration for aliquots of benzoic acid solution was performed in duplicate. Owing to the volatility of the acetone solvent and the attendant risk of leaving benzoic acid residues in the pipet, it was necessary to rinse the pipet with acetone and dry it between aliquots. The amount of benzoic acid (in mmol) in each aliquot (y-axis) was plotted against the corresponding titer of KOH solution in mL (x-axis). A regression analysis of the data was performed. For titrations where titrant is added at a constant rate, it is necessary to determine a system offset or blank. The system offset is a summation of all delays inherent in the system for the conduct of a titration under defined experimental conditions. These delays include contributions from kinetics of the chemical reaction between titrant and titrand, sensor response, mixing inefficiencies, and electronic transfer and computation of data. It is expressed as volume of titrant and is subtracted from the raw titration data. It is determined from the intercept of the linear regression curve on the y-axis. The determination of the system offset obviates the need to preneutralize the solvents used in the determination, because any residual acidity or basicity of the solvent is taken into account in this process. The titrant molarity is calculated from the gradient of the regression curve. This method has the advantage that it provides evidence of the linearity of the method response over the measurement range, as well as a high degree of confidence in the accuracy of the titrant standardization. Table 1 lists the makeup of the four solution strengths titrated for the preparation of the calibration curve.

TABLE 1
Makeup of Solutions for Preparation of Titrant Calibration Curve

Volume of benzoic acid in acetone solution (mL)	Volume of added acetone (mL)	Total volume of acetone (mL)	Volume of chloroform (mL)
25.00	0	25	2
20.00	5	25	2
15.00	10	25	2
10.00	15	25	2

Preparation and titration of test samples. Samples of new and used frying oils were supplied by a manufacturer of fried snack foods. In addition, a bottle of canola oil was purchased from a supermarket. For the new oils, approximately 10 mL of sample was accurately weighed into clean, dry 140-mL polypropylene titration vessels. A magnetic stirrer was provided, and the sample dissolved in 25 mL of acetone and 2 mL of chloroform dispensed into the vessel. For safety reasons, the acetone and chloroform were not mixed beforehand. For the used oil sample, approximately 10 mL was accurately weighed, and the titration solutions were prepared in the same manner. Statistically significant data on reproducibility of the procedure were obtained by analyzing the new canola oil and the sample of used frying oil in replicate. A comparison of the new catalytic end-point procedure employing the base-catalyzed reaction of acetone and chloroform with the previously published procedure using acetone only was made by titrating the same sample of used frying oil in replicate in this solvent alone.

Preparation and titration of oleic acid solutions. The method was tested for accuracy by titrating a sample of high-purity oleic acid (reported assay ~99%, determined by GC). Approximately 0.8 g of oleic acid was accurately weighed and transferred quantitatively into a 200-mL volumetric flask with acetone and made to volume with the same solvent. To each of the 25-mL aliquots, 2 mL of chloroform was added prior to titrating to the exothermic end point. The volumetric pipet was rinsed with acetone and dried before taking each aliquot to prevent carryover.

RESULTS AND DISCUSSION

Standardization of titrant. In the work reported here, the KOH titrant was determined to have a strength of 0.1007 mol/L. The system offset was determined to be 0.079 mL. The coefficient

TABLE 2
Analysis of Oil Samples and Oleic Acid Using Catalyzed End Point Thermometric Titrimetry

Sample	Catalyzed end point indicator method	Number of replicate analyses (*n*)	% w/w FFA (as oleic acid)	SD, as % w/w oleic acid	CV (% RSD[a])
Used fryer oil	Acetone only	5	2.731	0.040	1.5
Used fryer oil	Acetone/chloroform	10	2.603	0.003	0.11
Fresh fryer oil	Acetone/chloroform	2	0.029, 0.027[b]	—	—
Fresh canola oil	Acetone/chloroform	9	0.053	0.001	2.1
Pure oleic acid, ~99%	Acetone/chloroform	7	99.28	0.15	0.15

[a] RSD, relative SD.
[b] Two determinations only.

of correlation (R^2) for the linear calibration curve was found to be 1.00000. This standardization is valid for the batch of titrant and the titration conditions used in these experiments.

Results of analysis. Condensed results of experiments are listed in Table 2. The acetone/chloroform indicator system gives highly reproducible results. In the comparison of the two indicator systems using the same sample of fryer oil, the new acetone/chloroform indicator system is seen to be more reproducible than the previously published acetone indicator system. The result obtained from the analysis of the pure oleic acid sample is in close agreement with the label value of "~99%," which had been obtained by GC, and confirms the accuracy of the new procedure. The analysis of the new, unused oils demonstrates that the procedure is capable of providing reproducible results with oils of low FFA content. It may be noted that the precision of the acetone/chloroform indicator titrations approaches the best results obtainable in titrations of fully dissociated ionic species in aqueous solutions. Such reproducibility is rare in nonaqueous titrations.

Titration plots. Figure 1 illustrates a comparison of the thermometric titration curves obtained from the same sample using the acetone indicator system and the new acetone/chloroform indicator system. The temperature plot for the acetone/chloroform indicator titration A-A is read from to the y_1 axis, the acetone-only indicator titration B-B is read from the y_2 axis. Both y axes have the same span of 1°C. The end-point inflection for the acetone/chloroform titration is much sharper and much more pronounced than that for the acetone-only indicator titration, which is displaced toward higher titrant consumption. It can easily be seen why slightly lower results are obtained with the acetone/chloroform indicator method under the same experimental conditions. Further evidence of the sharpness of the end-point inflection for the acetone/chloroform indicator titration is seen in Figure 2, where the second-derivative plot is presented along with the solution temperature plot.

Safety of titration. Acetone and chloroform mixtures are known to react vigorously when basified. For that reason, mixtures were not prepared before the titration, and the reagents were added separately. However, there was no evidence of uncontrolled heat evolution either during or after the titration. In fact, the rate of heat evolution dropped away sharply shortly after the end point. This may be seen in Figure 3, which illustrates a combined plot of the solution temperature and the first derivative as a function of titrant addition.

The continued addition of titrant after the end point appears to have a quenching effect on the acetone/chloroform

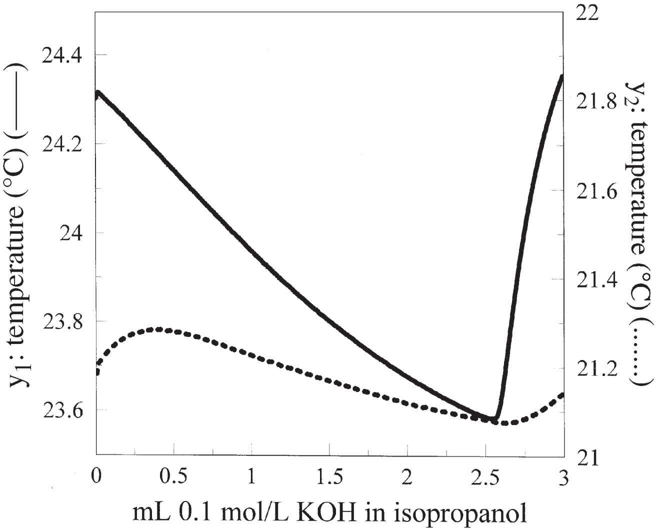

FIG. 1. Comparison of temperature plots of acetone-only and acetone/chloroform indicator thermometric titrations of FFA content of the same used fryer oil. Acetone only (-----); acetone/chloroform (——).

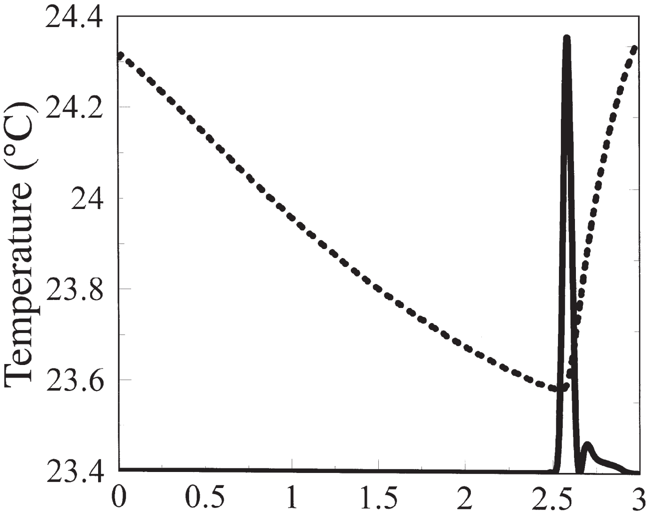

FIG. 2. Combined temperature and second-derivative plots for an acetone/chloroform indicator thermometric titration of FFA content of a used fryer oil. Temperature (-----); second derivative (——).

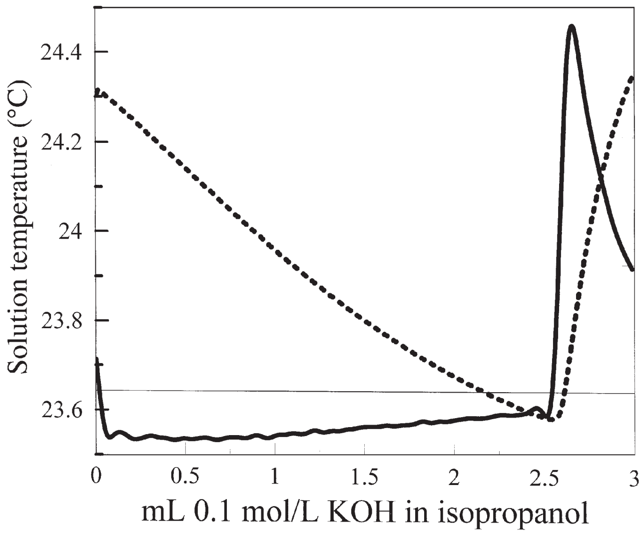

FIG. 3. Combined temperature and first-derivative plots for an acetone/chloroform indicator thermometric titration of FFA content of a used fryer oil. Temperature (-----); first derivative (——).

reaction. Titration residues were disposed of by pouring into a container of water.

The amount of chloroform used in each determination (2 mL) is quite small. In preliminary experiments, it was found that relatively sharp end points could still be obtained by using 1.0 and even 0.5 mL of chloroform, although there was a slight increase in the analyzed concentration of FFA and a loss in precision of the determination. Increasing the amount of chloroform to 5 mL also caused end-point rounding and loss of precision.

Although chloroform and the 2-trichloromethyl-2-propanol product of reaction (2) are not innocuous, the small amount of chloroform used and the application of safe laboratory work practices should permit the general use of this procedure. The compact automatic buret and magnetic stirrer combination may be easily accommodated in a fume hood. It may also be noted that the titration is performed in a beaker with a closely fitted lid, through which the combination titrant delivery-thermometric probe is inserted. Aside from preventing disturbance of the titration from stray air currents, loss of volatile components is also minimized.

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