A Fully Robotic Method for the Determination of Acid Values in Olive Oil Without Titration

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ABSTRACT: A method for the determination of acid values (AV) in olive oil without titration is proposed, based on pH measurements on an oil sample emulsion in a suitable reagent and fully automated using a robotic station. The robot performs the weighing of the sample, adds the reagent, emulsifies both, and measures the pH of the emulsion. The data are acquired and treated by the computer. After optimization, two versions of the measurement step (interpolation within a calibration curve and addition of standard) are developed and applied to 51 oil samples with AV values that also have been determined by the standard method based on potentiometric titration. Both versions are statistically compared with the standard method, and no significant differences are found. Excellent correlation ($R > 0.99$) between all three methods demonstrates the usefulness of the fully automated approach that works at a sample rate of 15 sample h^{-1} with relative standard deviations lower than 2.5%.

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KEY WORDS: Acid values, free acidity, olive oil, robotic automation.

Titratable acidity or acid value (AV) corresponds to the total concentration of titratable acids in a sample. This parameter is an important characteristic of the quality of a number of products such as vegetable oils (this is one of the major parameters in establishing the quality of batches of raw vegetable oil prior to acceptance), juices, wines, petroleum, motor oils, polyesteric resins, and plasticizers. Throughout the harvest period of vegetable-oil raw materials, the contribution from different sources is abundant, thus causing overload in routine laboratories because of the slowness of the AV determination.

The majority of national and international standard methods for AV determination in oil are based on acid-base titration in nonaqueous systems (1–3). The methods thus proposed are time- and labor-consuming, have relatively high detection limits, and are far from fully automated. The major drawbacks involved in these methods are potentiometric monitoring in nonaqueous media and preliminary extraction of the analytes, mandatory in very dirty matrices (1). Other shortcomings of the standard methods are the use of toxic solvents such as diethylether and methyl-isobutyl-ketone as well as nonaqueous alkaline titrants, which are sensitive to carbon dioxide contamination from the atmosphere.

Several methods for AV determination in oils without titration based on the use of pH metric (4–6) and spectroscopic techniques with (7) and without solvent (8,9) have been proposed. Among these methods, those based on pH measurements without titration seem to be the most efficient because of their simplicity, rapidity, low-cost setup, and easy automation. The method reported by Tur'yan *et al.* (10) is based on the formation of an emulsion of the oil sample with a solution of triethanolamine in a 1:1 water/isopropanol mixture. Quantitative extraction of the free fatty acids from the oil into the polar-basic solution is achieved, thus giving rise to a precise pH monitoring, easily correlated with the amount of the analytes in the sample (11–13).

Full automation of the steps involved in AV determination (namely, weighing, addition of the reagent, stirring if required, and monitoring) is difficult using an alternative to robots. Robotic stations were used for full automation of the determination of target parameters in olive oil (14–16). These methods show the usefulness of this type of automation for processes in which the first steps are the bottleneck of the overall process.

This paper describes a fully automated robotic method for the determination of AV based on extraction of free fatty acids from olive oil samples into an immiscible phase and subsequent AV determination by monitoring the pH of the sample-reagent emulsion using a combined glass-calomel electrode as proposed by Tur'yan *et al.* (10). Two different versions of the method were carried out and are discussed.

EXPERIMENTAL PROCEDURES

Instruments and apparatus. The robotic station consisted of a Zymate II Plus robot (Zymark, Hopkinton, MA) and the following peripherals in a circular arrangement (see Fig. 1).

Master laboratory station (MLS). The proposed method uses two MLS (MLS 1 and MLS 2), which consist of three syringes, intended to dispense liquids. One of them (MLS 2) is used in conjunction with the dilute and dissolve unit and only two of its three syringes (A and B) were used by connecting both to the reservoir and dilute and dissolve dispenser (syringe A: reagent solution; syringe B: distilled water). Each

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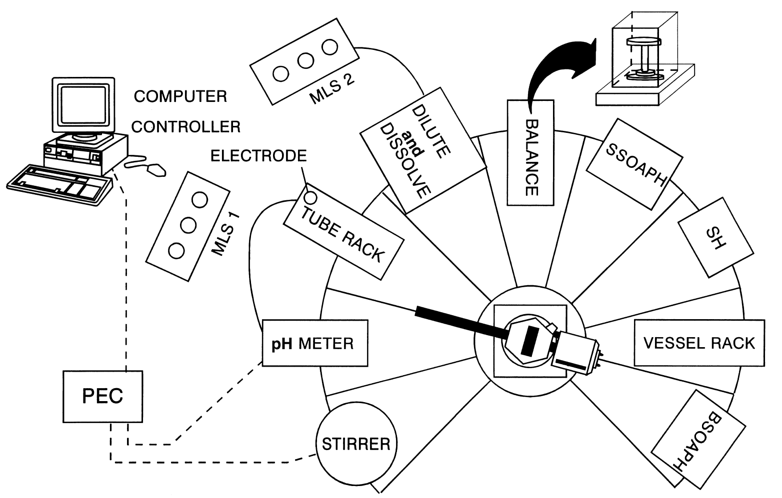

FIG. 1. Robotic station arrangement for the determination of acid values in olive oil without titration. BSOAPH, big-size object all-purpose hands; SSOAPH, small-size object all-purpose hands; MLS, master laboratory station; SH, syringe hands; PEC, power and event controller.

of the three syringes of the MLS 1 was connected to both the reservoir and dispenser tube (syringe A: 4.64×10^{-3} M HCl solution; syringe B: 5.79×10^{-3} M HCl solution; syringe C: 0.1 M KOH solution).

Small-size object all-purpose (SSOAP), big-size object all-purpose (BSOAP), and syringe hands (SH). The SSOAP hand allows the robot to seize centrifuge tubes and objects of similar size. For the SSOAP hand to be able to handle a tube of 1.5 mm o.d., that is, the usual diameter of a dispenser tube, an empty cartridge must be pierced at the bottom and the dispensing tube inserted through the hole over a length of *ca.* 5 cm. The BSOAP hand allows the robot to seize 100 mL-precipitate vessels and objects of similar size. The SH has a syringe which allows it to aspirate volumes between 0.2 and 1.0 mL.

Power and event controller (PEC). The PEC module acts as an interface between the controller, peripherals, and robot.

System V controller. This module sends orders to the rest of the units, including the robot. It acts as an interface between the computer and peripherals (those belonging to the robot station and the external modules). One of the abilities of the System V controller is to control additional units such as a peristaltic pump, a photometer, a microwave digester, and switching valves. The RS232 outputs of these units allow them to be operated by the System V controller *via* the PEC.

Balance. This module includes two wires which open the balance door on contact and close it on separation. The wires are connected to a PEC switch in such a way that issuing a command brings them into contact ("on" position) to open the balance door. The balance plate for tube weighing was modified as shown in the detail in Figure 1 (near the top of the balance), thus allowing the precipitate vessel to be maneuvered to and from the balance by the robot.

In addition, an Agimatic-N stirrer (Selecta, Barcelona, Spain), a Metrohm combined pH glass electrode (Switzerland), and a Radiometer PHM-64 research pH meter (Copenhagen, Denmark) were used. The System V controller was interfaced to a Netset 286/400 personal computer.

Reagents and solutions. Proposed method. The reagent solution consists of 0.20 M triethanolamine (Merck, Darmstadt, Germany) + 0.02 M KNO_3 (Merck) in a 1:1 vol/vol isopropanol (Panreac, Barcelona, Spain)/water mixture with a pH adjusted at 11.30 ± 0.05 with a 0.1 M KOH (Merck) aqueous solution. A 1 M (F = 0.966) HCl (Panreac) aqueous solution was used for preparing two more diluted solutions (5.79×10^{-2} M and 4.64×10^{-3} M) in reagent solution. A 0.1 M KOH (Panreac) aqueous solution.

Standard method. Isobutyl-methyl-ketone ($d = 0.8$) and a 0.1 N KOH solution in isopropanol were standardized with benzoic acid.

Standard method. Between 5 and 10 g of dried oil is weighed in a 150-mL glass vessel; 50 mL of isobutyl-methyl-ketone is added. Then, the electrode is plunged into the vessel, and the titration is carried out using the 0.1 N KOH aqueous solution as titrant.

The AV value is calculated from Equation 1:

$$\mathrm{AV} = \frac{V \times 56.11 \times M}{m} \quad \text{mg KOH g}^{-1}\text{ oil} \qquad [1]$$

where V is the volume of titrant (in mL) consumed in the titration, M is the molarity of the KOH solution (0.1 M), and m is the weight of the sample (in g).

Proposed robotic method. The robot catches a 100-mL glass vessel from the vessel rack which is carried to the balance and tared. The robot then selects the centrifuge tube containing the first sample and sets it in an inclined position above the vessel placed in the balance, thus permitting about 6 g of oil to be added before bringing the 100-mL vessel below the dilute and dissolve liquid dispenser and adding 50 mL of reagent solution. Then, the robot places the vessel on the stirrer, and the content is stirred for 5 min. Meanwhile, the robot prepares the electrode for measurement by introducing it into a drier tube. Next, the robot turns the stirrer off, introduces the electrode into the vessel and measures the pH of the emulsion (pH_1); the datum is acquired by the computer. The aforementioned steps are the same in the two versions of the method. Subsequently, in Version I the pH datum is interpolated within the calibration curve previously run, and the AV of the sample is delivered by the computer. In Version II, the SH of the robot aspirates 0.5 mL from the 0.966 M HCl solution contained in a centrifuge tube placed in the tube rack and adds it to the emulsion. The robot takes the electrode again, dries it, introduces it into the vessel, and measures the pH of the emulsion (pH_2). From the two data of pH, with and without standard addition, the AV of the sample is calculated by the computer.

Calibration and standardization. Before starting the abovementioned procedure, the robot standardizes the HCl and runs the calibration curve.

Calibration. The robot catches a 100-mL glass vessel from the vessel rack, takes it to the dilute and dissolve module, sets it below its liquid dispenser, adds 50 mL of reagent solution, and places it on the stirrer. The robot uses two HCl standard solutions for running the calibration curve. First, it gets the dispenser containing 4.64×10^{-3} M HCl, sets it above the vessel, adds 14 mL of the solution in 2-mL portions, and measures the pH after each addition. Then it gets the dispenser containing 5.79×10^{-2} M HCl and repeats the same steps. The controller collects all the pH values and runs the calibration line (pH vs. –log [HCl]).

Adjustment of the reagent pH. A 1000-mL glass vessel placed on the stirrer contains the reagent solution. The robot gets the electrode and introduces it into the solution. It selects the 0.1 M KOH dispenser, sets it above the glass vessel, and adds the necessary volume to adjust the pH of the reagent solution to 11.30 ± 0.05.

Potentiometric standardization of the HCl solution. The robot standardizes the 1 M HCl aqueous solution, adding the standard solution (aqueous Na_2CO_3) by using a SH and measuring the pH after each addition.

RESULTS AND DISCUSSION

Robotic steps. After a computer program was developed for both maneuvering the different peripherals by the robot and collecting the data from them, the various steps were optimized as follows:

In order to add about 6 g of sample, the robot selects the test tube containing the oil, sets it above the glass vessel in the balance, inclines the tube until the oil starts to go out (increase of the vessel weight), and keeps it in this position for 2 s. Then the controller obtains and evaluates the weight. If this is lower than 5 g, the process is repeated once more in the same manner. If the weight is between 5 and 6 g, the robot reduces the pouring time to 1 s, and if it is higher than 6 g, the robot finishes the weighing step and the computer stores the datum.

The natural pH of the reagent solution is about 11.0. In order to extend the linear range in the calibration line, the pH of this solution must be 11.30 ± 0.05 (10). The adjustment of this value is carried out by the robot. This operation is repeated every day in order to avoid systematic errors due to pH alterations.

Depending on the acidity of the sample, 2 to 5 min is required for quantitative extraction of free fatty acids from the oil to the reagent solution. After 5 min stirring, the combined electrode is plunged into the emulsion, and the pH is monitored at 1-min intervals. The computer acquires the datum when the same pH value coincides in three consecutive measurements.

Finally, the robot rinses and washes the electrode after monitoring the pH of the emulsion. In the rinsing procedure, the robot brings the electrode and plunges it into a test tube containing distilled water. Then the robot develops the washing procedure in which it takes the electrode to the dilute and dissolve dispenser and delivers 10 mL of distilled water; after this the robot puts the electrode in a waiting rack.

Data treatment. The principles on which the method is based can be found elsewhere (10). Two versions of the method were used for subsequent comparison of the results: Version I based on interpolation within a calibration curve and Version II based on the standard addition method.

Version I. The calibration curve was run as described in the Experimental Procedures section, and its characteristic parameters are: n, 11; equation, $y = 1.04x + 6.21$; linear range, 1.78×10^{-4}–0.93×10^{-2}; r, 0.997; confidence limits [tS: t, Student's t ($n - 2 = 9$, $\alpha = 0.05$) and S, standard deviation]: intercept, 0.06; slope, 0.03. The studied wide linear range amply encompasses the AV of any type of edible oil (usually between 0.2 and 4.5 mg of KOH g^{-1} of oil). The measurement of the pH in the reagent-oil emulsion enables the concentration of HCl from the calibration line which corresponds to the sample pH to be obtained and then the calculation of AV by Equation 2 according to the mass (m) of the sample,

$$AV = \frac{56.11 \times V \times [HCl]}{m} \quad (\text{mg KOH g}^{-1}\text{ oil}) \qquad [2]$$

where 56.11 is the molecular mass of the KOH, V is the volume (in mL) of reagent solution added (50 mL), and [HCl] is the concentration of HCl obtained from the calibration line according to the pH of the sample emulsion.

Version II. In this case, the pH for the reagent-oil emulsion was monitored (pH_1), then a volume of the acid standard solution was added to the emulsion, and the pH was monitored again (pH_2). Equation 3 shows AV calculation,

$$AV = \frac{56.11 \times N_{st} \times V_{st}}{(10^{\Delta pH} - 1 \times m} \quad (\text{mg KOH g}^{-1}\text{ oil}) \qquad [3]$$

where N_{st} is the concentration of the HCl standard solution

(0.966 M), V_{st} is the volume of HCl added (0.5 mL), and ΔpH is the difference between pH_1 and pH_2.

Precision study. A study of the reproducibility of the proposed method was performed using eight samples of AV = 2, which were measured on different days by the two versions. The precision of the method, expressed as relative standard deviation (RSD) was 1.5 and 2.5% for Versions I and II, respectively.

Application of the method and statistical comparison. The applicability of the two versions of the method was checked by applying them to 51 samples of olive oil supplied by two laboratories. A correlation plot of the results obtained is shown in Figure 2A, where r^2 is the correlation coefficient; a is the intercept [deviation expressed as the product between the Student's t-test ($n - 2 = 49$, α-confidence interval = 0.05) and the SD; b is the slope (deviation expressed as before; F_{calc} is the Fisher's F calculated for one sample with AV = 2 ($n = 8$); S_I (0.03) and S_{II} (0.05) are the SD of the two versions. As the slope is close to 1, the intercept is close to 0, and F_{calc} is lower than the F_{tab} (tabulated value for $n = 7$ and $\alpha = 0.05$). No significant differences between both versions can be concluded from these data. The results obtained with the robotic method were consistent with those provided by the manual method as the parameters obtained from their comparison, shown in Figure 2B, confirm the usefulness of the fully automated alternative.

The advantages and disadvantages of the two versions of the proposed method are as follows: the main advantage of Version I (usage of the calibration line) is its simplicity for large batches of samples. However, the calibration line must be corrected whenever temperature or pH-electrode characteristics are changed. Version II of the method lacks this drawback because neither temperature nor pH-electrode characteristics change during the short interval between pH_1 and pH_2 measurements. Despite the fact that temperature control is not necessary in Version II, two pH measurements and the intermediate addition of the standard solution are needed, making Version II more complicated than Version I. The throughput in both instances is 15 samples h^{-1}. The statistical comparison of both versions shows no significant differences in the results they provide; this assertion is also clear from Figure 2A. The precision of both versions (RSD less than 2.5%) is sufficient for monitoring the AV in routine laboratories of any type of edible oil industry.

Figure 2B shows that excellent correlation also exists ($r = 0.997$) between the fully automated method proposed here and the conventional method. The proposed method allows a reduction of time and labor consumption in comparison to the standard method (1–3) and other methods (8,9). The last, yet significant, advantage of the proposed assembly is its unattended capability for a 24-h working day in a robotic station, eliminating human intervention, decreasing overload created in routine laboratories in the determination of this common parameter.

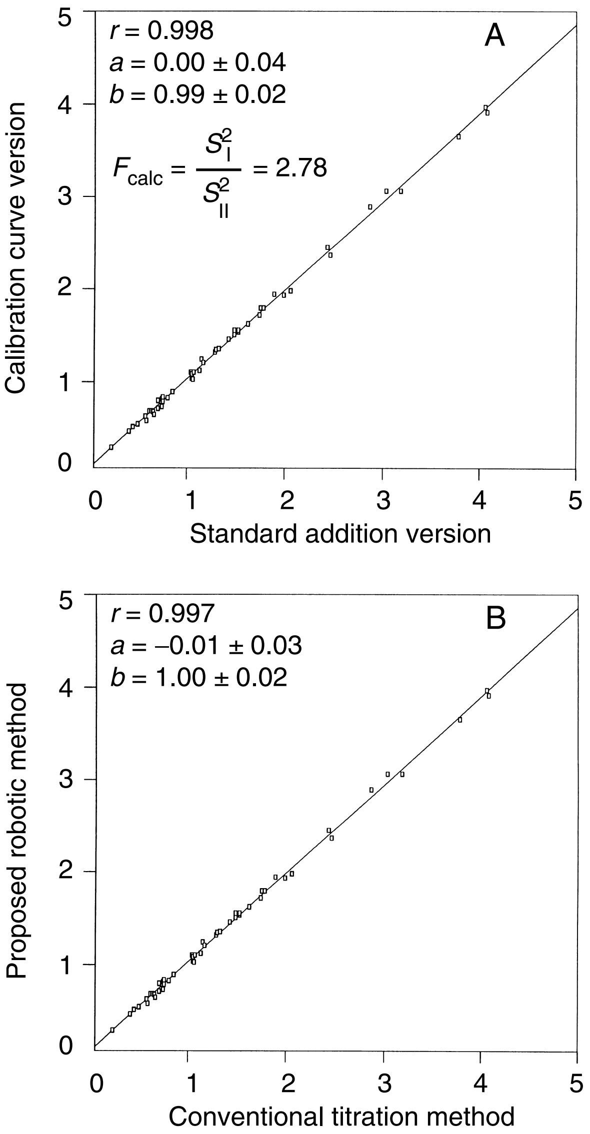

FIG. 2. Correlation plots of acid values determined by the two versions of the proposed method (A) and by the proposed and the conventional methods (B). See *Application of the method of statistical comparison* section for explanation of the statistical data.

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REFERENCES

1. Pritchard, J.L.R., *Analysis of Oilseeds, Fats and Fatty Foods*, edited by J.B. Rossel and J.L.R. Pritchard, Elsevier Applied Science, London and New York, 1991, pp. 69–75.
2. Animal and Vegetables Fats and Oils Determination of Acid Value and of Acidity, International Organization for Standardization, Switzerland, 1983, ISO 660-1983 (E).

3. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 4th edn., edited by D. Firestone, American Oil Chemists' Society, Champaign, 1989, Method Ca 5a-40.
4. Ackman, R.G., Gas–Liquid Chromatographic Analysis of Fatty Acids and Glycerides in Foods, *Food Sci. Technol. 53*:47–63 (1992).
5. Ballesteros, E., M. Gallego, and M. Valcárcel, Automatic Method for Online Preparation of Fatty Acid Methyl Esters from Olive Oil and Other Types of Oil Prior to Their Gas Chromatographic Determination, *Anal. Chim. Acta. 282*:581–588 (1993).
6. Ballesteros, E., S. Cárdenas, M. Gallego, and M. Valcárcel, Determination of Free Fatty Acids in Dairy Products by Direct Coupling of a Continuous Preconcentration Ion-Exchange-Derivatization Module to a Gas Chromatograph, *Anal. Chem. 66*:628–634 (1994).
7. Ismail, A.A., F.R. Van de Voot, G. Emo, and J. Sedman, Rapid Quantitative Determination of Free Fatty Acids in Fats and Oils by Fourier Transform Infrared Spectroscopy, *J. Am. Oil Chem. Soc. 70*:335–341 (1993).
8. Blumenthal, T.K., M.M. Blumenthal, R.F. Stier, and J.R. Stockler, paper presented at AOCS 84th Annual Meeting & Expo, Anaheim, California, April 28, 1993.
9. AOAC International, *The Referee 17*:N.11,4 (1993).
10. Tur'yan, Ya.I., O. Yu. Berezin, I. Kuselman, and A. Shenhar, pH-Metric Determination of Acid Values in Vegetable Oils Without Titration, *J. Am. Oil Chem. Soc. 73*:295–301 (1996).
11. Lapshina, T.M., Ya.I. Tur'yan, and S.I. Danilchuk, pH-Metric Method for the Determination of Acid Numbers of Oils, *J. Anal. Chem. of USSR 46*:833–840 (1991).
12. Tur'yan, Ya.I., S.I. Danilchuk, T.M. Lapshina, and L.M. Makarova, Inv. Cer. USSR SU 1688158 A1 (C1. GO1 N 33/02, C 11B1/00); 01.07.91. Appl. 4232029, 20.04.87 (1987).
13. Tur'yan, Ya.I., O.E. Ruvinskii, and S.Ya. Sharudina, Physico-Chemical Verification of pH-Metric Method of Determination of Acid Numbers Without Performing Titrations. Aqueous Systems, *J. Anal. Chem. of USSR 46*:661–667 (1991).
14. García-Mesa, J.A., M.D. Luque de Castro, and M. Valcárcel, Determination of the Oxidative Stability of Olive Oil by Use of a Robotic Station, *Talanta 40*:1595–1600 (1993).
15. García-Mesa, J.A., M.D. Luque de Castro, and M. Valcárcel, Determination of Bitterness in Virgin Olive Oil by Using a Robotic Station, *LRA 5*:29–32 (1993).
16. García-Mesa, J.A., M.D. Luque de Castro, and M. Valcárcel, Coupled Robot-Flow Injection Analysis System for Fully Automated Determination of Total Polyphenols in Olive Oil, *Anal. Chem. 65*:230–234 (1993).

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