JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Direct Automatic Determination of Bitterness and Total Phenolic Compounds in Virgin Olive Oil Using a pH-Based Flow-Injection Analysis System

JOSÉ A. GARCIA-MESA* AND RAQUEL MATEOS

Centro Venta del Llano, IFAPA, Ctra. Bailén-Motril Km. 18.5, E-23620-Mengíbar (Jaén), Spain

Flavor and taste are sensorial attributes of virgin olive oil (VOO) highly appreciated by consumers. Among the organoleptic properties of VOO, bitterness is related to the natural phenolic compounds present in the oil. Sensorial analysis is the official method to evaluate VOO flavor and bitterness, which requires highly specialized experts. Alternatively, methods based on physicochemical determinations could be useful for the industry. The present work presents a flow-injection analysis system for the direct automatic determination of bitterness and total phenolic compounds in VOO without prior isolation, based on the spectral shift undergone by phenolic compounds upon pH variation. This system enables a complete automation of the process, including dilution of the sample and its sequential injection into buffer solutions of acidic and alkaline pH. The variation of the absorbance at 274 nm showed a high correlation with bitterness and the total phenolic content of VOO, due to the close relationship between these two parameters. Thus, the proposed method determines the bitterness and phenolic compounds, with results similar to those from reference methods (relative errors ranging from 1% to 8% for bitterness and from 2% and 7% for phenolic compounds). The precision evaluated at two levels of both parameters ranged between 0.6% and 1.5% for bitterness and between 0.7% and 2.6% for phenolic compounds.

KEYWORDS: FIA; bitterness; phenolic compounds; virgin olive oil; spectrophotometric determination; pH

INTRODUCTION

Virgin olive oil (VOO) is a natural fruit juice obtained directly from olives without any further refining process. Its flavor and taste are characteristic and different from those of other fats and oils. Among the sensorial attributes of virgin olive oil, bitterness is an appreciated organoleptic property related to the natural phenolic compounds (*1*−*4*). However, if the intensity of this attribute is very high, it can provoke rejection by the consumer despite the positive effects on the oil's oxidative stability and the potential nutritional benefits derived from the high phenolic content of bitter olive oils.

The official method to analyze the bitter taste of olive oil, as well as the rest of its sensorial attributes, is by a panel of tasters, which requires highly specialized experts (*5*). On the other hand, there is well-established evidence of a significant correlation between bitterness and phenolic compounds (*1*, *6*, *7*). Thus, Gutiérrez et al. proposed an instrumental method (*8*) for the determination of bitterness, based on the extraction of the bitter constituents from VOO by solid-phase extraction and the measurement of the absorbance of the extract at 225 nm, constituting the bitterness index (K_{225}).

The literature contains numerous rapid and/or automatic methods for the determination of bitterness and/or phenolic compounds in olive oil. For example, García-Mesa et al. automated the manual method described by Gutiérrez et al. (*8*) by flow-injection analysis (FIA). This method is based on in-line solid−liquid extraction using C_{18} as the sorbent, reversing the order of the cleanup and elution stages (*9*). Another version, completely automated, developed by the same authors, involved the use of a robotic station where the robot mimics the analyst in the analytical procedure (*10*).

Flow-injection analysis has also been applied to methods based on the use of the Folin−Ciocalteau reagent for the determination of polyphenols in olive oil (*11*). Thus, different FIA systems have been proposed to determine total polyphenols in olive oils by liquid−liquid extraction without phase separation (*12*), using ultrasound to increase the effectiveness of mass transfer in these types of systems (*13*). Alternatively, immobilization of the Folin−Ciocalteau reagent in the detector flow cell with the help of an anionic resin has been used for integrated derivatization, retention, and detection of extracted polyphenols (*14*). These three FIA methods of liquid−liquid extraction without phase separation are based on the iterative change of the system flow direction. In a different approach, Ortíz-Boyer et al. proposed an FIA system of liquid−liquid

* To whom correspondence should be addressed. Phone: +34 953 366 366. Fax: +34 953 366 380. E-mail: josea.garcia.mesa.ext@juntadeandalucia.es.

10.1021/jf070235v CCC: $37.00
Published on Web 04/21/2007

extraction without phase separation in which the successive segments of the aqueous and organic phases passed through the flow cell of a diode-array detector (*15*). Finally, a system coupled to the robotic FIA has been proposed, the robot performing the liquid−liquid extraction and the FIA system the derivatization and detection steps (*16*).

On the other hand, rapid and/or automatic methods based on the use of electrochemical biosensors have been described. These methods allow direct determination of bitterness and/or phenolic compounds in oil (*17*−*19*) or oil extracts (*20*−*21*), where the use of an array of voltametric sensors has been proposed for the discrimination of bitter compounds (*21*). Flow-injection analysis has been adapted in some of the aforementioned electrochemical (bio)sensor methods for their automation (*17*−*20*).

Recently, our group developed a new methodology for the direct spectrophotometric determination of bitterness in olive oil without prior isolation of phenolic compounds (*22*). This new method, based on the spectral shift of VOO phenolic compounds in a pH gradient, is carried out directly on the oil dissolved in an adequate buffer, without the need to isolate the polyphenolic compounds from the oil matrix, which implies a drastic methodological simplification.

In the present work we report on an automated system for the determination of bitterness and phenolic compounds in virgin olive oil by flow-injection analysis. The proposed method is based on the spectral shift undergone by the phenolic compounds at two different pH values, allowing the direct determination of both parameters without prior isolation of the analytes.

MATERIALS AND METHODS

Virgin Olive Oil Samples. A total of 25 different VOOs were obtained by extraction from olives in the laboratory using an Abencor oil mill (Abengoa, Spain), comprised of a hammer mill, a thermobeater, and a vertical centrifuge. Olives were crushed, and the resulting paste was kneaded at 28 °C for 30 min (*23*). The oil was collected by centrifugation of the paste. After 30 min of decanting, the oil samples were filtered and stored at 4 °C until analysis.

Analytical Materials, Reagents, and Solutions. All solvents and reagents were of analytical grade unless stated otherwise. Doubly distilled water was used throughout the experiments. Potassium hydroxide, 1-propanol, *n*-hexane, methanol, and phosphoric, acetic, and boric acids were from Panreac (Barcelona, Spain). SPE cartridges packed with C_{18} phase were from J.T. Baker (Phillipsburg, NJ).

Britton−Robinson acid buffer (BR-buffer) stock solution was prepared to provide a final concentration of 5×10^{-3} M phosphoric acid, 5×10^{-3} M boric acid, and 5×10^{-3} M acetic acid. The solution prepared in this way had a pH of 2.12 (*24*).

Solutions used as reagents in the FIA system consisted of a mixture of 1-propanol and BR-buffer (11:1, v/v). Acidic and alkaline solutions at pH 5.0 and 13.5, respectively, were obtained by adding potassium hydroxide (0.6%) to the mixtures of propanol and BR-buffer. All solutions were filtered through a 0.45 μm pore size filter (Whatman Inc., Clifton NJ).

Apparatus. Spectrophotometric measurements were made using a spectrophotometer system consisting of a deuterium−halogen light source (DH-2000-BAL, Mikropack, Ostfildern, Germany), an HR-2000 spectrophotometer (Ocean Optics, Dunedin, FL), and a couple of fiber optics connected to a CUV-ALL-UV four-way cuvette holder equipped with a 1 cm quartz flow cell (Hellma, Germany). A Gilson Minipuls 3 peristaltic pump (Middleton, WI) was used as a propelling device.

Three automatic valves from VICI AG International (Switzerland) were used: a 10-port 2-position valve for sample dilution (DV), a 6-port 2-position valve for sample injection (IV), and a 6-position dead-end-path valve for reagent selection (SV). All three valves were actuated with the respective actuator control modules.

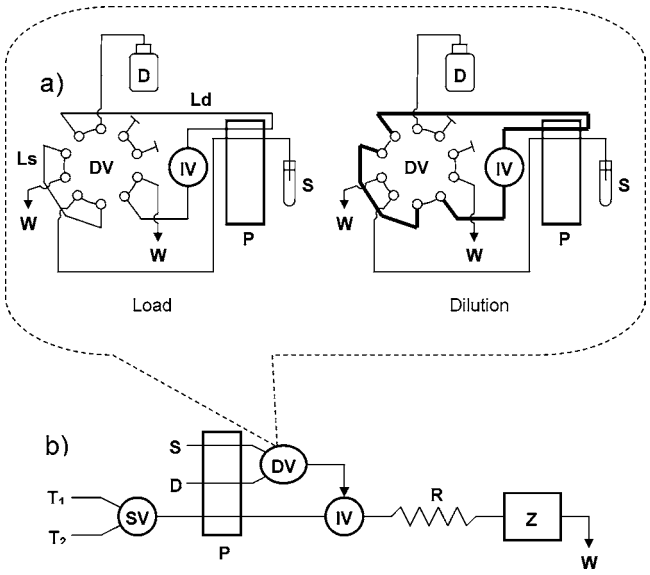

Figure 1. Flow-injection manifold proposed in the present work: (**a**) dilution subsystem; (**b**) main subsystem. Key: DV, dilution valve; IV, injection valve; SV, selection valve; P, peristaltic pump; D, solvent; S, sample; W, waste; L_s, sample loop; L_d, solvent loop; T_1, acidic BR-buffer; T_2, alkaline BR-buffer; R, reactor; Z, detector.

All connecting and reactor tubes (0.5 mm i.d.) were made of Teflon. The solvent-resistant pump tubing was from Watson-Marlow Ltd. (Cornwall, England).

The spectrophotometer was connected to a PC equipped with OOIBase32 software. A USB 6008 data-acquisition module and a suitable Labview 7.1 program (National Instruments, Austin, TX) were used for module control.

The pH of each buffer solution was measured with a CyberScan 2100 pH meter (Eutech Instrument, Singapore).

Spectrophotometric measurements of hydroalcoholic solutions corresponding to K_{225} and the determination of total phenolic compounds were made using an HP-8452 diode-array spectrophotometer (Hewlett-Packard, Palo Alto, CA).

Analytical Procedures. *Manual Determination of K_{225}*. Bitterness was evaluated by the determination of K_{225} according to the method proposed by Gutiérrez et al. (*8*). A sample (1.00 ± 0.01 g) of virgin olive oil was dissolved in 5 mL of hexane and passed over the C_{18} column previously activated with methanol (6 mL) and washed with hexane (6 mL). After sample elution, 15 mL of hexane was passed to eliminate the matrix, and the retained compounds were eluted with methanol/water (1:1) to 25 mL in a volumetric beaker. The absorbance of the extract was measured at 225 nm against methanol/water (1:1) in a 1 cm cell. The results were expressed at 1% (w/v) concentration (K_{225}).

Manual Determination of Total Phenolic Compound by the Folin−Ciocalteau Method. Total phenolic compounds were determined in oil extracts according to the method proposed by Vazquez et al. using the Folin−Ciocalteau reagent and measuring the absorbance at 726 nm (*11*).

Manifold for Automated Determination of K_{225}. **Figure 1** shows a scheme of the flow-injection manifold used. In the load position, the sample (S) is directly aspirated through a pump tube (**Figure 1a**), filling the sample loop (L_s) of the dilution valve (DV). In parallel, a stream of 1-propanol (D) passes through the solvent loop (L_d) of the DV and the sample loop of the injection value (IV). Once the sample loop is filled, the corresponding actuator is activated to switch this valve to the dilution position, whereupon the content of the sample loop is inserted into the stream of 1-propanol, which in this dilution position of the DV circulates in a closed circuit. The oil−solvent mixture reaches equilibrium after 300 s, at which time the sample is ready to be injected into the main FIA system (**Figure 1b**). Two injections of diluted sample are introduced into the buffer current. First, the selection valve (SV) is situated in the position of acid buffer (T_1, pH 5.0) for the first injection of the diluted sample. The sample is partially dispersed throughout the

length of the reactor (100 cm) and reaches the detector, where the absorbance is monitored at 274 and 800 nm. After the corresponding FIA peak is reached, the SV is switched to the position of alkaline buffer (T_2, pH 13.5) and a new sample dilution is made by actuating the DV in the way previously indicated. Next, the second diluted sample is injected into the stream of alkaline buffer, giving the corresponding FIA peak at the indicated wavelengths. The difference in height between the two peaks resulting under these conditions (measured in absorbance units) is calculated by the following expression: $\Delta A_{274\ nm} = A^*_{274\ nm}$(alkaline) $- A^*_{274\ nm}$(acid), where $A^*_{274\ nm}$(alkaline) $= A_{274\ nm}$(alkaline) $- A_{800\ nm}$(alkaline) and $A^*_{274\ nm}$(acid) $= A_{274\ nm}$(acid) $- A_{800\ nm}$(acid). The value of $\Delta A_{274\ nm}$ thus calculated is related to K_{225} and the total content of phenolic compounds.

RESULTS AND DISCUSSION

FIA Configuration. The aim of the present work was the development of an automated method for the direct determination of bitterness in virgin olive oil based on FIA. In addition, taking into account the relationship between bitterness and the phenolic content of olive oil, we evaluated the potential application of this FIA system for the determination of the total polyphenolic content of these oils in comparison with the Folin−Ciocalteau method.

The method for the direct determination of bitterness (*22*) is based on the spectral shift of the phenolic compounds of VOO caused by a pH gradient. In the manual analysis, the oil dissolved in 1-propanol was mixed with BR-buffer [5×10^{-3}M] at pH 5.0. Next a pH gradient was generated by the addition of an aqueous solution of sodium hydroxide at a constant flow rate under continuous magnetic stirring. The reaction was followed at 274 nm, where spectral profiles present a maximum in the alkaline medium, the intensity of this maximum being proportional to the bitterness of virgin olive oil. The reaction reached its highest value 300 s after the addition of the alkaline solution. The absorbance difference between the final and initial times highly correlated ($r = 0.988$, $p < 0.0001$) with the VOO bitterness as evaluated by the bitterness index (K_{225}). Simultaneously, the reaction was monitored at 800 nm to compensate for the slight turbidity that could occur by the addition of the aqueous alkaline solution.

In this manual bitterness determination, complete spectra were continuously recorded as the pH of the medium increased. However, only the absorbance values at the initial time (corresponding to pH 5.0) and after 300 s (at pH 13.5) were necessary for the calculations. On this basis, the FIA system proposed here was designed to carry out two single absorbance measurements, at acidic and alkaline pH.

An important factor that had to be taken into consideration was the possible influence of the lipid matrix in the absorbance values at acidic pH. At acidic pH, the absorbance of phenolic compounds in the olive oil was negligible; however, the lipid matrix could pose an important and variable contribution to the total absorption spectrum of the oil sample. Determination of the absorbance at 270 nm of olive oil is known to be a measurement of the overall state of secondary oxidation of this oil (*25*). Therefore, the use of a single blank sample is ruled out, since it would not reflect the degree of oxidation of the oil and its potential contribution to the overall absorbance. This fact makes it necessary to measure the absorbance at acidic pH for each sample. If this interference of the matrix had not existed, it would have been possible to use an even simpler system, in which only measurement of the absorbance at alkaline pH would have been necessary. Thus, in the case of samples with low oxidation levels (e.g., recently obtained extra virgin olive oil samples) the influence of the matrix may be minimal, permitting the omission of the acidic pH injection, thereby simplifying and streamlining the FIA system.

With the above considerations, the design of the FIA system used included the injection of the sample into a current of acidic buffer (for blank correction) followed by a second injection into a current of alkaline buffer (for analyte measurement). Thus, the system designed (**Figure 1b**) had a valve (SV) to select the buffer into which the sample was injected. At position 1 of the SV, the sample was injected into an acid current at pH 5.0 (T_1), and the corresponding FIA peak was recorded. Next, the valve was set at position 2, and the sample was injected again into a stream of alkaline buffer (pH 13.5, T_2), giving a second FIA peak. The difference in height between the two peaks was correlated with the K_{225} value as well as with the total content of phenolic compounds in the test oil. All the absorbance measurements were referenced at 800 nm to minimize the slight turbidity arising from working with a medium formed by an oil−propanol−water mixture.

Variable Optimization. (a) Main Manifold. The variables of the main FIA system (**Figure 1b**) were optimized to reach suitable performance and robustness of the system. A compromise among the speed of analysis, sensitivity, and reproducible dispersion of the sample was pursued.

A univariate method was applied for the optimization of the system, taking into account that the main limiting factor was the immiscibility between the sample and aqueous buffers. The main criterion selected was the repeatability of the FIA peaks obtained. Thus, an upper limit of 3% (expressed as the relative standard deviation) was selected.

The first variable studied was the selection of the most suitable solvent able to achieve a reproducible dispersion of the oily sample into the aqueous buffer. Several solvents were assayed such as 1-butanol, 1-propanol, and tetrahydrofuran. Finally 1-propanol was selected to dissolve small quantities of oil in aqueous buffer, constituting the hydroalcoholic carrier (T_1 and T_2).

The injection volume (IV, main FIA system) was studied in the range between 25 and 100 μL, 25 μL (the physically lowest possible value with the valve used) being enough to reach a satisfactory sensitivity. The oil concentration in the solvent (1-propanol) was selected as a compromise between the maximum analytic sensitivity and the miscibility of the sample with the hydroalcoholic carriers, since the higher the oil concentration the greater the turbidity in the system. Finally, a concentration of 7% (w/v) was used for the FIA analysis.

Considering the above-cited precision limit (3%), the flow rate of the buffers and the reactor length were optimized to shorten the analysis time.

The buffer concentration was optimized to obtain the lowest turbidity without loss of sensitivity. The turbidity resulting from the partial dispersion of oil in the hydroalcoholic carrier was inferred from the peak's height in alkaline medium at 800 nm, since the turbidity was higher in this medium than in the acidic solvent. Starting from a value of 0.0125 M, this concentration was gradually decreased until a value of 0.005 M, where the turbidity observed at 800 nm was negligible. The pH values for acidic (pH 5.0) and alkaline (pH 13.5) buffers were selected according to previous results (*22*). The selected values ensured the maximum sensitivity, i.e., the maximum difference in the peak height in both media.

Table 1 presents the ranges of the variables studied as well as their optimal values. Under the above experimental conditions, the sampling frequency of the main flow system was 12 injections h^{-1}.

Table 1. Ranges Assessed and Optimal Values for the Variables Influencing the Performance of the FIA System

variable	range studied	optimal value
oil concentration	1–10%	7%
sample loop length (DV)	10–50 cm	25 cm
flow rate of buffer	0.1–0.5 mL min^{-1}	0.25 mL min^{-1}
buffer concentration	0.005–0.0125 M	0.005 M
reactor length	25–200 cm	100 cm
injection volume (IV)	25–100 μL	25 μL
flow rate of solvent	0.3–1.0 mL min^{-1}	1.2 mL min−1

(b) Dilution Manifold. To date, several procedures have been proposed to dilute olive oil prior to injection into a flow system. These methods are based on the continuous mixture of the oil with a carrier through a mixture reactor of appropriate length (*26*−*28*). In these systems, the degree of dilution is determined by the relative flow rate of the oil and carrier. An alternative was proposed by García-Mesa et al. (*29*) in a procedure to determine the peroxide index in olive oil. In this method, the undiluted oil sample was injected directly into a carrier current, and a part of this resulting current was continuously discarded so that only a fraction of the oil initially injected reached the detector. In this case, the dilution level was determined by the percentage of the carrier flow discarded.

In the present work, a system of physical dilution of oil has been developed that, by a closed-loop flow system, mimics the weighing of the sample and its dilution with a solvent to a known volume, as done in manual procedures. The operation of the dilution system is outlined in **Figure 1a**. In the "load" position of the DV, the oil completely fills the sample loop (L_s) while the solvent fills the dilution loop (L_d), both the excess solvent and the oil being directed toward a waste container. Upon switching the dilution valve to the "dilution" position, both the sample and dilution loops connect, establishing a closed circuit where the oil is dispersed in the carrier solvent until reaching equilibrium. In this case, the respective volumes of the sample and solvent loops determine the dilution level. The mixture thus obtained is used to fill the sample loop of the IV of the main FIA system (**Figure 1b**).

This procedure of automatic dilution of the oil is slower, as it requires a waiting period to reach equilibrium in the sample−solvent mixture, but it is notably more robust than the methods previously reported. In these methods (*26*−*29*), the flow of the oil and solvent, and hence the resulting dilution, can be affected by the unequal wear undergone by the pump tubes used to propel the oil and the organic solvents. On the contrary, with the proposed system, the possible wear of the pump tubes would not affect the dilution level since the volumes of the L_s and solvent L_d are fixed. As noted above, the optimal concentration of the oil sample in the solvent (1-propanol) was 7%, as a suitable value to maximize the sensitivity of the method without compromising the reproducible dispersion of the oil sample in the hydroalcoholic carrier. The volume of the pump tube, connecting tubes, and sample IV determined the total volume of the solvent loop. To minimize the time of the sample dilution step, this volume was selected as low as possible. The value of the solvent loop in this experimental setup was 700 μL. Once the volume of the solvent loop was selected, the sample loop volume was optimized. To reach the optimal 7% dilution level, a 25 cm length sample loop was necessary in the DV.

To study the dissolution process, the dilution manifold shown in **Figure 1** was modified by substituting the IV by the spectrophotometer flow cell. This manifold made possible monitoring of the sample dispersion when this subsystem was switched to the dilution position. In **Figure 2** a typical oil dilution profile when the DV is switched to this position is shown. In this case, a wavelength of 410 nm was selected in an attempt to monitor the dissolution process measuring the concentration of olive oil carotenoids, with an absorbance maximum at 410 nm.

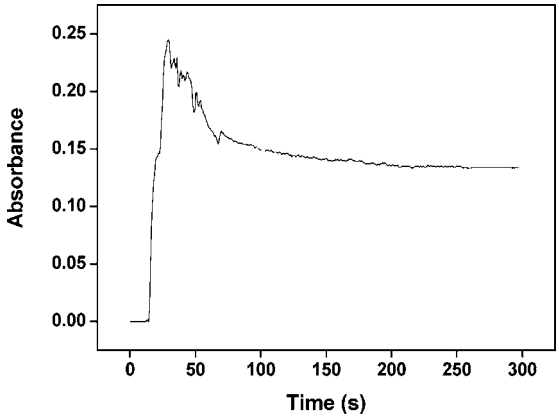

Figure 2. Dissolution profile of a virgin olive oil sample in the dilution system.

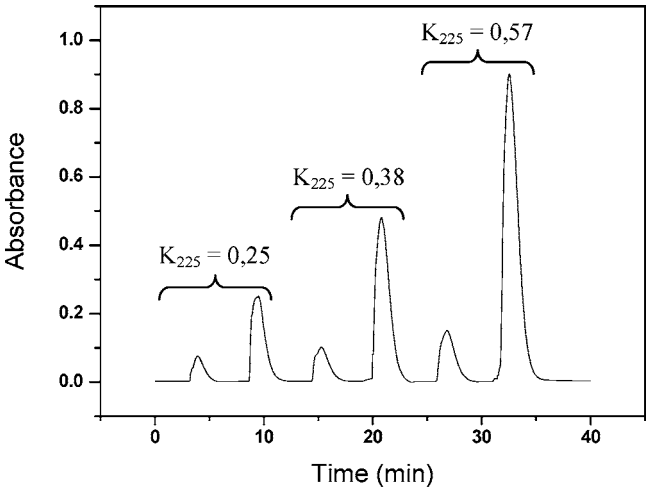

Figure 3. FIA-grams obtained during the analysis of three virgin olive oils with low (K_{225} = 0.25), medium (K_{225} = 0.38), and high (K_{225} = 0.57) bitterness.

The solvent flow rate was optimized to shorten the dilution time. This flow rate had the operational limit of incidental bubble formation, with the consequent malfunction of the system. With these considerations, the optimal value was 1.0 mL min^{-1}.

As indicated in the Materials and Methods, the time required for oil dissolution in the dilution system, considering the loop volumes and flow rate in the dilution system, was 5 min (see **Figure 2**).

The time required to fill and empty the dilution system after injection of the sample in the main system reduced the sampling frequency of the overall system to 5 samples h^{-1} (taking into account that every analyzed sample required two injections, in acidic and alkaline buffers). This time was enough to avoid cross-contamination between consecutive samples.

Validation of the Method. *Determination of Bitterness in VOO*. Seventeen VOOs with different contents of bitter compounds were submitted to the overall proposed procedure. In **Figure 3** FIA-grams obtained during the analysis of three samples of low, medium, and high bitterness are shown. Two peaks, the first at acidic pH and the second at alkaline pH, constitute the FIA-gram of each sample. The K_{225} of the selected

Table 2. Predicted and Reference Values of K_{225} and Total Phenolic Compounds

sample	K_{225}(ref)[a]	K_{225}(pred)[b]	PP(ref)[a]	PP(pred)[b]
1	0.57 ± 0.02	0.554 ± 0.002	1141 ± 55	1100 ± 6
2	0.35 ± 0.01	0.331 ± 0.001	500 ± 26	529 ± 5
3	0.34 ± 0.01	0.320 ± 0.001	489 ± 24	522 ± 3
4	0.40 ± 0.01	0.395 ± 0.004	720 ± 36	739 ± 11
5	0.33 ± 0.02	0.323 ± 0.002	514 ± 26	549 ± 2
6	0.29 ± 0.01	0.267 ± 0.001	403 ± 20	390 ± 3
7	0.27 ± 0.01	0.263 ± 0.004	371 ± 26	379 ± 11
8	0.31 ± 0.02	0.293 ± 0.001	479 ± 25	454 ± 3

[a] Reference values, mean ± SD ($n = 3$). [b] Predicted values, mean ± SD ($n = 3$).

samples of VOO were determined by the conventional method (*8*). The response of $\Delta A_{274\,nm}$ was checked by linear-regression analysis in the K_{225} range assayed. The response was linear in the K_{225} range evaluated, from 0.19 to 0.60, giving an equation of $y = 1.44x - 0.18$ ($n = 17$), where y represents the difference in peak height between alkaline and acidic media ($\Delta A_{274\,nm}$) and x is K_{225}. A linear-regression coefficient of $r = 0.992$ ($p < 0.0001$) was obtained, and this equation was used to quantify K_{225} in VOO.

Eight VOO samples, different from the oils mentioned above, were submitted to the overall procedure to validate the proposed method, and the calibration curve was used to calculate bitterness in these samples (analyzed in triplicate). Good agreement was found between the two values, with relative errors within 1% and 8%. Reference bitterness values in the samples were those calculated by the conventional method.

As a measure of the precision of the method, within-laboratory repeatability was evaluated with replicates ($n = 11$). The precision, expressed as the relative standard deviation, was 1.5% at the $K_{225} = 0.19$ level and 0.6% for the $K_{225} = 0.57$ level.

Determination of Total Polyphenols in VOO. As an evaluation of the potential use of the proposed method for the determination of the total phenolic content in VOO, the relation between $\Delta A_{274\,nm}$ and total phenolic compounds was tested. The total phenolic compounds of selected VOO samples were analyzed by the Folin−Ciocalteau method (*11*). The phenolic content of the 17 samples used above for calibration covered a wide range of phenolic concentrations, from 292 to 1363 (expressed as mg kg^{-1} of caffeic acid). The relation between $\Delta A_{274\,nm}$ and total phenolic compounds showed a high correlation ($r = 0.974$, $p < 0.0001$) in the range evaluated, giving an equation of $y = (5.6 \times 10^{-4})x - 0.01$ ($n = 17$), where y represents the difference in absorbance between alkaline and acidic media ($\Delta A_{274\,nm}$) and x is the total phenolic concentration. As described for the K_{225} determination, this equation was used to predict the content of phenolic compounds in the eight VOO test samples. **Table 2** summarizes the predicted and reference values of the total phenolic compounds, where reference values were those measured by the Folin−Ciocaletau method. Again, there was good agreement between both values, with relative errors ranging from 2% to 7% and a precision of 2.6% at the lowest polyphenolic concentration (360 mg kg^{-1}) and 0.7% at the highest (1194 mg kg^{-1}) level.

In summary, the procedure described here allows the direct automated determination of bitterness as well as the total phenolic content in virgin olive oils. The FIA system is based on the spectral shift of the phenolic compounds in VOO brought about by a pH change. The designed FIA system obviates prior isolation of the analytes from the lipid matrix and the dilution step of oil in a solvent, permitting complete automation of the process. The method showed satisfactory sensitivity, accuracy, and precision for the samples analyzed. Another redeeming feature of this new method is its robustness. This aspect is based on the use of a new dilution procedure for olive oil in flow systems and the absence of sorbents or (bio)sensors of limited life span.

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Received for review January 26, 2007. Revised manuscript received March 20, 2007. Accepted March 22, 2007.

JF070235V