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Determination of extra virgin olive oil acidity by FIA-titration

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Abstract

A direct determination of extra virgin olive oil acidity by an automated flow injection titration is proposed. The oil sample (280 µl) is directly injected, without dilution, in the carrier stream, a n-propanol solution flowing at 2 ml/min and containing potassium hydroxide 1 mM as titrant and phenolphthalein 5 10^{-5} M as indicator. In the mixing chamber (1.5 ml) the sample mixes and reacts with the carrier stream. By an optical fiber beam, connected to a spectrophotometer set at 562 nm, we are able to appreciate continuously the phenolphthalein absorbance (Abs) decrease. The area of the peak "ΔAbs vs. time" has a linear correlation with the logarithm of the sample acidity. Several extra virgin olive oil samples have been analysed. The correlation curve between the official (O) and proposed methods (P) is $P=(0.02\pm0.02)+(1.02\pm0.03)O$, with $r^2=0.997$ and $s_r=0.033$; the accuracy and precision, expressed as mean error and RSD, are both $<5\%$; the number of samples analysed per hour, the solvent and the sample consumption, in comparison with the official procedure, are respectively 12–60 versus 7–8, 2–10 ml versus 100–150 ml and 0.5 ml versus 10–20 ml. These features and the simple procedure indicate how the proposed method could represent an interesting alternative to the European community official method for the determination of free fatty acids in oil. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Extra virgin olive oil; FIA titration; Acidity degree

1. Introduction

Free fatty acid content is one of the most important parameter featuring the quality of an olive oil and it is often determined to classify and/or evaluate oil.

European Commission Regulation No. 2568/91 (ECC, 1991) classifies olive oils and suggests a manual procedure for the determination of free fatty acids: the sample, dissolved in a mixture of equal parts by volume of ethyl ether (95%) and ethyl alcohol, is titrated with an ethanolic solution of potassium hydroxide using phenolphthalein as indicator. The procedure is slow (7–8 samples per h) and solvent consuming [100–150 ml per sample; Linares, Luque de Castro & Valcárcel (1989)].

European Commission Regulation No. 2568/91 (ECC, 1991) also suggests to classify the olive oil according to the acidity degree (A.D.), reported as grams of oleic acid per 100 g of oil. According to this regulation extra virgin olive oil must have an acidity less than 1 A.D., virgin olive oil less than 3.3 A.D. and *lampante* virgin olive oil more than 3.3 A.D.

Therefore, it is important to know this value for each oil stock and moreover it is highly desirable to have an automated procedure less dangerous than the suggested one.

The determination of acidity in extra virgin olive oils is a procedure often necessary during the oil production in order to evaluate the price and the quality of the olive oils. Therefore, a simple and portable apparatus for evaluating the extra virgin oil acidity is an important tool for such application.

Flow injection analysis (FIA) has been proposed by some authors (Lineares et al., 1989; Nuoros, Georgiou & Polissiou, 1997; Zhi, Rpeíos & Valcárcel, 1996). FIA is easily applied to automation of aqueous solution reactions. The features of the FIA as sampling procedure made it suitable for routine application in many areas, such as clinical, pharmaceutical, water soil and environment analysis (Karlberg & Pacey, 1989; Schaller, Lenhardt, Weber & Euler, 1988), food and feed analysis (Ferreira, Lima & Rangel, 1994; Karlberg & Pacey, 1989) and process and quality control (Frenzel, 1988; Karlberg & Pacey, 1989). Moreover, in literature we find several applications of FIA titrations in aqueous solution (Karlberg & Pacey, 1989; Lopes de Conceição, Correia dos Santos, Simões Gonçalves & Santos, 2000; Saurina, Hernández-Cassou & Tauler, 1995).

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When FIA technique is applied with an organic solvent as carrier, attention has to be given to the pumping system as reported by Georgiou and Kupparis (1988), to the absorption–desorption processes at the tube walls of the FIA manifold, that could affect the shape of peaks (Nuoros et al., 1997) and to the viscosity gradient formed during the dispersion of the sample in the organic solvent carrier stream.

In this paper we described a simple, fast, easy to use, solvent saving automated determination of extra virgin olive oil by FIA technique using a n-propanol solution containing potassium hydroxide as titrant and phenolphthalein as indicator. The proposed method is based on the linear relation between the logarithm of analyte concentration and the area of the FIA peak. A portable prototype was realised and the results obtained are reported.

2. Materials and methods

2.1. Apparatus

The scheme of the system is depicted in Fig. 1. The peristaltic pump is a Gilson Miniplus 3 (Gilson Medical Electronics, France); the Polytetrafluorethylene (PFTE) tubing of the carrier stream has a 0.025 inches i.d; the injection valve is a Sample injecting valve Rheodyne 5020 (Rheodyne, CA, USA); the mixing chamber is a Stirred Mixer Pye Unicam PU4046; the flow cell is constructed in Teflon and contains the sensor, an optic fibre beam connected to a Metrohm 662 Photometer (Metrohm, Milan, Italy) connected to a 80486 Personal Computer; peak integration was performed with Microcal™ Origin™. This program allows the user to perform base line corrections.

2.2. Reagents

N-propanol was obtained from J.T.Baker (Milan, Italy), potassium hydroxide from Merck (Darmstadt, Germany), phenolphthalein and linoleic acid from Carlo Erba (Genoa, Italy) and Triton X-100 from Sigma (Milan, Italy).

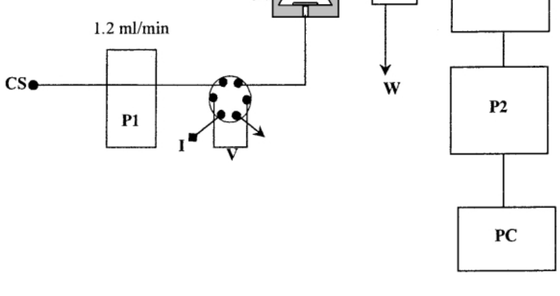

Fig. 1. Scheme of the system. CS, carrier stream; P1, pump; V, valve; I, injection; M, mixing chamber; C, cell; W, waste; F, optic fibres beam; S, spectrophotometer; P2, potentiometer; PC, personal computer.

The carrier solution of potassium hydroxide 1 mM and phenolphthalein 5 10^{-5} M was prepared daily by dilution from 0.1 M solutions prepared weekly by weighing and dissolving in n-propanol the appropriate amounts (maintained at 4°C).

Standards were obtained by adding linoleic acid to a low acidity oil (<0.2 A.D. by official method), according to the following formula: $X = M \cdot [1-(A_e-100)/(A_s-100)]/0.914$, where: X = linoleic acid added (ml); M = oil weight (g), A_e = final acidity to the standard (A.D.), A_s = oil basic acidity of the standard (A.D), $0.914 = d_{oil}$ (g/ml).

The acidity of the oil was measured according to the European Community official method. When very low acidity standards (<0.15 A.D.) were needed, linoleic acid was added to an oil neutralised as follows: n-hexane was added to the oil (1:2), then the solution was neutralised by mixing it in a separator funnel with an aqueous solution of Na_2CO_3 0.1 N (1:1, 2–3 times). After the separation, anhydrous Na_2SO_4 was added to the oil solution. After filtration the solution was left open in a hood to evaporate n-hexane.

Oil samples were obtained from the local food regional laboratory (A.R.P.A.T., Azienda Regionale Protezione Ambiente Toscana); the sample acidity was determined following the official method for free fatty acids determination.

2.3. Procedure

The flow rate and the wavelength are chosen, respectively, at 2 ml/min and 562 nm.

First, we determine the absorbance minimum and the base line absorbance value allowing, respectively, n-propanol and the carrier stream to flow for 3 min, then standards and samples are analysed.

The procedure is the same for both standards and samples. The oil is directly injected by the injection valve (~0.5 ml), into the stream; in the mixing chamber it reacts with the carrier, increasing the acidity along the sample zone and changing the phenolphthalein colour from red to colourless. Therefore, we obtain a negative absorbance peak, which has a linear relationship with the logarithm of the acidity of the sample as reported by Karlberg et al. (1989).

3. Results and discussion

3.1. Experimental parameters optimisation

N-propanol was used as solvent: it rapidly dissolves the oil samples substituting the mixture of ethanol and ethyl ether.

Phenolphthalein is soluble in n-propanol and, since its λ_{max} is 562 nm, spectral interferences are eliminated, as reported by Nuoros et al. (1997). A phenolphthalein concentration of 5 10^{-5} M is low enough to allow low free fatty acids concentration changing the indicator colour and high enough to measure high ΔAbs peaks.

Linoleic acid was chosen for preparing the standards as it is one of the major constituent of the olive oil. Standards were prepared in olive oil because dispersion in FIA technique depends on viscosity and density as reported by Nuoros et al. (1997).

During preliminary experiments we tested different loop volumes (80–180–280–500 μl), flow rates (between 0.5 and 10 ml/min) and titrant concentrations (0.5–5 mM): all these parameters affect the acidity range. All these parameters have been optimised for extra virgin olive oil samples in the range 0–1.0% of acidity. Two ml/min flow rate is the best compromise between analysis time (1–5 min according to sample acidity) and solvent consumption. One mM potassium hydroxide concentration (using a 2 ml/min flow rate and a 280 μl loop) is low enough to appreciate low acidity samples and, at the same time, it is high enough not to be affected from atmospheric carbon dioxide adsorption and to allow fast measurements.

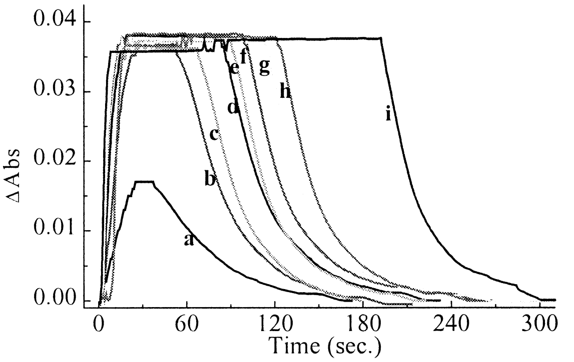

Fig. 2. FI titration peaks. a: 0.12; b: 0.20; c: 0.25; d: 0.30; e: 0.35; f: 0.40; g: 0.45; h: 0.50; i: 2.00 (all expressed as acidity degrees [A.D.]).

Concerning the data acquisition, the calculation of peak area (instead of the peak height), allows us to obtain a larger interval of linearity between the value measured (the peak area) and the logarithm of sample acidity.

All experiments reported in this paper are performed under optimised conditions, set as follows: flow rate 2.0 ml/min; valve loop 280 μl; mixing chamber 1.5 ml; carrier potassium hydroxide 1 mM and phenolphthalein 5 10^{-5} M in n-propanol. In Fig. 2 we report an example of ΔAbs vs. time (s) recorded for nine different samples.

We sometimes observed baseline variations during a series of experiments: we found that this phenomenon was due to deposits in the valve. To overcome this problem, at the end of a series of experiments, a Triton X100 aqueous solution (1 g/l) was flowed for 15 min and then n-propanol for 5 min to clean the line and to eliminate deposits that could be formed in the valve or in the cell. In this way the phenomenon has been completely eliminated.

3.2. Olive oil samples

In Table 1 we report the results of a classical experiment performed under optimised conditions on nine standards with different acidity ranging between 0.18 and 0.75 A.D.

In the first column of the table we report the sample acidity values (A.D.) determined by official method, in the second the sample acidity values determined by the calibration curve and the relative error for each sample, (|[(Ar−Ap)·100/Ar]|). In the last columns of the table we report the acidity values added, the values recovered by the proposed method and the recovery percentage. We also report the data of the calibration curve between the peaks area and logarithm of the acidity degree measured by the official method.

Table 1
Statistics results relative to the analysis of nine standards with different acidity degree[a]

Sample acidity/A.D.[b] (by reference procedure)	Sample acidity/A.D. (by proposed procedure)	Error (%)	Added acidity/A.D.	Recovered acidity±S.D.	Recovery (%)
0.180	0.178	1.2	0.020	0.018±0.005	90
0.200	0.198	1.0	0.07	0.071±0.002	101
0.250	0.251	−0.3	0.120	0.13±0.01	105
0.300	0.306	−2.0	0.170	0.17±0.01	101
0.350	0.352	−0.5	0.220	0.218±0.001	99
0.400	0.398	0.5	0.270	0.262±0.003	97
0.450	0.442	1.7	0.320	0.341±0.004	107
0.500	0.521	−4.1	0.570	0.55±0.02	97
0.750	0.732	2.4			
		Mean = 1.5			**Mean = 100±5**

[a] Calibration curve (PeakArea$_{(A.U.)}$ = $a + b$.log(Acidity$_{(A.D.)}$): $a = 1.00.104 \pm 2.10^2$, $b = 9300 \pm 100$, $r^2 = 0.998$, $S_r = 111$.

[b] A.D., acidity degrees.

[c] Error = (|[(Ar-Ap)*100/Ar]|) where Ar = acidity by reference method and Ap = acidity by proposed method.

Table 2
Statistics results relative to the analysis of nine samples[a]

Acidity/A.D. (by reference procedure)[b]	Acidity/A.D. (by proposed procedure)[b]	Error (%)
0.14	0.140±0.001	+0.3
0.21	0.202±0.001	+3.8
0.40	0.411±0.002	−0.4
0.50	0.559±0.003	−12
0.61	0.656±0.003	−7.6
0.74	0.816±0.005	−10
0.85	0.905±0.005	−6.5
0.98	1.000±0.006	−2.1
1.22	1.216±0.008	0.30
		mean <5%

[a] Calibration curve (PeakArea$_{(A,U.)}=a+b$.log(acidity): $a=6780\pm70$, $b=7410\pm190$, $r^2=0.999$, $S_r=117$.

[b] Correlation curve of results by proposed (P) and reference method (R) ($P=a+bR$).: $a=0.02\pm0.02$, $b=1.02\pm0.03$, $r^2=0.997$, $S_r=0.033$.

Relative error of the proposed procedure is always <5% (mean error = 1.5%) and the recovery percentage of linoleic acid, added to the oil, ranges between 90 and 107% (mean recovery = 100±5%). The measurements have a mean RDS <3% ($n=3$).

Single sample analysis time and solvent consumption depend on sample acidity: for extra virgin olive oils (acidity <1%) the analysis time ranges between 1 and 5 min and the carrier consumption between 2 and 10 ml. Therefore the proposed method has effective time and solvent saving and it appears as an interesting alternative to the official method.

We applied the proposed method to the analysis of extra virgin olive oil samples (Table 2). The calibration curve, $y=a+b\cdot\log(\text{acidity})$, was obtained with four standards (obtained from a 0.0 A.D. oil) and then we analysed nine extra virgin oil samples: all the measurements have been performed three times.

Relative error, $|[(Ar-Ap)\cdot100/Ar]|$, is always <10% (mean error = 5%) and the statistics of the correlation curve between proposed and reference method ($a=0.02\pm0.02$, $b=1.02\pm0.03$, $r^2=0.997$, $s_r=0.033$) indicate a good agreement in the range 0.1–1.25 A.D. The precision of the measurements, expressed as RSD, is always less than 3%.

4. Conclusions

The FIA procedure proposed allows determination of free acidity in olive oils faster than the official method (12–60 versus 7–8 samples per hour).

The proposed method reduces the solvent consumption (2–10 ml versus 100–150 ml per sample), the sample consumption (0.5 ml versus 10–20 ml per analysis) and eliminates the use of dangerous ethyl ether.

The statistics indicate a good correlation of results by reference and proposed method, the precision expressed as RSD is always <5% and the accuracy of the acidity determinations as mean error with respect to official method is 5% for nine samples.

Considering these experimental data, the simplicity and the automation of the FIA, we can affirm that this procedure can be used as a useful alternative to the official method for the determination of olive oil acidity in the range 0.1–1.3 A.D.

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