OLIVE OIL ANALYSIS BY FLOW INJECTION

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ABSTRACT

Automated analytical methods were developed for the quality control of olive oil, including the determination of acidity, peroxide value (PV), iodine value (IV), 2-thiobarbituric acid reacting substances (TBARS), iodine value (IV) and anisidine value (AV). The developed flow injection methods exhibit the following advantages: high analysis rates (20-100 h^{-1}); full automation that results in increased precision and accuracy; low cost; low solvent consumption and elimination of chlorinated solvents, that renders the developed methods environmental friendly; low sample consumption (0.0012-0.2 ml per analysis); good agreement with time consuming official methods; and protection of reagents from light and atmospheric oxygen.

INTRODUCTION

Flow injection (FI) is easily applied in automation of wet chemistries that do not involve organic solvents. However, when samples and/or reagents are not water soluble special consideration should be given to suitable pumping systems[1], refractive index[2],

density and viscosity[3] gradients formed during the dispersion of the sample in the organic solvent carrier stream. Also absorption-desorption processes at the tube walls of the FI manifold, known to affect the shape of FI peaks[4], are distinct when using organic solvents. FI methods concerning the analysis of non aqueous samples, especially edible oils are scarce[5].

In our laboratory we develop flow injection automated methods for the quality assessment of olive oil. The developed methods are based on homemade analysers, providing automated data acquisition and control. Reagents in organic solvents are continuously pumped by a peristaltic pump through PTFE micro-tubes of 0.8 mm inner diameter (coils). Micro-quantities of olive oil samples are automatically injected in the flow using a chromatography injection valve. Samples are mixed with the reagents and incubated while flowing through a mixing chamber or a mixing coil to the spectrophotometer.

For methods based on slow reactions, a parallel flow injection (PA-FI) multichannel analyser based on a stream selection valve (SSV) and ten incubation coils has been developed. While stored samples are incubated, new samples are injected and mixed with the reagents. Then, incubated samples are aspirated in the spectrophotometer by flow reversal. After measurement, samples are driven to waste. Automated methods have been developed for the determination of acidity, peroxide value (PV), 2-thiobarbituric acid reacting substances (TBARS), iodine value (IV) and anisidine value (AV).

Acidity and peroxide value correlate well with olive oil sensory characteristics. Therefore, olive oil pricing is based on the determination of these two parameters. Hydrolytic and oxidative rancidity are the processes that create free fatty acids and fatty acid peroxides. Advanced oxidative rancidity creates secondary oxidation products that are mostly low molecular weight aldehydes. These secondary oxidation products are determined by the 2-thiobarbituric acid value and the anisidine value. Secondary oxidation products are usually determined in thermally stressed oils, and oils that have been stored for a long time. Iodine value is used for the detection of olive oil adulteration with inexpensive seed oils.

EXPERIMENTAL

Analyser construction and operation

The FI analysers developed are depicted in Figures 1-5. Details concerning their construction are given elsewhere[6-10]. Here, the operation of the parallel flow injection analyser used in the determination of PV, IV and TBARS will be briefly discussed. The operation (timing sequence) of the analyser is shown in Figure 6. Briefly, the following steps are involved:

1. Injected samples pass through the detector and then are diverted by the stream selection valve to the incubation coils. During this cycle the pump operates in the forward direction to load all ten incubation coils for the first time.
2. After all coils are loaded, the pump reverses the flow and the first stored sample that has been incubated for ten minutes is aspirated to the detector for measurement.
3. The pump changes the flow to forward direction and the measured sample is washed out, while the injector loads the next (eleventh) sample.
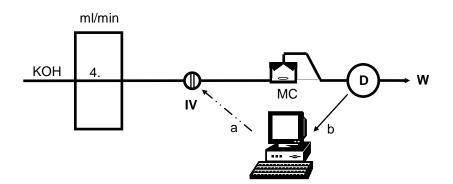

Figure 1. Flow injection analyzer developed for the determination of olive oil acidity. **KOH:** 7.5×10^{-4} M KOH and 4.0×10^{-5} M phenolphthalein in *n*-propanol; **IV:** injection valve; **MC:** 1.1 ml Plexiglas mixing chamber; **D:** spectrophotometer, λ=562 nm; **W:** waste; a: digital control signals; and b: analog data.
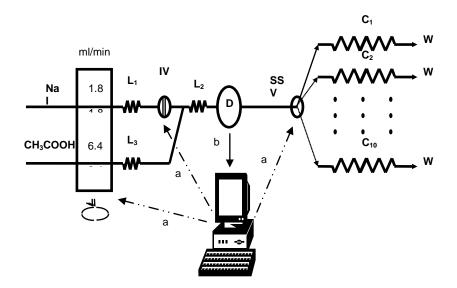
Figure 2. Automated multichannel Parallel Flow Injection analyser for the determination of olive oil peroxide value. **NaI:** 1.0 % w/v NaI in *n*-propanol; **CH₃COOH:** 43.2 % v/v acetic acid in *n*-propanol; **L₁, L₂** and **L₃**, 10, 70 and 60 cm coils respectively; **IV:** injection valve; **D:** spectrophotometer, λ=360 nm; **SSV:** ten position stream selection valve; **C₁-C₁₀:** 100 cm incubation coils; **W:** waste, a: digital control signals; and b: analog data.

4. Next sample (eleventh) is injected and driven through the detector to the incubation coil.
5. Steps from 2 to 4 are repeated until all samples are analyzed.

The time required for storing a single sample in the incubation coil varies between 30 and 60 s. According to the incubation time required for each determination, the number of incubation channels can be varied from 7 to 10. During the TBARS determination, all ten incubation
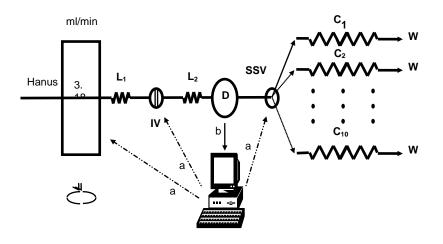
Figure 3. Automated parallel flow injection multichannel analyzer developed for the determination of olive oil iodine value. **Hanus:** 6×10^{-3} M IBr in acetic acid; **L$_1$, L$_2$**: 10 and 70 cm coils, respectively; **IV:** injection valve; **D:** Spectrophotometer, λ=392 nm; **SSV:** ten position stream selection valve; **C$_1$-C$_{10}$:** 100 cm incubation coils; **W:** waste; a: digital control signals; b: analog data.
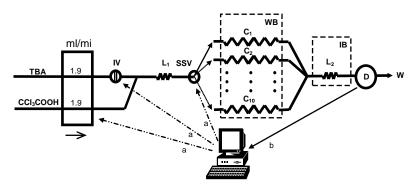

Figure 4. Laboratory-made parallel flow injection analyser for the determination of 2-thiobarbituric acid reacting substances in olive oil. **TBA:** 2.0×10^{-2} M 2-thiobarbituric acid in n-propanol; **CCl$_3$COOH:** 0.10 M in n-propanol; L$_1$ and L$_2$: 50 cm coils; **IV:** injection valve; **D:** spectrophotometer, λ=532 nm; **SSV:** ten position stream selection valve; **C$_1$-C$_{10}$:** 110 cm incubation coils; **WB:** water bath, 95$\pm$1 $^{\circ}$C; **IB:** ice bath; **W:** waste, a: digital control signals; and b: analog data.

coils are filled with olive oil samples during 5 min. Then, to minimize reagent consumption, the peristaltic pump stops for 25 min to facilitate an incubation time of 30 min.

The use of the PA-FI analyser overcomes the one 'sample at a time' disadvantage of the FI technique permitting incubation times up to 30 min while the sampling rate is more than 20 samples per hour.
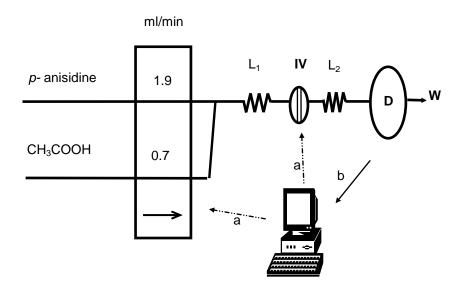

Figure 5. Automated two line flow injection analyser for the determination of olive oil anisidine value. *p-* **anisidine:** 4×10^{-2} M *p*-anisidine in propanol-2; **CH$_3$COOH:** glacial acetic acid, **L$_1$** and **L$_2$**, 100 and 50 cm coils respectively; **IV:** injection valve; **D:** photometer, $\lambda = 350$ nm; **W:** waste, a: digital control signals; and b: analog data.
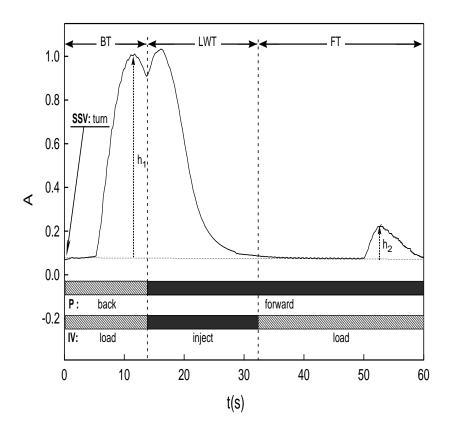
Figure 6. Timing sequence of the Parallel Flow Injection analyser. Recording obtained by injecting an olive oil of 40 peroxide value. **BT:** back time, 14 s; **LWT:** load-wash time, 18 s; **FT:** forward time, 17 s; **ST:** stop time, 11 s; **SSV:** stream selection valve; **P:** pump; and **IV:** injection valve.
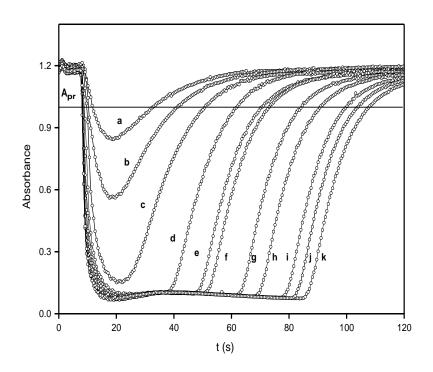
Figure 7. FI titration peaks. Standards of a) 0.100, b) 0.150, c) 0.277, d) 0.400, e) 0.800, f) 1.00, g) 2.00, h) 3.00, i) 6.00, j) 8.00 and k) 10.0 acidity degrees. Peak widths measured : a) 16.5, b) 31.7, c) 42.6, d) 48.7, e) 60.9, f) 64.4, g) 75.4, h) 81.1, i) 91.9, j) 97,0 and k) 101 s respectively. Peak widths were calculated at the A_{nr}=1.000 absorbance level.

RESULTS AND DISCUSSION

Determination of olive oil acidity by flow injection titrimetry[6]

The homemade FI analyser shown in Figure 1 was used. The analyser is based on a single line manifold using the FI titration technique. Olive oil samples are injected and dispersed in the titrant (mixed phenolphthalein, KOH solution) while flowing through a mixing chamber (MC). The absorbance of the reaction

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mixture is continuously monitored at 562 nm, the λ_{max} of the basic form of the indicator. The resulting negative absorbance peaks indicate the transition base (red)-acid (colorless)- base (red) of the indicator. The analysis rate achieved by the FI method is 30-100 samples per hour. The analytical range is 0.15-8.0% acidity degrees (AD, % w/w free fatty acid content expressed as oleic acid). Typical FI peaks acquired during the determination of acidity determination are shown in Figure 7. Results obtained by the proposed FI method compare well with those obtained through the official European Community titrimetric method (0.3-3.6% relative difference for the analysis of 32 olive oil samples). Advantages of the proposed FI method in comparison to the official one are depicted in Table 1.

Determination of olive oil peroxide value by flow injection parallel multichannel analysis[7]

The PA-FI multichannel analyser shown in Figure 2 was used. The developed method is based on monitoring, at 360 nm, the absorbance of iodine liberated by olive oil peroxides. Olive oil samples are injected in a NaI stream and acidified by merging with an CH_3COOH stream. As the two reagents used are incompatible, separate flow lines are used for each reagent. An analysis rate of 100 samples per hour is achieved while each sample is incubated for 5 min. With the proposed analyser design the drawback of "one sample at a time" is overcome as 7 samples reside simultaneously in the analyser, resulting in high analysis rates. The analytical range is 2.5-80 peroxide value (PV, meq O_2/kg oil). Results obtained by the proposed method compare well with those obtained by the official European Community titrimetric method (0-5.6% relative difference for the analysis of 27 olive oil samples). Other advantages of the proposed PA-FI method in comparison with the official one are shown in Table 1.

Determination of olive oil iodine value by flow injection parallel multichannel analysis[8]

The home-made parallel flow injection multichannel analyser shown in Figure 3 was used. Olive oil samples are diluted 1:50 in *n*-propanol and injected in an acetic acid Hanus stream. Using the developed PA-FI analyser, an analysis rate of 60 samples

per hour is achieved while samples are incubated for 10 min. The IBr absorbance in the carrier stream is continuously monitored at 392 nm. Injected samples consume IBr resulting in two negative absorbance peaks (Figure 8) for each sample, corresponding to the first and second pass through the spectrophotometer. The analytical range is 9–125 iodine value (IV, g I_2/100 g oil) and results obtained by the proposed method compare well with those obtained by the Association of Official Analytical Chemists Hanus titrimetric method (0.1-5.1 % relative difference for the analysis of 25 olive oil samples). Table 1 highlights the merits of the proposed method in comparison to the official method.

Determination of 2-thiobarbituric acid reacting substances by flow injection parallel multichannel analysis[9]

Malondialdehyde (MDA) is one of the secondary oxidation products of polyunsaturated fatty acids. Quantitation of MDA is based on reaction with 2-thiobarbituric acid (TBA) that is not specific. As other oxidation products react with TBA, the term 2-thiobarbituric acid reacting substances (TBARS) has been coined. The analyser developed for the TBARS determination is depicted in Figure 4. Olive oil is injected in a TBA stream and then is acidified by merging with a CCl_3COOH stream in a mixing coil. The resulting mixture is diverted through the SSV to the first incubation coil. All ten incubation coils that are immersed in a water-bath at 96 ^{o}C are loaded in 5 min. Then the flow is stopped for 25 minutes. After sample incubation, the flow starts, the reaction mixture is cooled by passing through an ice-bath and subsequently measured in the spectrophotometer at 532 nm. The developed method achieves an analysis rate of 20 samples per hour, while samples are incubated for 30 min. The analytical range is $(4-110) \times 10^{-4}$ M MDA and the proposed method compares well with a manual method (relative difference 0-5.6% for the analysis of 30 olive oil samples). The comparison of the proposed PA-FI method and the manual method is presented in Table 1.
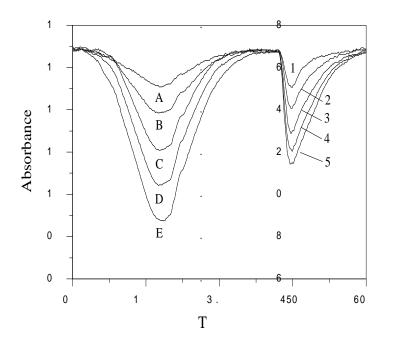
Figure 8. FI peaks obtained during iodine value determination. Standards of (A,1) 23, (B,2) 44.5, (C,3) 67.5, (D,4) 89.5 and (E,5) 111.5 IV. (A-E) Reversed flow and (1-5) Forward flow. Back time: 18s; Load-wash time: 18 s; Forward time: 25 s.

Determination of olive oil anisidine value by flow injection[10]

The homemade analyser used for the determination of olive oil anisidine value is depicted in Figure 5. The method developed is based on monitoring the reaction product of *p*-anisidine and the aldehydic secondary oxidation products from olive oil. As *p*-anisidine is not compatible with acetic acid when using propanol-2 solvent, two flow lines were used for premixing the reagents while flowing through a 100 cm coil. The absorbance is continuously monitored at 350 nm. The analysis rate achieved is 80 samples per hour. Results obtained compare well with those obtained through the official method (0.5-6.8% relative difference for the analysis of 28 olive oil samples). The comparison of the proposed FI method and the manual method is presented in Table 1.

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CONCLUSIONS

The developed flow injection methods exhibit several advantages. They are fast achieving analysis rates of 20-100 h^{-1}. Full automation results in increased precision and accuracy. The analysis cost is low, since small quantities of organic solvents are consumed. The low organic solvent consumption and the elimination of chlorinated solvents render developed methods environmental friendly. Good agreement with time consuming official methods is accomplished. The PA-FI analyser developed allows automation of methods that require long incubation time without loss of sampling rate. Table 1 presents the advantages of the developed methods over the official ones.

ACKNOWLEDGMENTS

Table 1. Comparison of the developed flow injection methods with the official methods

	Analysis time	Solvent consumption	Olive oil consumption
1. Acidity			
FI method	36-120 s[a]	3-7 mL n-propanol	0.175 ml
E.C.[b] method	10-15 min	50 mL ethanol, 50 mL ethyl ether	10-20 g
2. Peroxide value			
FI method	43 s	3.4 mL n-propanol, 3 mL acetic acid	0.02 ml
E.C.[b] method	10-15 min	10 mL chloroform, 15 mL acetic acid	0.3-5 g
3. Iodine value			
FI method	60 s	3.1 mL acetic acid	0.0012 ml
AOAC[c] method	30 min	25 mL acetic acid, 10 mL chloroform	0.25 g
4. TBARS			
FI method	3 min	1.7 mL n-propanol, 0.2 mL trichloroacetic acid	0.04 ml
manual method[d]	>45 min	6 mL acetic acid, 4 mL CCl$_4$, 5 mL trichloroacetic acid	0.05 ml
5. p-anisidine value			
	45 s	1.4 ml propanol-2, 0.4 ml acetic acid	0.04 ml
	10 min	25 ml isooctane, 1ml acetic acid	0.5 g

[a]Analysis time and therefore n-propanol consumption depend on sample concentration.

[b]European Communities.

[c]Association of Official Analytical Chemists.

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