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Talanta 65 (2005) 1056–1058

Talanta

www.elsevier.com/locate/talanta

Short communication

# Chemiluminescent evaluation of peroxide value in olive oil

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Received 24 May 2004; received in revised form 4 August 2004; accepted 24 August 2004
Available online 5 October 2004

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**Abstract**

A method is described for the evaluation of the peroxide value (PV, meq. $O_2$ kg$^{-1}$) in olive oil. The method is based on the chemiluminogenic energy-transfer reaction of bis(2,4,6-(trichlorophenyl)oxalate (TCPO) with hydrogen peroxide or total peroxides in the presence of Mn(II) as catalyst and 9,10-dimethylanthracene as fluorophore. The procedure developed allows the evaluation of PV within the range of 0.6–100 meq. $O_2$ kg$^{-1}$ (CL intensity = 1.76 × PV (meq. $O_2$ kg$^{-1}$) + 23.2, $r^2 = 0.994$, $n = 9$) and relative standard deviation within the range 1–5% by using a simple manual measurement.

*Keywords:* Peroxide value; Olive oil; Chemiluminescence; Delios S; TCPO

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## 1. Introduction

Peroxide value (PV) is a measure of total peroxides in olive oil expressed as meq. $O_2$ kg$^{-1}$ oil and hence it is a major guide of quality. A wide variety of analytical methods for the determination of PV have been established. The official EU method is based on the titration of iodine liberated from potassium iodide by peroxides present in the oil [1]. The appropriate amount of sample is dissolved in 10 mL of chloroform, the solution is then acidified with 15 mL of acetic acid and 1 mL of saturated potassium iodide is added. After incubation for 5 min, iodine is titrated with sodium thiosulphate standard solution. This method is empirical, requires large amounts of organic solvents, accuracy depends on a variety of experimental parameters such as timing and protection of the reaction mixture from light and atmospheric oxygen [2]. A limited number of alternative methods and techniques have been proposed for the measurement of PV. PV within the ranges 10–100, 2.5–80 and 1–100 have been determined by Fourier transform near-infra-red spectroscopy [3] or by flow injection analysis with spectrophotometric [4] or Fourier-transform infra-red spectroscopic detection [5], respectively. Although peroxides can be determined by chemiluminescence (CL), little attention has been given to the application of CL in olive oil. The inhibition of leukocyte 5-lipoxygenase by phenolic compounds [6], the adulteration of extra virgin olive oil with seed oil [7] and the antioxidant activity of aqueous extracts of olive oil and seed oil [8] have been studied by CL and the peroxide content of soybean oil has been evaluated by thermoluminescence [9].

Since hydrogen peroxide is one of the reagents of peroxyoxalate chemiluminescent reactions (PO-CL) in non-aqueous media, it was decided to investigate whether this reaction can be used to detect and quantify hydrogen peroxide or total peroxides in oil samples. For this purpose, an oil substrate based on Delios S was used for all measurements. Delios S is a stable model oil mixture of triglycerides of caprylic (C-8) and capric (C-10) acids (70:30) which is used in the flavour industry, as well as for cosmetic and pharmaceutical skin care preparations, well-fatting emulsions and skin oils.

Furthermore, it was decided to develop a simple procedure, which would enable the successful measurement of PV with simple instrumentation. Hence, the method was

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doi:10.1016/j.talanta.2004.08.041

optimised for batch analysis and the final procedure can be applied in field analysers allowing estimation of the quality of olive oil by the producer. To our knowledge, the proposed method is the first application of chemiluminescence in estimating the quality of olive oil.

## 2. Experimental

### 2.1. Apparatus

CL measurements were carried out using a Jenway 6200 Fluorimeter with the excitation lamp off and glass cuvettes of optical path equal to 10 mm.

### 2.2. Reagents

Acetonitrile (ACN) (Merck, Germany) and ethyl acetate (EtOAc) (Fluka Chemika, Germany) were of the highest purity available. Bis(2,4,6-(trichlorophenyl)oxalate (TCPO), manganese (II) chloride tetrahydrate and imidazole were obtained from Sigma (St. Louis, MO, USA), 9,10-dimethylanthracene was obtained from Aldrich (Germany). All chemicals were of analytical grade. Delios S (CASR number: 73.398-61-5) is a mixture of triglycerides of vegetable medium-chain saturated fatty acids (Caprylic (C-8)/Capric (C-10) acids–70:30), was gracefully offered by Vioryl S.A., Greece.

Aqueous solutions were prepared with de-ionised water from a Labconco Water Pro$^{TM}$ system (Kansas City, Missouri, USA).

### 2.3. Solutions

According to previous work [10], a mixed solvent of ethyl acetate saturated with water (SatEtOAc) and ACN (9:1) (solvent A) was used for the preparation of all solutions and oil dilutions. Solutions containing $5 \times 10^{-4}$ M 9,10-dimethylanthracene (DMA), $4.5 \times 10^{-3}$ M imidazole (IMI) and $4.5 \times 10^{-4}$ M TCPO were prepared by weighing 5 mg of DMA, 15 mg of IMI and 10 mg of TCPO and diluting each one to 50 mL with solvent A. 150 μM manganese(II) chloride was prepared by weighing 15 mg of manganese(II) chloride and diluting to 500 mL with solvent A.

## 3. Procedures

### 3.1. Preparation of oil samples

One gram of Delios S was mixed in a round bottom flask with 26 μl of an aqueous solution of $H_2O_2$, which was prepared by 1:2 dilution of $H_2O_2$35% (11.63 M) and 3–4 mL of methanol. The mixture was vigorously stirred on a rotary evaporator at room temperature for 30 min under vacuum in order to achieve homogenous distribution of the peroxide and purge methanol. The resulting mixture contained 100 mmol $H_2O_2$/kg (200 meq. $O_2$ kg$^{-1}$ oil) and was used as stock solution for all further studies.

### 3.2. Dilution of oil samples

Three hundred milligrams of oil sample was transferred in a 10 mL volumetric flask and diluted with solvent A. 0.50 mL of this solution was further diluted with solvent A in a 10 mL volumetric flask.

### 3.3. PO-CL assay

PO-CL with TCPO was selected since all dilutions of oil should be carried out in non-aqueous solvents. IMI was chosen due to its buffer and catalyst behaviour [11]. The choice of fluorophore and concentrations of all reactants has been described previously [10]. 1.8 mL of $5 \times 10^{-4}$ M DMA, 250 μl of $4.5 \times 10^{-3}$ M IMI, 40 μl of 150 μM manganese(II) and 25 μl of oil solution were transferred into the glass cuvette and the mixture was vortexed for 10 s. Two hundred microliters of $4.5 \times 10^{-4}$ M TCPO solution were quickly introduced into the cuvette, the mixture was shaken vigorously and the cuvette was inserted into the instrument for manual measurement of the CL intensity which reached a maximum signal after 1–3 min and remain constant for around 10 s.

## 4. Results and discussion

Various solutions of Delios S spiked with different amounts of hydrogen peroxide were measured by PO-CL. Initially the rate of the reaction was very slow, under the conditions selected, and the average time for reaching a plateau of emission intensity was around 15 min. The introduction of metal ions, as described in the literature [12], was expected to increase the reaction rate. Various metal ions were tried (Al(III), Cr(III), Mn(II), Fe(II), Co(II), Ni(II), Cu(II), Zn(II), Sn(II) and Pb(II)). The final choice was Mn(II) due to the stable CL signals and prolonged plateau (for 10 s) recorded. Therefore, the time for reaching a plateau was reduced to 1–3 min.

### 4.1. Figures of merit

The PV value of a series of Delios S solutions spiked with hydrogen peroxide were determined iodimetrically [1] and by the PO-CL method proposed. PV values can be determined in the range 0.6–100 meq. $O_2$ kg$^{-1}$ with regression line:

CL intensity = $1.76 \times \text{PV}$ (meq. $O_2$ kg$^{-1}$) + 23.2, $r^2 = 0.994$, $n = 9$ and relative standard deviation within the range 1–5%.

The PV of model oil samples were measured by the official EU method [1] and the results were compared with the PO-CL method (Table 1). Recoveries were within the range 80–106% with average 93%. The PV values of commercial

Table 1
Comparison of PV values of model oil samples by the official and the PO-CL methods

| PV (meq. $O_2$ kg$^{-1}$) | | Recovery (%) |
|---|---|---|
| Official EU method | Proposed PO-CL method (±S.D., *n* = 3) | |
| 10.0 | 9.5 ± 1.0 | 95 |
| 8.0 | 7.8 ± 1.1 | 98 |
| 5.0 | 4.0 ± 0.5 | 80 |
| 4.0 | 3.8 ± 1.2 | 95 |
| 1.6 | 1.7 ± 1.4 | 106 |
| 0.6 | 0.5 ± 0.9 | 83 |

Table 2
PO-CL and PV values of virgin unspiked and spiked olive oils

| Olive oil | PV (meq. $O_2$ kg$^{-1}$) | | Recovery (%) |
|---|---|---|---|
| | Expected | Found (±S.D., *n* = 3) | |
| Unspiked olive oil | – | 10.0 ± 0.9 | – |
| Spiked olive oil | | | |
| | 21.4 | 21.3 ± 1.6 | 99 |
| | 24.6 | 22.2 ± 1.8 | 88 |
| | 27.5 | 28.7 ± 2.1 | 104 |
| | 32.0 | 28.5 ± 2.1 | 88 |
| | 50.0 | 54.0 ± 2.8 | 108 |
| | 60.0 | 58.9 ± 3.1 | 98 |

olive oil spiked with hydrogen peroxide were also measured and compared to the PO-CL method (Table 2) showing good comparison.

## 5. Conclusions

The PO-CL method described is the first CL method for the evaluation of total peroxides as an analytical index for the quality of olive oil. The instrumentation required is very simple and the measurement can be made manually and away from the laboratory.

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