

Circular dichroism: a new approach to study olive oil

P. SALVADORI*, F. MAZZINI*, A. MATTEI**, F. MAROTTA**

* DIPARTIMENTO DI CHIMICA E CHIMICA INDUSTRIALE, UNIVERSITÀ DI PISA - ITALY

** CARAPELLI FIRENZE S.P.A. - TAVARNELLE VAL DI PESA (FI) - ITALY

The present work describes the first example of circular dichroism (CD) spectroscopy application to the study of olive oil. In respect of UV, CD gives meaningful and useful information showing strong absorptions in the 270-450 nm region due to optically active components, pigments (pheophytin A) and carotenoids (lutein) being the most contributing compounds. CD spectra variations observed in oil undergoing deodorization and photochemical stresses proved to be very promising to provide information on the oil treatments.

LA SPETTROSCOPIA DI DICROISMO CIRCOLARE: UN NUOVO APPROCCIO ALLO STUDIO DELL'OLIO DI OLIVA

Nel presente lavoro viene descritto il primo esempio di applicazione della spettroscopia di dicroismo circolare (CD) per lo studio dell'olio d'oliva. Rispetto alla tradizionale spettroscopia ultravioletta (UV), il CD fornisce informazioni addizionali nella regione spettrale 270-450 nm dove vengono evidenziati forti assorbimenti. I pigmenti (feofitina A-A') ed i carotenoidi (luteina) sono risultati i principali responsabili dello spettro di dicroismo circolare dell'olio di oliva. Sono state inoltre osservate variazioni significative negli spettri CD di oli sottoposti a deodorazione e fotoesposizione che risultano molto promettenti fornendo dati complementari agli spettri UV.

INTRODUCTION

Olive oil is composed of 98% triacylglycerols and 2% of non-glyceridic constituents [1]. These minor components are extremely important for peculiar properties of olive oil [2], such as the stability, flavour and bionutritional features. Many studies have been performed mainly by UV, HPLC and GC analyses, but the growing request of quality and origin guaranties promotes further research in this field. Therefore the employment of more specific, selective or never used before techniques is highly attractive towards a deeper insight into olive oil knowledge.

In the present paper we report the first example of the study of olive oil by circular dichroism (CD) which allows to obtain information about chiral molecular structures. Indeed CD spectroscopy is a form of light absorption spectroscopy that measures the difference in absorbance of right- and left-circularly polarized light due to a chiral substance [3]. While UV spectroscopy is related to the absolute square of the electric dipole transition moment, CD spectroscopy is due to the scalar product of the electric and magnetic transition dipole moment. As a consequence, it can be seen that electronic transitions gives always positive absorbance contributions in UV whilst they can be either positive or negative depending on the relative directions of the electric and magnetic dipole transition moments in CD. Therefore the latter gives a better resolution of spectra and provides informations on the stereochemistry of chiral molecules. CD has been used to study principal chiroptical analytes in different complex matrices like pharmaceutical mixtures [4] extracts of organic materials [5] and water soluble matrices [6], but not olive oil yet.

EXPERIMENTAL

Sample material and chemicals

Oil A sample is a spanish lampantino olive oil provided by Carapelli Firenze (Italy), stored protected from light and at

4°C. Lutein and pheophytin A-A' were extracted from crushed spinach leaves as reported by Schwartz et al. [7] and their purity assessed by comparison with UV/CD data reported in Literature [8]. (+)- α -tocopherol was purchased from Fluka. Samples for spectrophotometric analyses were prepared in dichloromethane (Carlo Erba, HPLC grade) solutions, excepting olive oil that was analyzed as it is.

Equipment

Circular dichroism spectra were obtained on a Jasco J-600 spectropolarimeter using a 0.2 cm path length cell. UV/VIS absorption spectra were obtained on a Varian Cary 4E spectrophotometer using a 0.2 cm path length cell. Sample concentration for CD spectra were 70 μ g/mL for lutein and pheophytin A-A' and 1 mg/mL for α -tocopherol using HPLC grade dichloromethane as a solvent.

Sample preparation

Spinach leaves were obtained from local sources. After thin layer chromatographic isolation from spinach extract, lutein and pheophytin A-A' were stored at -18°C under nitrogen before analysis. Light exposed olive oil sample were kept to direct light for three weeks. Deodorized samples were deodorized under nitrogen by increasing temperature up to 120°C in 5 h.

RESULTS AND DISCUSSION

Olive oil chiral compounds are represented by some volatiles, sterols, tocopherols, carotenoids and chlorophylls, but only the last three are detectable by circular dichroism without derivatization in ordinary spectral range. We preferred performing CD analyses directly on pure olive oil because the small amounts by which all these compounds are usually present make difficult their isolation. CD spectra were recorded between 700 and 250 nm: below 250 nm the low signal to noise ratio due to the high UV absorbance of conjugated dienes and trienes gives rise to

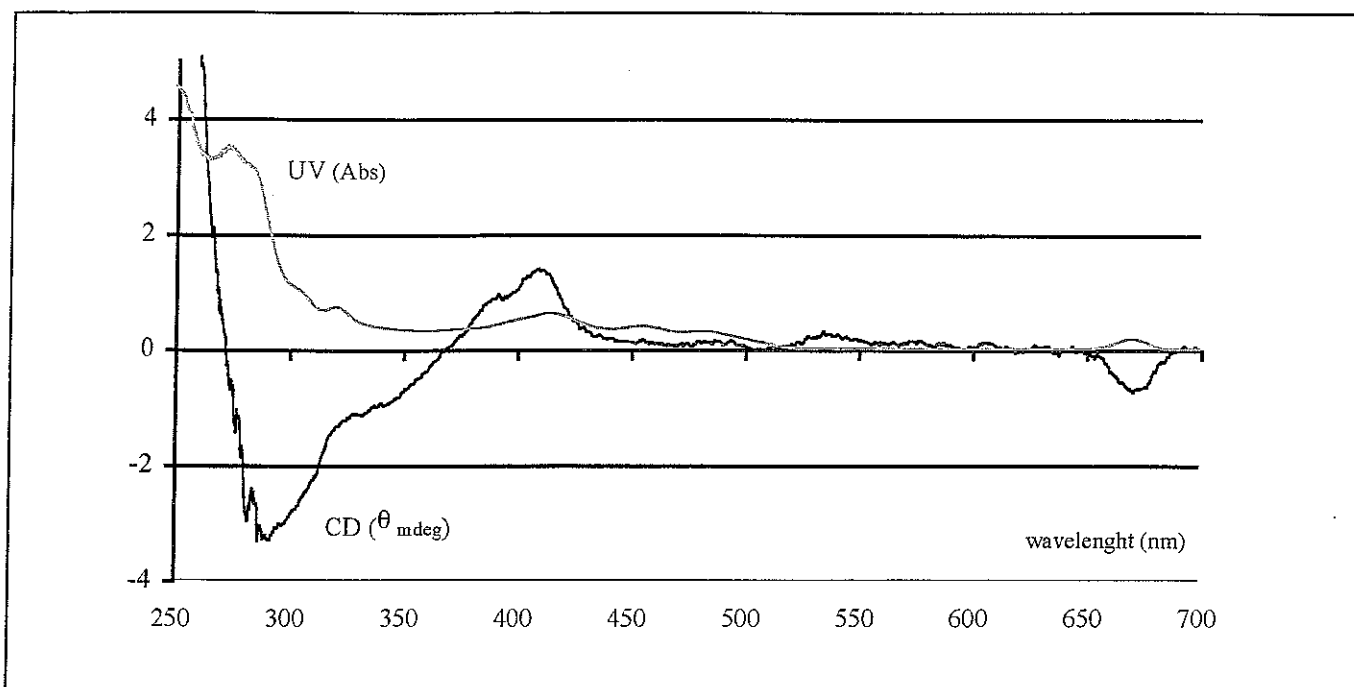


Fig. 1 - Oil A CD and UV spectrum

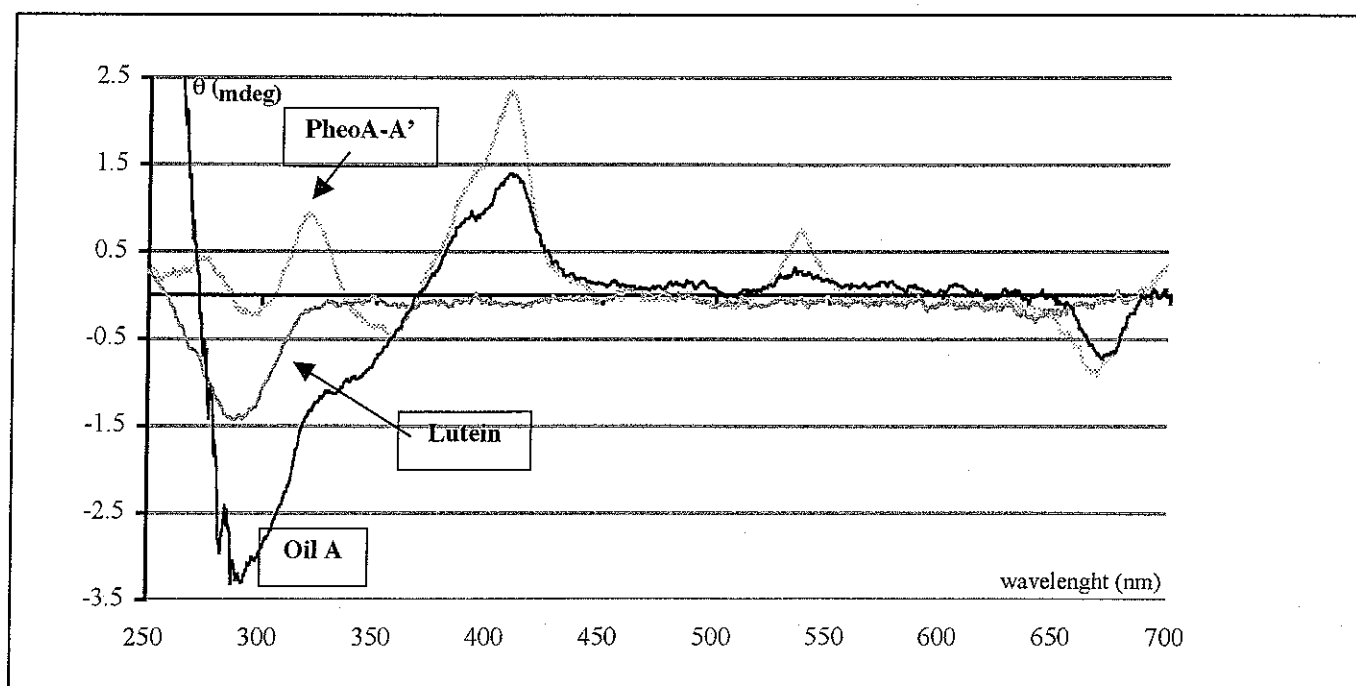


Fig. 2 - Qualitative comparison among CD spectra of olive oil A, Pheophytin A-A' and Lutein

meaningless signals. In Figure 1 we report the CD and UV spectra of oil A. CD spectrum showed well detectable bands in the 700-650, 420-390 and 340-290 nm regions.

From data reported in literature [8] and by comparison with lutein and pheophytin A-A' (extracted from spinach leaves mixture) CD spectra, we found that carotenoids and chlorophylls, despite the small quantity by which they are usually present in olive oil (10-40 mg/kg), are the most contributing compounds, if not exclusively, to the CD spectrum, while tocopherols have a very little contribution around 290 nm.

In Figure 2 olive oil spectrum is reported against CD of lutein

and a mixture of pheophytin A and A', respectively the most abundant carotenoid and pigment in olive oil. The band centred on 670 nm is due only to absorption of chlorophyll derivatives, as that around 420-390 nm. Absorption in this region is reported [9] for lutein, but it is very low. Quite interesting is the 340-290 nm region, resulting from the sum of the two absorptions of carotenoids (lutein) and pigments (pheophytin) which give rise to the marked negative band around 290 nm and to the inflection point at 330 nm.

Therefore CD can represent a sensitive tool to evidenciate carotenoids and pigments variations, as we observed in two

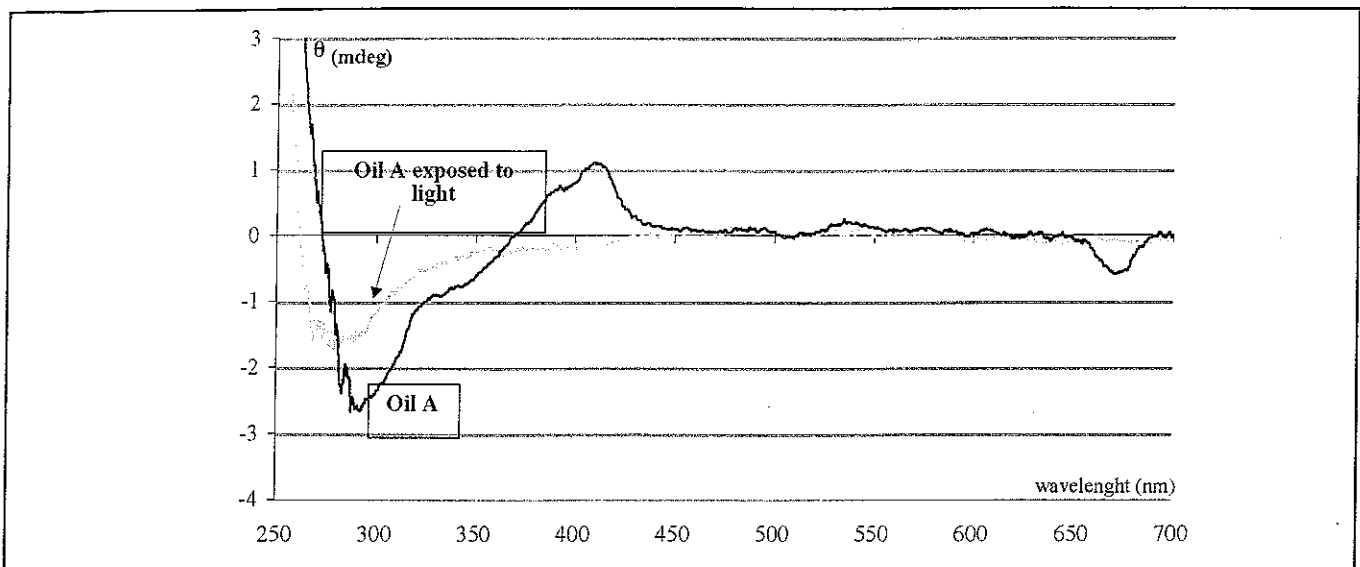


Fig. 3 - CD spectra of oil A and oil A exposed to direct light for 3 weeks

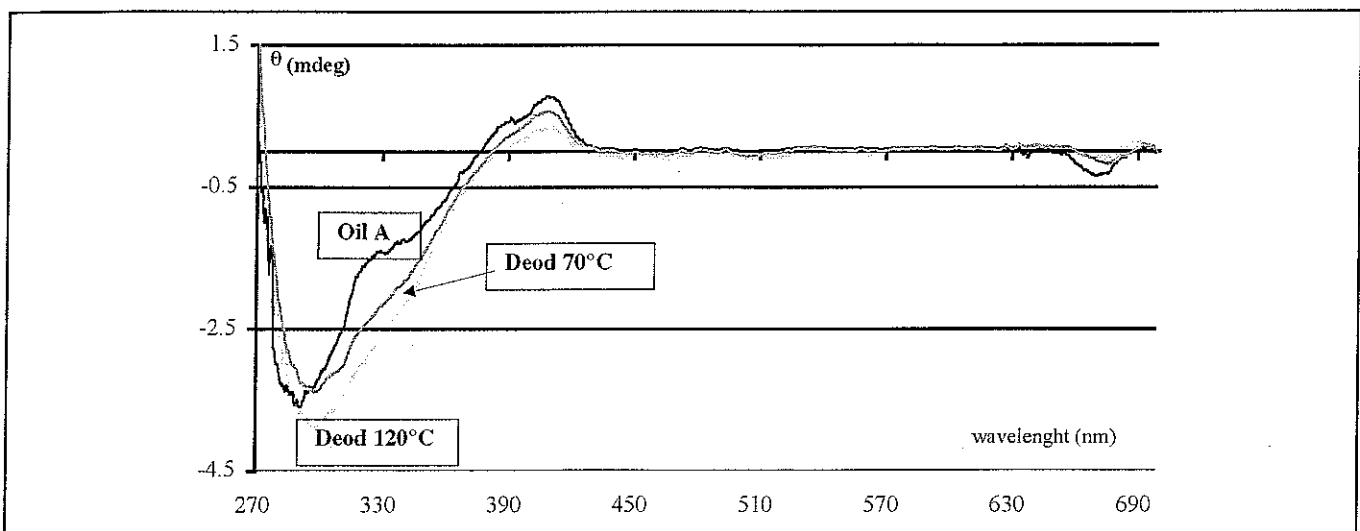


Fig. 4 - CD spectra of oil A and its deodorized samples at 70 and 120 °C

interesting kinds of stress to which olive oil samples have been undertaken: light exposure (Fig. 3) and deodorization (Fig. 4).

As shown in Figure 3, after exposing oil A to direct light for three weeks chlorophylls bands at 670 and 410 nm completely disappeared, as the inflection point at 330 nm, in accordance with previous studies [10]. The negative band remained around 290 nm is probably due to carotenoids survived to the stress by the action of tocopherols, accordingly to what recently reported in the photooxidation study by Tsimidou and Psomiadou [11].

Very interesting are the results obtained by a treatment of the same oil simulating a deodorization process. Differences from starting oil CD spectrum are clearly already evident in the deodorized oil at 70°C spectrum and increased in the one at 120°C, while UV spectra showed only the well known increment of the band at 270 nm relative to conjugated dienes and trienes [1]. The variations registered are probably due to changes in the relative contents of carotenoids and pigments [12], so that the sum of their contributions gives rise to the shape and intensity variation in the 280-350 nm region of CD spectrum. Moreover, it

has to be considered that degradation products of pheophytins can be formed during the heating process and show different absorptions. This topic is still under investigation.

CONCLUSIONS

Circular Dichroism proved to be a powerful tool in studying chromophoric chiral compounds of olive oil, giving additional informations in respect of UV spectroscopy. We found that carotenoids and pigments contribute almost exclusively to the CD spectrum, making this technique attractive in monitoring their variations under light stress, relating to storage conditions and time, and/or heat treatment, as deodorization processes. In particular, changes in the CD spectrum of oil deodorized in mild conditions are of interest in the difficult task of evidentiating this kind of adulteration, considering that at the present time there is no reliable method to identify certainly oil deodorized in mild conditions. Therefore they are worthy of further

investigations in order to better understand the limits of applicability of this new method.

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I componenti minori dell'olio di oliva, che ne costituiscono circa il 2%, rivestono un ruolo estremamente importante nella definizione delle sue peculiari proprietà edonistiche e bionutrizionali. La crescente richiesta di garanzie di queste qualità e dell'origine dell'olio di oliva spinge la ricerca all'impiego di tecniche di indagine sempre più specifiche e selettive. A questo scopo riportiamo il primo esempio di studio dei composti chirali dell'olio di oliva, come carotenoidi e clorofille, mediante la spettroscopia di dicroismo circolare (CD), una tecnica ottica che fornisce informazioni sulla stereochimica delle strutture molecolari, non ancora applicata all'olio d'oliva.

Le analisi sono state effettuate direttamente sull'olio tal quale, a causa delle piccole quantità con cui i composti in esame sono presenti. Lo spettro CD dell'olio A (lampantino spagnolo) riportato in Figura 1 ha evidenziato bande significative nelle regioni spettrali 700-650, 420-390 e 340-290 nm.

Dall'analisi dei dati riportati in Letteratura e dal confronto con gli spettri della luteina e di una miscela di feofitina A-A' (Fig. 2), responsabili di tali bande sono risultati i carotenoidi e le clorofille, con un minimo contributo da parte dei tocoferoli.

L'andamento dello spettro è quindi la risultante della relativa concentrazione di queste specie. In questo modo il CD può rappresentare un rapido strumento per la valutazione delle

variazioni di questi composti, come è stato da noi osservato sottoponendo l'olio ad esposizione diretta alla luce (Fig. 3) e deodorazione blanda (Fig. 4). Nello spettro CD dell'olio sottoposto a fotoesposizione è evidente la scomparsa dei segnali tipici delle clorofille, in accordo con studi precedenti. Dati promettenti sono emersi nello studio degli effetti della deodorazione. Differenze dallo spettro CD dell'olio di partenza sono già evidenti nello spettro dell'olio deodorato a 70°C ed aumentano in quello deodorato a 120°C, mentre i corrispondenti spettri UV mostrano solo il ben noto incremento della banda a 270 nm dei dieni e trieni coniugati. Le differenze registrate sono dovute con ogni probabilità a variazioni del contenuto in carotenoidi e pigmenti ed eventuale formazione di sottoprodotti provenienti dalla loro degradazione, in modo tale che la somma dei loro contributi dà luogo alla variazione dell'andamento dello spettro.

Il dicroismo circolare si rivela quindi come un promettente strumento per lo studio dei composti chirali cromoforici dell'olio di oliva, fornendo ulteriori informazioni rispetto alla spettroscopia UV. In particolare, questa tecnica è risultata interessante per il monitoraggio dei pigmenti e carotenoidi in relazione alle condizioni di conservazione ed ai trattamenti, come la deodorazione blanda.