OLIVE OIL CHARACTERIZATION BY VISIBLE MICRO RAMAN SPECTROSCOPY

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Abstract – Extra virgin (EV) olive oil has been characterized by means of micro-Raman spectroscopy. In order to overcome the natural fluorescence of oil a seed layer of Au nanoparticles (GNP) has been used on the top of microscope glass substrates that hosts the liquid sample during the measurement. This method enhances the Raman signal with respect to fluorescence and allows to obtain a deep insight on the chemical and structural state of the sample.

Keywords: Raman microscopy; Au nano-particles; Olive oil.

1. INTRODUCTION

The importance of olive oil in the Mediterranean diet and for health wellness has motivated the development of many techniques able to monitoring the quality and purity of this product. In particular this is important for preserving from adulteration and mislabelling the Extra Virgin (EV) olive oil obtained using only cold pressing without any thermal or chemical treatment. This product is highly demanded for its high quality and nutritional relevance. Among control techniques, micro-Raman spectroscopy (μ-RS) offers many advantages, because it can provides important information on chemical bonds, conservation state and possible adulteration without complicate or time expensive procedures. In the case of the EV olive oil the use of μ-RS is hampered by a huge fluorescent signal that hides the Raman response. Also the use of low energy light excitation (Infra-red laser) cannot completely overcomes this problem. Surface Enhanced Raman Spectroscopy (SERS) has been used fruitfully for materials with low Raman signal. This technique is based on the use of substrates with a special roughing coating of Au or Ag. In this work we have developed a simple method for the preparation of colloidal solution of GNPs that has been used as seed layer of the glass substrate for oil samples.

2. MATERIALS AND METHODS

2.1. Nanoparticles preparation

Gold nanoparticles were obtained by conventional citrate reduction method [1,2]. A 0.01% $HAuCl_4$ solution was reduced by 1% of sodium citrate with vigorous stirring at near boiling temperature. GNP size was controlled by adjusting the amount of sodium citrate. Solutions at various concentrations of GNPs with an expected diameter in the range of 20-70 nm were prepared. In this work GNPs with average size of 50 ±5 nm were used. The dimension of the GNPs was inferred from their light absorbance spectrum, that is significantly influenced by the surface plasmon resonance absorption band [1]. A drop of the GNP solution was posed on the microscope glass and left to dry for some hours. A small amount of the olive oil sample to analyze was thus deposed on the dried GNP solution.

2.2. Samples

In this study we have considered not commercial EV olive oils. They were produced in three different regions of the South Italy, namely Basilicata (from the locality of Chiaromonte), Puglia (Triggiano) and Campania (Bellona).

2.3. Micro-Raman spectroscopy

For the μ-RS measurements a Jobin-Yvon system from Horiba ISA was used, with a TriAx 180 monochromator, equipped with a liquid nitrogen cooled charge-coupled detector. The grating of 1800 grooves/mm allows a final spectral resolution of 4 cm^{-1}. The spectra were recorded in air at room temperature using a 17 mW He-Ne laser source (wavelength 632.8 nm). The spectrum accumulation time was 300 s. The laser light was focused to a 2 μm spot size on the sample through an Olympus microscope with 50× optical objective. Background signal and noise was reduced by using a data treatment based on wavelet algorithm [3]. The spectrum signal was decomposed in terms of sum of different wavenumber scaled elementary functions (named wavelets) and a hierarchical representation of the spectrum was thus obtained. Starting from the decomposed signal the spectrum has be reconstructed removing low and high frequency components due to background and non-correlated noise respectively. This data treatment method allows a reliable quantitative individuation of spectral detail also in the case of very weak signals, and it has been applied successfully to the Raman analysis of human single cell [4] and blood serum [5] The spectra obtained were analyzed in terms of convoluted Lorentzian functions by using a best-fitting routine of GRAMS/AI (2001, Thermo Electron) program, which is based on the Levenberg-Marquardt nonlinear least-square methods.

3. RESULTS AND DISCUSSION

The μ-RS when applied directly on a sample of EV olive oil results in a spectrum dominated by a huge broad mode due to fluorescence as reported in Fig.1 (curve a). The fluorescence is assigned to the chlorophyll content of natural oil and is centred at a wavelength of 675.5 nm [6,7]. When glass with GNP seed is used as substrate the fluorescence persists but the signal due to Raman scattering is clearly observed (fig.1, curve b). The presence of GNPs drastically reduce the fluorescence and enhance the Raman signal. This effect is due to the occurrence of Surface Enhanced Raman Spectroscopy (SERS) mechanisms [8]. Because of surface plasmon resonance, the metal surface of nanoscale particles can increase the electrical field when excited by laser resulting in a huge enhancement of the Raman scattering. In the Fig.2 it is reported the spectrum resulting after the removal of the background signal, performed by using a numerical process based on wavelets algorithms [3]. A fit of the spectrum in terms of sum of Lorentzian function was done in order to identify the main Raman modes occurring.

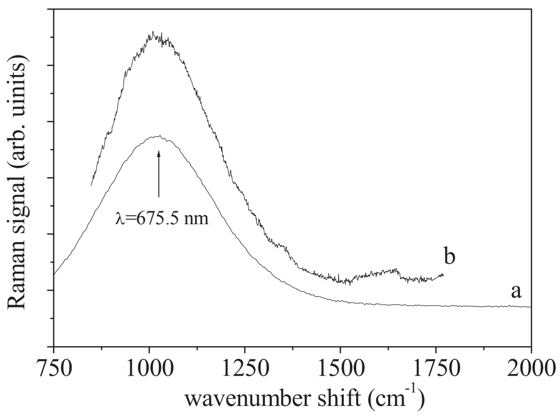

Fig. 1. Raman spectrum of EV olive oil on microscope bare glass (a) and on glass with GNP seed (b). Different y-axis scales were used for the two data set.

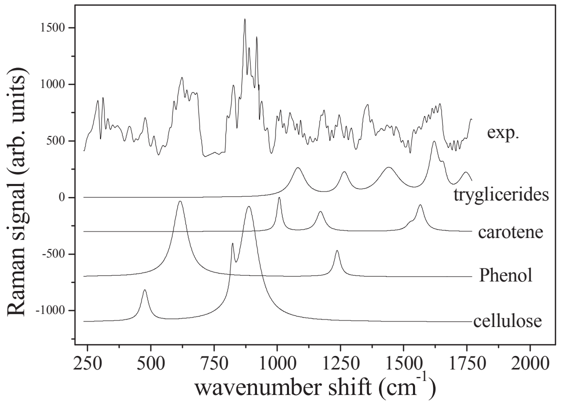

Fig. 2. Raman spectrum of EV olive oil on GNP seed/ glass substrate after background signal subtraction. The main spectrum components individuated are reported.

The spectrum in the range of 200-1800 cm^{-1} is reported in Fig. 2, together with a decomposition of the spectrum in the main Raman contributions found from the Lorentzian fit. The main contributions from vibrations of chemical bonds of triglycerides were individuated at wavenumber shifts equal to 1082 cm^{-1} (C-C stretching), 1265 cm^{-1} (in-plane C-H deformation in unconjugated cis- double bond), 1302 cm^{-1} (in-phase methylene twisting), 1441 cm^{-1} related to the deformation vibration of the group C-H, 1657 cm^{-1} (C=C), and 1747 cm^{-1} (C=O stretch of ester group). These modes are generally found in all kind of vegetable oils [9] even if the their relative intensity can change significantly depending on the oil origin.

The EV olive oil mainly consists of monounsaturated oleic acid (i.e. with only one C=C double bond with respect to other vegetable oils that have high content of linoleic acid characterized by another C=C double bond). Thus the weak intensity found for the 1265 and 1657 cm^{-1} modes is expected, because these modes are related to cis-(=C-H) vibration and cis-(C=C) vibrations respectively [9]. A peculiar property of the EV olive oil is the occurrence of a significant content of phenolic compounds and carotene, which are thought to endow it with many of health benefits [7,10]. The Raman contribution of these two components were clearly observed. Carotene is mainly related to chlorophyll and has Raman modes at wavenumber shift equal to 1008, 1150 and 1525 cm^{-1} [11].

Table 1. Experimental Raman modes and assignments

Mode (cm^{-1})	assignment	Ref.
616	In plane phenyl ring deformation	[12]
673	not assigned	
823; 887	Cellulose α-anomers and α-glycosides	[13]
1002-1008	Phenylinanine; carotene (C-CH$_3$ bend.)	[11]
1050	(C-C stretch. stretching in -(CH$_2$)- group	[11]
1082	Triglyc. C-C stretching	[9]
1150-1171	Carotene (C-C stretch.)	[11]
1237	phenol	[12]
1265	Triglyc. : Symm. rock in cis-double bond (=C-H)	[9]
1302	Triglyc. : (in-phase methylene twisting),	
1441	Triglyc. : Sciss. mode of meth. CH$_2$	[9]
1525	carotene	[11
1556	not assigned	
1620	not assigned	
1657	Triglyc. : cis- double- bond stretch. For insature C=C	[8]
1769	C=O stretch of ester group	[8]

The Raman modes at 616 and 1237 cm^{-1} were assigned to phenolic compounds (methylen bonds) [11]. The broad peak at about 900 cm^{-1} is argued to be related to cellulose content, due to a limited filtering of the sample [13]. Finally the Raman peak a 1350 cm^{-1} is probably related to CH$_3$ bond vibrations of oleic vibrations of oleic acid, even if the value is higher than the value of 1300-1320 cm^{-1} reported in literature [14]. The assignments of the detected Raman contributions are listed in Table I. An estimation of the relative amount of the main components in the oil can be obtained from the analysis of the Raman spectra. In this work we have compared not commercial EV olive oils produced in three different regions of the South Italy, namely in Basilicata (from the locality of Chiaromonte), in Puglia (Triggiano) and in Campania (Bellona). The sum of the intensity areas of the Raman modes that can be respectively attributed to carotene, oleic acid and phenol are reported in Fig. 3. The data have been normalized to the sum of intensities of the Raman modes assigned to triglycerides (see Table 1). Differences in the oil composition observed in Fig. 3, and in general the peculiar taste of each high quality oil, derive from many factors related to local terrain composition, climatic conditions and cultivation modality. Aging effects can also modify the relative composition of the oil. However these preliminary results seems to indicate a correlation between the carotene content and the components related to oleic acid and phenols.

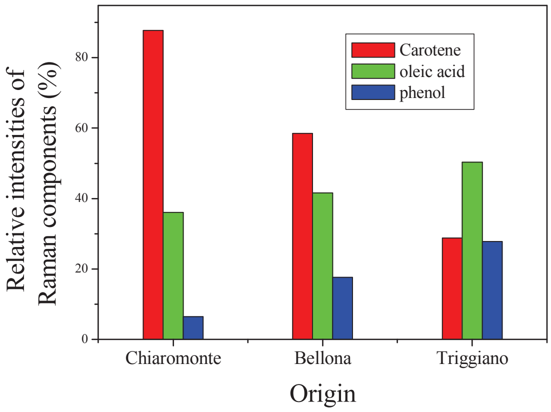

Fig. 3 Relative intensities of Raman mode assigned to Carotene (Red bars), Oleic acid (green bars) and phenol (Blue bars) bonds, for three EV olive oils.

A minor contents of carotene seems to correspond to an increase of oleic acid and phenols. Because these two last components have a strong influence on the oil taste, the analysis provided by the Raman spectroscopy has interesting implications for the development of analytical control procedures in the production of high quality EV olive oil.

4. CONCLUSIONS

Micro-Raman spectroscopy has been applied to the characterization of EV olive oil, confirming the validity of this technique as a rapid, non-destructive and reliable analytical technique for identifying bioactive components, such as phenols and carotenoids. A seed layer of colloidal GNPs has been employed for overcoming measurement difficulties related to huge fluorescence of olive oil. This method has induced a large amplification of the Raman signal allowing a deep insight in the composition and molecular structure of the investigated EV olive oil sample.

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