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Use of portable devices and confocal Raman spectrometers at different wavelength to obtain the spectral information of the main organic components in tomato (*Solanum lycopersicum*) fruits

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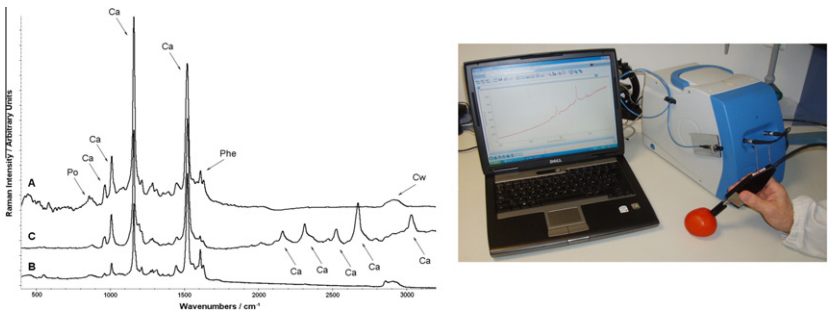
HIGHLIGHTS

- The three Raman instruments used provide different spectroscopic information.
- Portable instrument can give new options to carry out a wide range of *in situ* measurements in foodstuffs.
- Exhaustive assignation of the bands obtained in the Raman spectra of tomato fruit has been performed.
- Lycopene and β-carotene were differentiated in red tomatoes which have not been able to find in the bibliography.
- Phytoene/phytofluene was observed in tomatoes spectra.

GRAPHICAL ABSTRACT



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ABSTRACT

Tomato (*Solanum lycopersicum*) fruit samples, in two ripening stages, ripe (red) and unripe (green), collected from a cultivar in the North of Spain (Barrika, Basque Country), were analyzed directly, without any sample pretreatment, with two different Raman instruments (portable spectrometer coupled to a micro-videocamera and a confocal Raman microscope), using two different laser excitation wavelengths (514 and 785 nm, only for the confocal microscope). The combined use of these laser excitation wavelengths allows obtaining, in a short period of time, the maximum spectral information about the main organic compounds present in this fruit. The major identified components of unripe tomatoes were cutin and cuticular waxes. On the other hand, the main components on ripe tomatoes were carotenes, polyphenoles and polysaccharides. Among the carotenes, it was possible to distinguish the presence of lycopene from β-carotene with the help of both excitation wavelengths, but specially using the 514 nm one, which revealed specific overtones and combination tones of this type of carotene.

Introduction

Tomato (*Solanum lycopersicum*) is a species originally grown in South America [1]. This species is widely cultivated all over the world and it is the second most consumed vegetable nowadays [2]. The fruit of this plant has lots of benefits for human health as many studies have described. These benefits are attributed to the antioxidant compounds present in tomatoes, mainly to the organic polyenic molecules called carotenoids [3–6].

Structurally, carotenoids are usually long, aliphatic, C40 tetraterpenoids composed of eight C5 isoprenoid units giving place to a symmetrical molecule. Cyclic hydrocarbon saturated rings can also be linked at the end of the unsaturated aliphatic chains in some

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specific carotenoids. This natural pigment has an important characteristic attributed to its centrally located, extended conjugated double-bond system, which constitutes the light-absorbing chromophore that gives carotenoids their intense color [7,8]. This feature could be possible if the carotenoids had at least seven conjugated double bonds in their structures [9,10]. The electron-rich conjugated system of the polyene is responsible for the antioxidant activities of the carotenoids, both by quenching singlet oxygen and scavenging radicals to trigger chain reactions [11].

Chemically, carotenoids are classified into two major groups. The first one, carotenes, are the highly unsaturated hydrocarbon carotenoids such as lycopene, α-carotene, β-carotene, γ-carotene and ζ-carotene, which contain no oxygen and are usually orange and red. These molecules are particularly susceptible to oxidation because they are highly unsaturated. The second one, xanthophylls, are oxygen derivated carotenes and contain one or more oxygenated group on particular sites of the terminal rings. They are responsible for yellow and orange colors in these organic pigments [7,12].

Apart from these biosynthesised vegetable organic pigments, there are other antioxidant compounds in tomatoes. The proportion of them is lower in comparison with carotenoids, but they are also important. Among the mentioned compounds there are ascorbic acid, polyphenolic compounds, tocopherols (vitamin E), flavonoids, phytoene and phytofluene [4,5]. Furthermore, there are other compounds such as chlorophyll [13] and fatty acids, which are also present in these fruits [14].

Apart from these compounds, which are beneficial for human health, there are other organic components, which are essential to maintain the structure of these vegetables. The plant epidermis, which covers all aerial surfaces of plants, fruits and flowers, is mainly composed of a polyester matrix, cutin, and a mixture of soluble waxes. These fatty acids are embedded in the cutin matrix and deposited on the external surface forming an epicuticular layer [15–17]. From a biochemical and biophysical point of view, the two major components of this structure are cutin and waxes. The first one is a singular polyester of mainly C16 and C18 hydroxy fatty acids or diacids. The second one, consist of a heterogeneous mixture of very long-chain fatty acids and their monomeric derivatives, with carbon chain lengths ranging from C20 to C40 [17]. Furthermore, wax esters (primary fatty alcohols esterified to fatty acids) with chain lengths ranging from C36 to C70 are also present. As mentioned before, below this epicuticular wax layer there is a mixture of intracuticular waxes and cutin where polysaccharides such as pectins, cellulose and hemicelluloses are embedded [18]. The cuticle is thought to play important physiological roles in plants such as preventing water loss from aerial plant organs, protection against pathogens, mechanical damages, UV radiation and pollutants [15,16].

Spectroscopic techniques, especially Raman spectroscopy, are suitable analytical techniques to characterize carotenoids and other natural components (e.g., polysaccharides, lipids, phenolic compounds, etc.) of tomatoes. The use of different laser wavelengths offers the possibility of detecting more compounds, which is important for a better characterization of a complex matrix. Specifically, the Resonance Raman effect, achieved with a good selection of laser excitation wavelength (e.g. 514 nm laser), can be used to enhance the intensity of certain Raman bands in the Raman fingerprint area (900–1600 cm^{-1}) of carotenoids and can also help enhancing the intensity of Raman bands related to overtones and combination-tones (2000–4000 cm^{-1}). Overtones are defined as vibrational bands that are multiples of the fundamental transition modes, and combination bands are either the difference or sum of two fundamental bands [19]. These bands are fundamental vibrational modes that occur in the mid-high spectral region. They are particularly enhanced as a result of the multiplication of the most intense bands of the spectra [20]. Raman spectra, which Resonance effect is visible, could offer many slight changes that allow distinguishing a specific spatial conformation of certain carotenoids. The possibility of *in situ* and direct analysis in a non-invasive way and with no pre-treatment requirements make this technique a fast and easy-to-handle alternative to characterize the main components of fruits and vegetables [7].

Raman spectroscopy was used in this work to characterize the nature of the main organic components of unripe and ripe tomato fruits in a non invasive way. Moreover, Raman spectra which were obtained with two instruments (a portable device and a confocal microscope) and with different excitation laser excitation wavelengths (785 and 514 nm) were compared in order to evaluate the importance of an appropriate selection of laser excitation wavelengths. This procedure is essential to extract the maximum spectral information of the organic compounds present in these fruits. Additionally, a comparison of the performance between these instruments was carried out to assess if the portable spectrophotometer offers enough spectral information to indentify the analyzed organic compounds and to monitor them using an *in situ* strategy in tomato cultivars.

Material and methods

Samples

Several tomato fruits were collected in a crop located in Barrika, (Basque Country, North of Spain). Some fruits were selected and collected in an unripe stage (green color) and others in a ripe stage (red color). These fruits were collected strait from the plant and they were directly carried to the laboratory. After being washed with deionised water they were measured without any sample preparation or pretreatment.

Instruments

Raman analysis of green and red tomatoes was carried out using two different Raman instruments: a portable or hand-held instrument and a confocal Raman instrument. The portable instrument used was an innoRam® ($B_{\&}WTEK_{INC.}$, Newark, USA) spectrometer which implements a 785 nm laser excitation wavelength. Raman measurements with the portable instruments were performed on a home-made stage (MICROBEAM S.L, Barcelona, Spain) in which the Raman microprobe was assembled together with a micro-videocamera. This stage (of manual movement) allows performing microscopic Raman measurements (similar to a commercial microscope) focusing on the area of interest. In this case a long-range objective lens (Olympus, Tokio, Japan) of 20× was used. The spectral resolution of the instrument is 2 cm^{-1} and spectra were acquired with 25 s integration time and 12 accumulations.

As mentioned before, Raman measurements were also performed using another instrument; a Renishaw (Gloucestershire, UK) inVia confocal Raman spectrometer coupled to a DMLM Leica microscope. With this instrument, two different laser excitation wavelengths were used for the measurements: 785 and 514 nm. The power of 785 nm NIR laser excitation at the source (output power) is 350 mW, and about 150 mW (set as 100% of the laser power) at the surface of the analyzed area. The power of 514 nm argon ion excitation laser at the source is around 50 mW, and 20 mW (highest power) at the surface of the sample. In order to prevent possible thermal decomposition of carotenoids and further organic compounds of tomatoes the laser power was controlled with neutral density filters which were implemented in both instruments. After each measurement, the focused and measured area was checked. If the analyzed microscopic area was burned,