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Portable Raman spectroscopy for an in-situ monitoring the ripening of tomato (*Solanum lycopersicum*) fruits

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ABSTRACT

Ripening is one of the most important transformations that fruits and vegetables suffer, from an unripe to a ripe stage. In this study, it was followed up and analyzed the variations in the composition of tomato fruits at different ripening stages (green or unripe, orange or middle ripe, red or ripe and brown or overripe). The results obtained from the Raman measurements carried out showed a change in the composition of tomato fruits in the transit from green to brown. The analysis confirmed an increase of carotenoids from an unripe to a ripe stage of these fruits, being lycopene the characteristic carotenoid of the optimum ripe stage. The presence of chlorophyll and cuticular waxes decrease from the unripe to the ripe stage. Moreover, the relative intensity of phytofluene, a transition compound in the carotenoid biosynthetic pathway, is higher in the orange or middle ripening stage. The results obtained in-situ, without cutting and handling the tomato fruits, by means of a portable Raman spectrometer offered the same information that can be achieved using a more expensive and sophisticated confocal Raman microscope.

1. Introduction

The ripening of fruits and vegetables is a process that involves many changes in the final product [1]. Some of these changes are visible to the naked eye, e.g. the change of the colour [2]. However, there are other transformations that are not visible, such as molecular changes and reactions [3]. These transformations have been observed and described in many fruits. These ripening reactions involve changes in the texture, decrease of the firmness in the fruits and changes in the colour from green to red or yellow [4]. Furthermore, there is a change in the taste and aroma of these fruits [1]. Usually they become sweeter as the starch is transformed into sugar and the production of aromatic volatile compound is induced [5].

Among the wide variety of fruits that suffer from these ripening processes, tomatoes are one of the most important, because they have special properties that prevent many human diseases [3]. Among these properties, it can be highlighted that a high consumption of these fruits reduces the risk of suffering certain kinds of cancer [6–9]. They have also been associated with the improvement of the immune system [6,7] and the decrease in the risk of degenerative diseases like cardiovascular ones [6]. Moreover, they also decrease the risk of cataract [6,9].

These benefits come from some compounds called phytonutrients or phytochemicals. Tomato fruits are full of these vegetable natural compounds that act as antioxidants. They are not established as essential nutrients, but these natural pigments are biologically active chemicals present in the plants [10]. Moreover, they help humans to fight against different degenerative diseases and bring beneficial effects for the health [11]. Flavonoids, carotenoids, lutein, anthocyanins and terpenes are the most important compounds attributed to this group [10]. Among them, carotenoids are one of the most valuable compounds attributed to illnesses prevention. Carotenoids are naturally present as, yellow, orange and red organic pigments. These fat-soluble organic compounds are synthesized in plants, algae and some photosynthetic microorganisms. They are consumed by humans and animals in their diets, although they are unable to perform their de novo synthesis, but they are capable to transform and assimilate them [12–14].

However, green tomatoes present other substances and have different contents in carotenoids comparing them with the red tomatoes [15]. In this sense, some authors have claimed the importance to know which the differences among the components are in the different ripening stages, to asses which tomato provides more or less content in each compound [15]. But these differences in relative compound composition can be used to monitor the ripening process if one has an experimental technique capable to identify such compounds.

Although in the literature there are spectroscopic works related to the tomato ripening using different techniques such as SERRS [16], Raman imaging for the distribution of lycopene [17] even SORS for internal tomato maturity [18]; an in-situ analysis using portable Raman spectroscopy has become a challenge. In this way, Raman spectroscopy

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is a promising tool to observe these differences, as it has been shown recently through direct Raman measurements on the surface of tomato fruits [19], because most of the compounds present in the fruit are clearly identified. However, it was not demonstrated that changes in the compounds that take place during the ripening can be monitored through Raman measurements on the surface of the fruits without causing any damage on it.

The aim of this study was to evaluate the use of Raman spectroscopy to identify the variations of the compounds during the ripening process of a tomato fruit without causing any damage on it. As there are several Raman set ups in the market, a second objective to this work was to estimate if the main Raman features identified as the most promising to follow the ripening process, can be clearly identified using a portable Raman spectrometer. Thus, in a first step the use of a laboratory micro-Raman spectrometer which implements a 785 nm excitation laser will be used to identify all the compounds detected through direct measurements on the surface of tomato fruits at different ripening stages (green or unripe, orange or middle ripe, red or ripe and brown or overripe). In a second step a hand held Raman spectrometer, with the same 785 nm excitation laser, will be tested on the surface of the same tomatoes, and the results will be compared to evaluate its effectiveness.

2. Experimental

2.1. Samples

For the laboratory analyses, several tomato fruits were collected from an orchard located in Barrika, in the Basque Country (North of Spain). To carry out this study, "*Raf*" tomato variety was selected. Four groups of tomato fruits, at different ripening stages, and 2 samples per group were analyzed. In the first group green colour tomato fruits were collected, in the second group only orange colour tomato fruits, in the third group red colour tomato fruits (well ripened fruits) and in the last group brown colour tomato fruits (overripe) were included. All these fruits were collected from different plants and they were directly carried to the laboratory. After washing them with deionized water and in order to place each tomato sample in the confocal Raman microscope, a fragment from each tomato was extracted with a scalpel without peeling the tomato fruits.

2.2. Instrumentation

Raman analyses of the green, orange, red and brown tomatoes were carried out using two different Raman set ups. For the laboratory analyses, a Renishaw (Gloucestershire, UK) inVia confocal Raman spectrometer coupled to a DMLM Leica microscope was use. The measurements were performed using a 785 nm NIR laser. The laser power of the laser 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 of the tomato fruit. The laser power was controlled with neutral density filters implemented in both instruments in order to avoid possible thermal decomposition of carotenoids and further organic compounds of tomatoes. In the measurements carried out with the confocal Raman instrument, 50× long-range lenses were considered. The spectral resolution of the instrument is 1 cm^{-1} and spectra were acquired with 10 seconds integration time and 5 accumulations. In order to guarantee the representativeness of the acquired spectral information more than 25 spectra were acquired on each ripening stage.

To verify that tomato structure was not damaged after Raman measurements, microscopic observations were conducted before and after each Raman analysis with both, the confocal Raman microscope and the portable instrument.

The in-situ Raman analysis of green, orange, red and brown tomatoes was carried out using the innoRam® ($B_{\&}WTEK_{INC.}$, Newark, USA) portable spectrometer which implements a 785 nm laser. Raman measurements with the portable instruments can be performed in various modes: (a) directly with the probe on the hand and pressed smoothly on the surface of the tomato, (b) on a home-made stage (MICROBEAM S.L, Barcelona, Spain) in which the Raman microprobe can assembled together with a micro-videocamera, and (c) if required, this stage (of manual movement) allows to perform microscopic measurements by using a long-range objective lens (Olympus, Tokio, Japan) of 20× on its top. For this work, the measurements were performed directly on the tomato fruits, without collecting them from the plants. The spectral resolution of the portable spectrometer is 5 cm^{-1} and spectra were acquired with 25 seconds integration time and 12 accumulations. Once more, in order to guarantee the representativeness of the acquired spectral information, more than 25 spectra were acquired on tomato fruits at each ripening stage.

Most of the spectra were collected in the range 100–3000 cm^{-1}. A daily calibration using the 520.5 cm^{-1} silicon line was carried out to assure the instrument stabilization. Spectral acquisition with the confocal instrument was performed using the Renishaw WIRE 3.2 software. The Spectral acquisition with the portable instrument was carried out using the BWSpec™ 3.26 software ($B_{\&}WTEK_{INC.}$). Data treatment for both set of spectra was carried out using GRAMS/AI 7.02 software (Thermo Fisher Scientific Inc., Waltham, USA).

3. Results and Discussion

The first set of Raman measurements were performed with the laboratory confocal microscope. The spectra obtained on the surface of green tomatoes showed some bands which are suggested to be attributed to chlorophyll A [20,21]. The bands related to this natural green pigment are located ca. 744 (w), 915 (w), 985 (w) and 1325 (vw) cm^{-1} (see Fig. 1A and Table 1). Comparing these spectra with the ones obtained from the orange, red and brown tomatoes, it was observed a decreasing tendency of chlorophyll from green to brown tomato. In the orange tomatoes the presence of chlorophyll was still observed related to the appearance of the bands around 744 (vw) and 985 (vw) cm^{-1} (see Fig. 1B). However, in red and brown tomatoes these bands are not present (see Fig. 1C and D), which suggests that chlorophyll is not present in this ripening stage or Raman spectroscopy is not able to detect its low percentage. This decreasing tendency in the intensities of the Raman bands related to chlorophyll A, from green to brown tomatoes, attributed to the ripening process where the lost of this green pigment is observed, cannot be used to monitor the ripening stage due to its low relative intensities in comparison with the Raman bands of additional compounds.

Apart from the chlorophyll related bands, the spectra of green tomatoes showed the highest intensity of the bands around 1047, 1065, 1081, 1112, 1169, 1371, 1441, 1721, 2853, 2904 and 2921 cm^{-1} (see Fig. 1A and Table 1). These bands can be assigned to cuticular waxes [19]. In the measurements performed on the surface of orange, red and brown tomatoes, these bands showed a decreasing tendency in their intensities. The Raman spectra of unripe tomatoes showed higher intensity bands related to cutin and cuticular waxes. These compounds are known to have a structural function in the matrixes where they are present. Most of the bands related to these compounds are present in the green tomato and also in orange and red tomato, but in these two last ripening stages in a lower intensity. Moreover, these bands were not observed in the brown tomatoes.

When the tomato fruits are overripe there are some changes in the skin of the tomato, where cutin and cuticular waxes are located. Therefore, the absence of these compounds in the brown tomato may be related with the loss of the tomato skin firmness, consistency and softness [22] and Raman spectroscopy can detect its presence but also its absence, being an experimental indication of over-ripening.

Apart from the bands at 2853, 2904 and 2921 cm^{-1}, the Raman band at 1441 cm^{-1} in green tomato spectra has a stronger intensity than the same band in the orange, red and brown tomato spectra (see transition