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Citrus fruits freshness assessment using Raman spectroscopy

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ABSTRACT

The freshness of citrus fruits commonly available in the market was non-destructively assessed by Raman spectroscopy. Intact clementine, mandarin and tangerine species were characterised concerning their carotenoids skin Raman signalling in a time course from the moment they were acquired as fresh stock, supplying the market, to the physical degradation, when they were no longer attractive to consumers. The freshness was found to strongly correlate to the peel Raman signal collected from the same area of the intact fruits in a time course of a maximum of 20 days. We have shown that the intensity of the carotenoid Raman signal is indeed a good indicator of fruit freshness and introduced a Raman coefficient of freshness (C_{Fresh}), whose time course is linearly decreasing, with different slope for different citrus groups. Additionally, we demonstrated that the freshness assessment could be achieved using a portable Raman instrument. The results could have a strong impact for consumer satisfaction and the food industry.

1. Introduction

Many reasons point out the importance of consumption of citrus fruit for human health. It is well established that they are a rich source of vitamins, minerals and dietary fibre (non-starch polysaccharides) that are essential for normal growth and development, and overall nutritional well-being. Citrus fruit are also rich in phytochemicals, a group of non-nutrient bioactive compounds (including carotenoids). In addition, they contain no fats, sodium nor cholesterol (Economos & Clay, 1998).

Carotenoids are secondary metabolites synthesized by plants and algae that perform a wide range of functions in these organisms (Shumskaya & Wurtzel, 2013). Ikoma, Matsumoto, and Kato (2014) investigated the carotenoid biosynthesis and accumulation in citrus fruit. Carotenoids also have many beneficial effects for humans when acquired through plant-based food: protection of the skin against external and internal hazards (Darvin, Sterry, Lademann, & Vergou, 2011), protection against age-related macular degeneration (Meyers et al., 2014), protection against tumors (Nishino et al., 2000) and many other. There are more than 600 carotenoids known in nature, but only 40 appear in typical human diet (Kiokias, Proestos, & Varzakas, 2016).

High performance liquid chromatography (HPLC) alone or in combination with UV–Vis absorption spectrophotometry has been widely used for rapid and precise identification and quantification of carotenoids in various kinds and forms of plant-based food, including citrus fruit (Ağçam & Akyıldız, 2014; Bhaskarachary, Rajendran, & Thingnganing, 2008; Bhaskarachary, Sankar Rao, Deosthale, & Reddy, 1995; Biehler, Mayer, Hoffmann, Krause, & Bohn, 2010; Bureau & Bushway, 1986; Dias, Filomena, Camões, & Oliveira, 2009, 2014; Dugo et al., 2008; Gupta, Sreelakshmi, & Sharma, 2015; Hart & Scott, 1995; Meléndez-Martínez, Britton, Vicario, & Heredia, 2008; Rodrigo, Cilla, Barberá, & Zacarias, 2015; Scott & Eldridge, 2005; Varzakas & Kiokias, 2016; Xu, Fraser, Wang, & Bramley, 2006). However, the carotenoid content in citrus products (juices, pulp, extracts) has not been related with the freshness. Although HPLC has been the method of choice in many reports on citrus analysis, it has some limitations: it is an invasive method (i.e. the same sample can be analysed only once), there is a danger of carotenoid species loss due to isomerisation and destruction during sample preparation (Biehler et al., 2010; Varzakas & Kiokias, 2016), and, because of the inherent HPLC approach, it is not suited for prompt *in situ* analysis of food products. Nevertheless, the HPLC methodology for identification and quantification of carotenoids is constantly being improved (Dugo et al., 2008; Gupta et al., 2015). For discussion on some other spectrophotometric methods used for carotenoid characterisation see Biehler et al. (2010) and Darvin et al. (2011).

Portable Raman instruments are increasingly used for analysing and component identification in many fields of study. A search on the Web

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of Science platform found 177 scientific articles with the words "portable" and "Raman" in the title, published from the year 1992 onwards and concentrated in the past several years. Consistent Raman literature data covers a wide range of topics – from medical and food sciences to art and geological applications. Some proposed designs, their utility pertaining to food safety, and a brief explanation of the principles of Raman spectroscopy are described in studies by Feng et al. (2012), Fan, Wang, Wu, and Zuo (2013) and Sourdaine et al. (2015). Continuously improved portable Raman technology is gaining wider area of applications, being a convenient, sensitive and non-invasive tool for on-site control and assessment of a large variety of fruit and vegetables (e.g. in crops, storage facilities or market).

Carotenoids are particularly interesting in the context of this study because their Raman signal intensity increases rapidly as the laser line used for excitation approaches their electronic absorption of 470–490 nm (Cinta Pinzaru et al., 2015; Merlin, 1986; Tschirner et al., 2008). They have already been detected by means of Raman spectroscopy in various plant species, their tissues and extracts (Darvin, Gersonde, Albrecht, Sterry, & Lademann, 2007; de Oliveira, Castro, Edwards, & de Oliveira, 2009; Schulz, Baranska, & Baranski, 2005), and also in peel of citrus fruit (Feng, Zhang, & Zhu, 2013). Carotenoids presence in biological matrix can be inferred by the three prominent bands in Raman spectrum, termed ν_1, ν_2 and ν_3, which are usually observed in the 1536–1510, 1179–1151, and 1021–993 cm^{-1} spectral ranges, respectively, in a large variety of fruits and vegetables (Darvin et al., 2007; de Oliveira et al., 2009; Schulz et al., 2005). Although the optical properties and resonance Raman scattering of carotenoids and their benefits for consumption are widely investigated (Ermakov, Sharifzadeh, Ermakova, & Gellermann, 2005), their particular resonance Raman scattering behaviour was not exploited to probe any relationship with the freshness of food products, particularly citrus fruits

The aim of the present study was to prospect the relationship, if any, between the freshness of the citrus fruits species available on the common markets and their Raman spectroscopy output. More specifically, the freshness hypothesis should be related to the fruits aspect and colour and consequently, to the net carotenoids content of the outer peel. Furthermore, such prospect with portable instruments would allow the prompt, *in situ*, non-destructive and fast evaluation of the fruits quality and freshness in an objective and sensitive manner.

An important traceability issue is raised within this aim. The regulations regarding citrus fruits (UNECE Standard FFV-14 concerning the marketing and commercial quality control of citrus fruit, available (https://www.unece.org/fileadmin/DAM/trade/agr/standard/fresh/FFV-Std/English/14Citrus_2010e.pdf, accessed August 14, 2017) comprises the requirement regarding the "freshness", along with typical physical, biological and organoleptic characteristics. Specifically, the regulation requires the item called "colour", which "must be typical of the variety on at least one third of the surface of the fruit" (UNECE Standard, 2010). The usual control panels involve human appreciation, which, although qualified, still remains subjective. Therefore, our methodology proposed here, combining Raman spectroscopy tools with a new algorithm to relate spectral data with the freshness, might provide a useful, accessible, fast and appealing tool for the citrus freshness control.

2. Materials and methods

2.1. Citrus fruits

Specimens of mandarin oranges (genus *Citrus*) have been obtained as stocks of 0.5 kg from a freshly supplied local market. Three varieties commonly known as clementines (*Citrus clementina*), tangerines (*Citrus tangerina*) and mandarins (*Citrus reticulata*), have been selected. We acquired the samples under study as freshly received by the local supermarket. From the market point of view, the fruits were "the freshest", meaning just entered for consumer purchase. We used the market entering date as a starting date (Day 1). We used three replicates of each stock on each analysis session. The specimens were randomly chosen and the general criteria of any consumer have been applied, meaning that the fruits appeared appealing. Commercial regulations criteria were implicitly satisfied.

The clementine specimens were divided in two groups. One was kept for 20 days exposed to daily light at room temperature. The second group was preserved in dark conditions and was analysed in fresh state (Day 1), one week later (Day 7), and further, depending on the degradation of the individuals, the study spanned with the two sub-groups (Clementine 1 and 2) up to 20 days (Clementine 2). Fruits were individually wrapped in aluminium foil to prevent contamination and dehydration at room temperature. Randomly selected tangerines and mandarins were additionally considered during the study, under preservation in dark conditions at room temperature. Table S1 (Supplementary material) summarizes the samples investigated in this study and their respective treatment. The variability of the individual fruits stock concerning their provenance, transport and deposit conditions up to the market supply was not taken into consideration.

2.2. Micro-Raman spectroscopy

Raman spectra were obtained using a Renishaw InVia Reflex confocal Raman microscope. A cobalt diode pumped solid state (DPSS), air cooled laser operating at 532 nm was employed for excitation. Single point spectra were collected with a spectral resolution of 0.5 cm^{-1} and spatial resolution < 1 μm from a randomly defined area of about 15×15 mm which was marked on each fruit surface. The same marked area was investigated in a time course of 20 days, using the same acquisition conditions (20× objective, NA 0.35, WD 20 mm), 1 s integration time, 1 acquisition. The fruits size was selected from stock to fit the maximum working distance allowed by the lab-based instrument with the mobile XYZ microscope stage. Wire 3.4 and Origin 6.1 software was employed for data acquisition and processing.

The spectra of the clementine species were acquired under 100% (200 mW) laser power, while mandarins and tangerines required power lowering to 10% (20 mW) to prevent the detector saturation and consequently the peaks cut in the spectral response. For comparison purpose, and considering that the Raman signal strength is directly proportional to the incident laser power, the normalized data to the total laser power were further used. Our methodology to encompass Raman traceability was ensured by using cut citrus skin samples of the respective stocks taken under study. The small cut samples of controlled thickness, were horizontally deposited on the microscope slide to allow normal handling of the focal track and Raman data acquisition for optimal signal recording. The situation of the whole, intact citrus fruit handling, focussing and measuring on a lab-based Raman system was more difficult and required substantial Raman experimental skills for optimal data acquisition. Therefore, our methodology and results reported here, combining Raman tools with the proposed algorithm, might provide a useful tool for the citrus freshness control.

Automatic background subtraction has been applied for the working spectral range (100–1800 cm^{-1}) using a 10 point, end-weighted algorithm. Normalization to unit has been applied by dividing y data with the maximum limit of each set, i.e. Raman intensity of the C=C mode of carotenoids.

2.3. Portable Raman instrument

Two highly sensitive, high resolution portable Raman systems (i-Raman Plus, B & W TEK, 532 and 785 nm) with fiber optic probes (BAC 102 Raman trigger) were employed [http://bwtek.com/spectrometer-part-8-fiber-optic-probes/]. The pure signal from sample is selectively collected in back-scattering geometry due to the band-pass filter inserted in the optical path of the Raman probe. The dark subtracted