ARTICLE IN PRESS

Journal of Food Composition and Analysis xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

journal homepage: www.elsevier.com/locate/jfca

Original research article

Directional *versus* total reflectance spectroscopy for the *in situ* determination of lycopene in tomato fruits

Leonardo Ciaccheri[a], Lorenza Tuccio[a], Andrea A. Mencaglia[a], Anna G. Mignani[a], Ewelina Hallmann[b], Kalina Sikorska-Zimny[c], Stanislaw Kaniszewski[c], Michèl J. Verheul[d], Giovanni Agati[a,*]

[a] Istituto di Fisica Applicata "Nello Carrara" – CNR, Via Madonna del Piano, 10, 50019 Sesto Fiorentino (FI), Italy
[b] Department of Functional, Organic Food and Commodities, Faculty of Nutrition and Consumer Sciences, Warsaw University of Life Sciences (SGGW), Nowoursynowska 159c, 02-776 Warsaw, Poland
[c] Research Institute of Horticulture, Konstytucji 3 Maja 1/3, 96-100 Skierniewice, Poland
[d] Norwegian Institute for Agricultural and Environmental Research, Postvegen 213, Særheim, N-4353 Klepp Station, Norway

A R T I C L E I N F O

Keywords:
Food composition
Food analysis
Lycopene
Lycopersicon esculentum
Non-destructive assessment
Optical sensor
Partial least squares regression
Reflectance
Tomato

A B S T R A C T

Non-destructive tools for evaluating the lycopene content in tomatoes are of great interest to the entire fruit chain because of an increasing demand for beneficial health products. With the aim of developing compact low-cost reflectance sensors for lycopene determination, we compared Partial Least Squares (PLS) prediction models by using either directional or total reflectance in the 500–750 nm range. Directional reflectance at 45° with respect to the LED lighting direction was acquired by means of a compact spectrometer sensor. Total reflectance was acquired through a 50-mm integrating sphere connected to a spectrometer. The analysis was conducted on two hydroponic greenhouse cultivated red tomato varieties, namely the large round 'Dometica' (average diameter: 57 mm) and the small cherry 'Juanita' (average diameter: 26 mm). For both varieties, the spectral variance of directional reflectance was well correlated to that of total reflectance. The performances of the PLS prediction models were also similar, with R^2 of cross-validation between 0.73 and 0.81. The prediction error, relative to the mean lycopene content of full ripe tomatoes, was similar: i.e. around 16–17% for both varieties and sensors. Our results showed that directional reflectance measured by means of portable, low-cost and compact LED-based sensors can be used with an adequate precision for the non-destructive assessment of lycopene in tomatoes.

1. Introduction

Lycopene, which possesses high oxygen-radical scavenging properties, is the main antioxidant compound present in ripe tomato (*Lycopersicon esculentum* Mill.) fruits (Shi and Maguer, 2000). Because of this, its assumption can significantly reduce the risk of cancer and cardiovascular diseases (Leong et al., 2017; Müller et al., 2016; Rao and Agarwal, 2000). It should therefore be of large interest in producing tomatoes with the highest lycopene content and for quantifying this compound as a quality added-value.

Usually, the concentration of lycopene is determined destructively on sample extracts by means of unsafe solvents and time-consuming methods (Davis et al., 2003a; Fish et al., 2002). To improve this procedure, several non-destructive spectroscopic methods have been proposed (Baranska et al., 2006; Choudhary et al., 2009; Clément et al., 2008; Saad et al., 2016). Other more sophisticated techniques have concerned tomato spectral imaging, which in addition to predicting the lycopene content, can also provide information on its spatial heterogeneity (Liu et al., 2015; Polder et al., 2004). However, a standardized system and procedure for the rapidly estimating of lycopene is not yet available to tomato producers. This is a very important issue due to the large biological variability of tomato lycopene (Flores et al., 2017). Reflectance spectroscopy has been used for a long time to assess *in situ* fruit quality (Cozzolino, 2014; Nicolai et al., 2007). The method is rapid, non-destructive and can be applied both in the laboratory and in the field. The information that can be obtained from a reflectance

Abbreviations: PLS, partial least squares; RMSEC, root mean square error of calibration; RMSECV, root mean square error of cross validation

* Corresponding author.
E-mail addresses: l.ciaccheri@ifac.cnr.it (L. Ciaccheri), l.tuccio@ifac.cnr.it (L. Tuccio), a.mencaglia@ifac.cnr.it (A.A. Mencaglia), a.g.mignani@ifac.cnr.it (A.G. Mignani), ewelina_hallmann@sggw.pl (E. Hallmann), kalina.sikorska@inhort.pl (K. Sikorska-Zimny), stanislaw.kaniszewski@inhort.pl (S. Kaniszewski), michel.verheul@nibio.no (M.J. Verheul), g.agati@ifac.cnr.it (G. Agati).

https://doi.org/10.1016/j.jfca.2018.01.023
Received 12 May 2017; Received in revised form 19 December 2017; Accepted 27 January 2018
0889-1575/

spectrum depends both on the absorbance and scattering properties of the sample and the spectral range investigated. Highly pigmented fruits absorb visible light within the first few microns of tissue, then reflectance between 400 and 700 nm can provide data only on the superficial part of the sample. NIR, instead, penetrates deeper into the fruit tissues and can provide information on internal compounds and pulp structure. The specificity of reflectance spectroscopy can be limited when, as usual, overlapping bands are present in large numbers. For this reason, chemometrics can be used to extract information from the spectral data concerning sample quality features (Wang et al., 2015). The geometry of the irradiation and detection system is also important. In a directional-hemispherical system, irradiation occurs within a solid angle oriented with respect to the normal to the sample surface and the reflected radiation is collected over the entire hemispherical space above the sample surface. The bidirectional reflectance of samples can be fully characterized by using a spectrogoniophotometer that makes possible measurements as a function of the irradiation and detection angles (Combes et al., 2007).

Since fruit and vegetable surfaces are far to be Lambertian, the angle of detection with respect to the irradiation direction can significantly affect the reflectance values. Including or excluding the specular reflection is also fundamental especially for glossy samples (Combes et al., 2007).

In principal, collecting all the radiation reflected by the sample by using an integrating sphere, with an internal diameter ≥150 mm and a port fraction below 4%, should be ideal for obtaining the greatest accuracy and sensitivity. However, with the aim of developing a low-cost, user-friendly sensor that is as compact as possible, the choice goes for a simpler configuration (Mignani et al., 2015).

In this study, we compared directional *versus* diffuse reflectance spectroscopy for predicting lycopene content in tomatoes. The two nondestructive techniques were correlated to the actual content of lycopene extracted from the very same samples measured spectroscopically and quantified by means of HPLC. We used two tomato varieties of completely different sizes, in order to investigate whether this could have an effect on the method performance.

2. Materials and methods

The different steps of our study are represented in Fig. 1 and described hereafter.

2.1. Fruit material

Fully developed round tomatoes (*Lycopersicon esculentum* Mill. 'Dometica') and cherry tomatoes (*Lycopersicon esculentum* Mill. 'Juanita') were harvested at different ripening stages at the beginning of August 2014 from commercial greenhouses in southwestern Norway. Round tomatoes were harvested from Orre Gartneri (www.orregartneri.no) and cherry tomatoes from Lauvsnes Gartneri (www.lgartneri.no). The chosen tomato varieties are among the most representative of the fresh consumption Norwegian market that increased significantly during the last years (Verheul et al., 2015). All the different maturity stages ["green", "breakers", "turning", "pink", "light red" and "red" (USDA, 1975)] were represented (Fig. 2). Twenty-seven fruits for each variety, approximately 3 to 5 fruits per ripening stage, were used in the study. The plants were grown as hydroponic greenhouse cultures under optimal standard growth conditions in Norway (Verheul, 2012). The tomatoes were harvested from greenhouse compartments with and without the use of artificial light (280 μmol $m^{-2} s^{-1}$ PAR for maximum 18 h per day). The fruits from the 10–12 (Dometica) or 2–3 (Juanita) trusses were harvested. Global radiation outside of the greenhouse during the last 14 days before harvesting was, on average, 19.5 MJ $m^{-2} d^{-1}$. Daily mean temperatures in the greenhouse were 21 °C (Dometica) or 21.5 °C (Juanita). The plants were irrigated when needed using a standard nutrient solution for tomatoes, and an EC of 4.0 dS m^{-1} (Dometica) or 4.5 dS m^{-1} (Juanita) was maintained in the rock-wool slabs.

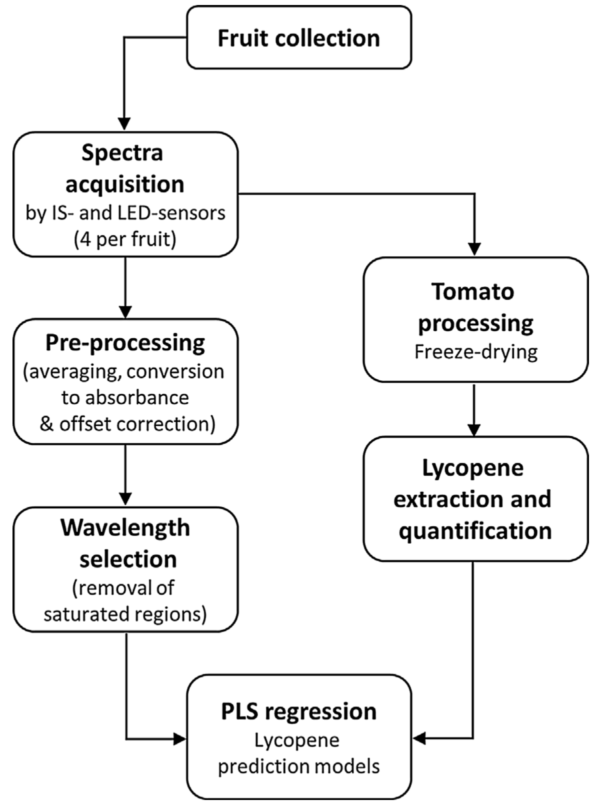

Fig. 1. Flowchart reporting the general steps of the data acquisition and statistical analysis. For details see Material and Methods.

2.2. Reflectance sensors

The directional reflectance spectra of the tomatoes were measured by means of the LED-based sensor (hereafter LED-sensor) described in Mignani et al. (2015). Briefly, it consisted of a group of LEDs for purposes of illumination and of a compact spectrometer for detection.

The light source was made up of three sets of four LEDs placed sequentially in a twelve-element circular-array. Each set consisted of a white LED and three other LEDs that emitted at 740 nm, 420 nm, and 680 nm, respectively. Lighting of the samples occurred at 45° with respect to the detection axis. This opto-geometric arrangement minimized the influence of any surface texture or roughness effects. The detector was an Ocean Optics STS-VIS spectrometer (Ocean Optics, Dunedin, FL, USA) with an optical fibre input, which operated in the 350–800 nm spectral range with a resolution of 1.5 nm. The sensor was USB-connected to a notebook and operated by means of a custom-made Labview® software interface. The zone of tomatoes sampled at the exit port of the sensor had a diameter of 5 mm.

The total reflectance spectra of tomatoes were acquired by means of the BRC112E-USB-VIS compact spectrometer (B&W Tek, Lübeck, Germany), which worked in the visible range (380–750 nm), and had a 25 μm slit and a resolution at 546 nm of 1 nm. This was connected by means of an optical fibre to a 50-mm integrating sphere (AvaSphere-50-LS-HAL, Avantes, Apeldoorn, The Netherlands), hereafter referred to as IS-sensor. An external halogen lamp (LS-1 Tungsten Halogen Light Source, Ocean Optics, Dunedin, FL, USA) connected to the integrating sphere by an optical fibre was used to illuminate directly the tomato samples at an 8° incidence angle. The IS-sensor sampled a zone of tomatoes of 10 mm in diameter.

The same day of harvesting, the fruits were immediately transported