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Fluorescence indices for monitoring the ripening of tomatoes in pre- and postharvest phases

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ABSTRACT

Greenhouse and climate chamber experiments were carried out to evaluate the ability of a portable multiparametric fluorescence sensor to monitor the ripening of tomato fruits (cultivar Cappricia) in pre- and postharvest phases. Fluorescence recordings were validated against established non-invasive optical methods based on reflection and remittance and against a visual colour classification scheme. Fruit ripening, as influenced by water supply (pre-harvest) and light quality (postharvest), was monitored by chlorophyll fluorescence indices (red and far-red fluorescence) after red and UV, red and green, or green and UV excitation. Chlorophyll breakdown was indicated by the fluorescence index NBI_R, which showed a negative and strong correlation with the reflection index a^*/b^* ($R^2 = -0.798$) and the remittance based stage-index ($R^2 = -0.754$). Characteristic curve patterns of the indices NBI_G, FLAV and Anth_RG enabled the pink (NBI_G, FLAV) and light red (Anth_RG) ripening stages to be defined and were well suited to detecting time-shifts in the ripening process. The potential of this technique for improved ripening monitoring and quality attribute determination in tomatoes is discussed.

1. Introduction

The ripening stage of tomato fruits is usually defined on the basis of their external colour, which changes due to the degradation of chlorophyll and the biosynthesis of lycopene and beta-carotene (Hobson and Grierson, 1993). The United States Department of Agriculture (USDA) established a colour classification system that is widely used to differentiate the ripeness of tomatoes (USDA, 1991). In practice, visual classification is time-consuming and may not always be accurate because the definition of colour is a subjective perception influenced by changing light conditions, particularly under greenhouse and field conditions (Hobson et al., 1983). With this background, extensive research has been conducted to develop optical sensors and determine their suitability as a fast and non-destructive method of assessing fruit quality attributes (Chen and Sun, 1991; Davis and Gardner, 1994; Hahn, 2002).

During ripening, the pigment composition of tomato fruits dramatically changes, affecting both the absorption and emission (reflection, remission, fluorescence) of light (Chen and Sun, 1991; Abbott, 1999). Techniques based on the determination of reflectance have found practical applications in postharvest fruit sorting. RGB colour cameras are traditionally used, although spectral determinations are considered to have higher discriminating power (Polder et al., 2002). Commonly, colorimeters measure the intensity of specular reflection and report readings on the L^* (lightness), a^* (red to green), b^* (blue to yellow) scale, a system defined by the Commission International de l'Éclavinge (Abbott, 1999; López Camelo and Gómez, 2004). In this context, absolute L^*, a^* and b^* values strongly depend on the tomato variety and environmental conditions, whereas parameters such as the a^*/b^* ratio and the chroma-index provide robust information on the progress of ripening (Johjima and Matsuzoe, 1995; Arias et al., 2000). Particularly, the a^*/b^* ratio has proven to be a good indicator for following the colour development of tomatoes (Koskitalo and Ormrod, 1972; Arias et al., 2000).

Another proposed technique, remittance VIS spectroscopy, has been successfully adopted to determine pericarp lycopene content

Abbreviations: Anth_RG-index, Anthocyanin-index: the decadic logarithm of the red-to-green excitation ratio of far-red chlorophyll fluorescence; CFL, compact fluorescence lamp; DAT, days after treatment initiation; FLAV-index, flavonol index; FRF_G, far-red fluorescence excited with green light; FRF_R, far-red fluorescence excited with red light; FRF_UV, far-red fluorescence excited with UV light; LED, light-emitting diode; NBI_G, nitrogen-balance index with green excitation light; NBI_R, nitrogen-balance index with red excitation light; NDVI, normalized difference vegetation index; NIR, near-infrared; R, remittance; RF_G, red fluorescence excited with green light; RF_R, red fluorescence excited with red light; USDA, United States Department of Agriculture.

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in tomatoes (Farneti et al., 2012; Seifert et al., 2014), and chlorophyll content in apples (Zude-Sasse et al., 2002; Kuckenberg, 2008) and bananas (Zude-Sasse, 2003) as well as carotenoid content in carrots (Zude-Sasse et al., 2007). Another method, fluorescence spectroscopy, has been proposed for the analysis of tomato ripening in the laboratory (Lai et al., 2007), whereas a hand-held multiparametric sensor has been used to characterize ripening and quality attributes of apples (Betemps et al., 2012), grapes (Cerovic et al., 2009; Ben Ghozlen et al., 2010; Agati et al., 2013) and oil palm bunches (Hazir et al., 2012a, 2012b). This portable system provides robust data on the pigment composition of leaves and fruits (Cerovic et al., 2002, 2009; Betemps et al., 2012; Müller et al., 2013) through the excitation of chlorophyll fluorescence with different wavelengths. In tomatoes, carotenoids that accumulate during the ripening process absorb UV and green excitation light, reducing the fluorescence emitted by chlorophyll (Lai et al., 2007). In contrast, red-light-induced fluorescence depends on the chlorophyll content but not on the presence of carotenoids or flavonoids (Buschmann et al., 2008).

Cultivation and environmental factors during pre- and postharvest strongly affect tomato fruit ripening and quality (Dumas et al., 2003; Brandt et al., 2006). In the pre-harvest phase, biotic and abiotic stress factors might accelerate maturation. Amongst others, water deficit might increase respiration and ethylene biosynthesis, triggering and/or promoting chlorophyll degradation and carotenoid accumulation in tomatoes (Abeles and Abeles, 1972; Tingey et al., 1976; Finger et al., 1995; Adams-Phillips et al., 2004; Cara and Giovannoni, 2008). Moreover, light signal transduction impacts the biosynthesis of carotenoids and flavonoids (see review of Adams-Phillips et al., 2004).

As early as 60 years ago, McCollum (1954) found that harvested tomatoes ripened in light had higher carotenoid levels compared to fruits excluded from light. Furthermore, light accelerates colour development (Jen, 1974; Thomas and Jen, 1975b). In addition to the impact of light intensity, light quality influences colour development in tomatoes, as the biosynthesis of carotenoids is mediated by phytochromes (Khudairi and Arboleda, 1971; Thomas and Jen, 1975b; Paynter and Jen, 1976; Liu et al., 2009). Early studies demonstrated that blue and red light accelerate the biodegradation of chlorophylls and the biosynthesis of carotenoids compared to fruits exposed to white light or far-red light (Jen, 1974; Thomas and Jen, 1975a,b). However, to our knowledge, no study has addressed the impact of the blue-to-red light ratio on the ripening progress of tomatoes.

The aim of our study was to evaluate the suitability of the multiparametric fluorescence technique as a tool for monitoring the pre- and postharvest ripening of tomato fruits. In the pre-harvest phase, we induced water deficit stress to accelerate fruit ripening; in postharvest, we exposed the fruits to different light conditions. The most promising and robust fluorescence parameters in the pre-harvest phase were selected and then validated in a postharvest trial. In the latter, sensor-based reflectance and remittance measurements were used as a reference.

2. Materials and methods

2.1. Pre-harvest analysis: maturation of vine-ripened fruits as influenced by water supply

The experiment was conducted from June to July 2013 in a commercial-like greenhouse at the Campus Klein-Altendorf research station (University of Bonn, Germany). The greenhouse is equipped with a gutter growing system. Seeds of the truss tomato (*Lycopersicon esculentum*) F1 hybrid Cappricia (Rijk Zwaan Distribution B.V., The Netherlands) were sown on 18 February 2013 into rockwool cubes (Grodan delta, Grodan, The Netherlands) and cultivated under supplemental lighting. Four weeks after sowing, the plantlets were transferred to rockwool slabs (Grotop Expert, Grodan, The Netherlands) with two plants per metre and 35 plants per row. The plants were cultivated under natural day length and light intensity conditions with average day and night temperatures of 21 and 18 °C, respectively. The trusses were thinned out to six fruits per truss. Water supply and fertilization were provided by drip irrigation. The plants were irrigated with a full standard nutrient solution mixed from two stock solutions. The irrigation setup was controlled by time (on average 5 min/h) and the daily irradiation sum (additional irrigation starting at 40 kilolux). When the first truss (from the bottom) started to ripen ('breaker'), treatments were initiated (113 days after sowing). The water deficit was implemented by reducing the amount of nutrient solution to 50% of the control treatment for a total of three weeks until fruits of the second truss reached the red stage. Prior to treatment initiation, the second truss of ten plants per row were tagged. Two fruits per truss in a previously defined fruit position were labelled for posterior *in situ* fluorescence recordings (Multiplex® 3, Force-A, France). Evaluations were performed at least once a week after treatment initiation.

2.2. Postharvest analysis: maturation of detached fruits under different light conditions

Tomato trusses of the cultivar Cappricia (Rijk Zwaan, De Lier, The Netherlands) were harvested at the 'mature green' stage from plants cultivated in the greenhouse at the Campus Klein-Altendorf research station (University of Bonn, Germany). Each truss consisted of six fruits. In the laboratory, two healthy and undamaged fruits were selected from each truss. In this step, fruits from the same position on the truss were chosen. Overall, 80 fruits of similar size and colour were selected and placed evenly, without touching each other, onto four plastic trays (20 fruits per tray). The tomatoes were numbered, and the fruit side facing upwards was labelled. After visual and sensor-based determination of fruit colour, the fruits were subjected to different light treatments. The light treatments were applied under controlled conditions in a custom-built climate chamber. Two different lighting systems were used: white compact fluorescence lamps (CFLs) (MASTER PL-L 4P, Philips, Amsterdam, The Netherlands) and light-emitting diode (LED) modules (a prototype optimized for our research purposes; Ushio Lighting Inc., Tokyo, Japan). The CFLs provide white light with main peaks at 435 nm, 545 nm and 612 nm, whereas the LED modules offer blue and red light with single peak at 445 nm and 665 nm, respectively (Hoffmann et al., 2015a, 2015b). The photoperiod was set to 14 h with day/night temperature of 20 °C/22 °C and relative humidity of 80%. The photon fluence rate of the different light compartments was set to 95 ± 5 μmol m^{-2} s^{-1}. For nine days, the trays were placed underneath either the CFLs or LEDs, and one tray was kept in the dark.

The following light treatments were conducted:

- 15% blue: 15% blue + 85% red light (LED)
- 75% blue: 75% blue + 25% red light (LED)
- white: 14% blue + 40% green + 46% red light (CFL)
- dark: control, no light

2.3. Visual characterization of fruit ripening

External fruit colour was assessed visually according to the *Standards for Grade of Fresh Tomatoes* established by the United States Department of Agriculture (USDA, 1991). For rating we used the following scale: