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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:

1 = green, 100% green
2 = breaker, a noticeable break in colour with less than 10% of colour other than green
3 = turning, between 10 and 30% red(ish) colour
4 = pink, between 30 and 60% red(ish) colour
5 = light red, between 60 and 90% red
6 = red, more than 90% red

2.4. Sensor-based characterization of fruit ripening

Three different sensor techniques were used to characterize colour changes during tomato ripening. Readings were carried out in a laboratory across the time-course of the experiment (days after initiation of treatments, DAT). In the course of the experiment, evaluations were taken regularly on the same fruit side, which was the one facing upwards (light-exposed fruit side).

2.4.1. Reflectance determinations

Tomato surface colour values were obtained with a portable spectrophotometer (CM-503d, Konica Minolta Inc., Tokyo, Japan). Readings were performed at the equatorial zone of the light exposed fruit side (one reading per sample and day). L^*, a^* and b^* readings of the CIELAB model were taken from a sensing area of 7 mm^2. Colour was expressed by the ratio a^*/b^*. This index represents the ratio of green-red to blue-yellow components of fruit colour and is widely used for tomatoes (Koskitalo and Ormrod, 1972; Arias et al., 2000).

2.4.2. Remittance determinations

Remittance (R) measurements were performed with a hand-held spectrophotometer (Pigment Analyzer 1101, Control in Applied Physiology GbR, Germany) according to Kuckenberg (2008). Each fruit was evaluated daily at the equatorial zone of the labelled light-exposed side. To monitor chlorophyll breakdown, the normalized difference vegetation index (NDVI; NDVI = $(R_{780} - R_{660})/(R_{780} + R_{660})$) was calculated. Redness of fruits according to the OECD ripening stages was evaluated by the Stage-index, a partial least squares algorithm based on the absorption of chlorophyll and carotenoids and absorption in the near infrared (NIR) region (Pflanz and Zude, 2008).

2.4.3. Fluorescence determinations

Fluorescence recordings were performed with a hand-held multiparametric sensor (Multiplex® 3, Force-A, Orsay, France) as described elsewhere (Ben Ghozlen et al., 2010; Leufen et al., 2014). Briefly, fluorescence is excited by light-emitting diodes (LEDs) in the UV-A (peak at 375 nm), green (peak at 518 nm) and red (peak at 635 nm) spectral regions. Fluorescence signals are detected in the blue (425–475 nm), red (680–690 nm) and far-red (720–755 nm) spectral regions. Recordings were taken from a constant distance of 0.10 m using a specific grid in front of the sensor. Fluorescence signals were recorded through an aperture 4 cm in diameter around the equatorial zone of the fruit (one reading per sample and measurement day). The following indices, as described by Ben Ghozlen et al. (2010), provided the most promising results:

Nitrogen Balance Index, excited with red: NBI_R = FRF_UV/RF_R
Nitrogen Balance Index, excited with green: NBI_G = FRF_UV/RF_G
Flavonol Index: FLAV = log (FRF_R/FRF_UV)
Anthocyanin Index: Anth = log (FRF_R/FRF_G)

2.5. Statistics

Statistical analyses were performed with SPSS statistical software (PASW statistics version 20.0, SPSS Inc., Chicago, USA). The data were checked for normal distribution and homogeneity of variance. Differences between the treatment groups in fruit colour according to the *Standards for Grade of Fresh Tomatoes* were determined by the Kruskal–Wallis-test and Mann–Whitney *U*-test. For the fluorescence readings taken in the pre-harvest phase, data were compared by the Kruskal–Wallis-test ($p \leq 0.05$). In the postharvest phase, means of the sensor-data were compared by analysis of variance (one-way ANOVA), and significant differences among the treatment groups were determined according to Duncan's multiple range test ($p \leq 0.05$). Correlations between selected parameters were tested with two-way Pearson correlation analysis. Graphs were drawn with SigmaPlot 11.0 (Systat Software Inc., Richmond, CA, USA).

3. Results

3.1. Pre-harvest monitoring: influence of water supply on tomato ripening

Ripening of the tomatoes, from the green to the red stage, took more than 20 days under normal growing conditions (Fig. 1). As indicated by visual assessment, fruit ripening was accelerated due to water deficit; significant differences ($p \leq 0.05$) between control and water-deficit fruits became apparent as early as 8 DAT (Fig. 1). The faster maturation was confirmed by fluorescence readings. Significant differences between the experimental groups were found for the parameters NBI_R, FLAV and Anth_RG at 8 DAT and for NBI_G at 10 DAT (Fig. 2). The fluorescence indices differed strongly in their temporal development. NBI_R decreased constantly during the ripening process, reaching its minimum at the red stage; NBI_G decreased until 13 DAT (water deficit) or 17 DAT (control), and then, it increased until the end of the experiment. In contrast, the indices FLAV and Anth-RG increased first, reaching a peak at 13 DAT (water deficit) or 17 DAT (control), and then decreased. In addition to the time-shift, the water deficit treatment also caused pronounced differences in the values of the minima and maxima. The peak maximum of the FLAV-index and peak minimum of NBI_G differed between fruits from control and water-deficit plants (Fig. 2), suggesting that the real peaks (maximum and minimum for the respective parameters) in the control treatments might have happened during the extended measurement interval from 13 DAT to 17 DAT and 20 DAT.

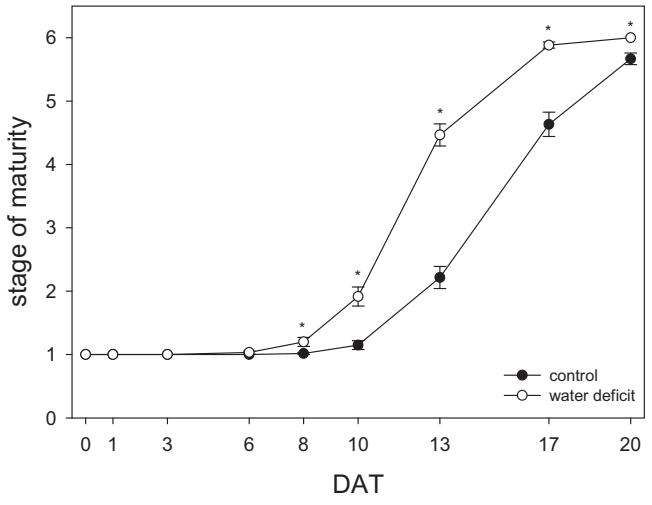

Fig. 1. Influence of water availability on the development of external fruit colour, assessed according to the *Standards for Grade of Fresh Tomatoes (USDA, 1991)*. Significant differences are indicated with asterisk according to Kruskal–Wallis-test; means ± SE; $n = 60$.

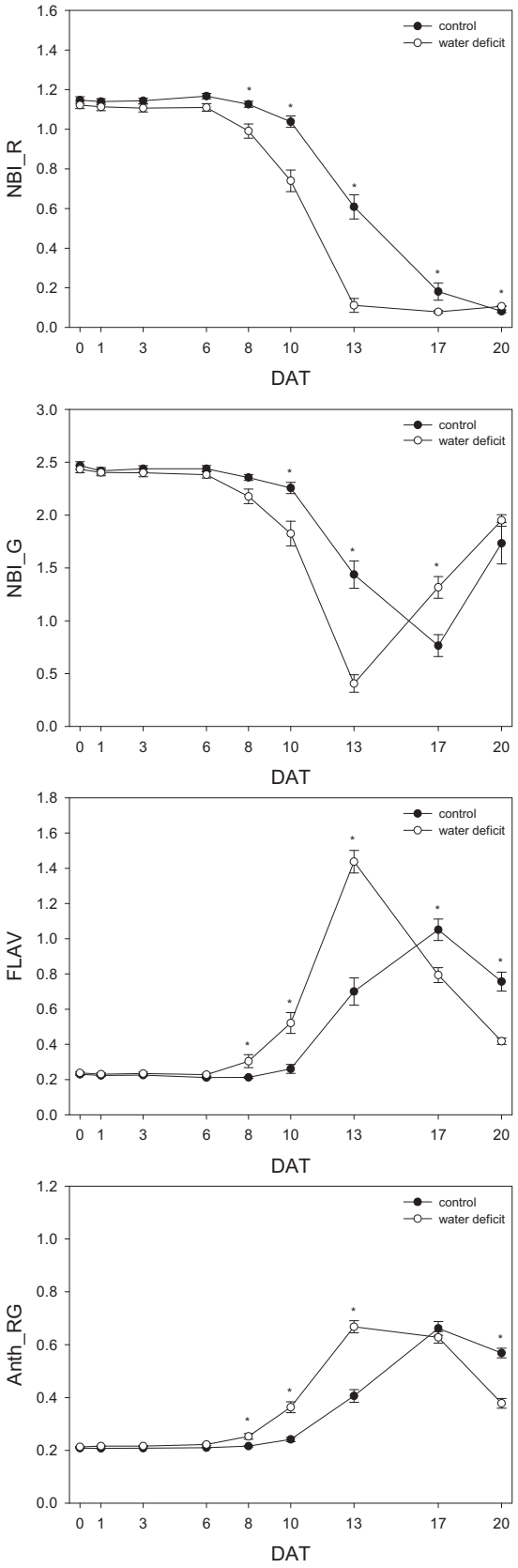

Fig. 2. Influence of water availability on the pre-harvest development of the fluorescence indices NBI_R, NBI_G, FLAV and Anth_RG. Significant differences are indicated with asterisk according to Kruskal–Wallis-test; means ± SE; $n = 60$.

3.2. Postharvest monitoring: influence of light quality on tomato ripening

In the postharvest experiment, we compared the impact of different light qualities on the maturation of tomatoes. The visual assessment indicated maturation from green to red within seven to nine days depending on the light conditions. In general, there was no difference between the different amounts of blue-light (15% or 75%) and there was only a minor difference compared to those fruits stored under white light. Fruits stored in the absence of light had a pronounced delay in their ripening process (Fig. 3). In general, this trend was confirmed by the fluorescence (NBI_R, NBI_G, FLAV, ANTH_RG), reflectance (a^*/b^*) and remittance (NDVI and stage) parameters (Figs. 4 and 5). All the sensor-based indices demonstrated that already at 1 DAT, the ripening process was significantly delayed in fruits stored in the absence of light.

Light quality affected the ripening development: until 4 DAT, all indices changed more quickly in fruits exposed to LED light (15% or 75% blue) than in those exposed to white light (Figs. 4 and 5). In general, the curve patterns of the four fluorescence indices (Fig. 4) were consistent with those determined under pre-harvest conditions in the greenhouse. In the postharvest phase, however, the minima of NBI_G and the maxima of FLAV were similar for all experimental groups. The FLAV-index increased until the turning to pink stage, reaching a peak at approximately 1.1 [rel. units], and then decreased until the end of the experiment. The Anth_RG index increased until a ripening stage of approximately 5.5 [rel units] (light red to red stage) and then decreased during the further course of the experiment. Of the evaluated fluorescence indices, NBI_R and FLAV were the most promising because of their more precise differentiation of the treatment groups.

3.3. Relationship between fluorescence, reflection and remittance parameters

Reflection and remission techniques were also successfully used to track the postharvest maturation of tomatoes (Fig. 5). In the case of the different light treatments, and in the situation of delayed ripening of fruits stored in the dark, the remission indices (NDVI and stage) could differentiate the treatments over a longer time-frame compared to the reflection index (a^*/b^*).

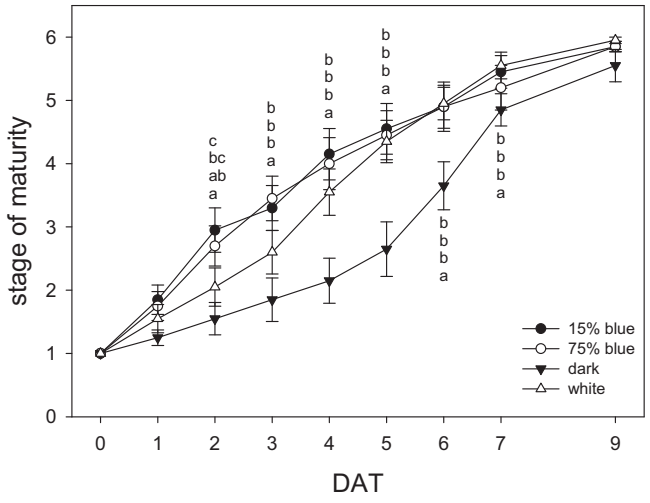

Fig. 3. Influence of light quality and darkness, on the postharvest development of external fruit colour, assessed according to the *Standards for Grade of Fresh Tomatoes (USDA, 1991)*. Means ± SE ($n = 20$) followed by the same letters (within the evaluation day) do not differ significantly according to the Duncan test ($p \leq 0.05$).

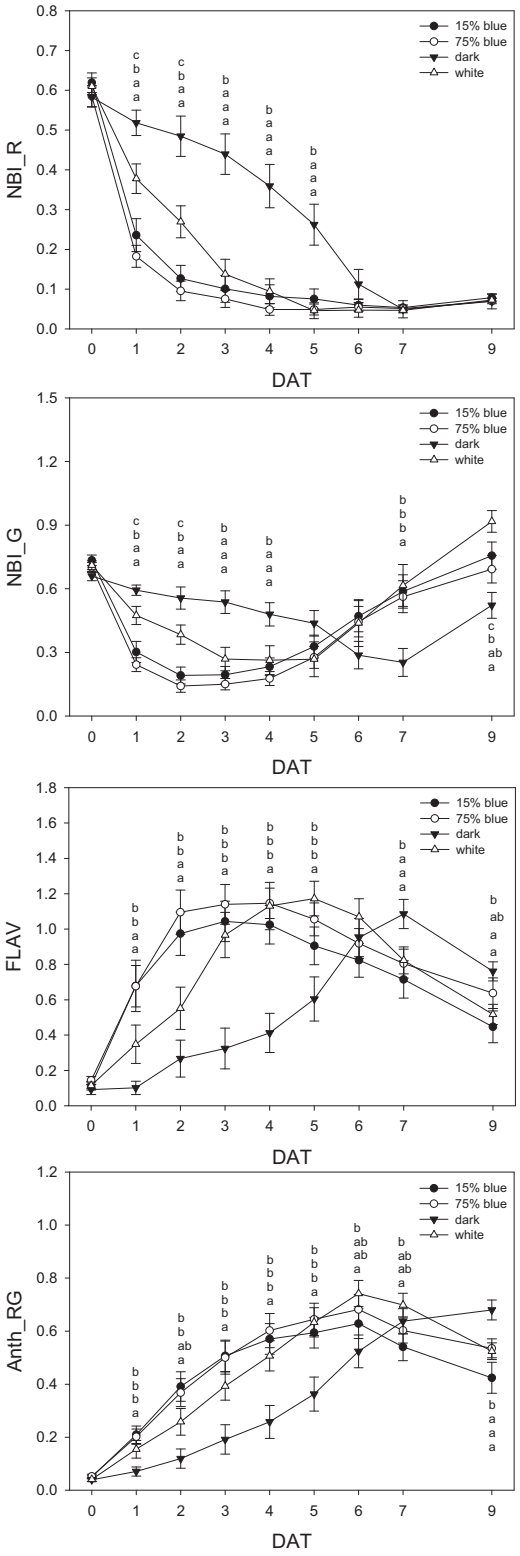

Fig. 4. Influence of light quality and darkness, on the postharvest development of the fluorescence indices NBI_R, NBI_G, FLAV and Anth_RG. Means ± SE ($n = 20$) followed by the same letters (within the evaluation day) do not differ significantly according to the Duncan test ($p \leq 0.05$).

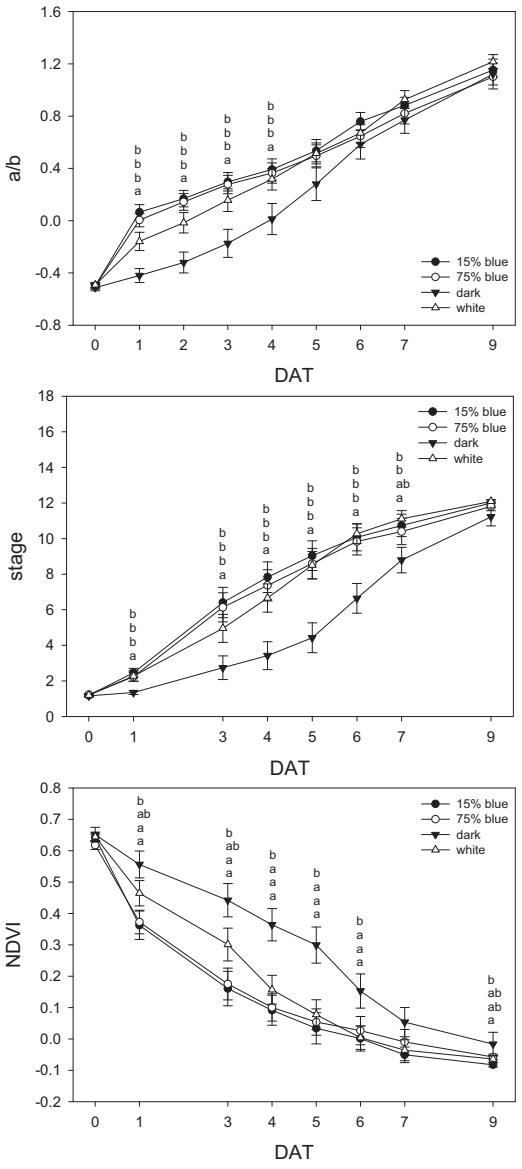

Fig. 5. Influence of light quality and darkness, on the postharvest development of maturity indices a/b, NAI and NDVI. Means ± SE ($n = 20$) followed by the same letters (within the evaluation day) do not differ significantly according to the Duncan test ($p \leq 0.05$).

Table 1
Pearson correlation coefficient (R^2) between the fluorescence-based indices (NBI_R, NBI_G, FLAV and Anth_RG) and the visually assessed stage of maturity, the remission indices *NAI* and *NDVI* and the reflection index a/b. $n = 740$ for the fluorescence-based indices, the a/b index and the stage of maturity. Statistical analysis considered all data collected over the whole experimental period. $n = 640$ for the remission-based indices (*stage*, *NDVI*).

Fluorescence index	Stage of maturity	a/b	Stage-index	NDVI
NBI_R	−0.782**	−0.798**	−0.754**	0.564**
NBI_G	0.013	0.055	0.082*	0.013
FLAV	0.465**	0.410**	0.393**	−0.346**
Anth_RG	0.844**	0.764**	0.831**	−0.637**

* Level of significance: $p \leq 0.05$.
** Level of significance: $p \leq 0.01$.

However, at the end of the experiment, only NDVI indicated significant differences between light-stored and dark-stored fruits (Fig. 5).

A Pearson correlation analysis was performed to elucidate mathematical relations between the independent parameters collected with the different sensors. Analyzed over the entire ripening process, the fluorescence indices based on red excitation (NBI_R, Anth_RG, FLAV) correlated best ($p \leq 0.01$) with the reflection and remittance indices (Table 1). The a^*/b^* index showed the highest

correlation with the fluorescence index NBI_R ($R^2 = -0.798$) followed by the Anth_RG index ($R^2 = 0.764$). Both Stage-Index and NDVI remittance-based indices correlated best with the Anth_RG index ($R^2 = 0.831$ and $R^2 = -0.637$). The indices a^*/b^* and NDVI showed no correlation, and the stage-index showed only a weak correlation with the fluorescence index NBI_G.

4. Discussion

In our study, we aimed to examine the suitability of a multiparametric fluorescence technique for monitoring the maturation of tomato fruits both pre- and post-harvest. Established methods such as visual classification according to the USDA standard, remittance spectroscopy and L^*, a^*, b^* readings were used as references. Recently, several studies have shown that chlorophyll fluorescence indices, based on excitation with different wavelengths, provide valuable information on the quality attributes of fruits such as apples (Betemps et al., 2012) and grapes (Cerovic et al., 2009; Ben Ghozlen et al., 2010; Agati et al., 2013). Now, we prove for the first time the suitability of this technique for monitoring tomato ripening. Because absolute fluorescence intensities are more sensitive to the undesirable influence of environmental factors, we focused on fluorescence ratios, which are more robust and less affected by changing conditions (Morales et al., 1998; Cerovic et al., 1999; Leufen et al., 2013). In our experiments, the indices NBI_R, NBI_G, FLAV and Anth_RG provided proper information on the ripening progress in the greenhouse and in the postharvest phase.

Changes in both the composition and distribution of pigments in the fruit during the ripening process affected several indices in distinct ways. Using a combination of different excitation lights and detecting either red or far-red fluorescence, characteristic maturation curve patterns were observed and defined. During transition from the green to red maturation stage, the light absorption spectrum significantly changes (Qin and Lu, 2008). Chlorophyll fluorescence emission decreases due to chlorophyll breakdown. As chloroplasts turn into chromoplasts, chlorophyll breaks down and is accompanied by the synthesis of carotenoids, first in the centre of the fruit and then in the pericarp (Hobson and Grierson, 1993; Bramley, 2002). This decrease in chlorophyll content is best indicated by the NBI_R index, which also has strong correlations with the stage index and the reflection index a/b. In contrast to the NBI_R index, the ratios FLAV and Anth_RG first increased and later dropped when fruits reached the pink (FLAV) or light red (Anth_RG) stage. Although in green fruits chlorophyll can be excited with full UV excitation light, in pink and red fruits, a major part of the UV is absorbed by epidermal flavonoids (primarily quercitin and kaempferol) and carotenoids (primarily lycopene) and does not reach the underlying chlorophyll molecules. Similarly, green light is increasingly absorbed by carotenoids during ripening, decreasing the chlorophyll fluorescence emission. In contrast, red excitation light is not absorbed by flavonoids and carotenoids; in consequence, UV and green light-induced chlorophyll fluorescence decreases faster at the beginning of the ripening process than does red light-induced chlorophyll fluorescence. However, evaluation of the absolute fluorescence intensities (*data not shown*) proved that there is almost a stagnation of FRF_UV when fruits reach the pink ripening stage. In contrast, RF_G and FRF_R decrease further, explaining the observed curve patterns of NBI_G and FLAV (Figs. 2 and 4). The Anth_RG index undergoes a similar process during which stagnation of FRF_G occurs at the light red stage, whereas FRF_R, which is not affected by the absorption of carotenoids, continues to decrease. However, products of chlorophyll degradation might affect the red and far-red emissions. Of particular note is pheophorbide *a*, which absorbs UV and red light and fluoresces red and far-red (Matile et al., 1999; Eichwurzel et al., 2000; Ashby et al., 2003).

Differences in the penetration profiles of the three different excitation wavelengths might also play a significant role in the temporal shifts observed in the NBI_G, FLAV and Anth_RG ratios (Figs. 2 and 4). As affected by the absorption properties of the different pigments and their localization in the tissue, the penetration depth of light into leaves and fruits depends on its wavelength (Qin and Lu, 2008; Brodersen and Vogelmann, 2010). Alba et al. (2000) demonstrated that the transmission of red light through the epidermis and outer pericarp of tomato increased 4-fold during the phase from immature green to turning stage, while the transmission of far-red light only changed slightly. Apart from the chemical nature, the quantity and distribution of pigment content in fruit, variations in tissue structure throughout the ripening process may impact the scattering properties of the fruit (Seifert et al., 2015) and, consequently, its fluorescence emission.

Of all the multiparametric fluorescence parameters, the indices NBI_R, NBI_G, FLAV and Anth_RG were the most reliable for monitoring the ripening progress in tomatoes. Prior to the harvest, biotic and abiotic stresses may have a strong impact on the initiation and coordination of ripening (Dumas et al., 2003; Brandt et al., 2006). Non-invasive methods can enable the fast and objective monitoring of such processes. As shown in the study of Pulupol et al. (1996), water deficit can increase respiration and ethylene biosynthesis in tomato fruits, promoting the ripening process and the colour change from green to red. Analogous to this, Zegbe-Domínguez et al. (2003) demonstrated that partial rootzone drying enhances tomato fruit ripening. Based on these publications and our preliminary experiments, we implemented water deficit irrigation by reducing the amount of nutrient solution to 50% of the control treatment for a total of three weeks, until the fruits of the second truss reached the red stage.

The multiparametric fluorescence system proved to be well suited to detecting stress-induced differences in the initiation of the ripening process. Thus, the fluorescence indices confirmed the visually assessed data, showing a faster change from green to red in fruits affected by the reduced water supply.

The results were validated in a second experiment in which the ripening of detached fruits was monitored. As early studies indicated that exclusion from light can lead to a significant delay in the ripening process (Jen, 1974; Thomas and Jen, 1975a,b) and that fruit-localized phytochromes mediate light-induced carotenoid biosynthesis under post-harvest conditions (Khudairi and Arboleda, 1971; Thomas and Jen, 1975b), different light treatments were initiated. The curve patterns of the four fluorescence indices were consistent with those determined under pre-harvest conditions in the greenhouse. With the fluorescence indices, the separation of the treatment groups achieved with the established reflectance (a^*/b^*) and remittance (NDVI and stage) parameters was confirmed, but at the final stage, an even more precise differentiation could be ensured. In particular, those indices utilizing red light for fluorescence excitation (NBI_R, Anth_RG, FLAV) correlated best ($p \leq 0.01$) with the reflection and remittance indices. The slightly faster transition from the green to red stage in fruits exposed to LED light compared to fruits exposed to CFL was more pronounced in the sensor-based indices than in the visually assessed data, illustrating once more the improved potential of sensor-based maturity characterization. Furthermore, the FLAV and Anth_RG fluorescence indices were better suited to detecting differences in the later phase of the ripening process.

So far, no study had addressed the impact of the blue-to-red light ratio on the ripening progress of tomatoes. Here, we could show that the transition from the green to red stage was not affected by the blue-to-red ratio, confirmed both by the visual and sensor-based data. However, the impact of the different light treatments

on the formation of carotenoids could not be estimated with the indices presented here. In this context, we assume that indices including fluorescence determination in the green region (500 nm and 600 nm) might provide additional information on the accumulation and contents of carotenoids.

In summary, the fluorescence indices presented here, recorded with a robust multiparametric fluorescence sensor, showed strong correlations with other already established non-destructive indices. In addition, the fluorescence indices enabled more precise differentiation of the experimental groups at the end of the experiment, indicating that these indices possess strong potential for improved monitoring of ripening and quality control in pre- and postharvest stages. In the scope of basic and applied research, fluorescence-based monitoring supports physiological and biochemical evaluations of the impact of pre-harvest growth conditions and postharvest storage conditions. Moreover, relevant fruit quality attributes might be monitored with this technique, as is already done for apples (Betemps et al., 2012). As an advantageous feature, the same sensor used to monitoring fruit ripening might also be used for physiological evaluations of the plant (Kautz et al., 2014a,b). Practical applications of the fluorescence technique might include defining the ideal harvest time precisely or use as a robust tool for fruit classification in high-speed sorting lines.

5. Conclusions

Using the multiparametric fluorescence technique, we monitored the maturation of tomatoes in pre- and postharvest phases with the indices NBI_R, FLAV, Anth_RG and NBI_G. Although NBI_R best indicated the degradation of chlorophyll, the indices NBI_G, FLAV and Anth_RG were well suited to following shifts in time to reach of the pink to light red ripening stage. Of the fluorescence indices, FLAV was the most promising parameter, enabling more precise differentiation of the treatment groups. The results obtained with the fluorescence technique showed comparable and, in many cases, superior quality compared to established reflectance and remittance methods; thus, this robust technique can be recommended for use in pre- and postharvest phases. Nevertheless, the precise relationships the fluorescence signals have with the biochemical compounds and quality attributes of ripe tomatoes still need to be explored.

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