Tomato fruit quality in relation to growing season, harvest period, ripening stage and postharvest storage

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ABSTRACT

The effect of two growing seasons (spring and fall), two harvest periods (early and late), four fruit ripening stages at harvest (S1, S2, S3 and S4, according to OECD gauge) and postharvest storage (0 or 16 days at 12 °C) on quality characteristics of tomato fruits was determined in order to investigate its one’s relative contribution. According to the results, all factors significantly affected most of the quality components, but not at the same magnitude. Ripening stage at harvest had the most significant effect in firmness, pH, and in the ratio soluble solids to TA, the growing season only in dry matter content, the storage on pigments’ content (chlorophyll, total carotenoids, lycopene and β-carotene) while the harvesting period was not the main factor in any of the quality traits determined. In conclusion, either the ripening stage at harvest or the time elapsed until consumption had the most significant effect on tomato fruit quality, but both could not be assessed at the time of consumption.

Keywords: Antioxidant; Carotenoids; Color; Firmness; Nutritional composition

INTRODUCTION

Tomato fruit ripening is a complex, genetically programmed developmental process that involves numerous metabolic changes leading in dramatic variations in physiological, biochemical and molecular level (Pék et al., 2010; Rugkong et al., 2010).

The ripening process of tomato is accompanied with the change of skin color (Brandt et al., 2006), induced by the disruption of chloroplasts’ structure and their subsequent transformation into chromoplasts. As a result, chlorophyll content is decreased, and carotenoid biosynthesis is simultaneously generated (mainly lycopene and β-carotene); thus, the color is converted from green to red (Wold et al., 2004; Klee and Giovannoni, 2011). Moreover, during ripening firmness is reduced (Brashlyanova et al., 2014; Hertog et al., 2004), due to the activity of polygalacturonase and glucosidase activities (Sabir and Agar, 2011) although it has also been shown to be affected by growing season and post-harvest storage.

Both skin color and firmness are the two most significant quality attributes that trigger consumer’s preference during purchase (Brandt et al., 2006; Tijskens and Evelo, 1994), although internal nutritional quality also plays a vital role in a repetitive selection (Magkos et al., 2003).

The nutritional quality of tomato fruits is affected by several preharvest and postharvest factors. Among the most important preharvest ones, the environmental conditions (air temperature, relative humidity and solar radiation), in which the tomato plants grow (Hertog et al., 2004; Giuntini et al., 2005; Kuti and Konuru, 2005; Brandt et al., 2006; Kacjan et al., 2011), as well as fruit ripening stage at harvest (Giovanelli et al., 1999; Wold et al., 2004; Pék et al., 2010) have been suggested to impose the greatest impact on the quality of fruits.

Among fruits harvested from plants grown in different seasons within the same year, a variation was observed in the dry matter and total sugars content as well as in the antioxidant capacity and total soluble phenols of fruits between the two productions (Anza et al., 2006; Toor and Savage, 2006), which was attributed to differences in temperature and light intensity (Dumas et al., 2003; Raffo et al., 2006). Fruits that were harvested at an immature stage and ripened off-vine, postharvest during storage,
eventually ended up with lower dry matter (Triglia et al., 2006), soluble solids content (SSC) (Kaur et al., 2006; Getinet et al., 2008), SSC/titratable acidity ratio (Bertin et al., 2000), total antioxidant capacity (Wold et al., 2004), as well as lycopene and beta-carotene (Giovanelli et al., 1999; Dumas et al., 2003; Kozukue and Friedman, 2003; Radzevičius et al., 2009), comparing to physiologically ripened fruits on vine.

Although differences in light and temperature that are proven to have a significant impact on the nutritional composition of tomato fruits do exist between different seasons in the same year, such as between Spring and Fall, there may also exist differences among the months in-between the same season, as a result of an interaction among different environmental conditions, vegetative developmental stage of the plant, as well as fruit load at the time of harvest. Therefore, harvest period may also be partially responsible for the fruit quality.

Although, all the above-mentioned factors (crop's growing season, the fruits' harvest period, the ripening stage, as well as the postharvest storage) have been extensively demonstrated to exhibit substantial impact on fruit quality whenever applied individually, there has never been published any report considering the simultaneous presence of all of them, which is indeed the common practice. Therefore, the aim of this study was to investigate the relative effect of these factors on changes of color and firmness, as well as of nutritional composition of tomato fruits occurring at harvest and postharvest.

**MATERIALS AND METHODS**

**Plant material and treatments**

Tomato plants cv. Nemesis F1 were grown following usual cultivation practices in a glass heated greenhouse, in two consecutive growing seasons, in Spring (from mid-February to late June) and in Fall (from mid-July to end of December). Air temperature inside greenhouse was monitored and min and max values are presented (Fig 1). Fruits were harvested in two harvest periods, early (98 days after transplanting-DAP) and late (134 DAP) in both growing seasons, respectively. In each harvest, fruits at four different maturity stages were collected, according to OECD color gauge, and particularly at the 4 (S1), 6 (S2), 8 (S3) or 10 (S4) stage (Fig 2). All fruits were handpicked early in the morning, transferred within 1 h to the laboratory in open plastic bags, and were wiped with wet paper to remove foreign particles from the surface. Half of the fruits in each ripening stage were stored at 12 °C for 16 days. The quality (color, the firmness and the nutritional composition) of the fruits from S1, S2 and S3 ripening stage was determined both at the day of harvest and after 16 days of storage, while quality of S4 fruits, corresponding to the ideal maturity stage for human consumption, was determined only on the day of harvest.

**Firmness**

Firmness was determined at two diametrically spots at the equatorial diameter of the tomato fruit using a Chatillon penetrometer (John Chatillon and Sons, New Gardens, NY) with a 9.5 mm length and 3.2 mm diameter probe attached.

**Color**

At harvest and at the end of storage the color was determined at two diametrically opposite spots at the equatorial diameter of the tomato fruit using a chromameter (Minolta CR-400, Minolta, Osaka, Japan), equipped with an 8-mm measuring head and a C illuminant (6774 K). The meter was calibrated using the manufacturer’s standard white plate. Color changes were quantified in the L*, a*, and b* color space. Hue angle \([(h°=180+\tan^{-1}(b*/a*))\] and chroma values \([C* = (a*^2 + b*^2)^{1/2}]\) were calculated from

![Fig 1. Daily maximum and minimum air temperature in the greenhouse, where the tomato plants cv. Nemesis F1 were grown the two growing seasons. The transplant dates in both growing seasons are depicted with the open arrows, while the harvests in each season are shown with the closed arrows.](image-url)
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a* and b* values. L* refers to the lightness, ranging from 0 = black to 100 = white; h° value is defined as a color wheel, with red-purple at an angle of 0°, yellow at 90°, bluish-green at 180°, and blue at 270°, and C* represents color saturation, which varies from dull (low value) to vivid (high value) (Lancaster et al., 1997).

**Nutritional composition**

Soluble solids content (SSC) was measured in the juice of the blended material using a portable Atago PR-1 refractometer (Atago Co. Ltd., Tokyo, Japan).

Dry matter content was determined after drying approximately 50 g of the blended material at 70 °C for 72 h and expressed as g kg⁻¹ FW.

DPPH radical scavenging activity was determined using a modified method of Brand-Williams et al. (1995). Sample homogenate, 5 g, was extracted with 25 mL methanol in ice, centrifuged at 5000 × g for 10 min and filtered through Whatman No. 1 paper. The supernatant was adjusted with methanol to 25 mL. The tomato extract, 200 μL, was added to 2800 μL of 0.1 mM methanolic DPPH, vortexed and kept in the dark at room temperature. The decrease in absorbance of the resulting solution was monitored at 517 nm for 30 min. The absorbance at 517 nm was read after 30 min. Ascorbic acid was used as the standard and the DPPH radical-scavenging activity was expressed as mg of Ascorbic acid equivalents antioxidant capacity (AEAC) per kg fresh weight (mg AEAC kg⁻¹ FW).

The pH and the titratable acidity were determined in the filtered extract by adding 100 mL distilled water into 10 g of blended material. The extract was titrated to pH 8.2 with 0.1 N NaOH, and the titratable acidity was expressed as percentage of malic acid.

Total carotenoids, b-carotene, and lycopene were determined according to the method of Lichtenthaler and Wellburn (1983) and D’Souza et al. (1992). One gram of the blended material was mixed with 10 ml of 100% acetone in plastic tubes, was tapped and placed in -20 °C for two days. The sample was thawed, vortexed, and centrifuged at 10000 × g for 10 min at 20 °C, and the supernatant was filtered through Whatman No. 1 paper in 25 ml volumetric flasks. Ten milliliters of 100% acetone were added in each tube, which were then vortexed at 150 × rpm for 10 min and re-extracted following the same procedure. Each extract was adjusted with 100% acetone to 25 mL. The absorbance of extracts was read at 450, 470, 503, 645 and 662 nm and 100% acetone served as blank.

For the individual determination of pigments, the following equations were used:

Total carotenoids (mg kg⁻¹ FW) = [(1000 × Abs₄₇₀) × (V / W) - (2.27 × 11.75 × Abs₆₆₅) + 2.35 × Abs₆₄₅ / W × V) - (81.4 × 18.61 × Abs₄₄₅ - 3.96 × Abs₆₆₂ / W × V)] / 227, where W = tissue weight (g) and V = extract volume (mL).

Fig 2. Representative tomato fruits cv. Nemesis F1 at four distinct ripening stages from the mature green (S1) and up to the dark red ripe (S4), as visually assessed during harvest.
Lycopene (mg kg\(^{-1}\) FW) = \((3.521 \times \text{Abs}_{503} - 0.587 \times \text{Abs}_{450})\) \times \text{V/W}, where \(W\) = tissue weight (g) and \(V\) = extract volume (mL).

\(\beta\)-carotene (mg kg\(^{-1}\) FW) = \((4.367 \times \text{Abs}_{450} - 2.947 \times \text{Abs}_{503})\) \times \text{V/W}, where \(W\) = tissue weight (g) and \(V\) = extract volume (mL).

Statistical analysis
Data were analyzed by analysis of variance in SPSS v.24 using a completely randomized design with 6 fruits per ripening stage in each harvesting period, growing season and storage period and the effect size of each factor was evaluated using \(h^2\) (eta squared) calculated as follows: \(h^2 = \text{SS factor}/\text{SS total}\), where SS = sum of squares. Means were separated by Duncan’s multiple range test at the 0.05 level.

RESULTS
Firmness and Color
Firmness and color (a/b parameter) were significantly affected by all factors, but most of the total variance was accounted for by differences between the ripening stage of fruits at harvest (\(h^2 = 41\) and 25), as well as by storage (\(h^2 = 20\) and 50\%, respectively) (Table 1). In particular, the more immature (S1) the fruit was harvested, the more firm (1.88 kg) and less red it was (Fig. 3a, 3b). After 16 days of storage at 12 °C, although fruits harvested at the S1 and S2 stages softened significantly (1.21 and 1.11 kg, respectively), they never became as soft as the S3 tomatoes were either at harvest (0.94 kg) or at the end of storage (0.85 kg) (Fig. 3a). Interestingly, at the end of storage, fruits that were initially harvested at the S1 stage became even more red than the S3 harvested tomatoes. Eventually, the S3 also developed a notable change in color, as well (Fig. 3b) ending up being even more red than the S1 harvested tomatoes. Although at the end of the storage the S3 fruits were equally soft as the S4 ones, they never became as dark red as the later ones.

Nutritional composition
Similarly to the color changes, pigments’ content was also affected by all factors but most of the total variance was accounted for by differences between storage (\(h^2 = 49 - 62\)) and by the ripening stage of fruits at harvest (\(h^2 = 16 - 20\)) (Table 1). The more immature the fruit at harvest, the lower the content of total and individual carotenoids it was (Fig. 4a, 4b, 4c). Storage of fruits at 12 °C for 16 days promoted the synthesis of carotenoids in fruits of all three maturity stages at harvest, resulting in a high pigments’ content even in S1 and S2 fruits at levels even beyond the one that S3 tomatoes had at harvest (Fig. 4a, 4b, 4c). Although \(\beta\)-carotene content in the stored fruits was the same irrespectively of the ripening stage at harvest (Fig. 4c), total carotenoids and lycopene was always significantly higher in S3 fruits (Fig. 4a, 4b), especially comparing to S1 fruits.

The dry matter, as well as soluble phenols content and antioxidant capacity of tomato fruits were significantly affected by all factors, but the season of growing the plants and the harvest period or their in-between interaction had the greatest impact on the above qualitative traits (Table 1). Fruits harvested during the spring season had a higher dry matter content than during Fall (data not shown), but in
both seasons, fruits collected at the late harvesting period always had a greater dry matter content comparing to the early period (Fig. 4). In the case of the total phenols content and antioxidant capacity, though, the interaction between plant growing season and harvesting period was mainly responsible for the differences observed, as long as both were at higher levels in fruits harvested in the late period of the spring season or in the early harvesting period of the Fall season (Fig. 5).

The soluble solids to titratable acidity ratio (SSC / TA) of tomato fruits was mainly affected by the ripening stage at harvest (h$^2$ = 46) and by the harvesting period (Table 1). S1 tomatoes had a lower SSC / TA ratio than S2 fruits, which in turn had lower ratio than S3 ones (Fig. 7), in both harvest periods. However, fruits of the same ripening stage at harvest had lower SSC / TA ratio in the late period, in comparison to the corresponding ones in the early period (Fig. 7). In addition, the more immature the fruits were harvested, the lower the SSC / TA ratio was and indeed; this ratio increased during storage even in S1 or S2 harvested fruits, but never reached the levels of the S3 ones (Fig. 8).

At last, pH was mainly affected by the ripening stage of fruits at harvest (h$^2$ = 65) (Table 1), indeed; the more ripe the fruit, the lower the pH level was (4.58, 4.63 and 4.75 for S1, S2 and S3 fruits, respectively, data not shown).

**DISCUSSION**

The ripening stage at harvest affected all the quality components of the fruits. Indeed, a/b color parameter, as well as pH, total carotenoids and lycopene content were higher while firmness and chlorophyll content were lower in S3 fruits, both before and at the end of storage, comparing to the S2 and S1 fruits (Figs. 3, 4, 5, 6, 7). The effect of the ripening stage at harvest (Dumas et al., 2003; Wold et al., 2004) on the nutritional composition
of tomato fruits is well documented. Although fruits harvested at the S1 stage has been reported to have a lower soluble solids and ascorbic acid content (Kaur et al., 2006; Opara et al., 2012), as well as dry matter, carotenoids and lycopene (Leonardi et al., 2000; Raffo et al., 2002) or other antioxidant compounds such as phenolics (Garcia-Valverde et al., 2013) than fruits harvested at the S3 stage, it is recommended that tomatoes should be collected from the plant while being at an immature stage of ripening (Kader, 2003), before reaching physiological maturity, in order to increase the product marketability, given that this practice renders the fruits more resistant to various postharvest conditions and ensures a longer storage duration.

The growing season of tomato plants (Spring or Fall) also affected significantly most of the characteristics determined, with the only exception of total carotenoids and lycopene content (Table 1). However, the most pronounced effect was exhibited only on the dry matter and the antioxidant capacity with the dry matter being increased in the fruits that were harvested during spring, and antioxidant capacity being greater in fruits produced in Fall (Figs. 4, 5). Significant differences in the growth and development of the tomato plants have been reported during a comparison among three growing seasons.

| Season | Dry matter (g kg\(^{-1}\) FW) | Harvest period | Soluble solids to titratable acidity ratio (SSC/TA) |
|--------|-------------------------------|----------------|-----------------------------------------------|
| Spring | Early                         | S1             | Higher                                        |
| Fall   | Late                          | S2, S3         | Lower                                         |

Fig 5. Dry matter content of tomato fruits harvested from plants grown in spring or fall, as an average of three ripening stages (S1, S2 or S3), two harvest periods (early or late) and two storage periods (0 or 16 days at 12 oC). Each column is the mean of 36 replications.

| Season | Total soluble phenols' content (mg GAE kg\(^{-1}\) FW) | Harvest period | Soluble solids to titratable acidity ratio (SSC/TA) |
|--------|------------------------------------------------------|----------------|-----------------------------------------------|
| Spring | Early                                                | S1             | Higher                                        |
| Fall   | Late                                                 | S2, S3         | Lower                                         |

Fig 6. Total soluble phenols’ content (A) and antioxidant capacity (B) of tomato fruits harvested from plants grown in spring or fall, as an average of three ripening stages (S1, S2 or S3), two harvest periods (early or late) and two storage periods (0 or 16 days at 12 oC). Each column is the mean of 36 replications.

| Season | Storage (days) | Soluble solids to titratable acidity ratio (SSC/TA) |
|--------|---------------|-----------------------------------------------|
| Spring | 0             | Higher                                        |
| Fall   | 16            | Lower                                         |

Fig 8. Soluble solids content to titratable acidity ratio of tomato fruits harvested at the S1, S2 and S3 stage and stored for 0 or 16 days at 12 oC, as an average of two growing seasons (spring or fall) and two harvest periods (early or late). Each column is the mean of 24 replications. Closed circles represent the mean value of 12 fruits harvested at the S4 stage in the early period of spring and fall cultivation.
(autumn, winter, summer) (Ashrafuzzaman et al., 2010) according to which, plant growth was greater and the number of inflorescences was increased in the winter cultivation; thus, inducing a subsequent higher yield due to the production of a greater number and mean weight of fruit per plant. At the same time, differences were also found in the nutritional composition of tomato fruits that were harvested from plants grown in Spring or Fall. In particular, lower firmness (Anza et al., 2006), as well as antioxidant capacity but higher dry matter content was observed in fruits harvested during spring, in comparison to the respective ones collected during Fall (Toor, Savage and Lister, 2006). These differences may probably be attributed to the fluctuation in daily temperature and solar radiation between the seasons.

The harvesting period (early or late) also affected most of the tomato fruit characteristics, with the only exception of β-carotene content, but never was the most crucial factor on any of the quality traits determined (Table 1). In contrary, differences in lycopene and soluble solids content were observed in fruits harvested in three consecutive periods from June to mid-July, but without these changes to be following a specific time trend (Brandt et al., 2006; Bertin et al., 2000). In our study, only the interaction between harvest period and the growing season had the main influence on the total soluble phenols content (Table 1). In particular, fruits harvested late in the spring and early in the Fall growing season had higher antioxidant capacity and total soluble phenols content (Fig. 6). The phenolic compounds content were also reported to be different between fruits that were harvested at the beginning and at the end of Fall, where the lowest average daily temperature during late Fall was considered to be responsible for the higher phenolics content in tomatoes (Riga et al., 2008). This latter interpretation is in full accordance with the findings of our study, as long as daily maximum air temperature started declining linearly after mid-September until the end of the Fall season and was substantially lower (by 5-10 °C) during the second season comparing to the spring one (Fig. 1). Similarly, differences were found in antioxidant composition (carotenoids, ascorbic acid and phenolics content) of red ripe cherry tomatoes, during six harvests in a season (April - March), but these were not associated either with temperature nor with solar radiation levels during cultivation (Raffo et al., 2006). The influence of the harvesting period in the qualitative characteristics of tomato fruits is directly related to climatic conditions during cultivation (Beckles, 2012), the total number of fruits per plant (Gautier et al., 2012), and possibly the developmental stage of the plants in which they form the fruits' morphological and qualitative characteristics (Dumas et al., 2003). The effect of harvesting period on the tomato fruit quality has been demonstrated in a similar study (Kowalczyk et al., 2011), according to which fruits that were harvested in July had higher dry matter and acidity values than those harvested in September, but without differences in pH and soluble solids content. In another study (Farneti et al., 2013) where fruits were harvested in five successive periods, once per month, from May to September, it was observed that the ratio of soluble solids to acidity was higher in tomatoes harvested in July and that fruits were firmer during harvesting in June.

Storage of fruits also affected all the determined characteristics, and indeed had the most significant effect in all pigments’ content (chlorophyll, total carotenoids, lycopene, β-carotene) (Table 1). Firmness and chlorophyll content decreased, while lycopene and β-carotene increased significantly in fruits of all ripening stages after 16 days of storage at 12 °C (Figs. 3, 4). The effect of the storage duration on the nutritional composition of tomato fruits has already been demonstrated (Raffo, 2017). Significant changes in organoleptic characteristics of tomato fruits during storage have been reported by De Katelaere et al., 2004, where a significant reduction in firmness was found in fruits from 13 different tomato genotypes harvested either in August or October, after a two weeks storage at 18 °C. Firmness of tomato fruits has been significantly decreased during a 2-week storage at 12, 17 or 22 °C, with the highest temperature inducing a greater loss of firmness (Hertog et al., 2004). Lycopene content increased in fruits harvested at all three different ripening stages, but significant differences were maintained between S1 and S3 fruits (Fig. 4b), indicative of the greater independence of lycopene synthesis to the on-vine or off-vine ripening process (fruit attachment or not on the tomato plant). An increase of lycopene content was also observed in tomato fruits during storage at 15 or 22 °C for 10 and 14 days, respectively (Toor and Savage, 2006; Javanmardi and Kubota, 2006).

Similarly to lycopene changes, at the end of the storage, the color parameter a / b was different only between S1 and S3 fruits, unveiling the high correlation between the red color development and lycopene synthesis, comparing to the respective β-carotene one (Figs. 3b, 4b, 4c).

The firmness of the S3 fruits was not affected by storage, suggesting that the transition from the commercial ripe stage (S3) to the desired consumption stage (S4), in terms of color, is not accompanied by a simultaneous softening of flesh (Fig. 3). Before storage, fruits that were harvested in the S2 stage had intermediate values of firmness compared to the S3 and the S1 fruits, with the latter being the firmest (Fig. 3a). However, after 16 days of storage at 12 °C, fruits that were harvested either in the S1 or in the S2 stage softened significantly but never became as soft as the S3 harvested fruits. The fact that although S3 harvested
fruits at the end of the storage became equally red as the S4 harvested ones, they never became as soft, implying that postharvest ripening process, in terms of softening, has a maximum limit which can only be reached when the fruit is attached to the plant.

In summary, at the end of storage, fruits that were harvested while being at the S2 or S1 stage, obtained the same color, firmness levels and most of the nutritional composition as the S3 fruits, but only the fruits harvested at the S2 stage also reached the same SSC / TA ratio as the S3 fruits, which implies that they were also of the same taste. As a result, although it seems impossible for the consumers to predict correctly the initial ripening stage of tomatoes during their expoal at the retail market, based on firmness and the color perception, this can only be accomplished only at the time of consumption and tasting of the fruits.

CONCLUSIONS

Ripening stage at harvest affected most of the fruit characteristics that were determined in this study (15 out of 16) and indeed had the most significant effect in firmness, pH, and in the ratio soluble solids to TA. The growing season of tomato plants (spring or Fall) significantly affected most of the components, with the exception of total carotenoids and lycopene, but had the most significant effect only in dry matter content. The harvesting period (early or late) also affected most of the determined characteristics but was not the main factor in any of the quality traits determined. The storage also affected all the determined quality characteristics, and indeed had the most crucial effect on pigments’ content (chlorophyll, total carotenoids, lycopene and β-carotene). Moreover, it is worth mentioning that consumers are not capable of assessing the actual ripening stage of tomatoes at the time of harvest based on fruit firmness and color, but only after consuming the product.

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Authors’ contributions

Dimitrios Kasampalis implemented the treatments, recorded the measurements in the greenhouse, performed the analytical procedures in the lab and prepared the manuscript.

Pavlos Tsouvaltzis designed the experiment, processed the data and prepared the manuscript.

Anastasios Siomos supervised the project and edited the submitted manuscript.

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