Content of lycopene and visible reflectance in case of conventional and elevated lycopene tomato cultivars.

Zoltán Pék[1], Hussein Daood[2], Andrea Lugasi[3], Lajos Helyes*[1]

[1]Institute of Horticulture and Technology,
Szent István University, Gödöllő
[2]Central Food Research Institute, Budapest
[3]National Institute for Food and Nutrition Science Budapest
* correspondence author:
Helyes.Lajos@mkk.szie.hu

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Abstract. This study examined the feasibility of using non destructive, spectrophotometrical method to predict one of the most valuable internal quality indices, lycopene in individual tomato fruits. An open field experiment was conducted to study the effect of irrigation and potassium supplement on the yield and lycopene content of processing tomato fruit. Three different treatments (regularly irrigated *RI*, irrigation cut-off 30 days before harvest *CO*, and rainfed *RF* unirrigated control) and two different K fertilisations (P) were applied. Regular irrigation significantly decreased the lycopene content of tomato fruits. The *CO* treatment resulted in the highest total lycopene without additional potassium supplement. Potassium supplement given at the time before fruit maturity significantly increased the lycopene concentration of cultivar Brigade, independently of irrigation. The closest correlation was at 700nm R^2=0.39 and R^2=0.50, between reflectance and the (all-E)-lycopene and the (9Z)+(13Z)-lycopene isomers respectively.

INTRODUCTION

Tomato fruit is an important dietary source of antioxidants, like ascorbic acid, β-carotene, lycopene and polyphenols (Helyes et al., 2007). Ecological conditions have great effect on carotenoid and antioxidants of tomato (Abushita et al., 2000). Colour is one of the most important quality components of tomato fruits. Amount of predominant carotenoid lycopene, which causes the red coloration of fruits, is well characterized by surface reflectance. Lycopene is accumulated mainly in deep red stage and colour is an indicator of lycopene level (Brandt et al., 2006; Helyes et al., 2006c).

Synthesis of lycopene is easily detectable by nondestructive method of colour measuring of fruit surface (Helyes et al., 2006b), also affected by varietal and ecological factors (Dumas et al., 2003; Tomlekova et al., 2007). Chlorophyll breaks down and carotenoids, mostly lycopene, accumulate during ripening (Biacs and Daood, 2006).

Lycopene occurs in various geometrical isomers. In most raw fruits, the (all-E)-isomer is quantitatively the most important, and within the relatively small proportion of (Z)-isomers, (5Z)-, (9Z)- and (13Z)-lycopene are usually predominant (Schierle et al., 1997). The present work eludicated the isomeric ratio of fresh tomato fruits, additionally the (all-E)/(9Z),(13Z)-isomer ratio influenced by technological traits.

MATERIALS AND METHODS

Samples

Open field experiment was carried out in the test sites of Szent István University, Gödöllő in 2008 and 2009. A determined conventional tomato variety Brigade F_1 and high lycopene cultivars, Triple Red F_1, and UG Red F_1 were investigated in the present study. The experimental field, which was 300 m^2, is brown forest soil, with mechanical composition are sand, sandy-clay and the subsoil water is below 5 m, therefore it cannot influence the water turnover.

Area of the experiment was 300 m^2 and the area of one plot was 25 m^2. Seeds were sown on the 7th of April 2008 and 2nd of April 2009 in greenhouse and transplanted on the 12th of May 2008 and on the 14th of May 2009 respectively. Tomato seedlings were planted out in twin rows, 0.4 m spacing inside the raw and 1.2 m between adjacent twin rows, the space between the plants in the row was 0.4 m. There were regularly irrigated (RI), irrigation cutoff (CO) plant stands and the rainfed (RF) control and two different K fertilisations (P). Drip irrigated water was given out according to potential evapotranspiration. National Meteorological Institute forecasts were used to calculate with the probable evapotranspiration.

Basic nutrition supply was given out when plants were transplanted with Agroblen 18-8-16 (nitrogene-phosphorus-potassium). Additionally more potassium fertiliser was applied with KNO_3 at fruit set, resulting a different potassium supply of 555 (+ K) kg ha^{-1}. Five harvested fruits in three replicates were chosen for preliminary spectrophotometric analysis. The spectrophotometric measurements were carried out using a Minolta spectrophotometer. Minolta chroma meter CM-512m3 is a multi-angle spectrophotometer, which uses geometry with D65/10° in both years.

Lycopene analysis in 2009

Lycopene contents were established from five fruits in three replicates of which lycopene content of fruits were analysed in the National Institute for Food and Nutrition Science in 2008. Lycopene was extracted from the tomato juice with a mixture of n-hexane, methanol and acetone (2:1:1) containing BHT. Optical density of the hexane extract was measured at 502 nm by a Perkin Elmer Lambda 3B UV Spectrophotometer (Perkin Elmer Co., Norwalk, USA) (Sadler et al., 1990). Lycopene concentrations were calculated by applying the molecular extinction coefficient of 158500 (Merck and Co., 1989). All parameters measured are referred to fresh weight of fruits. Lycopene contents were established from five fruits in three replicates of which lycopene content of fruits were analysed in the Central Food Research Institute in 2009. Five grams of fresh fruits were weighed and crushed in a crucible mortar in presence of quartz sand with gradual addition of 20 ml of methanol and then was let stand for 5 minutes. The supernatant was decanted carefully into a 100ml Erlenmeyer flask. The residues were further crushed and 50 ml of a 20:60 methanol-1,2-dichloroethan were gradually added. The whole mixture was quantitatively transferred to the Erlenmeyer flask by rinsing twice with 10 ml of the mixture of methanol-1,2-dichloromethan. After gentle hand shaking few drops of double-distilled water were added to enhance separation of polar (aqueous) and less-polar (solvent) phases. The solvent layer was separated in a separating funnel and dried over anhydrous Na_2SO_4.and then evaporated under vacuum at not higher than 40°C. The residues were re-dissolved in 5 ml of HPLC grade acetone and filtered through a Teflon PETF 0,45µm syringe filter before injection onto HPLC column.

HPLC instrument and conditions

Instrument: A Waters HPLC chromatograph consisting of a model 2695 separation module and 2696 photodiode-array detector was used for the analysis of carotenoids. The operation and data processing was performed by Empower software.

Column: Nucleodur ISIS C18, 3 µm, 150x4.6 mm was used

Mobile phase: The following gradient elution was applied:

		Solvents	
Time	Flow	%water	%acetone
0,01	0,7	20	80
3	0,7	20	80
8	0,7	12	88
12	0,7	12	88
20	0,7	5	95
25	0,7	5	95
28	0,7	20	80
30	0,7	20	80

Detection: Photodiode-array between 190 and 700 nm. For quantification, chromatogram was integrated at maximum wavelength for each peak using manual integration.

Calibration: Stock solutions for lycopene were prepared by dissolving 1 mg of standard materials (Sigma, St. Lo. USA) in 10 ml of petroleum ether using brown-coloured volumetric flask. Working solutions between 0 and100µg/ml were then prepared by dilution with HPLC acetone and injected onto the HPLC system. The recorded peak areas of standard solutions was Plotted versus concentrations to get the calibration curve using Microsoft xls 2007 program.

Identification: The peaks were identified by comparing their spectral characteristics and retention times with those of standard materials and with literature data when available (Bauernfeind, 1981).

Statistical analysis

The results were expressed as the average plus/minus significant differences at P=0.05. The statistical analysis was carried out by the t-student test, and the statistical analysis was made using the Statistica 9 software.

RESULTS AND DISCUSSION

CIELab colour parameters are the most commonly used parameters to indicate the development of red coloration of ripening tomato fruit. We established correlation coefficients of linear regression among a*, a*/b*, chroma and hue and fruit surface reflectance between 400-700 nm in 2008. The closest correlation between chroma and reflectance was at 630 nm (r^2=0.967; data not shown), so we used this frequency parameter to evaluate fruit surface colour. Table 1 shows the average lycopene content and surface reflectance at 630 nm of three hybrids tomato fruits. Regular irrigation significantly decreased the lycopene content of tomato fruits. Lycopene content is fundamentally determined by the genetic factors, but our results show contradictions. High lycopene hybrids (Triple Red F_1) with irrigation and (Ug Red F_1) without irrigation did not exceed lycopene content of normal lycopene hybrid (Brigade F_1). Reflectance data showed the same pattern, so we calculated linear regression between lycopene content and reflectance.

Table 1. Average lycopene content and reflectance at 630 nm of tomato fruits in 2008. (n=3; ±SD).

	Lycopene content (mg $100g^{-1}$)		Reflectance at 630 nm (%)	
Variety	RF	RI	RF	RI
Brigade F_1	$17.5±0.5^{a}$	$15.5±2.4^{ab}$	$28.5±1.6^{ab}$	$27.3±1.8^{a}$
Triple Red F_1	$22.5±1.8^{b}$	$15.0±0.7^{a}$	$29.6±0.8^{a}$	$27.7±1.6^{ab}$
Ug Red F_1	$14.9±1.7^{a}$	$19.2±1.9^{b}$	$25.0±1.9^{b}$	$29.8±1.3^{b}$

Data in the same column bearing the same superscript letter are not significant at P=0.05

Correlations are presented in Figure 1. The more lycopene content of fruits there was the higher reflectance values were reached at 630 nm. High lycopene hybrids resulted in more lycopene in tomato fruits. Triple Red F_1 reached the highest lycopene content (24.3 mg $100g^{-1}$), but not the highest reflectance (UG Red, 31.1%).

In 2009 we used the same three cultivars in the experiments, as in the previous years, supplementing the measurements with the determination of (all-E)-lycopene and (9Z)+(13Z)-lycopene isomer concentrations. The results are shown in Table 2.

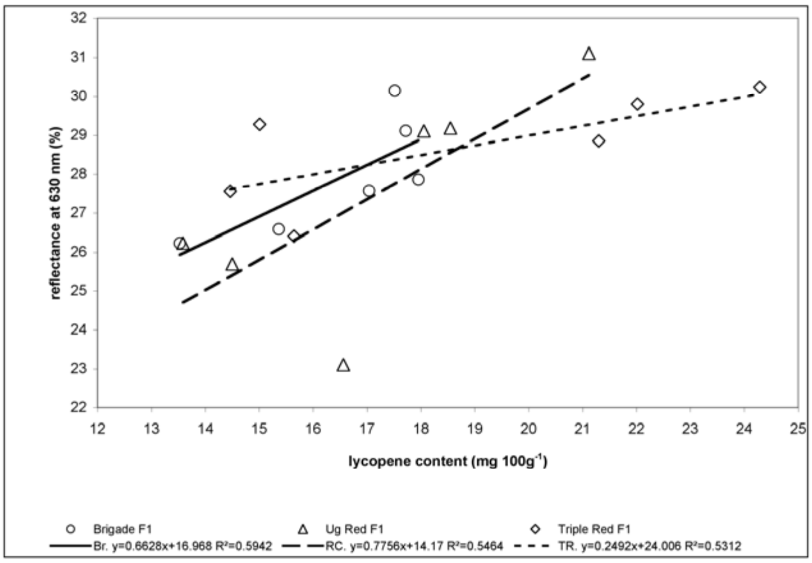

Figure 1.
Correlation between lycopene content and reflectance at 630 nm of tomato fruits, with function and correlation coefficient of linear regressions (n=6).

Table 2. Average carotenoid component of different types of tomatoes with different irrigation and potassium fertilization regime in 2009 (n=3).

	(all-E)-lycopene (mg 100g^{-1})	(9Z)+(13Z)-lycopene (mg 100g^{-1})	(all-E)+(9Z)+(13Z) lycopene (mg 100g^{-1})	(all-E)-lycopene (%)	(9Z)+(13Z) -lycopene (%)
Brigade RF	7.42±0.13^{d}	1.70±0.12^{d}	9.12±0.16^{d}	81.4%	18.6%
Brigade RF P	8.47±0.10^{e}	1.82±0.11^{de}	10.29±0.12^{e}	82.3%	17.7%
Brigade RI	4.04±0.10^{a}	0.71±0.11^{a}	4.75±0.01^{a}	85.0%	15.0%
Brigade RI P	5.74±0.25^{c}	1.15±0.11^{c}	6.89±0.21^{c}	83.3%	16.7%
Brigade CO	8.11±0.41^{e}	1.93±0.15^{e}	10.05±0.47^{e}	80.7%	19.3%
Triple Red RF	4.28±0.01^{b}	0.97±0.11^{b}	5.25±0.10^{b}	81.6%	18.4%
Triple Red RI	5.78±0.11^{c}	1.07±0.06^{bc}	6.84±0.08^{c}	84.4%	15.6%
UG Red RF	4.34±0.08^{b}	1.13±0.13^{bc}	5.47±0.21^{b}	79.5%	20.5%
UG Red RI	4.14±0.27^{ab}	0.94±0.08^{b}	5.09±0.35^{ab}	81.5%	18.5%

Data in the same column bearing the same superscript letter are not significant at P=0.05

In year 2009 the cultivars contained less lycopene at harvest than in 2008. This can be explained by the difference in weather conditions. In 2009 the temperature was higher on average and there was less precipitation at fruit maturity than in 2008 (data not shown). Among the cultivars Triple Red produced significantly higher total lycopene content than Brigade and UG Red.

The irrigation generally decreased the total lycopene content of fruits, except in cultivar Triple Red, where it caused significantly higher lycopene concentration. The CO treatment resulted in the highest total lycopene without additional potassium supplement. Potassium supplement given at the time before fruit maturity significantly increased the lycopene concentration of cultivar Brigade, independently of irrigation. The greatest effect of irrigation on lycopene could be measured in cultivar Brigade, where it reduced the concentration almost by 50%.

The major part of lycopene content is constituted by (all-E)-lycopene, changed between 79.5 and 85%, while the ratio of (9Z)+(13Z)-lycopene isomers ranged between 15.6 és 20.5%. Irrigation reduced (all-E)-lycopene, in cultivars Brigade and UG Red, while in the case of cultivar Triple Red the opposite effect could be measured. Irrigation only caused a significant reduction of (9Z)+(13Z)-lycopene isomers in the case of cultivar Brigade. Potassium increased all lycopene isomers, under unirrigated conditions it increased (all-E)-lycopene to the greatest extent, while under irrigated conditions (9Z)+(13Z)-lycopene.

In 2009 we also performed the correlation analysis between the spectral reflectance of fruits and their lycopene content. In the analysis we did not include the RF treatments of high lycopene hybrids. The results are shown on Fig 2. The measured reflectance values (400-700nm) showed a positive correlation at 630-700nm with the lycopene components, among which the closest was at 700nm R^2=0.39 and R^2=0.50, thus the (all-E)-lycopene and the (9Z)+(13Z)-lycopene isomers respectively.

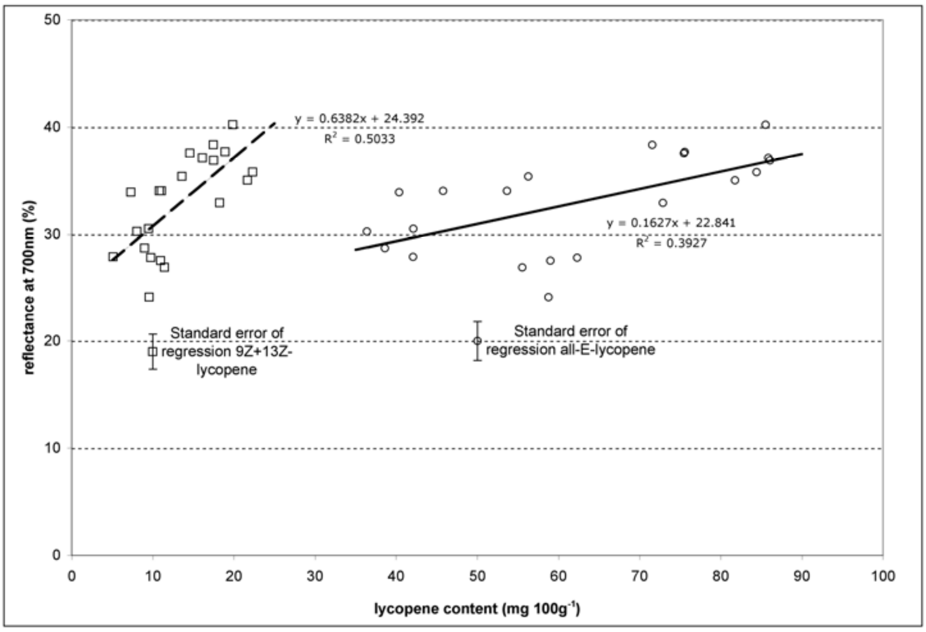

Figure 2.
Correlation between (all-E)-lycopene and (9Z)+(13Z)-lycopene
content and reflectance at 700 nm of tomato fruits,
with function and correlation coefficient of linear regressions in 2009 (n=21).

Conclusion

We managed to prove the quantitative and qualitative changes in lycopene content of raw tomato fruit samples as a result of irrigation and potassium supplement treatments. In the two consecutive years the lycopene content of fruits differed in all three examined cultivars, which was possibly a result of the difference in weather conditions. The lycopene content of high lycopene hybrids was higher under irrigated conditions, than that of the traditional cultivars. We have shown that irrigation cutoff which is an old technological trait used to speed up the rate of maturation, how effectively increases the lycopene content of raw fruits. In the case of (9Z)+(13Z)-lycopene isomers more beneficial to human health, a significant increase was caused by irrigation in high lycopene hybrids, compared to traditional cultivars.

Reflectance values measured in the visible range gave closer positive correlation with lycopene content above the 600nm range in both years. Based on the results of 2009 it can be concluded that the reflectance of lycopene isomers is possibly different. Besides this the correlations are not so close, so that the lycopene content of fruits could not be determined more accurately by the reflectance of fruit surface. Further spectrophotometrical experiments are required, to fine-tune a nondestructive method of lycopene isomer determination.

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