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Variation of Nutritional Value of Tomato in Post-Harvest Period

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Abstract

Screening tomato genotypes in terms of compounds that affect fruit quality in post-harvest period is an important task for breeders. It is important for selection of genotypes that are characterized by desirable traits, such as prolonged shelf life, the possibility of prolonged storing and fruit firmness and theirs including in breeding programs. Content of β-carotene, total acid and dry matter have been investigated in tomato fruits in post harvest period for ten tomato genotypes. The fruits have been picked 65 days after anthesis and kept for 60 days. During this time, the samples have been evaluated six times. On the basis of one-way and two-way ANOVA, group comparisons have been performed, by complex contrast comparisons. Regression analysis has been performed for each genotype and calculation of homogeneity of slope coefficients. According to the regression ANOVA concerning β-carotene content, the results are not significant for any genotype. There was significant total acid content decrease over time for two genotypes (Korona x L-10 F1 i Korona x L-12 F2). In rin genotypes, significant dry matter content decrease over time was found for parents and offspring generations (L -10 9.457, Korona x L-10 F1 31.946 and Korona x L-10 F2 20.074).

Key words: *lycopene, β-carotene, total acid, regression analysis, contrast comparisons*

1. Introduction

Tomato fruits contain carotenes, the substances with antioxidant activity that may reduce the risk of myocardial infarction [21] and prevent certain forms of cancer. Carotene pigments are essential for all photosynthetic organisms. They assist in harvesting light energy and protect photosynthetic apparatus against harmful reactive oxygen species that are produced by over excitation of chlorophyll. Carotene biosynthesis takes place in plastids, from central isoprenoid pathway [11]. Tomato breeding programs aiming to increase carotene content often include the introduction of "*tangerine*" mutant gene which increases β-carotene and lycopene production. "*Tangerine*" tomato fruits are characterized by high carotene and lycopene content and orange color [14].

Carotene pigment inheritance in tomato was for the first time studied by [28]. The classification based on color includes four types: red (*rrttbb*), yellow (*rrttbb*), β-orange (*RRTTBB*) and "*tangerine*" orange (*rrttbb*). Carotene biosynthesis may be affected by mutant genes [12]. Fruits of *rin* and *nor* tomato do not normal develop all other carotenoids. [6] and

at the same time differences in maturity influence the content of β-carotene, which is very low in non-mature fruits and grows with maturity [16].

Ripening inhibitor genes *rin (ripening inhibitor)*, *nor (non ripening)* and *alc (alcobaca)* alter carotene biosynthesis in the direction of carotene content decrease [17]. Tomato ripening is characterized by "climacteric pick" [26], in other words, seed reaches maturity before complete fruit coloration. Genotypes with ripening inhibitor genes (*rin, nor*) have prolonged shelf life. Heterozygous combinations between *rin* and *nora* mutants increase fruit firmness. The effect of the *rin*+/*rin, nor*+/*nora* genotypes on fruit firmness may be roughly expressed as the sum of the individual effects of each locus. The *rin* allele is more efficient than *nora* to keep fruit firmness [9].

Post harvesting period may be significantly prolonged. Some tomato varieties, depending on genotype, have different levels of maturity [2]. Carotene metabolism in post harvesting period is different for genotypes with and without "shelf life genes". B-carotene biosynthesis is faster than lycopene biosynthesis for fruits of genotypes with "shelf life genes", especially if picked in light green period [5]. An increase in β-carotene content has been found in genotypes picked in light red period of ripening by [4].

During marketing package period fruit quality does not remain the same, especially in terms of carotene, due to transition from initial pink to final red color [15], [19]. Quality changes and losses affect fresh tomato consumers. Therefore, obvious is the importance of breeding programs aimed to create commercial F1 hybrids with prolonged shelf life. Our aim was to screen tomato genotypes in terms of compounds (β-carotene, total acid and dry matter) that affect fruit quality in post-harvest period.

2. Material and methods

The plant material that has been tested belongs to the collection of the Institute for Vegetable Crops, Smederevska Palanka, Serbia. Four parental lines (Korona, R-83, L-10-*nor* and L-12-*rin*), F_1 and F_2 offspring from Korona x R-83, Korona x L-10 and Korona x L-12 crossings have been included in the study. F_1 and F_2 generations have been obtained in glasshouse conditions. Comparative field trial has been conducted during tomato growing season of 2010, applying standard agro technical procedures.

The following traits have been analyzed: shelf life (the period between fruit picking and rot), dry matter, β-carotene and total acid content dynamics. B-carotene content has been determined using spectrophotometer, acid content by acetic acid equivalent method and dry matter content by drying at 105ºC to constant weight.

The analyses have been performed at 10-day intervals, during 60 days. 10 fruits per parent and F_1 generation and 20 fruits per F_2 generation have been analyzed. The fruits have been picked from the first flower branch, 65 days after pollination.

Biometric data analysis

The transformed data have been processed by one-way and two-way ANOVA. LSD test was used for comparison of mean values [25]. The transformation included:

$$X_{ijk} = 2\arcsin\sqrt{Xijk}$$

$$X_{ijk} = 2\arcsin\sqrt{Xijk \pm 1/2n}$$

Sign + is used in case of $X_{ijk} \approx 0$, sign – in case of $X_{ijk} \approx 1$ and n is the number on the basis of which is calculated proportion (10 or 20). The transformed data have been processed by two-way ANOVA. B-carotene, total acid and dry matter content and dynamics analysis included 10 genotypes measured three times (usual ripening dynamics) and 6 genotypes measured six times (prolonged ripening). One-way ANOVA aimed to estimate the significance of factors and variance homogeneity was calculated:

$$\chi^2 = \frac{2.306 f (K \log Sp^2 - \sum_{i=1}^{k} \log Si^2)}{1 + [(K+1)/3Kf]}$$

$$\text{Sp} = \frac{\sum_{i=1}^{k} Si^2}{K},$$

with *f* representing the number of degrees of freedom for each variance and K representing the number of variances. If the calculated value exceeds the table value, the data are transformed according to log(x+1) formula, otherwise raw data are processed.

The data were processed by complex contrast comparison. Complex contrast with multiple degrees of freedom is a group of simple contrasts. It is usually defined as comparison among groups (s-number of groups):

$$M = g_1 \text{ with } g_2 \text{ with } g_3 \text{ with } \ldots g_s$$

with g_i representing i-th group composed of m_i treatment.

The hypothesis of equality of group means was tested:

$$\text{SS(M)} = \frac{1}{rgs} \sum_{i=1}^{r} \frac{Gi^2}{m_i} - \frac{\left(\sum_{i=1}^{s} Gi^2 \right)^2}{rgs \sum_{i=1}^{s} m_i}$$

with r representing the number of replications, g terms of measurements, $M=g_1$ with g_2 has (s-1) = 2-1=1 degrees of freedom.

$$\text{F} = \frac{\frac{SS(M)}{(s-1)}}{MSa}$$

F-table value for $v_1=1$ and $v_2=60$ degrees of freedom is 4.00 and 7.08 for 0.05 and 0.01 levels of probability, respectively.

Two groups (s=2) of treatments, namely genotypes without prolonged shelf life (4) and mutant genotypes (6) have been considered:

1. group g_1: Korona, R-83, Korona x R-83 F_1 and Korona x R-83 F_2, ($m_1=4$);
2. group g_2: L-10, Korona x L-10 F_1, Korona x L-10 F_2, L-12, Korona x L-12 F_1 and Korona x L-12 F_2, ($m_2=6$).

If the calculated F value exceeds the table value, the means of the observed groups are significantly different.

For the data concerning β-carotene, total acid and dry matter content and dynamics including 6 measurements, regression analysis has been performed (for each genotype). Homogeneity of regression coefficients (k-regression) has been tested:

Ho: $\beta_1 =: \beta_2 ..... =: \beta_k$

$$A_i = \sum y^2 - \frac{\left(\sum xy\right)^2}{\sum x^2} \quad \text{for i=1,2...k}$$

$$B = \sum Ai$$

$$C = \sum x_1^2 + \sum x_2^2 + \cdots + \sum x_k^2$$

$$D = \sum y_1^2 + \sum y_2^2 + \cdots + \sum y_k^2$$

$$E = \sum x_1 y_1 + \sum x_2 y_2 + \cdots + \sum x_k y_k \quad F = \frac{\left[D - (E^2 / C) - B\right]/(k-1)}{B \Big/ \left(\sum_{i=1}^{k} n_i - 2k\right)}$$

with $v_1=(k-1)$ and $v_2=(\Sigma n_i-2k)$.

3. Results and discussion

The content of metabolites changes during the growth the fruits. Screening tomato genotypes in terms of compounds that affect fruit quality is an important task for breeders. The best management strategy provides higher yield and fruit quality. β-carotene content is one of the most important parameters that affect tomato fruit quality [12], [13], [22]. Our results showed that carotene biosynthesis continues during postharvest period, which is in accordance to the results of [4] and [5]. The phenomenon is more pronounced in genotypes carrying *nor* and *rin* genes comparing to genotypes without prolonged shelf life. *Nor* genotypes had longer shelf life comparing to genotypes carrying *rin* genes (approximately three and four months after picking, respectively). Concerning the first measurement, significant differences for β-carotene content have been observed between mutants (0.546 and 0.276 mg% for L-10*nor* and L-12*rin*, respectively) and genotypes without prolonged shelf life (1.213 and 1.305 mg% for Korona and R-83, respectively). For parental genotypes without prolonged shelf life, the highest β-carotene content has been observed at the second measurement (1.330 and 1.259 mg% for Korona and R-83, respectively), and for mutants at the fourth measurement, 40 days after picking (1.190 and 1.017 mg% for L-10*nor* and L-12*rin*, respectively). The fourth measurement was characterized by rot of all genotypes without prolonged shelf life. Similar results have been found for F_1 and F_2 hybrids of genotypes with and without prolonged shelf life. Concerning the first measurement, F_1 hybrid of genotypes without prolonged shelf life Korona x R-83 had higher β-carotene content (1.311 mg%) when compared to mutant F_1 hybrids Korona x L-10 (0.455 mg%) and Korona x L-12 (0.398 mg%). Concerning F_1 and F_2 hybrids, the highest β-carotene content has been observed at the second measurement for genotypes without prolonged shelf life (3.870 and 4.619 mg% for F_1 and F_2 hybrids, respectively) and at the fourth measurement for genotypes with prolonged shelf life (1.912 and 3.022 mg% for *nor* F_1 and F_2, respectively; 4.514 and

4.200 mg% for *rin* F_1 and F_2, respectively). After the mentioned measurements, the decrease in β-carotene content was noted for both genotypes with and without prolonged shelf life (Table 1). Our results are in accordance to the results of Agar [1].

Table 1: Average values and post-harvest dynamics of β-carotene, total acid and dry matter content in tomato fruits

	Traits	β-carotene content (mg%)						Total acid content (%)						Dry mater content (%)					
	Measuring date	4.8.	14.8.	24.8	3.9.	13.9.	23.9.	4.8.	14.8.	24.8	3.9.	13.9.	23.9.	4.8.	14.8.	24.8	3.9.	13.9.	23.9.
1	P1 Korona	1,21	1,33	0,58	-	-	-	0,23	0,27	0,31	-	-	-	4,89	5,23	4,00	-	-	-
2	P2 R-83	1,31	1,26	0,24	-	-	-	0,41	0,28	0,43	-	-	-	5,19	5,2	4,54	-	-	-
3	F1 Korona x R-83	1,31	3,87	1,67	-	-	-	0,22	0,28	0,33	-	-	-	5,12	4,85	4,18	-	-	-
4	F2 Korona x R-83	1,63	4,62	2,62	-	-	-	0,27	0,31	0,37	-	-	-	5,41	4,40	3,83	-	-	-
5	P3 L-10 (*nor)*	0,55	0,67	0,75	1,19	0,20	0,16	0,32	0,36	0,43	0,37	0,31	0,22	5,67	6,21	5,51	5,50	6,37	6,87
6	F1 Korona x L-10	0,46	0,97	1,19	1,91	0,56	0,49	0,38	0,38	0,40	0,32	0,27	0,18	6,17	4,71	4,83	5,08	6,23	6,2
7	F2 Korona x L-10	0,30	0,54	2,63	3,02	0,46	0,35	0,28	0,32	0,38	0,35	0,34	0,30	5,99	4,8	5,66	5,56	5,10	6,06
8	P4 L-12 (*rin)*	0,27	0,70	0,72	1,02	0,56	0,13	0,33	0,37	0,40	0,31	0,29	0,19	4,34	4,9	5,03	4,71	5,11	5,60
9	F1 Korona x L-12	0,40	0,66	3,19	4,51	0,58	0,47	0,28	0,32	0,37	0,29	0,29	0,25	4,5	4,82	4,99	4,79	5,71	5,68
10	F2 Korona x L-12	0,31	1,13	3,62	4,20	0,36	0,30	0,31	0,36	0,38	0,27	0,21	0,16	4,18	4,68	4,64	4,96	4,83	5,30
	LSD	**0,05**	**0,01**		**0,05**	**0,01**		**0,05**	**0,01**		**0,05**	**0,01**		**0,05**	**0,01**		**0,05**	**0,01**	
	Measuring date (MD)	0,017	0,023		0,023	0,031		0,031	0,042		0,023	0,029		0,136	0,181		0,161	0,214	
	Genotypes (G)	0,031	0,041		0,023	0,031		0,067	0,089		0,023	0,029		0,289	0,384		0,161	0,214	
	MD x G	0,055	0,072		0,056	0,058		0,094	0,125		0,054	0,055		0,408	0,543		0,394	0,405	

The dynamics of β-carotene content over time was significantly different (154.95^{**}) between groups of genotypes with (six genotypes) and without (four genotypes) prolonged shelf life. Therefore, the difference included all ten genotypes and the first three measurements. Because χ^2 test showed that variance was not homogenous, the log transformation of data concerning β-carotene content was performed. Genotypes with prolonged shelf life were characterized by significant β-carotene content increase between the first and the third measurement (except for parents between the second and the third measurement), when compared to genotypes without prolonged shelf life (Table 2).

Average values for β-carotene in parental genotypes without prolonged shelf life are significantly higher comparing to *nor* and *rin* genotypes, which is in accordance to results of [18], [19], [17] and [4]. Fruits of genotypes without prolonged shelf life reached maturity ten days after picking; fruits of *nor* and *rin* genotypes reached full maturity 30 days after picking. Therefore, *nor* and *rin* genes retain usual tomato fruit ripening. Similar results were reported by [27] and [26].

Nor and *rin* genes affect ripening in different stages of fruit development [26], [12], which was confirmed by our research. Dynamics of β-carotene content increase is also different for *nor* and *rin* genotypes, in both our research and research performed by [26]. According to [24] *nor* genotype x genotype without prolonged shelf life hybrids were characterized by β-carotene content increase comparing to parent carrying *nor* gene.

During tomato fruit ripening, β-carotene biosynthesis precedes lycopene biosynthesis. Lycopene contributes to formation of red color [11]. Increase in carotene content is followed by chlorophyll content decrease [27]. In tomato genotypes without prolonged shelf life β-carotene biosynthesis is terminated in mature fruits [26], [12], [18]. In *nor* and *rin* genotypes lycopene is delayed or absent, in favor of β-carotene biosynthesis [17].

Total acid / sugar content ratio affects tomato fruit taste. Slightly acidic taste is most preferred by consumers. The trait is important for genotypes that will be used for processing (juices, puree). At the first measurement, parents without prolonged shelf life had lower total acid content (0.233 and 0.241% for Korona and R-83, respectively) when compared to parents with prolonged shelf life (0.316 and 0.333% for *nor* and *rin* parents, respectively). The fruits of genotypes without prolonged shelf life were characterized by total acid content increase from the first measurement to rot, while total acid content of nor and *rin* genotypes increased

from the first to the third measurement and decreased thereafter. The same results have been obtained for hybrids of both groups of genotypes. At the first measurement, F_1 hybrids with prolonged shelf life had higher total acid content (0.375 and 0.283% for *nor* and *rin* hybrids, respectively) comparing to F_1 hybrid without prolonged shelf life (0.216%); similarly to F_2 generation (Table 1). Higher total acid content was observed in fruits of tomato genotypes carrying *nor* and *rin* genes, when compared to genotypes without prolonged shelf life [26] and [7]. Buesher [7] measured higher total acid content in tomato juice of hybrid C-36 x *nor* comparing to C-36 (genotype without prolonged shelf life).

Table 2: One-way ANOVA for β-carotene, total acid and dry matter content for 10 tomato genotypes in 3 measuring dates

	4.08	14.08	24.08	Group comparisons	F (table value)/)		Homogeneity of variance χ^2	
					0,05	0,01	0,05	0,01
β-carotene content Genotypes	54,94**	62,34**	28,92**		2,40	3,45	5,99	9,21
Group comparisons				G1=13,22 G2 =14,9 154,95**	4,00	7,08	χ^2=23,162**	
Total acid content Genotypes	3,62**	2,94**	2,13		2,40	3,45	5,99	9,21
Group comparisons				G1=10,63 G2 =19,10 38,55**	4,00	7,08	χ^2=0,46	
Dry matter content Genotypes	3,26**	2,12	3,15*		2,40	3,45	5,99	9,21
Group comparisons				G1=170,51 G2 =274,9 7,23**	4,00	7,08	χ^2=0,46	

The dynamics of total acid content was significantly different between the genotypes with and without prolonged shelf life at the first (3.62^{**}) and the second (2.94^{*}) measurement. There was no difference between the groups at the third measurement. χ^2 test showed homogeneity of variance. Total acid content was significantly different (38.55^{**}) for genotypes with and without prolonged shelf life. The difference included all ten genotypes and three measurements (Table 2).

In our research, total acid content of *nor* and *rin* genotypes was decreased over time. Total acid content of genotypes without prolonged shelf life was increased over time. Agar [1] reported total acid content decrease in both genotypes with and without prolonged shelf life. Since temperature and irradiance affect final fruit quality [10], total acid content increase that has been observed in our research for genotypes without prolonged shelf life may be the consequence of fruit rot. For tomato which is kept fresh and then processed, [3], [23] investigated different storing conditions and dynamics of fruit quality. Significant differences in total acid content between genotypes without prolonged shelf life and *nor* and *rin* genotypes, in both moment of picking and during fruit picking, have been found by [20]. During fruit picking, higher total acid content has been found in *nor* comparing to *rin* genotypes.

Among parents, the highest dry matter content had *nor* (5.67%) and the lowest *rin* genotype (4.34%), concerning the first measurement. Dry matter content of genotypes without prolonged shelf life was between the two values. At picking, all genotypes had similar dry matter content. Comparing the first and the last measurement, dry matter content decreased for genotypes without prolonged shelf life and increased for *nor* and *rin* genotypes (Table 1).

Dry matter content was significantly different between the groups at the first (3.26^{*}) and the third (3.15^{*}) measurement. The variance was homogenous. Genotypes with and without prolonged shelf life were significantly different (7.23^{**}) concerning dry matter content (Table 2).

For *rin* genotypes, dry matter content increased over time, which is opposite to the results of Agar [1], who reported significantly faster dry matter content decrease in *nor* genotypes comparing to *rin* genotypes. Dry matter content decreased over time for genotypes without prolonged shelf life, which is in accordance to the results reported by [8] and [7]. Dry matter content can be manipulated by cultivation, in terms of water uptake, which can decrease dry matter content in fruits.

Two-way ANOVA showed significant differences among genotypes, measurements, as well as significant genotype x measurement interaction concerning β-carotene and dry matter content. Total acid content varied significantly among genotypes and measurements (Table 1).

One-way ANOVA showed that β-carotene content varied significantly among six genotypes with prolonged shelf life at six measurements. Variance was not homogenous. Total acid content varied significantly between *nor* and *rin* genotypes at the fifth and sixth measurement (3.85^{*}, 5.87^{**}, respectively). χ^2 test showed homogeneity of variance. Dry matter content varied between *nor* and *rin* groups at the first measurement only (5.04^{**}), Table 3.

Table 3: One-way ANOVA for β-carotene, total acid and dry matter content for 6 tomato genotypes in 6 measuring dates

	4.08	14.08	24.08	3.09	13.09	23.09	F (tab) 0,05	F (tab) 0,01	Homogeneity of variance χ^2/ 0,05	Homogeneity of variance χ^2/ 0,01
β-carotene content Genotypes	12,46**	10,84**	25,09**	28,22**	14,91**	33,86**			11,08	15,09 107,47**
Total acid Genotypes	1,84	0,97	0,49	1,57	3,85*	5,87*	2,81	4,34	11,08	15,09 2,12
Dry matter Genotypes	5,04**	2,59	1,3	0,60	2,57	4,61			11,8	15,09 0,49

The results of two-way ANOVA concerning β-carotene content showed significant differences between *nor* and *rin* groups of genotypes, measurements and significant genotype x measurement interaction. Total acid content varied significantly between groups of genotypes and between measurements. The same results have been obtained for dry matter content dynamics (Table 1).

The regression analysis concerning β-carotene content showed insignificant values for all genotypes tested, while total acid content decreased significantly over time in case of genotypes Korona x L-10 F_1 and Korona x L-12 F_2. Both *rin* parents and and offspring (L-10, Korona x L-10 F_1 and Korona x L-10 F_2) exhibited significant dry matter content increase over time (Table 4).

Table 4: Regression analysis for *nor* and *rin* genotypes

Genotypes	β-carotene content F (calculated)	Total acid content F (calculated)	Dry matter content	F 0,05	F 0,01
P3 L-10 (*nor*)	1,258	1,549	2,555		
F1 Korona x L-10	0,013	15,301**	0,604		
F2 Korona x L-10	0,001	0,122	0,062	7,71	
P4 L-12 (*rin*)	0,130	5,676	9,457*	21,20	
F1 Korona x L-12	0,003	1,139	31,946**		
F2 Korona x L-12	0,045	8,605*	20,074*		

Tomato fruit shelf life, as well as dynamics of β-carotene, total acid and dry matter content depends on genotype, in other words, depends on presence or absence of *nor* and *rin* genes. Twenty days after picking, all fruits of genotypes without prolonged shelf life (parents, F_1 and F_2 hybrids) decayed. Fruits of *nor* and *rin* genotypes started to decay thirty days after picking, and the great majority of fruits preserved after the last measuring date. Higher β-carotene and lower total acid content have been measured for genotypes without prolonged shelf life comparing to *nor* and *rin* genotypes. Dry matter content was similar for almost all genotypes included in the analysis. After picking, β-carotene content was increased and total acid content was decreased over time in *nor* and *rin* genotypes; due to fruit ripening. Opposite was for the genotypes without prolonged shelf life, due to fruit decay and rotting. Dry matter content was increased over time for all genotypes. Genotypes carrying *nor* and *rin* genes are characterized by desirable traits, such as prolonged shelf life, the possibility of prolonged storing and fruit firmness. Therefore, they should be included in breeding programs, in order to create tomato hybrids that could be stored for longer period and transported to higher distance, without significant change in quality.

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