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Effect of ripening on biochemical characteristics of tangerine tomatoes (*Solanum lycopersicum* L.)

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Conflict of interest. The authors declare no conflict of interest.

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Abstract

Relevance. When creating tomato varieties and hybrids, much attention is paid not only to the commercial component (yield, appearance and uniform ripening), but also to the taste qualities of the products. Salad tomato varieties with yellow, tangerine, and pink fruits are increasingly in demand. As a rule, these fruits do not store for a long time, so it is necessary to increase their storability and transportability. Fruits of this group of tomatoes have high antioxidant activity, which is due not only to the significant content of water-soluble antioxidants (such as ascorbic acid), but also carotenoids. The preservation of fruit largely depends on the dry matter content.

The aim of investigation is to study biochemical parameters of tangerine tomatoes fruits under different ripening conditions.

Materials and methods. Plants were grown in the greenhouse of Federal Scientific Vegetable Center. Biochemical characteristics of tomato fruits were studied during harvesting and after laying for storage in the milky ripeness phase according to the following indicators: dry matter, ascorbic acid, total content of water-soluble antioxidants, titratable acidity, monosaccharides, polyphenols and carotenoids.

Results. The percentage of dry matter in tangerine tomato fruits does not change during storage, even increases slightly. The dry matter content of fruits from the open field is slightly higher than that of fruits from the greenhouse. The content of sugars and ascorbic acid in fruits with tangerine fruits after laying for ripening decreases slightly. However, the content of these compounds is higher in mature fruits immediately after picking than in fruits after ripening. The total content of antioxidants during ripening decreases, though not significantly (in 1.1-1.7 times).

Keywords: tangerine tomatoes, dry matter, monosaccharides, ascorbic acid, total antioxidant content, total acid content, polyphenols, carotenoids.

Влияние дозаривания на биохимические показатели плодов томата (*Solanum lycopersicum* L.) оранжевой окраски

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Вклад авторов. Кондратьева И.Ю. –автор эксперимента, Кондратьева И.Ю. и Молчанова А.В. провели исследование, обработали и проанализировали данные. Кондратьева И.Ю. предоставила материал для изучения и консультационную помощь. Все авторы рассмотрели и одобрили это представление. Все авторы участвовали в написании статьи, прочитали и согласились с опубликованной версией рукописи.

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Резюме

Актуальность. При создании сортов и гибридов томата уделяется большое внимание не только коммерческой составляющей (урожайности, внешнему виду и равномерному созреванию), но и вкусовым качествам продукции, относящиеся к органолептическим показателям. Все больше востребованы салатные сорта томата с жёлтыми, оранжевыми и розовыми плодами. Как правило, такие плоды долго не хранятся, в связи с чем необходимо увеличить их лёжкость и транспортабельность. Плоды данной группы томата имеют высокую антиоксидантную активность, которая обусловлена не только значительным содержанием водорастворимых антиоксидантов (например, аскорбиновой кислоты), но и каротиноидами. Сохранность плодов во многом зависит от содержания сухого вещества.

Цель исследования – изучение биохимических показателей оранжевоокрашенных плодов томата при разных условиях дозаривания.

Материалы и методы. Растения выращивали в поликарбонатных теплицах лаборатории селекции и семеноводства паслёновых культур ФГБНУ ФНЦО. В лабораторно-аналитическом отделе был изучен биохимический состав плодов томата при уборке с поля и после закладки на хранение в фазе молочной спелости по следующим показателям: сухое вещество, аскорбиновая кислота, суммарное содержания водорастворимых антиоксидантов, титруемая кислотность, моносахаров, полифенолы и каротиноиды.

Результаты. Процент сухого вещества в оранжевоокрашенных плодах томата не изменяется при хранении, даже несколько повышается. Содержание сухого вещества в плодах из открытого грунта несколько выше, чем в плодах из теплицы. Содержание аскорбиновой кислоты в плодах с желто-оранжевой окраской плодов после закладки на дозаривание уменьшается в 1,2-1,4 раза, а сахаров в 1,4 раза. Однако в зрелых плодах, сразу после сбора, содержание данных соединений несколько выше, чем в плодах после дозаривания. Отмечена тенденция по снижению суммарного содержания водорастворимых антиоксидантов при дозаривании (в 1,1-1,7 раза).

Ключевые слова: оранжевоокрашенные сорта томата, сухое вещество, моносахара, аскорбиновая кислота, суммарное содержание антиоксидантов, полифенолы, каротиноиды

Introduction

The problem of year-round supply of the population with fresh vegetables, including tomatoes, is generally solved. Partly due to domestic production and to a greater extent due to import. The area under tomatoes in the open field in Russia is decreasing. In 2020, according to Russian Statistics, they were only 81.8 thousand hectares, which is 6.9 thousand hectares less than in 2015 [1]. However, a marked increase in the area of protected ground (according to estimates of the Ministry of Agriculture of the Russian Federation in 2018, about 300 hectares of winter greenhouses were commissioned in Russia) and an increase in the area of open ground under drip irrigation (they grew by more than 50 times and amounted to 51.0 thousand ha), which influenced the increased production of fresh domestic products. According to estimates of INTERAGRO, the yield of tomatoes in 2020 will be 560 thousand tons, import – 534 thousand tons. Thus, for the first time in many years, domestic production may displace import and reaches 51%, which is 3% higher than in 2019.

Increased production of domestic products will probably gradually replace imported products of tomato fruits and seeds. First of all, it is necessary to create competitive varieties and hybrids of tomato for both open and protected ground, to establish seed production and especially the creation of hybrids. In addition to, yield and high marketable qualities, it is necessary to focus more on organoleptic components, in terms of appearance and taste [2 – 5].

Salad tomato varieties with yellow, tangerine and pink fruits, whose coloration depends on the qualitative and quantitative composition of carotenoids such as lutein, β-carotene and ζ-carotene, which have positive effects on the human body, are increasingly in demand [6; 7]. In 16 tomato samples, high-performance liquid chromatography methods showed that high lycopene content was noted in red-colored samples, whereas three dominant carotenoids (δ-carotene, β-carotene and prolicopene) were detected in tangerine-colored tomatoes. Yellow-colored tomatoes showed lower carotenoid content compared to red and tangerine tomatoes (no lycopene and little β-carotene content) [8]. Deineka et al. [9] on tomato samples of red, pink, tangerine, and yellow coloration: trans-lycopene and its cis-isomers are the main carotenoids of red and pink fruits, protolycopene and other carotenes preceding its biosynthesis are typical for orange-colored fruits, while in yellow tomatoes a significant accumulation of lutein was noted. Despite these results, in terms of the content of carotenoids, sugars, and ascorbic acid in yellow-colored, tangerine-colored, and pink-colored tomato fruits, these varieties are in demand for dietary foods for people with digestive problems and children's diets [10 – 14]. But they do not store for a long time and are not transportable, due to the fact that simultaneously with the ripening of the tomato fruit there are processes leading to spoilage of the product, which, in turn, is caused by the cleavage of pectin by the enzyme polygalacturonase [15].

Fruit preservation depends on the dry matter content [16]. The higher the percentage of dry matter, the better the preservation of fruits. It is necessary to increase the storage period and create varieties more storable, without losing their taste qualities. We have already created a number of such varieties: tangerine-fruited – Charovnitsa, Osennyaya rapsodiya, Dolgonosik, Viking [17].

Therefore, the purpose of our investigation was to study the biochemical parameters of tangerine-colored tomato fruits under different conditions of ripening.

Material and Methods

The material for the study were selection samples of tomatoes from the laboratory of breeding and seed production of *Solanaceae* crops FSBSI FSVC with tangerine-orange color of fruits (Fig. 1, 2, 3, 4).

Breeding accession Line 1-21 is a medium-late, determinant. The fruit is oval in shape with an elongated nose, dense, lying, tangerine in color, weighing up to 200-250 g. Highly resistant to phytophthora. Intended for growing in greenhouses.

Cv. Rufina is an early maturing, determinant variety. The leaf is medium-sized, green. Inflorescence intermediate type, the fruit is round, slightly ribbed, medium density, weighing up to 90 g. The color of the immature fruit is green, the mature one is bright tangerine. The number of nests 4-5. Weight of the fruit up to 90 g. Marketable yield 14-18 kg/m^2. Resistant to tobacco mosaic virus and phytophthora.

Cv. Charovnitsa – medium early determinant bush, the height of the main stem to 60-70 cm. Variety medium-late maturity (100-115 days). The fruit is oval, dense, smooth, does not crack, weighing up to 100 g, original tangerine color, with good preservation of fruits after collection. The peduncle without articulation. The dense consistency of fruit makes it possible to use them for whole-fruit canning and pickling. Yields 50-60 t/ha. Resistant to phytophthora and tomato apical rot. Recommended for cultivation in a field and greenhouses.

Cv. Osennyaya rapsodiya is selected for the open field, medium-early, from the mass sprouts to maturity 97-100 days. The height of the main stem is 50-55 cm. The bush is determinant. Oblivionality is average, the leaf average, light green. The shape of the fruit is rounded, weighing 130-160 g. The color of the fruit in the technical ripeness is green, without the green spot at the stalk, in the biological ripeness – tangerine. The yield is 55-65 tons per hectare. Drought tolerant and cold hardy. Relatively resistant to phytophthora, fruits do not crack, not affected by apical rot.

Standard agricultural techniques for tomato crop were used. Samples were grown in unheated greenhouses. Seedlings were planted in cassettes (5x5 cm cell) on March 25, planting in the ground on April 25. Scheme of planting a two-line 70x50x35 cm. Field experiments, phenological observations, and yield record were conducted according to the Methodical instructions on selection of tomato varieties and hybrids for field and greenhouse [18].

Fruits for ripening were placed in the milk-ripe phase of maturity in plastic crates of 25 x 40 cm with holes on the entire surface of the walls. The ripening was carried out indoors, at a room temperature not exceeding 18ºC. Fruits were laid visually healthy, without damage, collected in dry weather. Fruits were laid in two layers, boxes were not closed. Illumination of the room was natural. Counting and biochemical analysis were performed after fruit ripening in two weeks after storing.

Analysis of biochemical composition in fruits with yellow-tangerine coloring of fruits of promising lines was carried out in two stages – the first analysis of fruits collected mature, the second analysis of fruits collected in the milk-ripe stage and put in for ripening.

The biochemical composition of tomato fruits was studied according to the following indicators: determination of the total content of water-soluble antioxidants – by the method of Maximov et al. [19], ascorbic acid (AA) was the standard; AA content – by the Sapozhnikova and Dorofeeva method [20]. Dry matter content – by drying the sample to a constant weight [21], titratable acidity was determined by the Andryushchenko method [22], monosaccharides content was conducted by cyanide method [23]. Taste Index was calculated according to Navez et al. [24] using the formula TI=TA+TS/(20xTA), (where TA – titratable acidity, TS – sugar content).

Polyphenol content was determined using a spectrophotometer using the Folin-Ciocalteu reagent [25] in alcoholic extracts of dried tomato fruits (70% ethanol, heating to 80 ˚C for 1 h). Gallic acid was

Рис. 1. Томат, Линия №1-21
Fig.1. Tomato b.a. Line №1-21

Рис. 2. Томат, сорт Руфина
Fig. 2. Tomato cv. Rufina

Рис.3. Томат, сорт Чаровница
Fig. 3. Tomato cv. Charovnitsa

Рис. 4. Томат, сорт Осенняя рапсодия
Fig. 4. Tomato cv. Osennyaya rapsodiya

used as a standard. The results were expressed in mg-eq of gallic acid/g dry weight.

The content of the sum of antioxidants was determined by titrating 1 ml of 0.05 N $KMnO_4$ solution in 0.24 M H_2SO_4 medium with the analyzed solution (extract of dried tomato fruits in 70% alcohol) until the permanganate solution discolored [25]. Gallic acid was used as a standard [26]. The results were expressed in mg-eq of gallic acid/g dry weight.

Determination of carotenoid composition was carried out according to the following procedure. Before the analysis, fruits were washed, dried with filter paper and homogenized. From 0.5 to 1.5 g of the resulting mixture (depending on the brightness of fruit color) was extracted with acetone (3x5 ml), using a glass powder for better extraction of carotenoids while grinding samples in a mortar. To the combined acetone extract, about 9 mL of hexane and then 50-60 mL of distilled water were added. The aqueous layer was separated and the washing of the organic layer with water was repeated 4-5 times until the harsh smell of acetone disappeared. The hexane layer thus obtained was quantitatively transferred to a 10 ml pycnometer, brought to the mark with hexane and filtered on a folded filter through a layer of anhydrous sodium sulfate. The solution was stored without bright light and analyzed within 6 h after preparation [27; 28].

Statistical processing of the experimental data was performed according to Dospekhov [29] using the Excel statistical program. Data were processed by analysis of variance and mean separations were performed through the Duncan multiple range test, with reference to 0.05 probability level.

Results and discussing

Tomato varieties and hybrids with high dry matter content (more than 6%) are in demand for the preparation of juices and tomato paste. In the process of ripening, the fruits of such varieties do not lose their marketable appearance, remaining dense [30]. Knowledge of the dynamics of dry matter accumulation in tomato samples of different ripeness groups during the early phases of ontogenesis makes it possible to use the specific breeding material more purposefully in a short time of the field season, which will significantly accelerate the process of creating new hybrids and tomato varieties with specified parameters [16].

The comparative analysis of dry matter content in mature fruits immediately after harvesting and in fruits after 20 days storage (fruits laid in the milk phase of maturity) showed that this indicator in tomato fruits after storage has not changed, except for the cv. Rufina. The percentage of dry matter was higher in ripe tomatoes – 7.5 %, and in the laid in storage significantly lower – 5.9 %. The fruits of this variety are more dense than other varieties, which apparently led to less intensive moisture loss. Probably, the conditions of growing in protected ground on the quality of fruits after storage had no significant influence (Table 1).

For the content of monosaccharides a different pattern was noted: the maximum is shown for samples cv. Rufina and Line 1-21 before putting in storage. In 20 days after storage in these samples there was a significant decrease in the content of this parameter: from 3.0%...3.4% to 2.1%...2.4%, while in the cv. Charovnitsa and cv. Osennyaya rapsodiya these values were comparable with each other, both before storage and after (Table 1).

At the same time, the correlation coefficient between dry matter content and monosaccharides was high and was $r=0.75$ at $p<0.02$ (Fig. 5).

The chemical diversity of natural antioxidants makes it difficult to separate, identify, and quantify individual antioxidants from complex food/biological substances. Nevertheless, the indicator of total antioxidant activity is often more informative in assessing the healing and beneficial effects of biological complexes due to the synergistic action of

Table 1. Dry matter content and content of monosaccharides in tangerine-colored tomato fruits before and after storage

Sample	Matured fruit		After storage	
	Dry matter, %	Monosaccharides, %	Dry matter, %	Monosaccharides, %
b.a. Line №1-21	6.3±0.20 b	3.3±0.09 a	6.8±0.12 a	2.4±0.04 b
Rufina	7.5±0.21 a	3.0±0.04 a	5.9±0.03 b	2.1±0.02 b
Charovnitsa	5.3±0.01 c	2.0±0.03 c	5.1±0.01 c	1.8±0.03 c
Osennyaya rapsodiya	4.9±0.02 c	2.2±0.04 b	4.9±0.09 c	1.7±0.01 c

Values with similar letters do not differ statistically according to Duncan's test at p<0.05.

individual antioxidants [31]. Therefore, the content of water-soluble antioxidants in vegetable crops is currently one of the most important indicators [25]. We measured both the total content of water-soluble antioxidants and one of the indicators – L-ascorbic acid.

The ascorbic acid content was the highest in mature tomato fruits of cv. Rufina and cv. Charovnitsa, and in Line 1-21, whereas in cv. Osennyaya rapsodiya it was significantly lower by 1.3 times. After three weeks of storage, the ascorbic acid content in tomato fruits of all samples was 1.2-1.4 times lower than in mature tomatoes collected from the field and immediately analyzed (Table 2). The same pattern was shown for the sum of water-soluble antioxidants for all samples except the cv. Osennyaya rapsodiya – the amount of antioxidants was comparable both in fruits after storage and in those immediately collected in the field.

The calculated correlation coefficient between ascorbic acid content and the sum of water-soluble antioxidants was also high – r=0.86 (Fig. 6) due to the fact that ascorbic acid makes the main contribution to antioxidant activity.

One of the indicators of the taste characteristics of tomato fruits is the total acidity – titratable acidity (calculated per malic acid). The highest percentage of acidity in tomato fruits was found in the cv. Rufina, the fruits of which were collected from the field and immediately analyzed. A 1.2-fold decrease in titratable acidity was observed in Rufina fruits put in storage. The least amount of organic acids was determined in the cv. Charovnitsa in both variants of the experiment. Apparently, the varietal factor determines the variation in the content of organic acids in the studied tomato varieties in different variants (Table 2). It is known that the ratio of sugars and acids in tomato

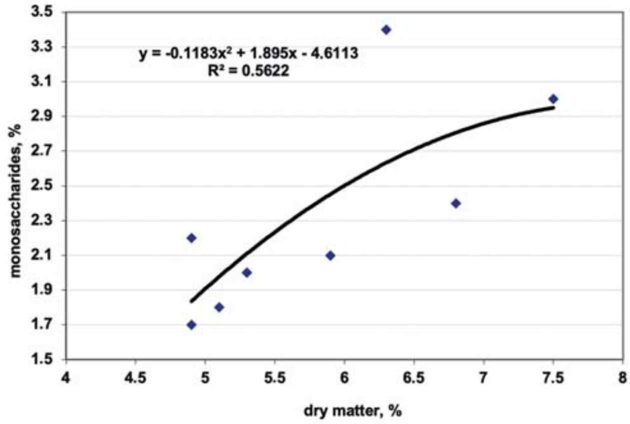

Fig. 5. Relationship between monosaccharides and dry matter content in tomato fruits (r=0.75; p<0.02)

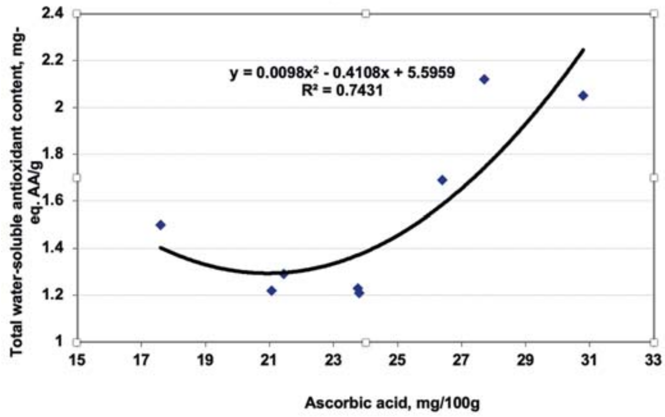

Fig. 6. Relationship between ascorbic acid content and the total water-soluble antioxidant content in tomato fruits (r=0.86; p<0.001)

fruits is an important criterion for evaluation of taste qualities – taste index (TI).

Taste index of fruits harvested mature was significantly higher in Line 1-21 compared to cv. Rufina, cv. Charovnitsa and cv. Osennyaya rapsodiya by 1.5 times. Whereas after storage, the taste index in the Line 1-21 and cv. Osennyaya rapsodiya decreased by 1.3-1.4 times and their values became comparable to the other varieties. In cv. Charovnitsa and cv. Rufina taste index remained unchanged. This may be explained by the fact that the tomato fruits harvested mature, receive water and the necessary organic and mineral substances from the mother plant, while the harvested fruits in the phase of ripeness and put in storage, do not receive nutrients.

Among secondary metabolites, polyphenols appear to be the most important due to their high antioxidant activity and anticarcinogenic properties [32]. In addition to these properties, such compounds also exhibit a synergistic effect with other natural antioxi-

Table 2. Content of ascorbic acid, the total water-soluble antioxidants and titratable acidity in tangerine tomato fruits before and after storage

Sample	Matured fruit			After storage		
	Ascorbic acid, mg/100g	Total water-soluble antioxidant content, mg-eq. ascorbic acid/g	Titratable acidity, % (in terms of malic acid)	Ascorbic acid, mg/100 g	Total water-soluble antioxidant content, mg-eq. ascorbic acid/g	Titratable acidity,% (in terms of malic acid)
b.a. Line №1-21	26.4±0.1a	1.69±0.01b	0.287±0,003c	21.5±0.44b	1.22±0.005b	0.305±0.01b
Rufina	30.8±0.9a	2.05±0.03a	0.403±0,004a	21.5±0.88b	1.29±0.005b	0.345±0.01b
Charovnitsa	27.7±0.4a	2.12±0.02a	0.275±0,013c	23.8±0.88b	1,23±0.005b	0.261±0.01c
Osennyaya rapsodiya	23.7±0.9b	1.21±0.01b	0.311±0,01b	17.6±0.01c	1,05±0.015c	0.351±0.01b

Values with similar letters do not differ statistically according to Duncan's test at p<0.05.

Table 3. Taste index of tangerine-colored tomato fruits before and after storage

Sample	Matured fruit	After storage
b.a. Line №1-21	0.62a	0.45b
Rufina	0.42b	0.36c
Charovnitsa	0.41b	0.39b
Osennyaya rapsodiya	0.40b	0.30c

Values with the same letters are not statistically different according to Duncan's test at p<0.05

dants [25; 33]. In addition, researchers from Estonia determined the total content of polyphenols and flavonoid content in plants of the Solanaceae family – tomato, eggplant, chili pepper and potato [34]. It was shown that the highest content of polyphenols was found in eggplant – 9.00 mg/g and 6.60 mg/g respectively, followed by chili pepper – 4.80 mg/1 g and 2.40 mg/1 g) respectively, and in tomato – 3.6 mg/1 g in terms of tannic acid.

In terms of polyphenol content, 2 samples – Line 1-21 and cv. Rufina analyzed after laying for ripening. The fruits showed the highest amount of antioxidants compared to the fruits harvested and analyzed immediately. Whereas in other experimental variants the values of this indicator were comparable (Table 4).

The amount of antioxidants in fruits after storage increased by 1.3-1.5 times. At the same time, a high correlation coefficient between these two parameters – r=0.92 - was also shown (Fig. 7). In many publications, a high correlation coefficient between these two parameters was observed on various plant objects. It has been shown that phenolic compounds make a major contribution to the antioxidant activity due to their antioxidant-reduction properties, which allow them to act as reducing agents, hydrogen donors and neutralizers of singlet oxygen [35].

One of the most important tasks in the breeding of cultivated varieties of tomato with a high content of carotenoids in the fruit. When stored for ripening, the carotenoid content was in the range of 8.8...12.6 mg%. At the same time, the lowest content (8.8 mg%) was observed in the fruits of tomato Line 1-21. In the remaining samples, the values were comparable (Fig. 8). After ripening, carotenoid content increased in Line 1-21 (1.5-fold) and cv. Osennyaya rapsodiya (1.4-fold). In the other tomato samples, the carotenoid values before storage and after ripening were comparable.

The content of ascorbic acid and the amount of water-soluble antioxidants increased significantly during storage of orange-red tomato fruits. Apparently, this is associated with an increase in respiration intensity during ripening, and with the increase of ROS activity. Similar results were also reported by Mandol et al. (2004) [36].

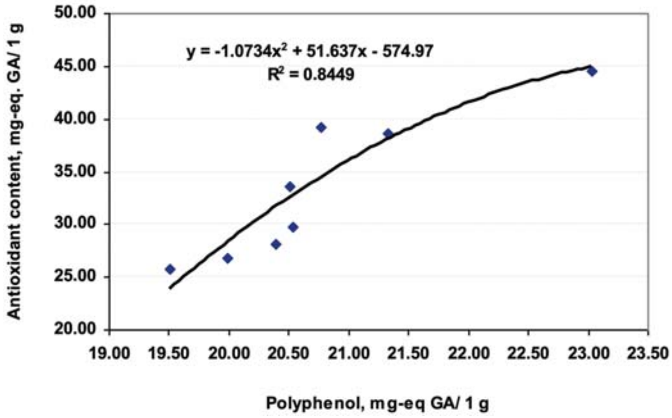

Fig. 7. Relationship between the content of polyphenols and antioxidant content in tangerine-colored tomato fruits (r=0.92; p<0.001)

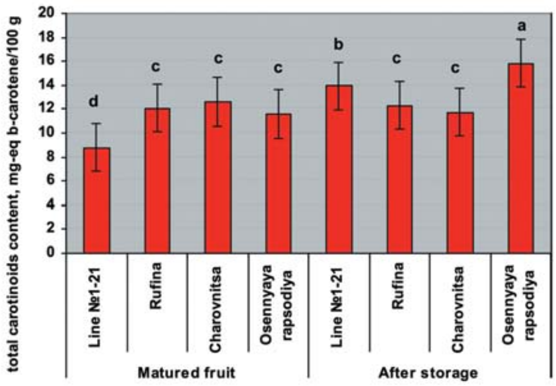

Fig. 8. Sum of carotenoid content in the tangerine-colored tomato fruits before and after storage Values with similar letters do not differ statistically according to Duncan's test at p<0.05

They showed that the ripening process of tomato fruits is accompanied by an increase in oxidative stress due to a significant increase in the amount of ROS. Varieties with short storage time are more susceptible to oxidative stress than long storage varieties due to the enzyme activity decrease and the increase in ascorbic acid content.

The increase in the ascorbate content during storage, is known to be accompanied by β-carotene increase [37], and a decrease of lycopene [38] as a result of lycopinepsilocyclase and lycopinbetacyclase activity. In the orange-colored tomatoes in the present work, only two varieties demonstrates the total carotenoid content increase during stoorage (line 1-21 and Osennyaya rhapsodya).

The increase in polyphenols and antioxidant amount during tomato storage was shown both in our study and in the investigation of Wrzodak et al. (2015 a, b) [39, 40] on plants treated with 1-MCP before storage. The authors attributed this result to the fact that 1-MCP is an ethylene inhibitor, which reduces the rate of ripening of tomato fruits, reduces rotting and weight loss. But the analysis of

Table 4. Polyphenol and antioxidant content in the alcohol extract in the tangerine-colored tomato fruits before and after storage

Sample	Matured fruit		After storage	
	Polyphenol, mg-eq Gallic acid/g	Antioxidant content, mg-eq. Gallic acid/g	Polyphenol, mg-eq Gallic acid/g	Antioxidant content, mg-eq. Gallic acid/g
b.a. Line №1-21	20.54 ±0.09b	29.81±1.24b	23.04±0.17a	44.58±0.74a
Rufina	19.51±0.49b	25.76±3.06c	21.33±0.63a	38.63±0.81b
Charovnitsa	19.98±0.12b	26.87±0.12c	20.51±0.25b	33.54±1.43b
Osennyaya rapsodiya	20.39±0.12b	28.08±0.62c	20.77±0.23b	39.20±4.30b

Values with similar letters do not differ statistically according to Duncan's test at p<0.05 publications showed that tomato fruit ripening is a complex multi-code process with many branching biosynthesis pathways, and, for example, the MYB protein family transcription factors can affect both carotenoid accumulation and chromoplast biogenesis, but also negatively regulate polyphenol accumulation by suppressing gene expression [37].

Conclusion

Thus, the analysis of tangerine-colored tomatoes after storage revealed that, the fruits of Line 1-21 significantly increased the total antioxidant activity, ascorbic acid and decreased the percentage of dry matter, indicating the possibility of using these tomatoes as a donor for taste qualities and long storage period in the breeding process. Biochemical parameters of tomato fruits of cv. Rufina, cv. Charovnitsa and cv. Osennyaya rapsodiya varieties did not change after storage. The content of carotenoids in genotype Line 1-21 and cv. Osennyaya rapsodiya varieties significantly increased after storage, while in other varieties it remained unchanged.

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