



Research article

The effect of postharvest ultraviolet irradiation on the content of antioxidant compounds and the activity of antioxidant enzymes in tomato

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ABSTRACT

The effect of different doses of long-wavelength UV-A (320 nm–400nm) irradiation on physicochemical and antioxidant characteristics of tomatoes grown on the territory of the Russian Federation was studied. The obtained results show that this kind of processing does not cause deterioration of qualitative parameters of vegetables (texture, color, soluble solids content, titratable acidity). It was established that the total content of phenolic compounds and flavonoids increases (p-value<0.05) in tomatoes at all the investigated wavelengths (353 nm, 365 nm and 400 nm), while the content of chlorophylls reacts ambiguously: at some wavelengths, it increases, at other, it decreases. The maximum increase in antioxidant activity, as compared to untreated samples, is observed in tomato samples irradiated for 360 min within the range of 365 nm. For different types of tomatoes, the increment for common content of phenolic compounds is – 42.9–55.0 %, carotenoids – 24.0–56.0 %, flavonoids – 28.0–33.0 %, β -carotene – 70.9–71.6 %, lycopene – 62.6–69.0 %, lutein – 64.8–72.0 % from original. The studies reveal some potential of post-harvest ultraviolet irradiation (A-range) of tomatoes to increase their antioxidant activity. However, more research is needed to confirm this fact and the possibility to develop some technology.

1. Introduction

Free radical formation contributes to the natural processes of metabolism in organisms. In turn, these active forms of oxygen are neutralized by antioxidant forces of the organism. In case its functioning fails, free radicals accumulate causing damage to biomolecules (proteins, lipids, DNA, etc.) which results in the development of various diseases such as cancer, atherosclerosis, cardiovascular disease, neurological, pulmonary, hormonal, and other diseases [1, 2]. Research confirms that high consumption of fruit and vegetables containing vitamin E, vitamin C and carotenoids, phenolic compounds including flavonoids and phenolic acids reduces the incidence of various dangerous diseases such as cancer and cardiovascular disease [3, 4, 5]. Therefore, natural plant foods are considered a potential source of natural highly active antioxidants such as phenolic compounds of various plant extracts or products [6, 7, 8, 9, 10, 11, 12, 13]. Having

similar properties, the so-called Mediterranean diet features tomatoes and tomato products as key components.

Global production of tomatoes (*Solanum lycopersicum* L.) which are cultivated as vegetables exceeds 100 million tons per year. In terms of human health, tomatoes are a source of many nutrients and secondary metabolites, minerals, vitamins C and E, β -carotene, organic acids, etc. [14, 15]. In addition to providing pro-vitamins, enzymes, dietary fiber, they are powerful antioxidants (containing lycopene, phenolic compounds, and others), preventing the formation and neutralizing various forms of free radicals [16].

Most of the nutritional components of tomatoes found *in vivo* are caused by their genetic characteristics, natural and climatic growth conditions. Biochemical composition changes throughout the growth stage, especially after ripening, harvesting and storage [17]. Processing changes the antioxidant potential of tomatoes [6]. However, there are studies showing that compounds with antioxidant activity such as

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lycopene or carotene may exhibit an increased capacity accumulation after food products are processed [18, 19]. To optimize the processing conditions and to discover methods to increase the antioxidant capacity of food products, it is crucial to understand the biochemical basis of these apparently contrasting studies, to determine the degree of biochemical changes as well as components obtained in the course of industrial processing.

In most of previously conducted studies, antioxidants were measured mainly in fresh tomatoes or processed products [20]. A number of studies of flavonoid content and lycopene peel of tomatoes [21] reported that most of the flavonols in tomatoes were present in the peel.

The work within which ultraviolet irradiation of berries [22], fruit [23] and vegetables, especially tomatoes [24, 25] has led to an increase in antioxidant capacity is especially interesting. UV-C-radiation used as a post-harvest processing method inhibited spoilage and increased the amount of ascorbic acid and the total content of phenolic compounds in tomatoes by more than 20 %, however there is no information on the change of individual phenolic compounds in tomatoes.

After the UV treatment, tomato and tomato product samples retain many physicochemical properties (total solubility of solids, pH, water activity, color, titrated acidity and brightness) with reduced microbiological damage to the surface. Despite the negative impact of UV-irradiation on the lycopene and ascorbic acid content, on the whole, tomato's antioxidant activity did not decrease. The difference in the chemical composition and antioxidant activity of tomatoes of different varieties and growth territories has been observed [26]. There is also a difference in the response of plants to the content of antioxidant substances in the ultraviolet irradiation at different wavelengths. It is known that shorter wavelength irradiation requires a decrease in the duration of irradiation, otherwise the damage from irradiation both for plants and their fruit will be greater than possible benefits. UV-A irradiation refers to the safe part of the spectrum. It has been established that its various types cause enhanced synthesis of carotenoids and anthocyanins, prolonged exposure reduces synthesis of chlorophyll, while short-term exposure leads to an increase in synthesis of some biologically active substances [26, 27, 28, 29].

In the present study, the effect of long-wave ultraviolet irradiation on the quality characteristics, including antioxidant, of tomatoes grown on the territory of Russia was studied.

2. Materials and methods

2.1. Materials and chemicals

Fresh red ripe tomatoes of 'Budenovka', 'Bull Heart' and 'Gina' varieties (grown in Krasnodar region, Russian Federation) were bought at commercial facilities in Kemerovo in July 2016–2018. Mid-sized tomatoes (250 ± 15 g) with smooth, nitidous and rather solid skin without visible damages and efflorescence were chosen.

The study used citric acid, hydrochloric acid, metaphosphoric acid, sulfuric acid, sodium hydroxide, acetone, methanol, ethanol, hexane (LLC Component-Reactiv, Russia); gallic acid, Ethylenediaminetetraacetic acid (EDTA), PVP (pyrrolidinovalerophenone), N, N'-dimethylformamide (DMF) (Fluka/Sigma-Aldrich (Sigma-Aldrich Rus, Moscow, Russia) and all other chemicals of analytical or higher grade.

2.2. Sample preparation

Fresh tomatoes were irradiated at ambient temperature with UV-A lamps at wavelengths of 353 nm, 365 nm and 400 nm, after which their physicochemical properties were monitored: texture, color, soluble solids content, titratable acidity, content of antioxidant substances. Long-wave range irradiation was chosen for its smallest penetration through the membranes and the lowest negative impact on biological objects [30, 31, 32]. The ultraviolet source was placed at a distance of 50 cm from the test objects. The intensity of illumination during treatment was 15–20 klx. 36 tomatoes of each variety were used in the study. Fresh vegetables of same varieties not exposed to UV-A range were the control.

Tomatoes were put in a UV-A chamber (width: 700 mm, depth: 580 mm, height: 732 mm) one by one directly under the UV-A tubular lamp (Sylvania F15W/T8/BL350, Philips TLD 15W/05, TLD 15W/03, Germany, rated power 15 W, emission spectrum 315–400 nm, 315–460 nm, 380–470 nm, respectively) to distribute the irradiation dose. The UV intensity was determined using a UV radiometer (TKA-PKM 12, Russia) calibrated at 353 nm, 365 nm and 400 nm. UV irradiation was applied continuously for 10 min, 180 min and 360 min in the following doses: 0.33, 0.28 and 0.28 W m⁻², respectively for UV sources. All tomato samples (control and irradiated) were kept for 36 h at a temperature of 4 °C until the study of physicochemical characteristics. Further, all the

Table 1. Indicator values for fresh ripe red tomatoes (total content of phenolic compounds, chlorophylls, carotenoids, flavonoids) subjected to UV-A-irradiation at different exposure times and different wavelengths.

Samples	Operation modes									
	Control	10 min			180 min			360 min		
		353 nm	365 nm	400 nm	353 nm	365 nm	400 nm	353 nm	365 nm	400 nm
Total content of phenolic compounds (mg/kg)										
A	310.5 ± 21.7 ^a	326.0 ± 22.8 ^a	335.3 ± 23.5 ^a	319.8 ± 22.4 ^a	347.8 ± 24.3 ^b	378.8 ± 26.5 ^b	332.2 ± 23.3 ^a	366.4 ± 25.7 ^b	481.3 ± 33.7 ^b	357.1 ± 25.0 ^b
B	287.5 ± 20.1 ^a	293.2 ± 20.5 ^a	310.5 ± 21.7 ^a	301.8 ± 21.1 ^a	307.6 ± 21.5 ^a	382.3 ± 26.8 ^b	330.6 ± 23.1 ^b	324.8 ± 22.7 ^b	411.1 ± 28.8 ^b	350.7 ± 24.6 ^b
C	304.9 ± 21.3 ^a	314.0 ± 22.0 ^a	335.3 ± 23.5 ^a	320.1 ± 22.4 ^a	332.3 ± 23.3 ^b	381.1 ± 26.7 ^b	353.6 ± 24.8 ^b	347.5 ± 24.3 ^b	469.5 ± 32.9 ^b	378.0 ± 26.5 ^b
Total content of chlorophylls (mg/kg)										
A	15.7 ± 1.1 ^a	14.9 ± 1.0 ^a	16.9 ± 1.2 ^a	15.5 ± 1.1 ^a	14.6 ± 1.0 ^a	17.7 ± 1.2 ^b	15.3 ± 1.1 ^a	14.3 ± 1.0 ^a	18.7 ± 1.3 ^b	15.3 ± 1.1 ^a
B	15.3 ± 1.1 ^a	15.3 ± 1.1 ^a	15.7 ± 1.1 ^a	15.3 ± 1.1 ^a	15.1 ± 1.1 ^a	16.9 ± 1.2 ^b	16.0 ± 1.1 ^a	15.0 ± 1.1 ^a	17.6 ± 1.2 ^b	16.6 ± 1.2 ^b
C	14.8 ± 1.0 ^a	14.8 ± 1.0 ^a	15.8 ± 1.1 ^a	15.2 ± 1.1 ^a	15.1 ± 1.1 ^a	16.4 ± 1.2 ^b	15.7 ± 1.1 ^a	15.5 ± 1.1 ^a	17.0 ± 1.2 ^b	15.9 ± 1.1 ^b
Total content of carotenoids (mg/kg)										
A	68.5 ± 4.8 ^a	68.5 ± 4.8 ^a	85.6 ± 6.0 ^b	73.2 ± 5.1 ^a	70.5 ± 4.9 ^a	99.9 ± 6.7 ^b	75.9 ± 5.3 ^a	72.6 ± 5.1 ^a	106.8 ± 7.5 ^b	78.7 ± 5.5 ^b
B	69.1 ± 4.8 ^a	69.8 ± 4.9 ^a	73.9 ± 5.2 ^a	72.5 ± 5.1 ^a	73.2 ± 5.1 ^a	80.2 ± 5.6 ^b	77.4 ± 5.4 ^a	76.7 ± 5.4 ^a	85.7 ± 6.0 ^b	80.1 ± 5.6 ^b
C	68.8 ± 4.8 ^a	70.8 ± 5.0 ^a	74.3 ± 5.2 ^a	72.2 ± 5.1 ^a	73.6 ± 5.2 ^a	77.0 ± 5.4 ^a	75.0 ± 5.3 ^a	75.7 ± 5.3 ^a	87.3 ± 6.1 ^b	78.4 ± 5.5 ^b
Total content of flavonoids (mg/kg)										
A	73.2 ± 5.1 ^a	73.6 ± 5.2 ^a	79.07 ± 5.53 ^a	78.3 ± 5.5 ^a	77.6 ± 5.4 ^a	93.0 ± 6.5 ^b	84.9 ± 5.9 ^b	80.5 ± 5.6 ^a	97.3 ± 6.8 ^b	88.6 ± 6.2 ^b
B	73.0 ± 5.1 ^a	75.2 ± 5.3 ^a	81.02 ± 5.67 ^a	78.1 ± 5.5 ^a	77.4 ± 5.4 ^a	89.8 ± 6.3 ^b	83.2 ± 5.8 ^b	82.5 ± 5.8 ^b	94.2 ± 6.6 ^b	89.1 ± 6.2 ^b
C	73.4 ± 5.1 ^a	74.9 ± 5.2 ^a	79.31 ± 5.55 ^a	77.1 ± 5.4 ^a	78.6 ± 5.5 ^a	85.2 ± 6.0 ^b	82.3 ± 5.8 ^b	83.0 ± 5.9 ^b	94.0 ± 6.6 ^b	84.5 ± 5.9 ^b

Sample A – 'Budenovka', sample B – 'Bull Heart', sample C – 'Gina'. The data are expressed as mean ± standard deviation (n = 3). Values followed by lower case letters in lines are significantly different (P ≤ 0.05) by LSD post-hoc test.

Table 2. Indicator values for fresh ripe red tomatoes (β -carotene, lycopene, lutein) subjected to UV-A-irradiation at different exposure times and different wavelengths.

Samples	Operation modes									
	Control	10 min			180 min			360 min		
		353 nm	365 nm	400 nm	353 nm	365 nm	400 nm	353 nm	365 nm	400 nm
β-carotene (mg/kg)										
A	0.62 \pm 0.03 ^a	0.64 \pm 0.03 ^a	0.70 \pm 0.04 ^a	0.66 \pm 0.03 ^a	0.89 \pm 0.04 ^b	0.96 \pm 0.05 ^b	0.87 \pm 0.04 ^b	1.02 \pm 0.05 ^b	1.10 \pm 0.06 ^b	1.00 \pm 0.05 ^b
B	0.57 \pm 0.03 ^a	0.58 \pm 0.03 ^a	0.63 \pm 0.03 ^a	0.56 \pm 0.03 ^a	0.69 \pm 0.03 ^a	0.78 \pm 0.04 ^a	0.70 \pm 0.04 ^a	0.84 \pm 0.04 ^b	0.90 \pm 0.05 ^b	0.82 \pm 0.04 ^b
C	0.78 \pm 0.04 ^a	0.84 \pm 0.04 ^a	1.00 \pm 0.05 ^a	0.83 \pm 0.04 ^a	0.94 \pm 0.05 ^a	1.18 \pm 0.06 ^b	1.00 \pm 0.05 ^a	1.06 \pm 0.05 ^b	1.40 \pm 0.07 ^b	1.16 \pm 0.06 ^b
Lycopene (mg/kg)										
A	32.0 \pm 1.6 ^a	34.1 \pm 1.7 ^a	41.1 \pm 2.1 ^a	37.1 \pm 1.9 ^a	36.1 \pm 1.8 ^a	48.0 \pm 2.4 ^b	39.2 \pm 2.0 ^a	38.0 \pm 1.9 ^a	53.1 \pm 2.7 ^b	42.0 \pm 2.1 ^a
B	18.1 \pm 0.9 ^a	20.0 \pm 1.0 ^a	22.9 \pm 1.1 ^a	21.0 \pm 1.1 ^a	21.1 \pm 1.1 ^a	26.1 \pm 1.3 ^b	24.1 \pm 1.2 ^a	21.0 \pm 1.1 ^a	30.0 \pm 1.5 ^b	25.1 \pm 1.3 ^a
C	40.0 \pm 2.0 ^a	41.2 \pm 2.1 ^a	47.8 \pm 2.4 ^a	42.0 \pm 2.1 ^a	43.0 \pm 2.2 ^a	63.1 \pm 3.2 ^b	48.0 \pm 2.4 ^a	45.1 \pm 2.3 ^a	64.9 \pm 3.3 ^b	50.1 \pm 2.5 ^a
Lutein (mg/kg)										
A	1.16 \pm 0.06 ^a	1.27 \pm 0.06 ^a	1.34 \pm 0.07 ^a	1.28 \pm 0.06 ^a	1.35 \pm 0.07 ^a	1.58 \pm 0.08 ^b	1.41 \pm 0.07 ^a	1.43 \pm 0.07 ^a	2.02 \pm 0.10 ^b	1.54 \pm 0.08 ^b
B	1.25 \pm 0.06 ^a	1.37 \pm 0.07 ^a	1.53 \pm 0.08 ^a	1.39 \pm 0.07 ^a	1.39 \pm 0.07 ^a	1.75 \pm 0.09 ^b	1.55 \pm 0.08 ^a	1.51 \pm 0.08 ^a	2.12 \pm 0.11 ^b	1.64 \pm 0.08 ^b
C	1.24 \pm 0.06 ^a	1.41 \pm 0.07 ^a	1.46 \pm 0.07 ^a	1.43 \pm 0.07 ^a	1.44 \pm 0.07 ^a	1.75 \pm 0.09 ^b	1.60 \pm 0.08 ^b	1.46 \pm 0.07 ^a	2.05 \pm 0.10 ^b	1.8 \pm 0.09 ^b

Sample A – 'Budenovka', sample B – 'Bull Heart', sample C – 'Gina'. The data are expressed as mean \pm standard deviation ($n = 3$). Values followed by different letters in lines are significantly different ($P \leq 0.05$) by LSD post-hoc test.

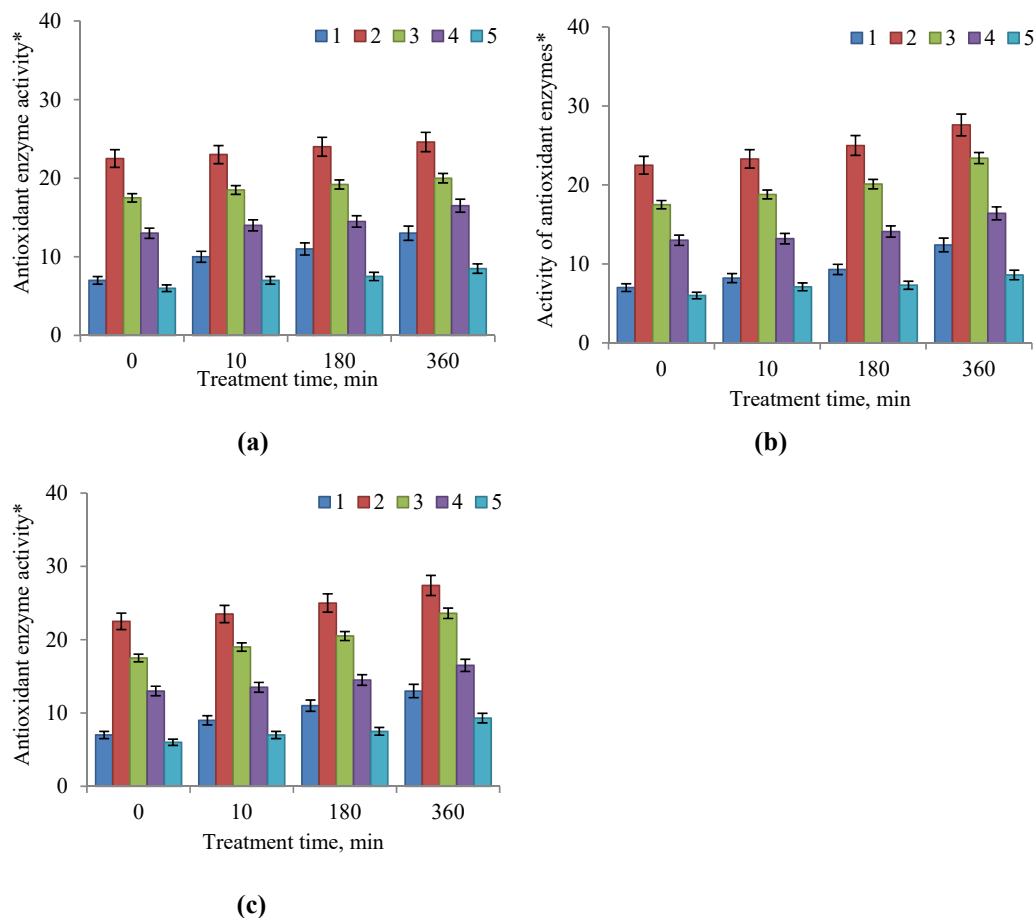


Figure 1. Antioxidant enzyme activity in ripe tomatoes of a 'Budenovka', b 'Bull Heart' and c 'Gina' varieties depends on the duration of UV-A radiation (averaged data at wavelengths of 353 nm, 365 nm and 400 nm are provided for each variety): 1 - catalase, 2 - peroxidase, 3 - polyphenoloxidase, 4 - superoxide dismutase, 5 - phenylalanine ammonia lyase (bars in graphs indicate the standard deviation of three samples). * - catalase activity expressed in micromoles of decomposed $\text{H}_2\text{O}_2 \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ of tomato weight; peroxidase activity in formed micromoles purpurogallin $\cdot \text{min}^{-1} \cdot \text{g}^{-1}$ of tomato weight; polyphenol oxidase activity in formed micromoles of purpurogallin $\cdot \text{min}^{-1} \cdot \text{g}^{-1}$ of tomato weight; superoxide dismutase activity in micromolar of cinnamic acid $\cdot \text{min}^{-1} \cdot \text{g}^{-1}$ of tomato weight; activity of phenylalanine ammonia-lyase - in $\text{U} \cdot \text{h}^{-1} \cdot \text{mg}^{-1}$ protein.

vegetable samples were cut and homogenized with Omnimixer (Ultra-Turrax T25, Staufen, Germany) and stored at $-80\text{ }^{\circ}\text{C}$ until the analysis.

2.3. Analytical methods

Mass fraction of total soluble solids (TSS) was determined by placing 0.5 mL of fruit homogenate purified by centrifugation ($4000 \times g$, 10 min) on hand prism refractometer Atago PR1. The results are presented in $^{\circ}\text{Brix}$ [33].

Titrate acidity was determined by titration of 10 mL fruit homogenate to a pH of 7.6 using 0.1 M sodium hydroxide and expressed in g/L of citric acid with a digital pH-meter (pH-150M, Gomel'skiy Zavod Izmeritel'nykh Priborov, Russia).

The firmness of tomatoes was evaluated by puncture strength (N) with a digital penetrometer PCE-PTR 200 (PCE Instruments UK Ltd, Hampshire, UK). Tomatoes were compressed with a constant speed of 0.95 mm/s, the diameter of the puncture was 5 mm.

The color of tomatoes was determined using Minolta Chroma Meter model CR-200B (Konica Minolta, Tokyo, Japan) at three different points located in the equatorial area [34]. The colorimeter had a beam diameter of 8 mm, three response detectors set at 0 viewing angle and a CIE standard illuminant C with diffuse illumination. This illuminant was accepted as having a spectral radiant power distribution closest to reflected diffuse daylight. Color changes were documented over the duration of the experiment. L values indicate lightness (black [L = 0] and white [L = 100]), a values indicate redness-greenness (red [a = 100] and green [a = -100]), b values indicate yellowness-blueness (yellow [b = 100] and blue [b = -100]). Chroma (C) ($C = \sqrt{a^2 + b^2}$) measures color saturation or intensity and the hue angle ($h = 1/\tan(b/a)$) determines the red, yellow, green, blue, purple, or intermediate colors between adjacent

pairs of these basic colors. A lower hue value indicates a redder product [35].

2.4. Total phenolic content

The total polyphenolic content was determined using a colorimetric assay and the Folin-Ciocalteu reagent (Sigma, St. Louis, USA) by the reagent method [36].

The samples were subjected to hydrolysis procedure [37]. In order to do this, 1 ml of 1M HCl was added to 2 g of the sample and shaken for 1 min, then incubated at $37\text{ }^{\circ}\text{C}$ for 30 min. Next, 1 ml of 2M NaOH was added to 75 % of methanol, after which the resulting mixture was shaken for 2 min then incubated at $37\text{ }^{\circ}\text{C}$ for 30 min. Then, 1 ml of 0.75 M metaphosphoric acid was added and the sample was stirred for 2 min and then centrifuged at $5000 \times g$ for 10 min. The supernatant was removed and transferred to a 10 ml volumetric flask. The pellet was resuspended in 1 ml of acetone: water (1:1, v/v) was shaken for 1 min and centrifuged at $5000 \times g$ for 10 min. Both supernatants were combined and the final volume was adjusted to 10 ml with acetone: water (1:1, v/v). Results are expressed in mg of gallic acid equivalent/kg.

2.5. Total photosynthetic pigments content

Chlorophyll content was evaluated by the method of Moran and Porath [38], using the formula proposed by Inskeep and Bloom [39]. 100 mg of the sample mass was crushed and placed in a tube with 10 ml of DMF and stored for 24 h at $4\text{ }^{\circ}\text{C}$. Colored supernatant was used to estimate the amount of chlorophyll by measuring absorbance at 647 nm and 666 nm on a spectrophotometer with DMF as control for chlorophyll.

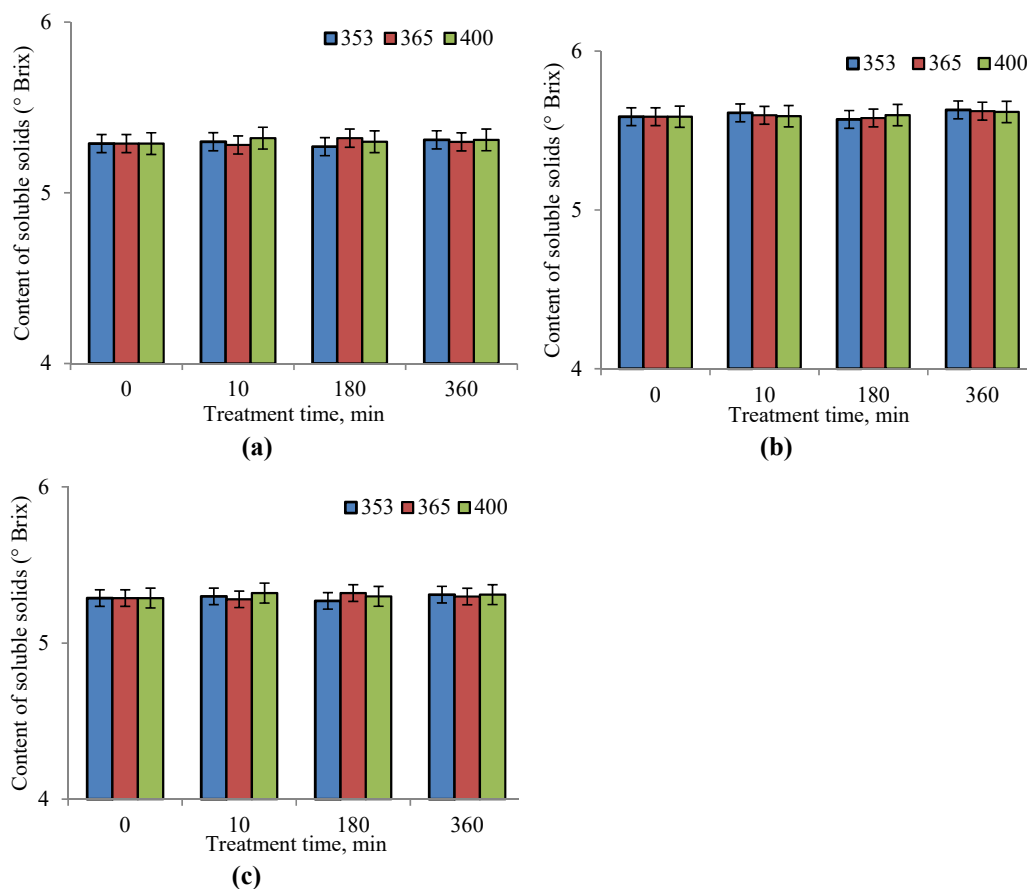


Figure 2. The content of soluble solids in fresh ripe red tomatoes of a 'Budenovka', b 'Bull Heart' and c 'Gina' varieties is dependent on the duration of UV-A radiation with a wavelength of 353 nm, 365 nm and 400 nm (bars in graphs indicate the SE of three samples).

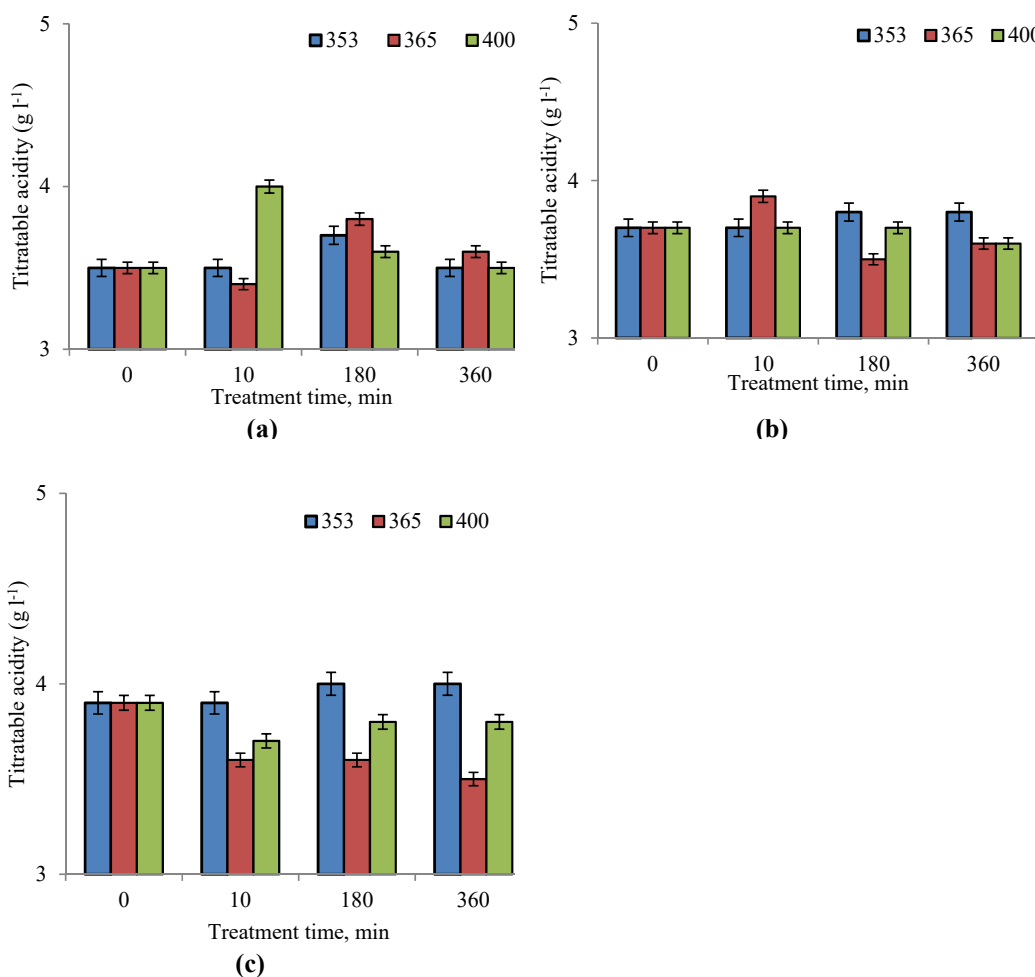


Figure 3. Change of titratable acidity in fresh ripe red tomatoes of a 'Budenovka', b 'Bull Heart' and c 'Gina' varieties is dependent on the duration of UV-A radiation at a wavelength of 353 nm, 365 nm and 400 nm (bars in graphs indicate the SE of three samples).

Flavonoids were extracted and analyzed by the method of Mirecki and Teramura [40]. 100 mg of the sample was placed in 80 % of acidified methanol (methanol: water: HCl 80:20:1) for 12 h in the dark at 4 °C to extract flavonoids and light absorption was measured at 315 nm.

The total carotenoids were determined by the colorimetric method. 2 g of the sample was extracted for 30 min with 50 ml of a triple solvent (hexane/ethanol/acetone 50/25/25). After filtration, the solvent was washed three times with 25 ml of distilled water. Absorbance at 450 nm was determined using a spectrophotometer Shimadzu UV 1800 (Shimadzu Corporation, Japan). The results were obtained using the Beer-Lambert law and are expressed in mg/l.

2.6. Antioxidant enzyme activity

Catalase (CAT, EC 1.11.1.6) activity was measured by the method of Machly and Chance [41]. One gram of the sample was homogenized in 10 ml of 0.1 mM sodium phosphate buffer, pH 7, and centrifuged at 4 °C for 10 min at 10,000 g. 1 ml of aliquots supernatant of enzyme extract was added to the reaction mixture containing 1 ml of 0.01 M H₂O₂, 3 ml of 0.1 M sodium phosphate buffer, pH 6.8. The reaction was terminated after 5-min of incubation at 20 °C by adding 10 ml of 1 % H₂SO₄. Acidified medium with or without the enzyme extract was titrated with 0.005N KMnO₄.

Peroxidase (POX, EC 1.11.1.7) activity was determined by the method of Kumar and Khan [42] and superoxide dismutase (SOD, EC 1.15.1.1) activity by the method of Beauchamp and Fridouich [43]. One

g of the sample was homogenized for 20 ml in cold extraction buffer containing 2M MgCl₂, 1 mM EDTA, 10 mM of β-mercaptoethanol, 7 % PVP, and 10 mM of sodium metabisulfite. The homogenate was filtered through two layers of cheesecloth and centrifuged at 10,000 × g for 5 min and the supernatant was adjusted to 20 ml with the same buffer, the resulting mixture was used as the enzyme source.

The polyphenol oxidase (PPO, EC 1.14.18.1) activity was determined by the method of Kumar and Khan [42]. Polyphenol oxidase assay mixture contained 2 ml of phosphate buffer (pH 6.0), 1 ml of 0.1 M catechol 3.6 and 0.5 ml of enzyme extract. The mixture was incubated for 5 min at 25 °C and then quenched by addition of 1 ml 2.5 N of sulfuric acid. Purpurogallin absorbance was measured at 495 nm. Enzyme activity was expressed in units. One unit was defined as the amount of generated purpurogallin which enhances the absorption of 0.1 per min under assay conditions.

To determine the phenylalanine ammonia-lyase (PAL, 4.3.1.5) [44], 500 mg of the sample was homogenized in 5 ml of cold 25 mM mercaptoethanol, and centrifuged at 12000 × g for 20 min. The supernatant was used for analysis. 0.2 ml of the aliquot enzyme extract was mixed with 0.5 ml of borate buffer and 1.3 ml of distilled water. The reaction was initiated by adding 1 ml of a 0.1 M solution of phenylalanine and incubated for 30–60 min at 32 °C. After the incubation, the reaction was stopped by adding 0.5 ml of 1 M of trichloroacetic acid and the absorbance at 290 nm minus control. The reaction rate was expressed as micromoles formation of trans-cinnamic acid per mg protein per min.

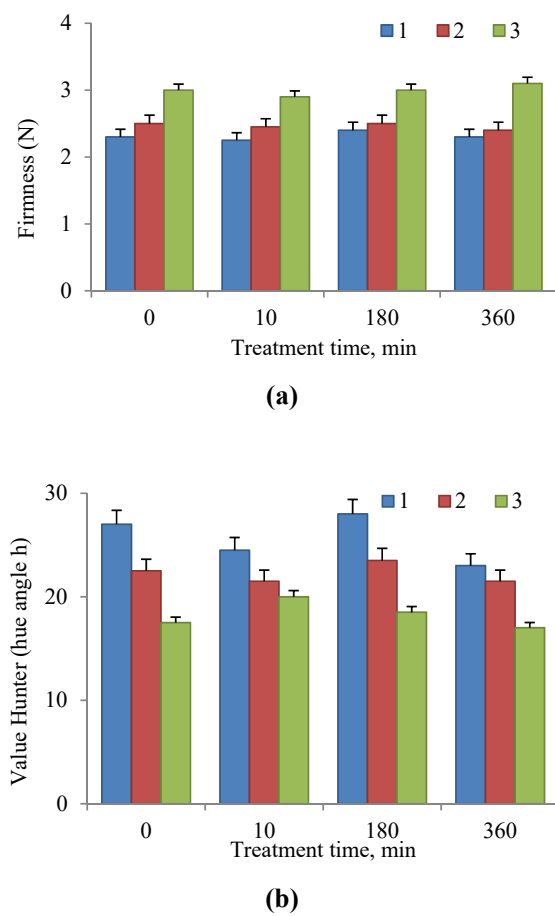


Figure 4. Changes in firmness a and dependence of color b of fresh ripe red tomatoes on the duration of UV-A radiation: variety 1 is 'Budenovka', variety 2 is 'Bull Heart', variety 3 is 'Gina' (averaged data at wavelengths of 353 nm, 365 nm and 400 nm are provided for each variety) are represented (bars in graphs indicate the SE of three samples).

2.7. Statistical analysis

Each experiment was repeated three times and data were expressed as means \pm SE. Data processing was carried out by standard methods of mathematical statistics. Homogeneity of the sampling effects was checked using the Student's t-test. The data were subjected to analysis of variance (ANOVA) using Statistica 10.0 (StatSoft Inc., 2007, USA). Differences between the means were considered significant when the confidence interval was less than 5% ($P \leq 0.05$).

3. Results and discussion

It was established [25, 45, 46, 47] that immediately after the irradiation, there was no effect on the accumulation of total phenolic compounds. Moreover, in the first hours after the irradiation, even a decrease could be observed both for antioxidant activity and the content of individual antioxidant substances. Santin et al. [47] assumed that in the first hours after the UV-B treatment, phenolic compounds already present in the tissues of tomato peel began to break down, a decrease in the amount of total phenolic compounds occurred 24 h after the treatment. However, their number increased 36 h after the irradiation, explained by the expression of genes responsible for the production of metabolites. Radiation activated specific reactions and after that, newly synthesized metabolites began to accumulate. Metabolic changes became noticeable 36 h after the treatment. During the post-irradiation storage of tomatoes (up to 30 days), the content of antioxidants in the ripe red tomatoes

decreased, however, mature green tomatoes showed the accumulation of substances responsible for the color. Since we used red tomatoes in our work, we decided not to consider the dynamics of their antioxidant characteristics during the post-irradiation storage (it is not necessary to wait for the increase of these characteristics). On the other hand, after the irradiation, it was necessary to wait until the accumulation process started. Analyzing the available works of different authors, the process was stopped after 36 h.

The ripe red tomatoes were irradiated with ultraviolet radiation (wavelength 353 nm, 365 nm and 400 nm) for 10 min, 180 min and 360 min to examine the markers of antioxidant activity dynamics (Tables 1 and 2, Figure 1). The content of phenolic compounds and the total content of chlorophylls, carotenoids, flavonoids; content of individual compounds (lycopene, lutein, β -carotene); antioxidant enzyme activity (catalase, peroxidase, superoxide dismutase, polyphenoloxidase, phenylalanine ammonia-lyase) were determined 36 h after the irradiation.

Increased UV-A irradiation time within the considered wavelength range (353 nm, 365 nm and 400 nm) increased the overall content of phenolics, carotenoids, and flavonoids in ripe tomatoes. There were practically no changes in the content of phenolic compounds, as well as in the total content of chlorophylls, carotenoids, flavonoids of different modes of irradiation lasting for 10 min ($P = 0.982$ – 0.997). The change in the values of the total chlorophyll and carotenoid content was also not significant in the case of irradiation for 180 min at a wavelength of 353 nm and 400 nm ($P = 0.889$ – 0.919). It should be emphasized that at the emission wavelength of 365 nm, the average chlorophyll content increase reached 7.98–19.02 %, 2.02–15.00 % and 6.98–14.97 % for 'Budenovka', 'Bull Heart', and 'Gina' varieties, accordingly.

The total content of phenolic compounds in tomatoes irradiated at a wavelength of 353 nm for 360 min has increased by 13.0–18.0 % compared to the control, when irradiated at a wavelength of 365 nm, it went up by 42.9–55.0 % when irradiated at a wavelength of 400 nm – by 15.0–24.0 %. A similar trend was observed for the content of carotenoids and flavonoids. Carotenoid content in fresh red-ripe tomatoes irradiated at a wavelength of 353 nm for 360 min increased by 6.0–11.0 % compared to the control, when irradiated at a wavelength of 365 nm, it rose by 24.0–56.0 %, when irradiated at a wavelength 400 nm – by 14.0–16.0 %. Flavonoid content in fresh tomatoes irradiated at a wavelength of 353 nm for 360 min increased by 9.5–13.0 % compared to the control, when irradiated at a wavelength 365 nm – by 28.0–33.0 %, when irradiated at a wavelength 400 nm – by 15.0–22.0 %.

After the UV-A irradiation, the content of individual antioxidant compounds (β -carotene, lycopene, lutein) increased in the samples, while the indicator reached its peak at a wavelength of 365 nm (Table 2). Significant changes ($p < 0.05$) against the control were observed for the whole group of studied antioxidant substances subjected to irradiation for at least 180 min at a wavelength of 365 nm, and up to 360 min at a wavelength of 400 nm only for β -carotene and lutein. After 360 min of UV radiation at a wavelength of 365 nm, β -carotene increased by 70.9–71.6 % vs. the original, lycopene – by 62.6–69.0 % and lutein – by 64.8–72.0 %.

Ripe tomato irradiation led to an increase of antioxidant enzyme activity regardless of the wavelength (Figure 1). After 360 min ($p < 0.05$) of exposure, catalase activity increased by 20.0–34.7 % on average, peroxidase activity – by 18.2–34.8 %, polyphenol oxidase activity – by 23.8–38.1 %, superoxide dismutase activity – by 23.8–42.7 %, and phenylalanine ammonia-lyase activity – by 27.6–60.5 %.

In conclusion, the UV irradiation did not show any effect on the physical and chemical properties (Figures 2, 3, and 4) of ripe tomatoes of the studied variety (the color, texture, size, titratable acidity and the amount of soluble solids in the tomato).

Changes in the magnitude of firmness and the content of total soluble solids for all three varieties of ripe tomatoes irrespective of continued irradiation was not significant ($p > 0.984$) without exceeding 5.0 % and 1.0 % of the initial value. Changes in the magnitude of titratable acid

composition was to 0.5 g/l and was primarily due to oxidative processes in the tomato samples instead of radiation. Prolongation of UV irradiation of ripe tomatoes was uniquely reflected in the Hunter value – parameter h ($p < 0.05$). For the 'Budenovka' variety, average deviation from the initial value ranged from 3.8 % to 9.6 %, for 'Bull Heart' it was 4.5 %, for 'Gina' – from 2.9 % to 11.4 %, the obtained values corresponded to one segment of the spectrum.

Similar results were obtained by other authors who studied the effect of UV-irradiation of other spectra (UV-B and UV-C-irradiation) on antioxidant qualities of tomatoes [25, 27, 45, 46, 48, 49, 50, 51, 52]. The cumulative undisturbed antioxidant activity of the samples' compounds was established with a significant increase in some of them. As the works of other authors show, green vegetables need time counted in days. Antioxidant characteristics improve along with the softening of irradiated samples. A similar picture is observed without irradiation, but at a slower rate. Antioxidant substances with a positive accumulation dynamics are determined primarily by the genetic characteristics of selected tomato varieties, climatic and geographical conditions of growth, ripeness level, and only then – by the length, duration of ultraviolet irradiation and storage conditions. There was a common trend in the activity of antioxidant enzymes (catalase, peroxidase, polyphenol oxidase, superoxide dismutase, phenylalanine-ammonium-lyase) for tomato samples with a different duration of irradiation. All irradiated samples demonstrated growth in this respect, especially after more than 3 h of treatment.

Irradiation of ripe red tomatoes led to a rather rapid increase in antioxidant activity (36 h) and the need for their rapid processing or consumption. Thus, the rate of antioxidant compound accumulation after irradiation is inversely proportional to the degree of maturity of the vegetable.

The studies [25, 27, 45, 46, 47, 48, 49, 50, 51, 52] of UV-B and UV-C irradiation of tomatoes during storage (up to 30 days) did not show visible physical damage to the samples. In our work, we used safer UV-A irradiation, therefore, the study of this aspect with long-term storage looks inexpedient. Physicochemical parameters of irradiated tomatoes were also evaluated 36 h after treatment. Similar results were obtained in our research. Therefore, the applied irradiation (regardless of the wavelength) did not affect the quality characteristics of tomatoes.

4. Conclusions

Vegetables and fruit are traditionally considered the sources of nutrients, vitamins and antioxidants. After UV-A-irradiation, tomatoes grown on the territory of the Russian Federation (Krasnodar region) retained many of the physicochemical properties (total solubility of solids, pH, color, titrated acidity) with a non-negative dynamics of aggregate antioxidant characteristics. Post-harvest UV treatment at a wavelength of 365 nm had a significant positive effect on the content of biologically active compounds (lycopene and common phenolic compounds) and the antioxidant activity of components of red-ripe tomato samples. Under the irradiation conditions lasting for not less than 180 min, UV-A radiation produced a safe effect that stimulated the accumulation of photosynthetic pigments, particularly carotenoids, activated the antioxidant enzyme activity and induced the synthesis of flavonoids. Stimulation of the antioxidant enzyme activity (catalase, peroxidase, polyphenol oxidase, superoxide dismutase, phenylalanine ammonia-lyase) indicated highly efficient antioxidant system in fresh vegetables. Increased antioxidant activity of the processed ripe red tomatoes (365 nm, 360 min) persisted for 2–3 days after the treatment. As soon as this period ends, antioxidant characteristics will not exceed the original ones. The obtained regularities can become the basis for further studies of the effect of UV-A-irradiation on the quality characteristics of other fruit and vegetables grown on the territory of this country, and the development of technologies to increase the antioxidant activity of fresh fruit and vegetables.

Declarations

Author contribution statement

Lyubov Dyshlyuk: Conceived and designed the experiments; Analyzed and interpreted the data.

Olga Babich: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Alexander Prosekov, Svetlana Ivanova, Valery Pavsky: Analyzed and interpreted the data; Wrote the paper.

Tatiana Chaplygina: Performed the experiments; Analyzed and interpreted the data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

Chemical compounds studied in this article: Catalase (PubChem CID: 8434); Peroxidase (PubChem CID: 86062779); Superoxide dismutase (PubChem CID: 72941490).

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