Study of the Antioxidant Potential of UV-Treated Vegetables

Svetlana Ivanova 1,2,* and Alexander Prosekov 3

1 Natural Nutraceutical Biotesting Laboratory, Kemerovo State University, Krasnaya Street 6, 650043 Kemerovo, Russia
2 Department of General Mathematics and Informatics, Kemerovo State University, Krasnaya Street 6, 650043 Kemerovo, Russia
3 Laboratory of Biocatalysis, Kemerovo State University, Krasnaya Street 6, 650043 Kemerovo, Russia
* Correspondence: pavvm2000@mail.ru; Tel.: +7-384-239-6832

Abstract: The effect of UV-A irradiation (353 nm, 365 nm, and 400 nm) on the antioxidant properties of fresh vegetables (cucumbers, bell peppers, zucchinis) was investigated. This type of processing was found to increase the total content of phenolic compounds and flavonoids in vegetables. With a UV irradiation of 360 min at a wavelength of 365 nm, the greatest increase in antioxidant activity occurred in vegetable samples vs. untreated control samples. The total content of phenolic compounds increased by 34–58% and the content of flavonoids by 26–53% for various vegetable varieties. There was an increase in the activity of antioxidant enzymes (catalase up to 86%, peroxidase from 38%, polyphenol oxidase up to 74% depending on the variety of vegetables) after 360 min of exposure (p < 0.05). The results of the conducted studies indicate that post-harvest ultraviolet irradiation of vegetables has the potential to control the antioxidant characteristics of vegetables; however, additional research is needed to form a complete mechanism of this effect and create a technology for vegetable processing.

Keywords: cucumbers (Cucumis sativus); bell pepper (Capsicum annuum); zucchinis (Cucurbita pepo); UV-A-radiation; antioxidant activity; phenolic compounds

1. Introduction

Studies on the prevention of early aging of the human body caused by an excess of free radicals in biological fluids have expanded in recent decades. An excess of free radicals leads to pathological changes in the human body and the emergence of dangerous (cardiovascular, oncological, etc.) diseases [1]. Radicals interact especially actively with membrane lipids containing unsaturated bonds and change the properties of cell membranes. A natural antioxidant system containing enzymatic and non-enzymatic substances protects a healthy body from the effects of free radicals [2]. A decrease in the activity of the natural human antioxidant system and, consequently, an increase in the concentration of free radicals in the body is associated with many adverse factors (radioactive and ultraviolet radiation, environmental degradation, alcoholism, smoking, drug addiction, constant stress, consumption of contaminated food, uncontrolled intake of certain drugs, etc.).

The harmful effects of free radicals in the case of oxidative stress can be reduced by regular consumption of certain foods, drinks, drugs, and dietary supplements with antioxidant activity. Plant products rich in polyphenols, vitamins, carotenoids, and other antioxidants are the most promising for improving the antioxidant status of the human body because of their widespread distribution, availability, valuable properties, gentle effect on the body, and low toxicity [3].

Vegetables, such as cucumbers, capsicum (especially sweet) peppers, and zucchinis, require the least amount of care and are grown and eaten in many countries. These vegetables have beneficial properties for human health and contain a large number of substances with antioxidant activity [4]. Individual biologically active substances accumulate in vegetables during their ripening; however, there are ways to accelerate the production of...
antioxidants using various technologies, as well as slow down the aging of vegetables during transportation and storage [5–7]. One of these technologies is ultraviolet or LED irradiation [8,9]. There are approaches to the irradiation of both the plants themselves in the process of growth and their fruit [10–12] to increase the antioxidant activity of vegetables, enhance their palatability, and preserve their structure. However, when using UV light to process fruits and vegetables, several factors must be strictly observed, including the type of product, its maturity, variety, spectral region of UV radiation, dose, intensity, and irradiation scheme, since failure to comply with these parameters will lead to overheating, damage, and burns of fruits and vegetables, and the biosynthesis of pigments, vitamins, polyphenolic compounds, flavonoids, and other biologically active substances (BAS) can be reduced. Prolonged UV irradiation causes negative changes at the DNA level in biologically active substances in fruits and vegetables [13].

Ultraviolet (UV) radiation includes the region of the electromagnetic (EM) spectrum between visible light and X-rays (100–400 nm) and can affect vegetables and fruits at the cellular level. Excessive irradiation through the effects of oxidation processes promotes the degradation of molecules due to the oxidation of membrane lipids and inhibition of important cellular enzymes. This effect is associated with a difference in the structure of the cell wall peptidoglycan, which can affect the penetration of radiation. This process is affected by the complexity and genetic usefulness of cells and the low content of pyrimidine in DNA, which increases the probability of absorption of photons [14].

The content of antioxidants in fresh vegetables strongly depends on the external conditions of cultivation and storage; therefore, research aimed at developing methods for managing the biologically active characteristics of fresh vegetables is relevant. In this study, we investigated the influence of the intensity of ultraviolet irradiation of fresh vegetables (cucumbers, bell peppers, and zucchinis) available to the general population in Russia on their antioxidant properties.

The scientific novelty of the study lies in the fact that the accumulation of phenolic compounds and, in particular, flavonoids, in the tissues of cucumbers, bell peppers, and zucchinis exposed to UV radiation was investigated. The key parameters leading to an increase in the antioxidant activity of vegetables (vegetable type, variety, spectral range of UV radiation, dose, intensity, and irradiation scheme) were determined. It was established that UV irradiation causes a significant accumulation of phenolic and flavonoid compounds in vegetables, which makes it possible to achieve high antioxidant activity values. It was hypothesized that the increased bioavailability of these compounds triggers a number of protective reactions. These include the activation of glucanases and chitinases, which are involved in the binding of free radicals, which causes the induction of genes associated with the biosynthesis of phenolic compounds, such as phenylalanine ammonia-lyase, polyphenol oxidase, and peroxidase. A significant increase in antioxidant activity was expected due to the inactivation of genes encoding antioxidant enzymes, such as superoxide dismutase [13].

2. Materials and Methods

2.1. Materials and Chemicals


Cucumbers of medium size (length 8–9 cm, weight 85–95 g) were chosen with a smooth, bright green skin with moderate spotting. Bell peppers were of medium shape (90–150 g) and a color from yellowish to red, depending on the variety. The zucchinis were 20–25 cm, weighing 500–600 g, and the skin was smooth, dense, and slightly ribbed. Depending on the variety, the vegetables were golden, pale cream, or dark green with light green stripes.

Reagents and chemicals of analytical or higher grade purchased from Fluka/Sigma-Aldrich (Sigma-Aldrich Rus, Moscow, Russia) were used in the study.
2.2. Sample Preparation

Fresh cucumbers, peppers, and zucchini were treated with UV-A lamps at room temperature, then the antioxidant activity of the obtained samples was measured. The smallest penetration through cell membranes, and consequently the least negative impact on the objects of biological nature \cite{10,15} became the main factors in choosing long-wave radiation as an impact in our study. UV-radiation lamps were placed at a distance of 0.5 m from the studied objects. During the irradiation of the objects, the illumination intensity was 15–20 klx. A total of 36 vegetables of each variety were processed in each series. Non-irradiated fresh vegetables of the appropriate variety were considered as a control.

The dimensions of the UV-A chamber for processing vegetables were as follows: width 700 mm, depth 580 mm, height 732 mm. To evenly distribute the radiation dose, the vegetables were placed one by one under a UV-A tubular lamp Sylvana F15W/T8/BL350 (Osram Sylvania, Wilmington, MA, USA), Philips TLD 15W/05, TLD 15W/03 (Signify Philips, Eindhoven, The Netherlands) with a nominal power of 15 watts and a radiation spectrum of 315–400 nm, 315–460 nm, and 380–470 nm. UV radiometer TKA-PKM 12 (TKA, Moscow, Russia) calibrated at 353 nm, 365 nm, and 400 nm was used to measure the intensity of ultraviolet radiation. UV treatment was carried out continuously (10 min, 180 min, and 360 min) and the UV radiation doses were 0.33, 0.28, and 0.28 W m\(^{-2}\), respectively. Before the study of physicochemical characteristics, controls and processed samples of vegetables (cucumbers, bell peppers, and zucchini) were kept in the refrigerator at a temperature of 4 \(^\circ\)C for 36 h. Next, all vegetable samples were sliced and homogenized using an Omni mixer (Ultra-Turrax T25, Staufen, Germany) and stored at \(-80\ \degree\)C until the analysis. The choice of parameters was based on previous studies \cite{10}.

2.3. Total Phenolic and Flavonoid Contents

The colorimetric method and a method using chemical reagents \cite{10,16} were used to determine the total content of phenolic in vegetable samples. The samples were hydrolyzed. A total 1 mL of 1M HCl was added to 2 g of the sample and shaken for 1 min, then incubated at 37 \(^\circ\)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 \(^\circ\)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.

Flavonoids were isolated and studied by the Mirecki and Teramura method, similar to \cite{17}. A total of 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 \(^\circ\)C to extract flavonoids and light absorption was measured using a UV-1280 spectrophotometer (Shimadzu, Kyoto, Japan) at a wavelength of 315 nm.

2.4. Antioxidant Enzyme Activity

Measured by the method described in \cite{10}, catalase activity was determined (CAT, EC 1.11.1.6). Homogenization of the sample (1 g) was carried out in 10 mL of sodium phosphate buffer (0.1 mM) at pH 7 and centrifuged for 10 min (10,000 \(\times\) g) at a temperature of 4 \(^\circ\)C. To the reaction mixture containing 0.01 M H\(_2\)O\(_2\) (1 mL), 3 mL 0.1 M sodium phosphate buffer (3 mL), an aliquot of the enzyme extract supernatant liquid (1 mL), pH 6.8 was added. Incubated at 20 \(^\circ\)C, 5 min, with the addition of 10 mL H\(_2\)SO\(_4\) (1%), the reaction was stopped. Acidified medium with and without the enzyme extract was titrated with 0.005N KMnO\(_4\).

The activity of peroxidase (POX, EC 1.11.1.7) and superoxide dismutase (SOD, EC 1.15.1.1) was measured in the same way as earlier in our study \cite{10}. The sample (1 g) was homogenized in 20 mL of a cold extraction buffer containing MgCl\(_2\) (2M), EDTA (1 mM), \(\beta\)-mercaptoethanol (10 mm), PVP (7%), and sodium metabisulfite (10 mm). The homogenate was filtered through a double-layer cheesecloth, then centrifuged (10,000 \(\times\) g) for 5 min.
The mixture obtained from the supernatant liquid by bringing up to 20 mL with the buffer described above was used as an enzyme source.

### 2.5. Statistical Analysis

Each experiment was repeated at least three times and the data are represented by the sum of mean and permissible deviation. The homogeneity of the sample distribution was tested using the Fisher and Student’s criteria. The analysis of variance (ANOVA) was carried out using Statistica (StatSoft Inc., 10.0, 2007, Tesla, WV, USA). The differences between the samples were considered significant with $p < 0.05$.

### 3. Results

Natural phenols are compounds containing a phenolic group. This class includes monodiphenols, phenolic acids, lipidic phenols, polyphenols, and in particular flavonoids [18].

Ripe fresh vegetables (cucumbers, bell peppers, zucchini) were irradiated with UV-A radiation for 10 min, 180 min, and 360 min to study markers of antioxidant activity dynamics. The content of phenolic compounds and the total content of flavonoids, and the activity of antioxidant enzymes were determined 36 h after irradiation (Tables 1–3, and Figures 1–3).

#### Table 1. Dependence of the total content of phenolic compounds and in particular flavonoids in cucumber samples of different varieties (A—‘Erofei’, B—‘Rodnichek’, C—‘Makhaon’) subjected to UV-A irradiation of different intensities.

<table>
<thead>
<tr>
<th>Operation Modes</th>
<th>Total Content of Phenolic Compounds (mg/kg)</th>
<th>Total Content of Flavonoids (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A (mg/kg)</td>
<td>B (mg/kg)</td>
</tr>
<tr>
<td>Control 10 min</td>
<td>267 ± 19 a</td>
<td>245 ± 17 a</td>
</tr>
<tr>
<td>Control 180 min</td>
<td>277 ± 20 b</td>
<td>252 ± 18 b</td>
</tr>
<tr>
<td>Control 360 min</td>
<td>285 ± 20 b</td>
<td>256 ± 18 b</td>
</tr>
</tbody>
</table>

The data are represented by the sum of the mean and standard deviation ($n = 3$). Values in the rows followed by different-case letters differed significantly ($p < 0.05$) according to the results of the post-hoc test.

#### Table 2. Dependence of the total content of phenolic compounds and in particular flavonoids in bell pepper samples of different varieties (A—‘Fidelio’, B—‘Rapsodia’, C—‘Atlantik’) subjected to UV-A irradiation of different intensities.

<table>
<thead>
<tr>
<th>Operation Modes</th>
<th>Total Content of Phenolic Compounds (mg/kg)</th>
<th>Total Content of Flavonoids (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A (mg/kg)</td>
<td>B (mg/kg)</td>
</tr>
<tr>
<td>Control 10 min</td>
<td>571 ± 10 a</td>
<td>552 ± 39 a</td>
</tr>
<tr>
<td>Control 180 min</td>
<td>593 ± 42 a</td>
<td>578 ± 41 b</td>
</tr>
<tr>
<td>Control 360 min</td>
<td>620 ± 44 b</td>
<td>594 ± 42 b</td>
</tr>
</tbody>
</table>

The data are represented by the sum of the mean and standard deviation ($n = 3$). Values in the rows followed by different-case letters differed significantly ($p < 0.05$) according to the results of the post-hoc test.
Figure 1. Dynamics of activity of antioxidant enzymes (catalase, * micromoles of decomposed H$_2$O$_2$ min$^{-1}$·g$^{-1}$ of vegetable weight) of fresh (a) cucumbers, (b) bell peppers, (c) zucchini of different varieties (on average for each variety): 1—‘Erofei’; 2—‘Rodnichek’; 3—‘Makhaon’; 4—‘Fidelio’; 5—‘Rapsodia’; 6—‘Atlantik’; 7—‘Golda’; 8—‘Gorniy’; 9—‘Zebra’. Values followed by different-case letters differed significantly ($p < 0.05$) according to the results of the post-hoc test.
Figure 2. Dynamics of activity of antioxidant enzymes (peroxidase, * micromoles of decomposed H$_2$O$_2$·min$^{-1}$·g$^{-1}$ of vegetable weight) of fresh (a) cucumbers, (b) bell peppers, (c) zucchinis of different varieties (on average for each variety): 1—‘Erofei’; 2—‘Rodnichek’; 3—‘Makhaon’; 4—‘Fidelio’; 5—‘Rapsodia’; 6—‘Atlantik’; 7—‘Golda’, 8—‘Gorniy’, 9—‘Zebra’. Values followed by different-case letters differed significantly ($p < 0.05$) according to the results of the post-hoc test.
Figure 3. Dynamics of activity of antioxidant enzymes (polyphenoloxidase, * micromoles of decomposed \( \text{H}_2\text{O}_2 \cdot \text{min}^{-1} \cdot \text{g}^{-1} \) of vegetable weight) of fresh (a) cucumbers, (b) bell peppers, (c) zucchini of different varieties (on average for each variety): 1—’Erofei’; 2—’Rodnichek’; 3—’Makhaon’; 4—’Fidelio’; 5—’Rapsodia’; 6—’Atlantik’; 7—’Golda’, 8—’Gorniy’, 9—’Zebra’. Values followed by different-case letters differed significantly \( (p < 0.05) \) according to the results of the post-hoc test.
Table 3. Dependence of the total content of phenolic compounds and in particular flavonoids in zucchini samples of different varieties (A—‘Golda’, B—‘Gorniy’, C—‘Zebra’) subjected to UV-A irradiation of different intensities.

<table>
<thead>
<tr>
<th>Operation Modes</th>
<th>Total Content of Phenolic Compounds (mg/kg)</th>
<th>Total Content of Flavonoids (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Control</td>
<td>222 ± 16 a</td>
<td>226 ± 16 a</td>
</tr>
<tr>
<td>10</td>
<td>230 ± 16 a</td>
<td>242 ± 17 a</td>
</tr>
<tr>
<td>365</td>
<td>255 ± 18 b</td>
<td>262 ± 18 b</td>
</tr>
<tr>
<td>400</td>
<td>235 ± 16 a</td>
<td>246 ± 17 a</td>
</tr>
<tr>
<td>180</td>
<td>242 ± 17 a</td>
<td>253 ± 18 b</td>
</tr>
<tr>
<td>365</td>
<td>286 ± 20 b</td>
<td>289 ± 20 b</td>
</tr>
<tr>
<td>400</td>
<td>250 ± 18 a</td>
<td>257 ± 18 b</td>
</tr>
<tr>
<td>360</td>
<td>246 ± 17 a</td>
<td>260 ± 18 b</td>
</tr>
<tr>
<td>365</td>
<td>337 ± 23 c</td>
<td>330 ± 23 c</td>
</tr>
<tr>
<td>400</td>
<td>259 ± 18 b</td>
<td>264 ± 19 b</td>
</tr>
</tbody>
</table>

The data are represented by the sum of the mean and standard deviation (n = 3). Values in the rows followed by different-case letters differed significantly (p < 0.05) according to the results of the post-hoc test.

4. Discussion

When studying the effect of UV intensity on the antioxidant activity of fresh vegetable samples, it was found that an increase in the duration of treatment in all series of experiments led to an increased accumulation of the total content of phenolic compounds in samples of cucumbers, bell peppers, and zucchini. After a 10-min treatment of vegetables, no significant differences were found in the samples either in the content of phenolic compounds or flavonoids (p = 0.979–0.998).

The best indicators of antioxidant activity in vegetable samples were achieved at a wavelength of 365 nm. Depending on the variety, the average increase in the content of phenolic compounds was 45–55%, 34–58%, 55–58%, and 46–52% in samples of cucumbers, bell peppers, and zucchinis, respectively. Flavonoid content increased 28–33%, 29–38%, 28–53%, and 26–38% for tomato, cucumber, bell pepper, and zucchini samples, respectively.

Irradiation of ripe fresh vegetables (cucumbers, bell peppers, zucchinis) led to an increase in the activity of antioxidant enzymes in the samples, regardless of the wavelength (Figures 1–3). Catalase activity increased by 77–86%, 25–80%, 3–34%, and 30–34%, peroxidase activity increased by 9–23%, 30–31%, 33–38%, and 30–35%, and polyphenol oxidase activity increased by 14–35%, 24–29%, 28–74%, and 36–44%, respectively, after 360 min of exposure (p = 0.038–0.006). It is assumed that an increase in the content of phenolic compounds and, in particular, flavonoids, exposed to UV light resulted from an increase in their bioavailability due to the destruction of biopolymer macromolecules and sulfated compounds, which significantly increased their biological activity [19]. The expression of genes responsible for the production of phenolic compounds and, in particular, flavonoids, was manifested. UV radiation activated specific reactions, after which the accumulation of newly synthesized metabolites began.

Immediately after irradiation no significant effect on the accumulation of total phenolic compounds was found [20–22]. In the first hours after UV-B irradiation, in some cases, there was a decrease in both antioxidant activity and the content of individual antioxidant substances in fresh tomato samples. Up to 24 h after treatment, the total amount of phenolic compounds in the samples decreased; however, 36 h after irradiation, their amount increased. Radiation activated specific reactions (expression of genes responsible for the production of metabolites), and after that, newly synthesized metabolites began to accumulate. The antioxidant content of ripe red decreased during storage after irradiation (for up to 30 days), whereas mature green showed an accumulation of color-producing compounds.
Unripe tomatoes were exposed to UV-B radiation at doses of 10, 20, 40, and 80 kJ/m² in [23]. The best results were obtained at radiation doses of 20 and 40 kJ/m². Irradiation contributed to the accumulation of the total amount of phenols and flavonoids, and increased antioxidant activity. The maximum dose of UV-B irradiation (80 kJ/m²) contributed to the accumulation of lycopene but had a negative effect on the structure of the vegetables and other antioxidant components. In [24], tomatoes (variety 'Raf', chilled, after harvest) were treated with UV-A irradiation (wavelength 366 nm), UV-C irradiation (wavelength 254 nm) for 5 h, red-blue LED (light efficiency 25.4 µmol/m²/s) for 7 days at 6 °C. As a result, no significant changes in antioxidant activity were observed in tomato samples after UV irradiation. LED showed the best results (b-carotene increased 2 times, lycopene—14 times, total carotenoids—5 times, total phenolic compounds—1.2 times). On day 7 after UV-A irradiation, the content of total phenolic compounds in the samples decreased by 22%.

The study in [25] described the results of irradiation of different wavelengths (385 nm, 445 nm, 630–670 nm, and 730 nm) of cucumber seedlings (vegetables and leaves, variety Gribovchanka) for 50 days. The content of carotenoids and chlorophyll increased in the samples with a combination of irradiation technologies. Yellow bell peppers [26] were irradiated with UV-C (2.2, 4.4, 6.6 kJ/m²) at 12 °C for 15 days. When treated with UV-C irradiation (6.6 kJ/m²), the amount of carotenoids and flavonoids and the total antioxidant activity increased. The study in [27] investigated the possibility of irradiating lettuce, spinach, cabbage, basil, and bell pepper with various combinations of red and blue light (83/17; 91/9; 95/5). The highest average content of carotenoids increased in lettuce and spinach (irradiation 94/9), in cabbage, basil, and pepper (irradiation 83/17), and in antioxidant activity of spinach, lettuce, cabbage (83/17), basil, pepper (91/9). In [28], zucchinis were treated with UV-A irradiation (360 nm) for 6 h, and an additional temperature effect of 65 °C for 3 min. As a result, the content of individual antioxidant compounds (carotenoids, anthocyanins, flavonoids, β-carotene, lycopene, lutein) increased.

5. Conclusions

Plant foods, especially vegetables and fruit, are traditionally regarded as sources of nutrients, vitamins, and antioxidants. After UV-A irradiation, vegetable samples (cucumbers, bell peppers, and zucchinis grown in the Russian Federation) showed a positive trend in the total antioxidant characteristics. Our data showed that the post-harvest UV-A irradiation of vegetables increased the nutritional value of the samples by increasing the total content of phenolic compounds and flavonoids. Antioxidant enzymes were activated by post-harvest UV exposure at a wavelength of 365 nm for at least 180 min, confirming the high effectiveness of the antioxidant system of fresh vegetables. The increased antioxidant activity of the treated vegetables of ripe cucumbers, bell peppers, and zucchinis (365 nm, 360 min) persisted up to 3 days after treatment. The patterns obtained require further studies of the effect of UV-A irradiation on other qualitative characteristics of both the same vegetables and other fruit in order to develop technologies for increasing the antioxidant activity of fresh fruits and vegetables.

Author Contributions: Conceptualization, S.I.; methodology, S.I. and A.P.; analysis and interpretation of the data, S.I.; formal analysis, A.P.; writing—original draft preparation, A.P.; writing—review and editing, S.I.; project administration, A.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Prioritet 2030 program of Kemerovo State University.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are contained within the article.

Conflicts of Interest: The authors declare no conflict of interest.