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The Influence of Ozonation Carried Out during Vegetation on the Content of Selected Bioactive Phytochemicals and the Microbiological Load of Tubers of *Raphanus sativus* var. *sativus*

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Abstract: The aim of the study was to determine the effect of ozone gas fumigation on the mechanical, chemical, and microbiological parameters of radish tubers. Radish plants were grown in the ground in accordance with the principles of good agricultural practice and condition suitable for the soil and climatic conditions of south-eastern Poland. At the end of the growing season, 24 h before harvest, radish plants were exposed to a variable factor, i.e., fumigation with ozone gas at various doses (1 ppm for 1 and 5 min; 5 ppm for 1 and 5 min) in order to modify selected metabolic pathways of bioactive compounds. Then, 24 h after ozonation, radish tubers were harvested and placed in a climatic chamber with controlled conditions, i.e., 2 °C and 90% humidity. Laboratory analyses were performed during storage on days 1, 5, and 10. The ozonation used did not significantly improve the mechanical properties and water content of radish tubers. There was a beneficial effect of selected gaseous ozone doses (1 ppm for 1 and 5 min; 5 ppm for 1 min on the 10th day of storage) on the biosynthesis of selected bioactive compounds, i.e., ascorbic acid content, total polyphenol content, and antioxidant potential during storage. The most beneficial effects of the use of gaseous ozone were observed in the storage process in reducing the microbiological load of radish tubers. Among the ozonation doses used, the dose of 5 ppm for 5 min had the most beneficial effect on reducing the microbiological load. It reduced the number of yeasts and molds by 14.2% and aerobic mesophilic bacteria by 20.9% compared to the control sample on the last day of storage. Additionally, between the 5th and 10th day of storage, a significant effect of each ozone dose applied on reducing the occurrence of yeasts, molds, and mesophilic aerobic bacteria during tuber storage was noted.

Keywords: vegetables; radish; ozone; phytochemicals; microbial load

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1. Introduction

Radish (*Raphanus sativus* L.) is a plant from the Mediterranean region [1]; it belongs to the Brassicaceae family [2], just like cauliflower, oil radish, and cabbage. Mature plants have a small size, tuberous roots, and green leaves [3]. *Raphanus sativus* (*R. sativus*) tubers have culinary and medicinal uses. They are eaten raw or after heat treatment. Usually, raw radishes are used as an addition to many dishes [4]. Worldwide production of radish is approximately 7 million tons per year [5]. Japan is one of the largest radish producers in the world [6,7]. Radish cultivation depends largely on soil and climatic conditions suitable for optimal growth and development [8].

Raphanus sativus plants contain large amounts of saponins, flavonoids, polyphenols, glycosides, essential oils, vitamin A, and vitamin C. Due to their rich chemical composition and numerous related properties, these plants have been widely used in medicine [9]. Radish has a number of scientifically proven health-promoting properties. *Raphanus sativus* juice is used to treat coughs, rheumatic arthritis, and gallstones. Moreover, it has a diuretic effect [10]. In Arabic and Indian folk medicine, *R. sativus* was used to treat many diseases, such as jaundice, gallstones, liver diseases, indigestion, and stomach pain. The leaves, which are usually discarded, have strong antioxidant properties because they contain 10 times more vitamin C than the tubers [11]. In addition, the leaves and tubers have been used to treat infections with intestinal parasites. The properties of the tubers are responsible for antiscorbutic, antispasmodic, choleretic, and digestive properties. This plant contains raphanin, which has bactericidal and antifungal properties. It inhibits the growth of Staphylococcus aureus, *E. coli*, and streptococci. *R. sativus* is rich in antioxidants and minerals, such as calcium and potassium. However, raphanin is found in the internal tissues of the plant and, therefore, its effect on the plant surface is weakened [12]. These ingredients help lower high blood pressure and reduce the risk of heart disease. Additionally, *R. sativus* contains a large quantity of glucose–nolates–sulfur compounds that protect cells against mutations that cause cancer [13,14].

Plant production depends primarily on soil and climatic conditions and the agrotechnical treatments used. In order to obtain good quality raw material, constant conditions must be maintained during the vegetation process [15]. Moreover, there are many biotic and abiotic factors that influence plant raw materials [16]. Abiotic factors include ozone, which is called an elicitor [17]. Ozonation is a process that has a positive effect on reducing water losses in stored plant raw materials. Moreover, in the case of climacteric fruits, it reduces ethylene release [18–20]. The use of gaseous ozone in a controlled manner induces the plant defense reaction, and in response leads to increased production of bioactive compounds [21]. Ozone, through its oxidizing properties, disinfects the plant raw material, thus, extending its storage life [22]. Another method of improving the quality of plant raw materials is ultraviolet radiation (UV-C). The use of UV-C before harvesting strawberries is treated as an innovative method that leads to an increase in the content of bioactive compounds and resistance to diseases [23]. Moreover, ultraviolet radiation (UV-C) induces defense responses to pathogens in plant crops and is considered a method of disease control that does not pollute the natural environment [24].

As a result of damage caused during harvesting and processing of vegetables, pathogenic microorganisms penetrate inside them, causing putrefactive processes. Losses during storage of vegetables can be reduced by using chemical fungicides, but fungal populations often become resistant to such substances, which limits their effectiveness [25]. Moreover, concerns about the impact of their use residues on human health force the search for alternatives [26]. Taking into account consumer safety and the natural environment, one of the most effective technologies that has already been used in the food industry is ozonation. Moreover, ozone is already used to disinfect water in municipal pipelines, bottled water, sewage, hospitals, aquariums, and equipment used in aquaculture. [27]. In addition, ozone gas is used as an air disinfectant [28]. Ozone in the form of gas or dissolved in water is an antimicrobial agent in the post-harvest treatment of fruits and vegetables [29]. The main purpose of the use of ozone after harvesting vegetables is to inactivate the growth of bacteria [30], prevent fungal decomposition [31], reduce ethylene concentration during fruit storage [32], and destroy pesticides and chemical residues [33]. Ozonation is considered a non-thermal method of food preservation that does not pollute the environment and does not deteriorate its quality parameters [34]. Ozone gas used in low parts of the atmosphere does not pollute the air because it decomposes spontaneously. The effect of air pollution with ozone gas is significant during ozone generation as a result of photochemical smog [35].

Additionally, it has also been shown that ozonation can affect the mechanical properties of plant material [36].

The aim of the study was to determine the possibility of using ozonation to improve the quality of radish tubers grown in the ground, including intensification of the biosynthesis of selected bioactive compounds and mechanical and microbiological properties.

2. Materials and Methods

2.1. Plant Materials and Growth Conditions

2.1.1. Plant Materials in a Field Experiment

In the field experiment, the radish variety Saxa 2 (*Raphanus sativus*) was used for research. It is an early variety, and less than a month passes from sowing the seeds to harvesting. It is recommended for growing in the ground and under cover and is perfect for growing in early spring and autumn.

The field experiment was assumed for the following five variants:

- Control—no variable factors were used.

For the remaining variants, fumigation with ozone gas was carried out in the following doses:

- 1 ppm for 1 min.
- 1 ppm for 5 min.
- 5 ppm for 1 min.
- 5 ppm for 5 min.

2.1.2. Growth Conditions

In the experiment, the forecrop for radish was tomato. In autumn, pH was determined based on a soil sample. It was at a level appropriate for growing this vegetable and amounted to 6.5. Soil analysis showed that the soil's nutrient content was sufficient for growing radish. Plowing was carried out in late autumn, and pre-sowing cultivation was performed in early spring. At the beginning of April, radishes were sown at a depth of 1 cm in rows spaced 20 cm apart. The seeds were sown quite densely, in rows every 3 cm, using a manual precision vegetable seeder. After emergence, the seedlings were interrupted at the second leaf stage. At the end of the growing season, ozonation of plants in the ground was carried out in accordance with the methodology. The tubers were harvested 24 h after the fumigation treatment. The experiment was conducted in south-eastern Poland, Podkarpackie Voivodeship. The soil and climatic conditions at the place where the experiment was conducted (geographic coordinates: 50.03 N, 22.36 E) were suitable for growing radish. The soil and climatic conditions were the same for each variant of the experiment.

2.2. Ozonation of Radish Plants

The research work was preceded by an experimental determination of the plant tolerance threshold for ozonation treatment. For this purpose, plants of the same variety from other batches were exposed to ozone at concentrations of 1, 2, 5, and 10 ppm for 1, 3, 5, and 10 min. Based on preliminary tests, the doses of ozone used in field radish cultivation conditions were selected. A cover (dimensions: 1.2 m length, 0.5 m width, 0.4 m height) was placed over the rows of plants in the form of a foil tent adapted for growing low vegetables. A hose from the Korona L5 ozone generator (Scientific and Implementation Laboratory "Korona" Piotrków Trybunalski, Poland) was connected to the foil tunnel using flexible connections. In the fumigation process of radish plants, the concentration of ozone gas was measured with a 106 M 2B Technologies (Broomfield, CO, USA) ozone detector in the measurement range of 0–1000 ppm (Figure 1). The experiment for each variant was carried out in three independent repetitions. Ozonation was performed once, 24 h before harvesting the tubers. Then, after the fumigation process, the foil tunnels were removed from the plant rows. The next stage was the collection of radish tubers and placing them in conditions appropriate for their storage, i.e., at a temperature of 2 °C and 90% humidity for 10 days.

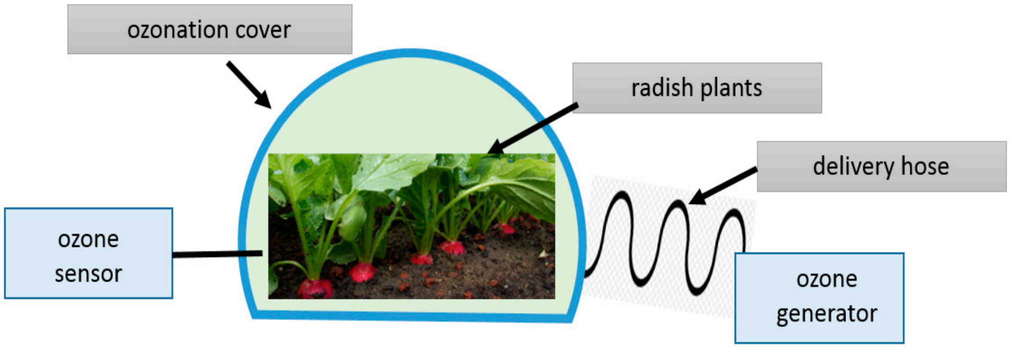

Figure 1. The process of ozonation of radish plants cultivated in the ground using a cover in the form of a foil tunnel.

2.3. Mechanical Properties—Compression Test

The tests for the resistance of radish tuber tissues to mechanical damage in the process of uniaxial compression included oval samples. The tests were carried out on fresh samples of similar size and material exposed to various doses of ozone and stored for 10 days. Measurements were performed in 36 repetitions for each experimental variant. The process of uniaxial compression of the tails was carried out on a Zwick/Roell Z010 testing machine, with the following operating parameters: initial force when compressing the sample F = 0.5 N, module speed during the compression test 0.5 $mm \cdot s^{-1}$.

2.4. Measurement of Water Content

Measurements of the water content of fresh radish tubers and on the 10th day of storage were carried out using a laboratory dryer (SLW 115 SMART). Material samples (cut tubers) were dried at 105 °C until dry mass was obtained. The drying process lasted 18 h.

2.5. Antioxidant Potential, Polyphenol Content, and Vitamin C

The antioxidant potential was determined by the 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) acid method, and the total polyphenol content was determined by the method described by Matłok et al. [37]. Vitamin C was determined using the titration method in accordance with the methodology presented by Matłok et al. [38].

2.6. Microbiological Analyses

Radish tubers were collected for microbiological tests on the 1st, 5th, and 10th day of storage. The numbers of mesophilic aerobic bacteria, mesophilic lactic acid bacteria, and yeasts and molds were determined according to the methodology described in the Supplementary Materials' (File S1) microbiological analyses [39].

2.7. Statistical Analysis

In order to check the significance of the effect of ozone gas on the mechanical, chemical, and microbiological parameters of radish tubers during storage, analysis of variance was used for each measurement with a significance level of $\alpha = 0.05$. These analyses were performed using STATISTICA 13.1 (TIBCO Software Inc., Hillview Avenue, Palo Alto, CA, USA).

3. Results and Discussion

3.1. Measurement of Water Content during Storage

The test results (Figure 2) of radish samples treated with ozone gas and samples not subjected to such treatment and stored in identical conditions (2 °C, 90% humidity) show the effect of the ozone dose on mass loss. The greatest weight loss of the control sample compared to the ozonated variants was observed 24 h after ozonation (water content measurement performed immediately after harvest). Ozonated tubers had a higher water content by approximately 2% compared to the control sample. The difference is statistically insignificant. On the fifth day of storage (120 h), ozonated tubers had a higher water content by approximately 0.5% compared to the control. However, on day 10 (240 h), insignificant differences were observed in the water content in radish tubers of all tested variants.

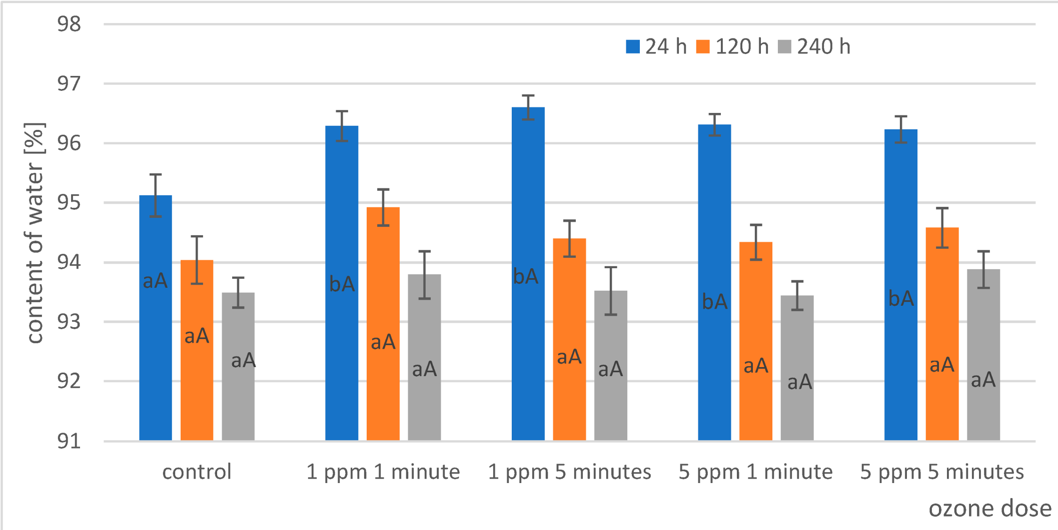

Figure 2. Water content in samples subjected to ozonation and in the control sample on subsequent storage dates of radish tubers (n = 3). Small letters—differences between test dates; capital letters—differences between ozone doses.

Reducing water losses in stored plant materials has also been reported in other studies. In the case of rhubarb [22] and cucumbers [40], a similar relationship was observed as a result of the use of ozone gas. The results of the study report that the use of ozone dissolved in water reduces the decrease in the fresh weight of artichoke heads compared to the control sample [41]. Other research results indicate that ozonation of fennel reduces its weight loss by as much as 47% compared to control during 20 days of storage [42]. Ozone gas with a concentration of 10 ppm applied to green asparagus had a significant impact on reducing weight loss during 30 days of storage of the tested raw materials [43].

3.2. Measurement of Mechanical Properties

The average values of the destructive force in the process of uniaxial compression of radish tubers after the application of ozone at various combinations of dose and exposure time, compared to the control, are shown in Figure 3. For each of the experimental variants, a decrease in the value of the destructive force was observed during the storage period. The largest decreases in destructive force during storage of radish tubers were recorded for the control. In the case of ozonated variants, regardless of the ozone dose, slight decreases in destructive power were observed during 120 h (5 days) of storage. On the fifth day of storage (120 h), the highest average value of the destructive force of 251.8 N was recorded for an ozone dose of 5 ppm for 5 min and was 8% higher than the control. In the last 10 days (240 h) of storage, the plants of the 5 ppm for 1 min (156.33 N) variant had the lowest destructive force, and the tubers ozonated with a dose of 1 ppm for 1 min (220.4 N) had the highest value.

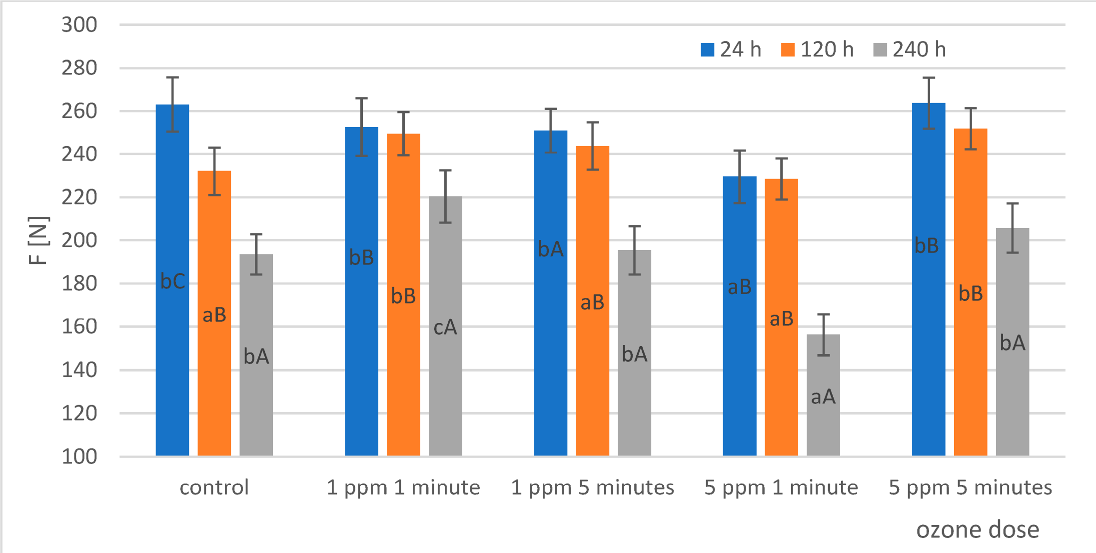

Figure 3. Average values of destructive force for radish tubers depending on the ozone dose used and storage time ($n = 3$). Lowercase letters indicate differences between variants with the test date. Capital letters indicate differences between test dates.

In the case of sea buckthorn fruit, an appropriately selected dose of ozone (10 ppm for 15 min) increased the fruit's resistance to mechanical damage. The use of a shorter ozonation time did not significantly affect the values of the destructive force of the tested raw materials [34]. Horvitz and Cantalejo [44] tested red paprika. They found that the hardness of non-ozonated paprika was lower after 14 days of storage.

3.3. Ascorbic Acid Content, Antioxidant Activity, and Total Phenolic Content

The content of ascorbic acid for each ozone dose tested decreased significantly after 24 h of storage. On the fifth day (120 h) after the ozone fumigation treatment, it was observed that for the ozone doses of 1 ppm for 1 min, 5 ppm for 1 min and 5 ppm for 5 min, an increase in vitamin C content was observed compared to the control. Only for a dose of 1 ppm for 5 min did the ascorbic acid content decrease within this period. On the 10th day of storage, a significant increase in vitamin C concentration was recorded in radish tubers for the ozone dose of 1 ppm for 1 min, 1 ppm for 5 min, and 5 ppm for 1 min compared to the control. For an ozone dose of 5 ppm for 5 min, no beneficial effect of this ozone gas on the vitamin C content in radish tubers was observed (Figure 4).

The process of using ozone gas at low concentrations can increase the ability of fruits or vegetables to synthesize vitamin C. Ozone can also slow down the decline in vitamin C content. The use of ozone gas at concentrations that are too high for a given type of plant raw materials can destroy antioxidant systems, and this also may contribute to the oxidation of vitamin C. Gaseous ozone with low concentrations of 1–6 ppm applied for 15 min significantly increased the content of ascorbic acid in garlic during storage compared to the control sample [45]. Research on artichoke heads after ozonation showed that the vitamin C content in the tested raw material decreased. The observed decrease in ascorbic acid content during cold storage of artichoke heads may be caused by the conversion of ascorbic acid to dehydroascorbic acid (DHAA), which is one of the main mechanisms of detoxification of harmful reactive oxygen species (ROS) [41]. Ascorbic acid is undoubtedly one of the most important antioxidants found in fresh vegetables. Research has shown that the total content of ascorbic acid in parsley and lettuce as a result of the use of ozone gas during storage has a decreasing tendency compared to the control sample [46]. In the case of studies on the effect of ozone gas on the vitamin C content in avocado fruits, significant changes in the concentration of the tested vitamin were noted depending on the ozone dose. Up to the 21st day of storage of the tested fruits, no significant effect of ozone gas on the content of the tested vitamin was observed. However, on day 28, a significant effect of the applied ozone doses on the vitamin C content in the tested fruits was noted compared to the control [47].

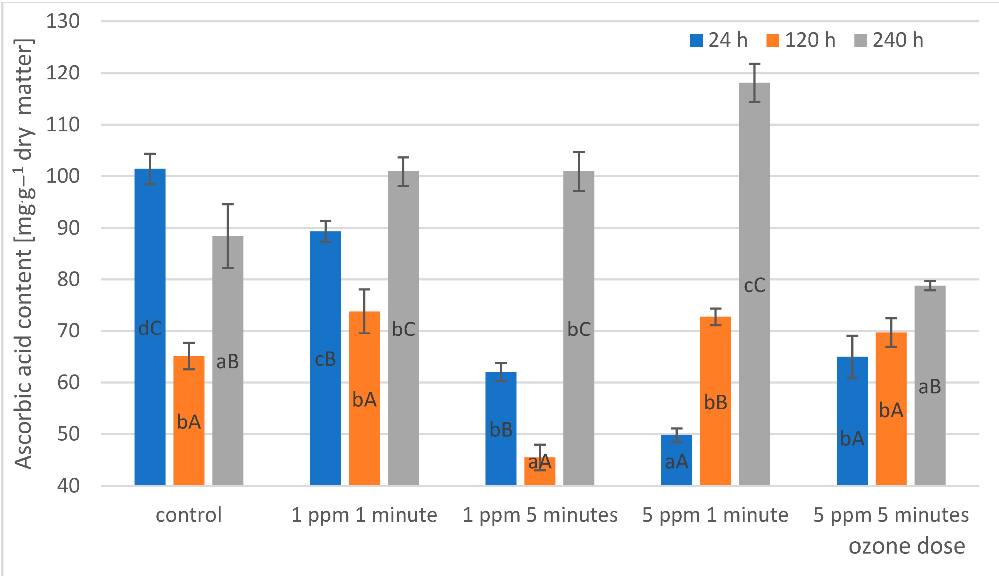

Figure 4. Content of ascorbic acid in radish tubers depending on the ozone dose and storage time (n = 3). Lowercase letters indicate differences between variants with the test date. Capital letters indicate differences between test dates.

The present study showed that selected ozone doses combined with the storage period significantly influenced the antioxidant activity of radish tubers (Figure 5A,B). The most beneficial effect was observed on the 10th day of storage after the ozone fumigation treatment for all ozone doses used. The antioxidant activity of radish tubers detected on that day increased by 7.1–48.2% compared to the control sample, depending on the measurement method used.

The research results indicate that the use of ozone gas under controlled conditions can increase the antioxidant capacity of fruits and vegetables. A good example is seen with pitaya fruits, for which an increase in antioxidant capacity was noted, and their antioxidant activity reached the maximum value after 2 days of storage after the use of ozone gas at concentrations of 2, 4, and 6 mg L^{-1} for 20 min [48]. Further examples of the beneficial effect of ozone gas on antioxidant activity are pineapple [49], tomatoes [50], blackberries, [51] or kiwi [52]. In the case of these studies, an improvement in the quality parameters of the tested raw materials was noted, as exemplified by the increased antioxidant activity of the tested fruits. In the case of vegetables, increased antioxidant activity was also noted as a result of the use of gaseous ozone. Studies of several artichoke varieties showed that the antioxidant activity of samples stored in an ozone atmosphere was higher than the control sample. The results showed that the tested varieties were able to control the resulting oxidative damage caused by ozone exposure [53].

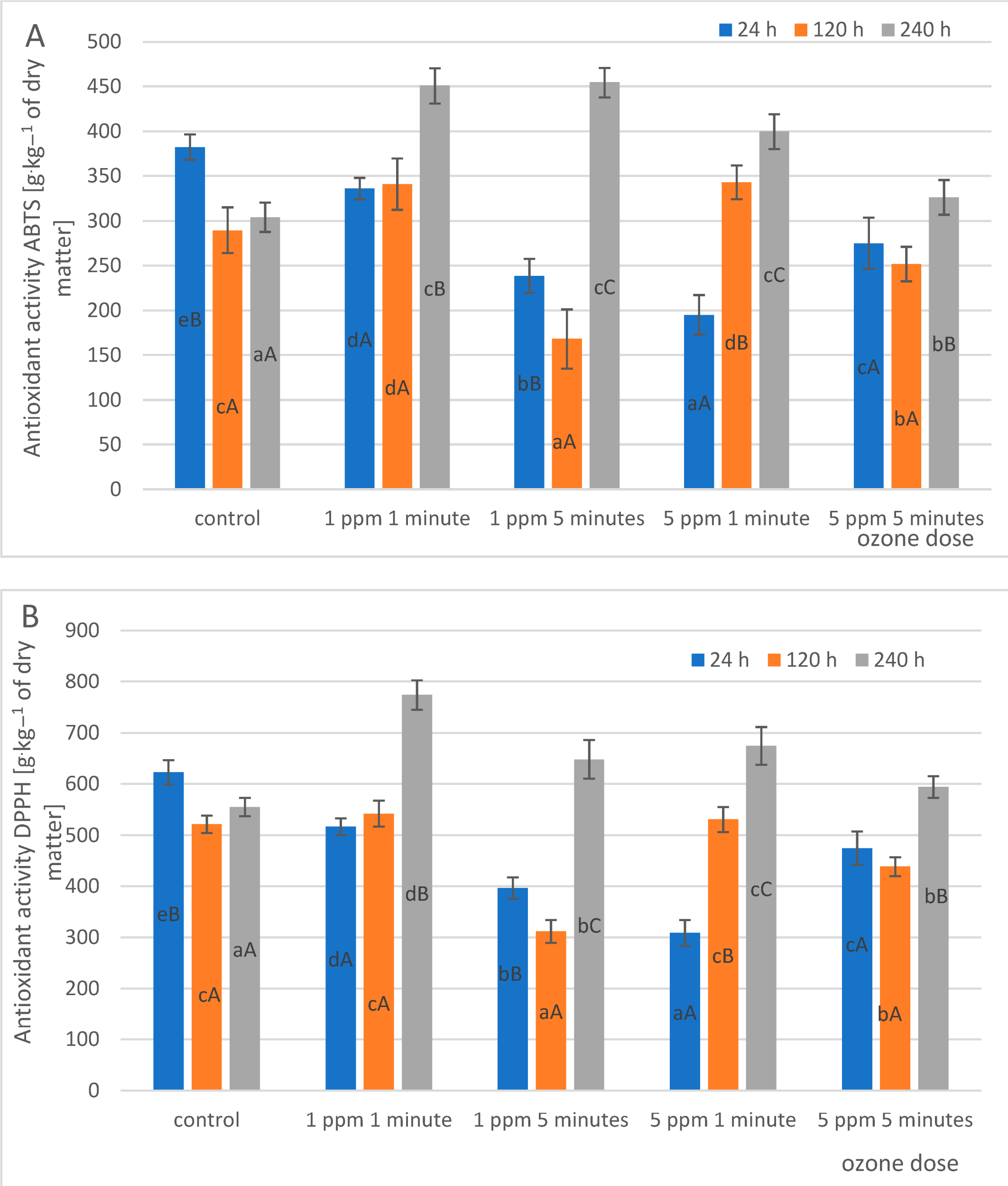

Figure 5. Antioxidant activity. ABTS test (**A**) and DPPH test (**B**) in radish tubers depending on the ozone dose and storage time (n = 3). Lowercase letters indicate differences between variants with the test date. Capital letters indicate differences between test dates.

A similar relationship as for antioxidant activity was found in the case of the total content of polyphenols in radish tubers (Figure 6). After fumigation with the tested ozone doses, this parameter decreased after 24 h (1 day) and 120 h (5 days) of storage compared to the control. However, on the 10th day of storage, the tested index increased by an average of 48.72% for the tested ozonated variants compared to the control.

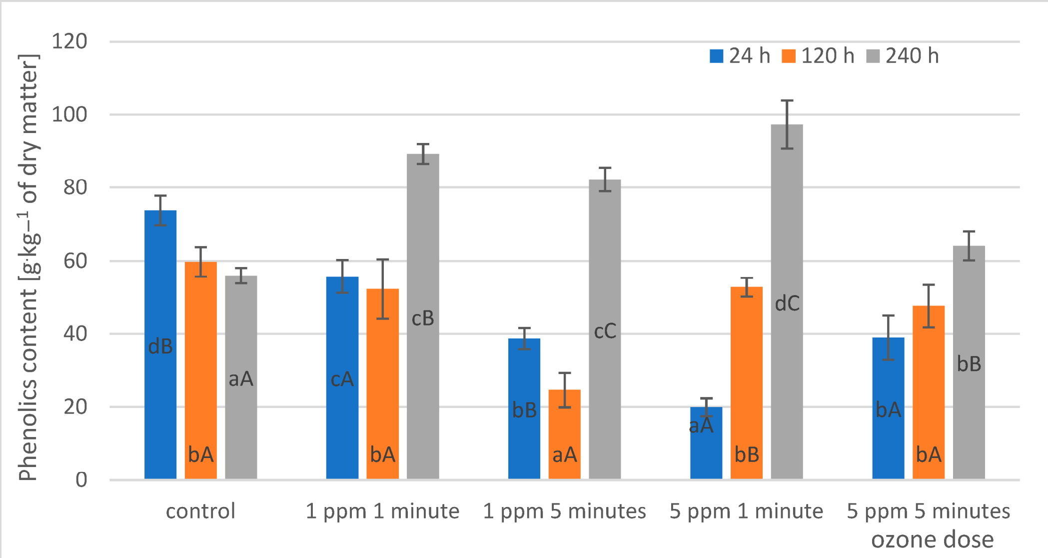

Figure 6. Total content of polyphenols in radish tubers depending on the ozone dose and storage time (*n* = 3). Lowercase letters indicate differences between variants with the test date. Capital letters indicate differences between test dates.

The ozonation process carried out under controlled conditions has a positive effect on the overall content of polyphenols, as exemplified by numerous scientific research results. Balawejder et al. [54] studied the effect of gaseous and water ozone on the content of polyphenols in apples. They found that the ozonation process carried out for 1 to 30 min had a positive effect on the tested parameter. Vettraino et al. [55] studied the effect of ozone gas on chestnut fruits. They found that after 90 days of chestnut storage, the polyphenol content in the ozonated variants was 40% higher than in the control sample. The research results prove that the stress factor ozone can activate the synthesis of polyphenols as a defense response. Scientists' research on the effect of ozone on artichoke heads has shown that the stress caused by the use of this gas has a positive effect on the overall content of polyphenols. The use of ozonated water and subsequent storage of vegetables in an ozone atmosphere significantly affect the concentration of polyphenols depending on the variety. The beneficial effect of ozone is also noticeable on the seventh day of storage of the tested raw material, taking into account the polyphenol content [41]. Glowacz et al. [56] report that differences in the content of polyphenols at different doses of ozone gas can be explained by increased phenylalanine ammonia lyase activity or decreased polyphenol oxidase and peroxidase activity.

Gaseous ozone penetrates the plants through gas exchange and dissolves in the water solutions available inside, generating various types of reactive oxygen species. The living organism activates defense mechanisms, which causes increased biosynthesis of polyphenols. In many cases, an increase in the content of other small molecular antioxidants was also observed during short-term exposure to relatively high concentrations of ozone [57].

In order to determine the impact of ozone gas fumigation on the microbiological quality of radish tubers, microbiological analyses were performed during their storage.

In the case of aerobic mesophilic bacteria, their fastest development in the stored raw material was recorded for the control sample (Figure 7). For the ozonated variants, an increase in the tested bacteria was also observed during storage, but it was significantly smaller during 10 days of storage, comparing the results of the tests on the ozonated variants and the control sample. The ozone dose of 5 ppm for 5 min had the most beneficial effect on the reduction in mesophilic aerobic bacteria, regardless of the test date. On the 10th day of storage, this dose reduced the number of tested bacteria by 20.9% compared to the control variant.

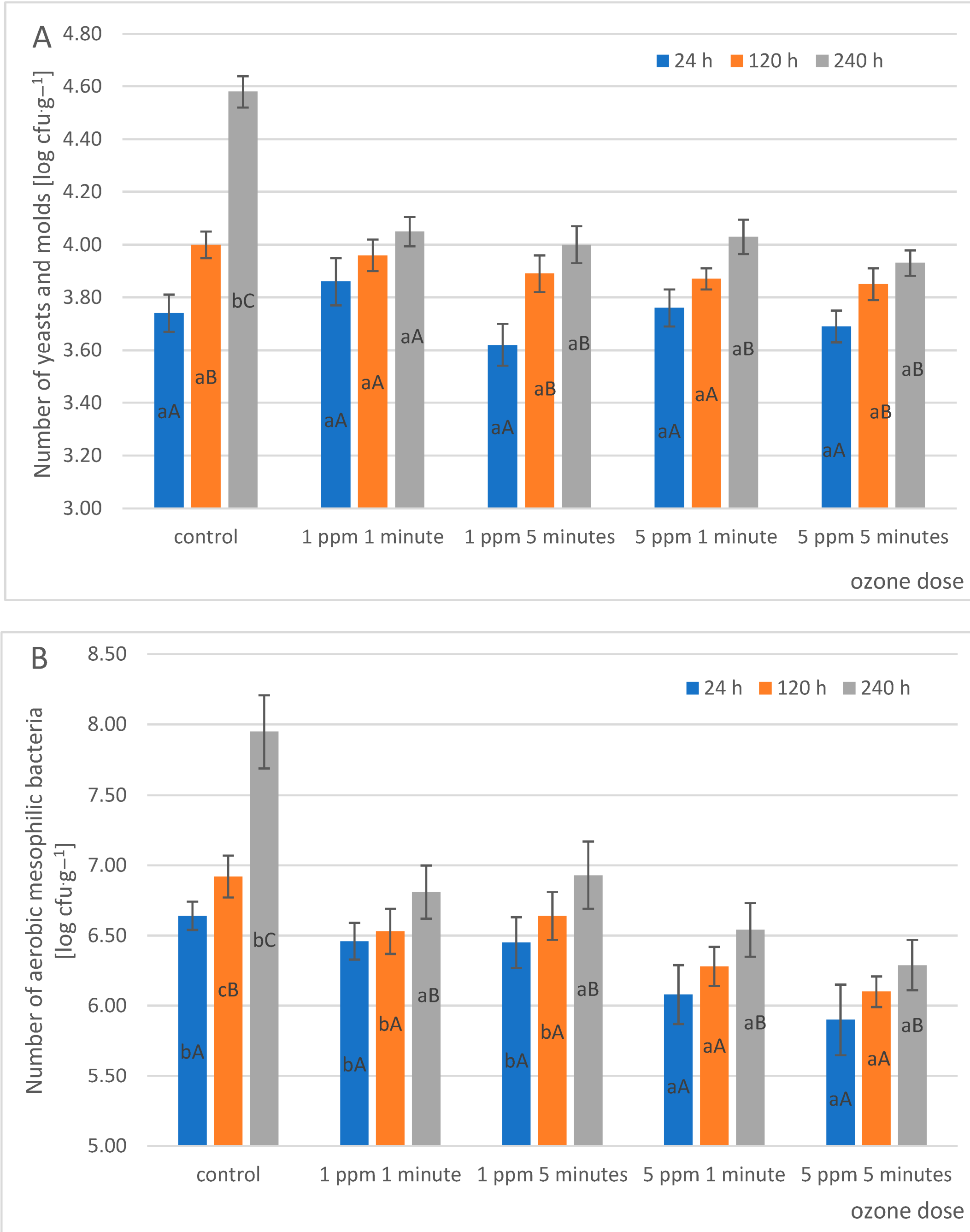

Figure 7. Microbiological load. (**A**) Number of yeasts and molds; (**B**) number of aerobic mesophilic bacteria) of radish tubers depending on the ozone dose and storage time (n = 3). Lowercase letters indicate differences between variants with the test date. Capital letters indicate differences between test dates.

The beneficial effects of the ozonation process were also observed in the reduction in the number of yeasts and molds in the stored raw materials. On the first day of storage of the raw materials, there was no beneficial effect from the dose of 1 ppm per minute and 5 ppm per minute on reducing the number of yeasts and molds in the tested raw materials compared to the control sample. On day 5 (120 h), for the ozone doses used, in addition to the dose of 1 ppm for 1 min, a beneficial effect of gaseous ozone fumigation on the reduction in the burden of yeasts and molds was observed. In turn, the ozone dose of 5 ppm for 5 min during radish storage had the most beneficial effect on the reduction in yeasts and molds. On the last day of storage, this dose reduced the tested microbiological load by 14.2% compared to the control sample.

Ozone (O_3), being a strong oxidizing agent [58], is used in the food industry due to its numerous advantages, in particular fighting microorganisms [59] and reducing mycotoxin contamination of plant raw materials [60]. Additionally, the use of ozone guarantees the absence of any residues in the material subjected to this process [61]. Moreover, ozone fumigation reduces the number of Gram-positive and -negative bacteria, as well as their spores [62,63]. There are a number of scientific reports in the literature that ozone reduces the number of mesophilic aerobic bacteria during storage of asparagus [43], rhubarb [22], marjoram [39], groats corn [64], or date palm fruit [65]. Moreover, ozone gas reduces the number of yeasts and molds on strawberry fruit [66,67], citrus fruits [68], dried figs [69], and berries [70].

Similarly to fruit, there have been numerous reports on the beneficial effect of ozonation on the microbiological load of vegetables. Ölmez and Akbas [71] reported that the use of ozonated water at a concentration of 2 ppm for 2 min was the optimal condition for disinfection of green leaf lettuce, taking into account the reduction in the microbiological load during cold storage.

Other studies on the microbiological load report that the average number of yeasts, molds, and aerobic bacteria in the tested cultivars of globe artichoke from the C3 Violet de Provence and Romanesco clones was reduced as a result of the ozonation process. For 7 days of storage, a beneficial effect of ozonation on the reduction in the tested microorganisms responsible for storage losses was noted [72]. In their study, Liew and Prange [73] reported that ozonation of carrots at a concentration of 60 ppm reduced the microbiological load of *Sclerotinia sclerotinia* by 50%. Storing carrots in an ozone atmosphere with a concentration of 15 ppm reduces the microbiological load without negatively affecting the physical properties of carrots.

The results of tests on the microbiological load of radish tubers resulting from the use of gaseous ozone correspond to the results of other studies in which gaseous ozone reduced the concentration of microorganisms by approximately 2 log.cfu^{-1} [74–76].

4. Conclusions

The paper describes the impact of the ozonation process carried out before harvesting radish tubers on their quality during 10 days of storage. Mechanical properties, content of selected bioactive compounds, and microbiological load were tested. The research shows that the use of gaseous ozone reduces the microbiological load and does not have a statistically significant effect on water loss and mechanical properties. Moreover, tests showed an increased vitamin C content after applying ozone at a dose of 5 ppm for 1 min on the 10th day of storage. In the tested raw material, the antioxidant activity and polyphenol content were higher after each ozone dose compared to the control sample on the 10th day of storage. The research results show that ozonation can be effectively used to improve the parameters determining the quality of radish tubers and to extend the shelf life of this raw material.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture13112153/s1, File S1: Mikrobiological analyzes.

Author Contributions: Conceptualization, methodology, investigation, visualization, and writing—original draft preparation, M.Z.; visualization and investigation, N.M.; methodology, T.P.; conceptualization, methodology, and review and editing, M.B. All authors have read and agreed to the published version of the manuscript.

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