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Ozone Treatment as a Process of Quality Improvement Method of Rhubarb (*Rheum rhaponticum* L.) Petioles during Storage

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Abstract: The aim of the study was to identify the effects of ozone treatment reflected by the microbial, mechanical properties and selected chemical parameters during the storage of rhubarb petioles. For this purpose, after they were harvested, rhubarb petioles were treated with gaseous ozone at concentrations of 10 and 100 ppm, for the duration of 5, 15 and 30 min. Subsequently, the plant material was stored at room temperature for 14 days. After this time, the raw material was subjected to a number of chemical and mechanical tests. It was shown that the rhubarb petioles treated with ozone at a rate of 100 ppm for 30 min were characterized by the lowest loss of water content. It was also found that, compared to the control, most samples subjected to ozone treatment presented better mechanical properties, as well as higher oxidative potential and contents of polyphenols and vitamin C. Based on these findings, it was determined that ozone treatment largely increases storage stability of rhubarb.

Keywords: rhubarb; ozone; shelf life

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1. Introduction

Rhapontic rhubarb (*Rheum rhaponticum* L.) is a plant in the buckwheat family (*Polygonaceae*). The genus *Rheum* comprises approximately 60 species [1]. Rhubarb is a perennial plant with edible leaf stalks (petioles) [2]. It is widely grown in Tibet and China [3,4], as well as the United Kingdom, New Zealand, USA and Canada. The popularity of the plant is associated with its varied uses in the food industry [5]. After the aboveground parts of the plant have been harvested, the leaf blades are removed from the stalks, which are then subjected to processing [1]. If the stalk tissues are injured in the process of cutting, the moisture content in the petioles is likely to decrease. The loss of water results in the deterioration of the commercial value and mechanical properties of the plant material [6]. This may be prevented by using storage at low temperatures and by monitoring moisture in the storage chamber. Another way that was shown to be effective in the case of rhubarb involves wrapping the petioles in aluminum foil. These methods, however, are difficult to apply in field conditions or in supply chains [7]. One of the methods offering a possible solution to the problem involves the application of ozone treatment. The process, in fact, was shown to be effective in the case of soft fruit. Researchers have reported that it is possible to significantly improve the shelf life of raspberries kept at room temperature if ozonation is applied directly after the fruit has been harvested [8]. Importantly, ozone treatment was also shown to be effective in the case of perishable vegetables [9], rhubarb being one of these. The major problems during storage and transport of rhubarb petioles are related to mechanical injuries, which constitute a gateway for tissue infection. These tissues are subject to a variety of static and dynamic stresses associated with the harvesting, loading, unloading, transport, sorting and other handling operations necessary in the process of preparing the end product [10]. By testing the mechanical properties of vegetables, it is possible to assess their quality relative to the production conditions, cultivar as well as duration and method of storage [11]. Development of storage diseases may be limited by applying chemical compounds, mainly fungicides, before harvesting. However, due to the existing limitations related to the use of crop protection agents during plant production, it is necessary to look for alternative methods to shield crops against pathogens. The methods usually applied should be environment-friendly. Ozonation is one of the technologies used in the industry to extend the shelf life of plant materials and to affect their quality by means of various mechanisms [12]. The ozone gas is applied because of its disinfectant properties, and it favorably affects plants capable of metabolism by promoting the activation of metabolic pathways, which is known as elicitation [8]. Ozone is also applied to remove residues of crop protection chemicals [13–15].

The aim of the study was to identify the effect of ozone treatment as a process of quality improvement method of rhubarb (*Rheum rhaponticum* L.) petioles during storage. The ozone effect was determined by mechanical, chemical and microbiological measurements.

2. Materials and Methods

2.1. Plant Material

The research material comprised petioles of rhapontic rhubarb (*Rheum rhaponticum* L.) of Raspberry Red variety, part of the earliest crop collected on 20 May 2020. Rhubarb petioles designated for the tests exceeded 30 cm in length and were at least 3 cm-wide in the central segment of the stalk.

2.2. Ozone Treatment of the Plant Material

A sample of 30 *Rheum rhaponticum* L. petioles was placed in an ozonation chamber, which was connected with the ozone generator Korona L5 (Scientific and implementation laboratory "Korona" Piotrków Trybunalski). The gaseous ozone concentration was measured using a Korona ozone detector, at a measuring range of 0–1000 ppm. The ozone treatment was conducted at a constant temperature of 20 °C in three replications. After the ozonation, the petiole samples were placed in a container and stored at a room temperature (mean 18 °C) for 14 days. After this period, further measurements of selected mechanical and chemical parameters were conducted.

2.3. Measurement of Water Content

Measurements of moisture content in fresh rhubarb petioles and at the end of the storage period were conducted using a laboratory drying oven (SLW 115 SMART). Three-centimeter-long sections of the stalks were picked from the samples of the material and subjected to drying at a temperature of 105 °C, until dry matter was obtained.

2.4. Measurement of Mechanical Properties

2.4.1. Indentation Test

Rhapontic rhubarb petioles were subjected to strength test using an indenter with a diameter φ = 6 mm (Zwick/Roell Z010 machine). The tests were performed on fresh stalks and the raw material previously subjected to ozone treatment at selected doses and stored for 14 days. The indentation test was performed in three locations of rhubarb petioles: bottom, the central and top part, in 36 replications for each test series. The resistance of the skin to the indenter was measured using the following working parameters: Fv = 0.05 N (initial force), V1 = 30 mm min^{-1} (crosshead return speed).

2.4.2. Compression of Independent Specimens

Tests for the resistance of rhubarb petiole tissues to mechanical damage in the process of uniaxial compression involved specimens with a cylindrical shape, a height of 10 mm and a diameter of 4 mm. The cylindrical specimens were cut along the axis and transversely to the axis of the petioles in the top, central and bottom parts. The tests were performed on fresh specimens and material subjected to treatment with various doses of ozone and stored for 14 days. The measurements were carried out in 36 replications for each variant of the experiment. The process of uniaxial compression of the petioles (independent specimen) was performed using Zwick/Roell Z010 testing machine, with the defined working parameters: initial force for specimen tension F = 2 N, speed of the module during compression test 0.5 mm·s^{-1}.

2.5. Antioxidant Potential, Contents of Polyphenols, Vitamin C Chemical Composition of HS-SPME

The antioxidant potential was determined by the 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) methods, the total polyphenol content by the method described by Matłok et al. [16]. The composition of the volatile fraction was determined by the headspace solid-phase microextraction (HS-SPME) method described by Matłok et al. [17]. Vitamin C was determined by the titration method in accordance with the methodology presented by Matłok et al. [18].

2.6. Microbiological Analyses

The rhubarb petioles were subjected to microbiological tests on the first day after the plants were treated with ozone. The enumeration of the number of mesophilic lactic acid bacteria in the raw material was determined using the method specified in PN-ISO 15214:2002 "Microbiology of food and animal feeding stuffs-Horizontal method for the enumeration of mesophilic lactic acid bacteria-Colony-count technique at 30 °C". In accordance with this method, the count of living mesophilic lactic acid bacteria is performed in a solid MRS medium incubated at a temperature of 30 °C for 72 h. The yeast and mold count in 1 g of rhubarb petioles was determined using the method described in PN-EN ISO 21527-1:2009 "Microbiology of food and animal feeding stuffs- Horizontal method for the enumeration of yeasts and moulds. Colony count technique at 25 °C". Enumeration of mesophilic aerobic bacteria in the relevant raw material was performed using plate count method, in accordance with the standard set forth in (Ae) (O) PB-77/LM, 4th edition dated 07.12.2015. Finally, the presence of endospore producing anaerobes was determined using the method described in (Ae) (O) PB-13/LM, 4th edition, dated 07.04.2014. The analyses were performed in 3 replications.

2.7. Statistical Analysis

To verify the significance of the effect of ozonation time on the quality parameters for each term of analysis, one-way ANOVA (analysis of variance) and Tukey's post hoc test was used at =0.05. These analyses were performed using STATISTICA12.5 PL from StatSoft [16].

3. Results and Discussion

3.1. Water Content

The findings of the tests (Figure 1) involving samples of rhubarb exposed to ozone and samples not subjected to such treatment and stored in identical conditions (18 °C) show effects of the concentration and duration of ozonation on the loss of weight. The largest weight loss amounting to 25.50% was observed in the control sample on the 14th day of storage, while the lowest weight loss of 12.39% was recorded in the sample subjected to ozone treatment at a rate of 100 ppm, for the duration of 30 min.

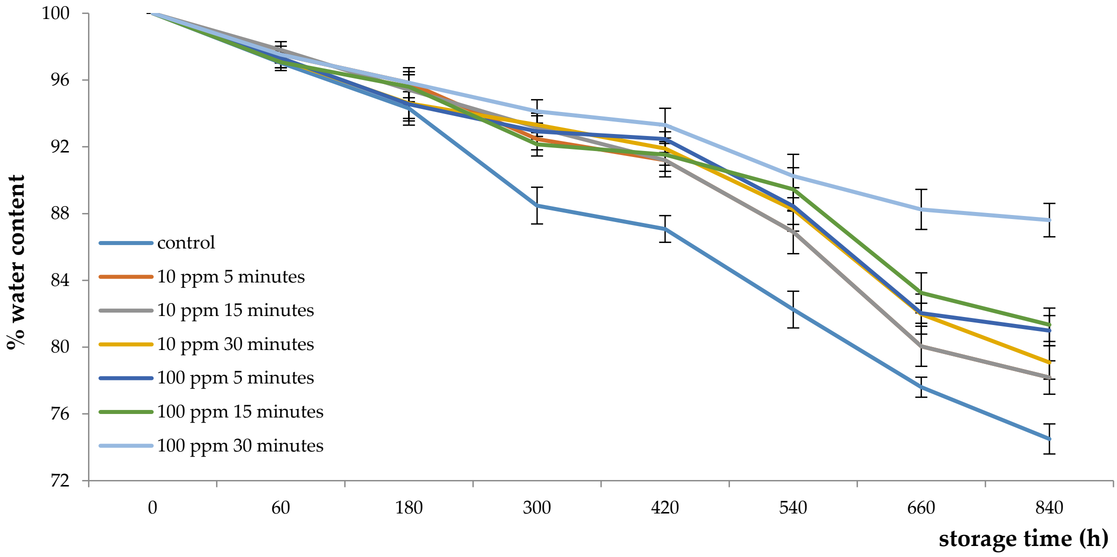

Figure 1. Water content in the samples subjected to ozone treatment and in the control sample on the consecutive dates related to storage of rhubarb petioles.

A mobile solution has been designed [19] that will allow for the ozone treatment after harvesting. It was proposed to equip the transport devices with appropriate chambers that would allow the ozone treatment to be carried out during the initial transport.

Transport lasting up to several days usually takes place at ambient temperature, leading to weight loss, mainly due to water loss. The ozonation process carried out under optimal conditions allows keeping 11.5% of the mass, translating into an increase in profit by 64.30 € t^{-1}, assuming the average price of rhubarb at the level of 559.30 € t^{-1}.

It should be assumed that the cost of ozonation, excluding the cost of equipment, is 0.5 kWh t^{-1} of electricity, which is 0.11 € t^{-1}. The cost of energy consumption was estimated on the basis of the power of the ozone generator used, which was used under the conditions of the experiment [19].

A different response of the raw material to ozone treatment was observed in the case of apples. After ozonation, apples have much worse preservation [20]. However, Antos et al. [20] reported a similar loss of weight in apples subjected to ozonation and in the control sample. Notably, however, the researchers applied periodic ozone treatment using ozone stream at a concentration of 1 ppm. This was due to the sensitivity of the fruit to a higher concentration, and the observed storage-related effect was associated with the impact of ozone on the pesticide residues in the stored fruit.

3.2. *Measurement of Mechanical Properties*

3.2.1. Indentation of Petioles

The results of the tests (Figure 2) show the highest resistance to indentation of the skin on the backside of the rhubarb petioles following harvest in the material treated with ozone at a rate of 10 ppm for 15 min, in the central part of the petiole—the value of indentation force amounting to 89.94 N. Ozone treatment applied at a rate of 10 ppm for 5 min did not produce an increase in the resistance of the skin to indentation, compared to the control.

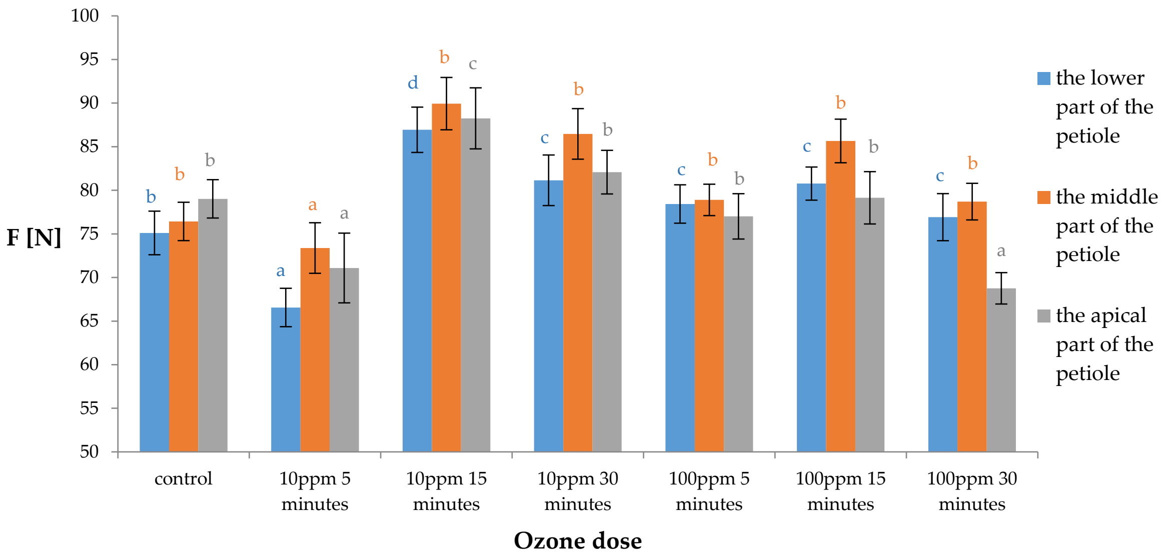

Figure 2. Mean values of the force needed to puncture the skin with φ = 6 mm indenter, on the backside of rhapontic rhubarb petioles. Differences in the results between the punching force values for individual variants are marked with different small letters, $p < 0.05$.

Irrespective of the ozone treatment variants, the central section of the petiole was characterized by the highest resistance to indentation in fresh rhubarb. After the dose of 10 ppm was applied for 15 min, the mean values of the force needed to puncture the skin on the back side of rhubarb petiole were similar in all the locations tested, and application of this variant made it possible to increase resistance to indentation, compared to the control. The mean values of indentation force in the control sample amounted to 76.85 N, and in the sample exposed to ozone at a rate of 10 ppm for 15 min–88.38 N.

The force needed to puncture the skin with φ = 6 mm indenter on the flat side of rhubarb petioles, after application of ozone in various combinations of dose and duration, compared to the control, is shown in Figure 3. The highest resistance of the skin to indentation on the flat side was found in the bottom part of the petioles in all the ozonation variants. The highest mean resistance of the skin to the indentation on the flat side of the petioles following harvest was observed for the treatment at a rate of 10 ppm for a duration of 15 min. Compared to the control (petioles not subjected to ozone treatment), resistance to indentation increased by 19%. Notably, the lowest ozone dose (10 ppm, 5 min) did not produce increased resistance to indentation of the skin on the flat side. Application of ozone treatment to improve storage stability of other raw plant materials was also reported to be effective. Migut et al. [21] reported that by applying ozone treatment to field cucumbers, it was possible to prolong their usefulness for processing, as reflected by their improved mechanical properties following the storage, relative to the control sample. The fruit subjected to ozone treatment were suitable for the souring process even after 22 days. Preliminary ozonation also affected the mechanical and organoleptic properties of pickled cucumbers [22]. It was established that irrespective of the cultivar and its purpose, cucumbers subjected to ozonation presented improved organoleptic and mechanical characteristics compared to the controls and contained no hollow spaces.

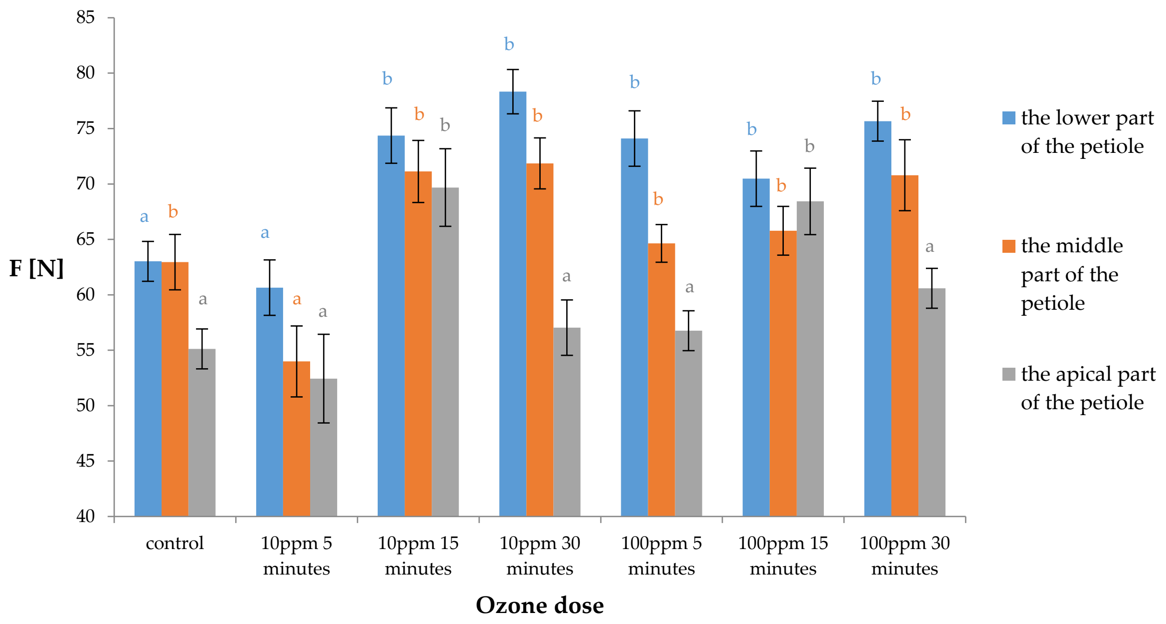

Figure 3. Mean values of the force needed to puncture the skin with φ = 6 mm indenter, on the flat side of rhapontic rhubarb petioles. Differences in the results between the punching force values for individual variants are marked with different small letters, $p < 0.05$.

3.2.2. Compression of Independent Specimens

Figure 4 presents the findings reflecting mean values of destructive force for samples cut out transversely to the axis of rhapontic rhubarb petioles after ozone treatment was applied at various combinations of doses and duration of exposition, compared to the control. Ozone treatment applied at a rate of 10 ppm for 5 min did not produce increased resistance of the material to damage compared to the control. In the case of the remaining doses and durations of exposure to ozone, the findings showed increased values of destructive force, compared to the control, in all the parts of rhubarb petioles subjected to testing. The highest mean values of destructive force for the top part of rhubarb petioles were identified after application of 100 ppm for 30 min. In the case of the central and bottom part of the petiole, the highest values of this force were found after the treatment at a rate of 100 ppm for 15 min.

The mean values of destructive force in the process of uniaxial compression of the specimens cut out along the axis of rhapontic rhubarb petioles, after ozone treatment was applied at various combinations of the dose and duration of exposure, compared to the control, are shown in Figure 5. When ozone treatment was applied at a rate of 10 ppm for 5 min, the tests did not show significantly higher values of destructive force compared to the control. When the remaining combinations of dose and duration of exposure to ozone were applied, the highest resistance to damage was found in the bottom segments of rhubarb petioles. Ozone treatment at a rate of 100 ppm for the duration of 15 min resulted in the highest mean values of destructive force in all of the rhubarb petioles parts subjected to testing, compared to the control. Gorzelany et al. [23] investigated the effects of ozone fumigation on the mechanical properties of field cucumbers. The cucumbers were first subjected to ozonation and then to a souring process. The researchers showed that ozone favorably affected the quality of the pickled cucumbers. The cucumbers subjected to ozone treatment prior to the pickling process were characterized by greater resistance of skin and mesocarp to damage, compared to the cucumbers which were processed without the preliminary ozonation. Ozonated parts of rhubarb petioles showed increased mechanical strength, which is associated with potentially less damage during transport, and this may contribute to increasing the profitability of the ozonation process by reducing weight loss due to water loss [24].

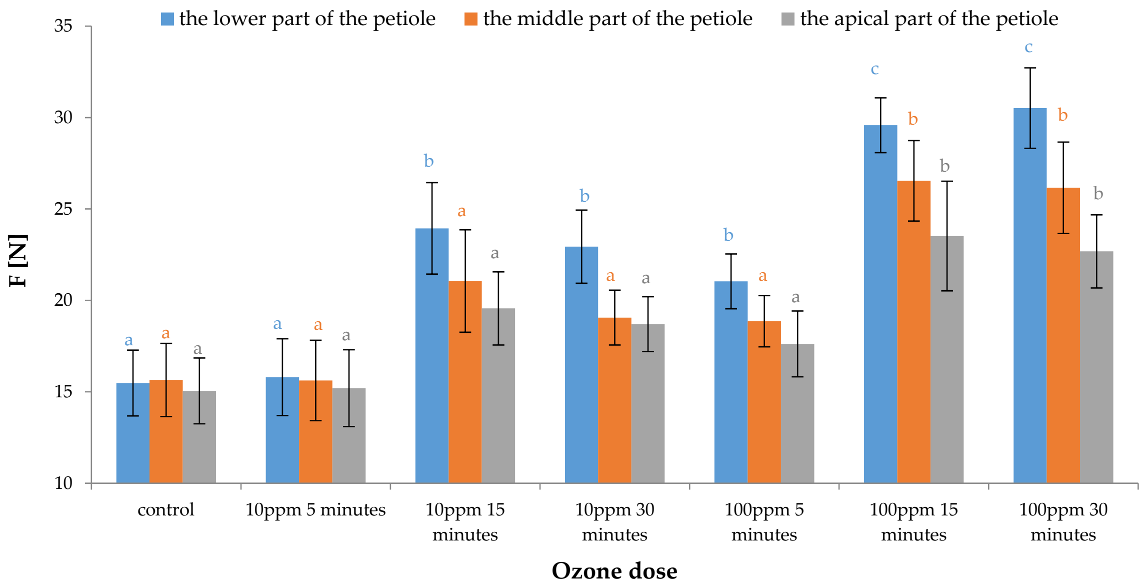

Figure 4. Mean values of destructive force identified for the specimens φ = 4 mm cut out transversely to the axis of rhapontic rhubarb petioles. Differences in the results between the values of the destructive force for individual variants are marked with different small letters, $p < 0.05$.

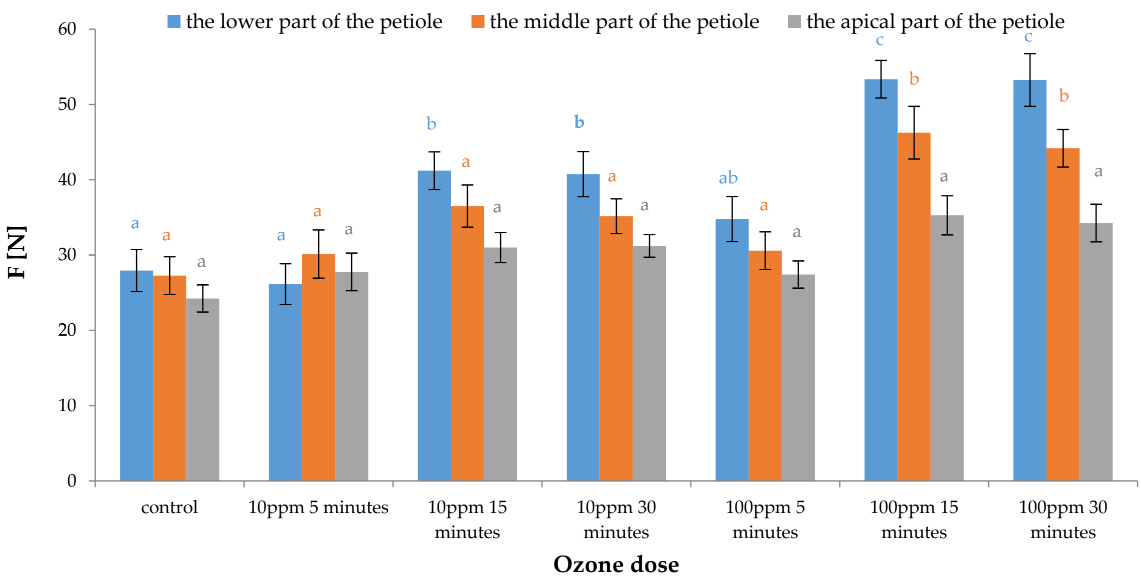

Figure 5. Destructive force identified for the specimens φ = 4 mm cut out along the axis of rhapontic rhubarb petioles. Differences in the results between the values of the destructive force for individual variants are marked with different small letters, $p < 0.05$.

3.3. Antioxidant Activity, Total Phenolic and Ascorbic Acid Content

Application of ozonation process in controlled conditions is associated with the occurrence of oxidative stress in the plant cell structures as a result of which enzymatic systems are activated, leading to the production of greater quantities of secondary metabolites affecting the final quality of the raw material [25].

The ozone treatment applied to rhubarb petioles immediately after harvest produced a change in antioxidant potential determined in relation to ABTS and DPPH radicals (Figure 6), and in the total contents of polyphenols (Figure 7) as well as the contents of vitamin C (Figure 8). Short exposure (5 min) of rhubarb petioles to gaseous ozone at a rate of 10 ppm led to an increase in antioxidant activity

(Figure 6A,B). On the other hand, the ozone treatment applied at the same rate and prolonged to 15 or 30 min resulted in a decreased antioxidant potential of the plant material investigated (Figure 6B). A similar relationship was observed when ozone was applied at a higher rate of 100 ppm. However, if the ozone treatment was continued for a duration of 30 min, there was a significant increase in the antioxidant potential of the raw material, compared to the control variant (Figure 6A). Likewise, 30 min exposure of rhubarb petioles to ozone applied at a rate of 100 ppm resulted in the highest increase in total polyphenols (Figure 7) and vitamin C (Figure 8), compared to the control.

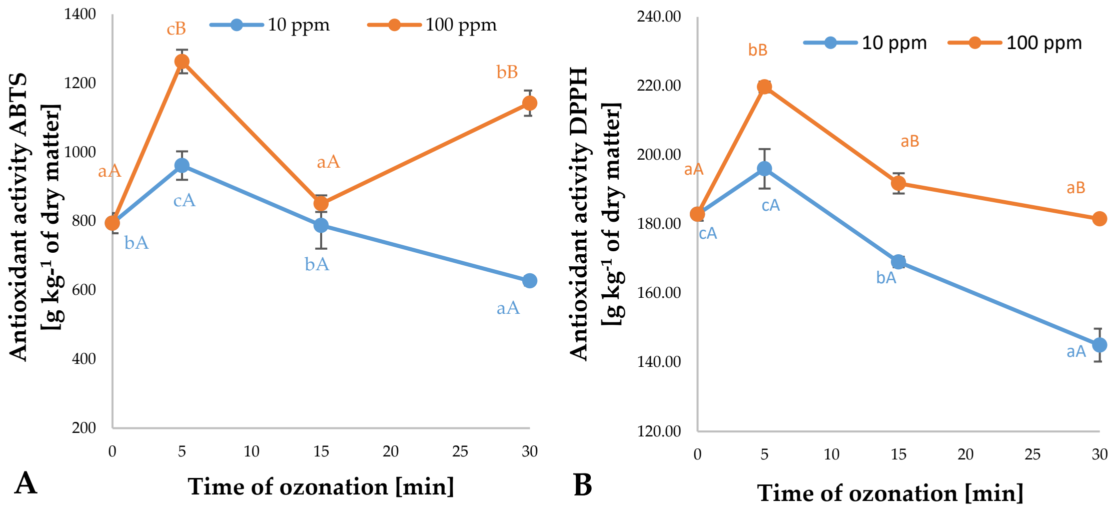

Figure 6. Antioxidant activity. ABTS test (part **A**), DPPH test (part **B**) in *Rheum rhaponticum* L. petioles, depending on the duration of ozone treatment (n = 3). The differences in the results between the ozonation time for individual variants are marked with different small letters and the difference between the measurement values for the tested variants is marked with different capital letters, $p < 0.05$.

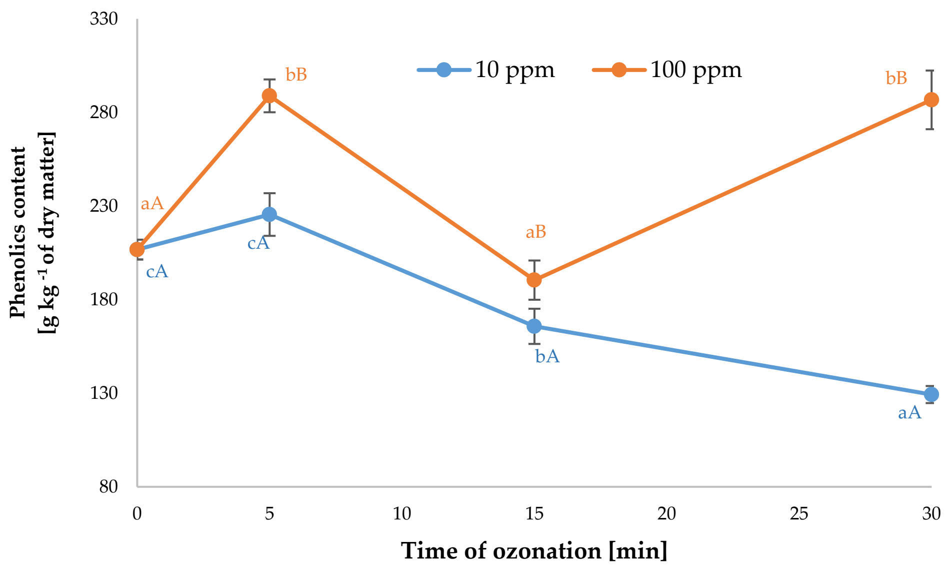

Figure 7. Total polyphenolic content in *Rheum rhaponticum* L. petioles, depending on the duration of ozone treatment (n = 3). The differences in the results between the ozonation time for individual variants are marked with different small letters, and the difference between the measurement values for the tested variants is marked with different capital letters, $p < 0.05$.

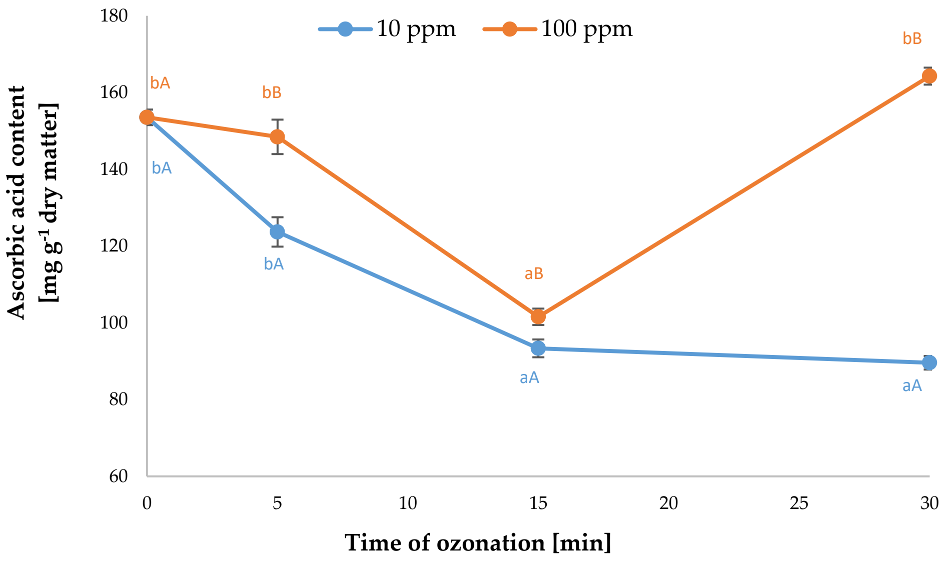

Figure 8. Ascorbic acid content in *Rheum rhaponticum* L. petioles, depending on the duration of ozone treatment (n = 3). The differences in the results between the ozonation time for individual variants are marked with different small letters, and the difference between the measurement values for the tested variants is marked with different capital letters ($p < 0.05$).

The use of the ozonation process on the petioles of rhubarb gives them higher concentrations of bioactive compounds than in natural conditions. After this process, this raw material has new properties that affect its usefulness in processing, including the production of functional food with a high content of bioactive compounds, especially antioxidants [26].

Increased contents of bioactive compounds in raw plant material subjected to ozone treatment were also reported by Piechowiak and Balawejder [25]. Their study showed that exposure of raspberries to ozone resulted in higher contents of polyphenols, vitamin C and antioxidant potential when the fruit were stored at room temperature. A similar relationship between the contents of selected bioactive compounds and ozone treatment was demonstrated for raspberries by Onopiuk et al. [27] and for high blueberry by Piechowiak et al. [8]. Yeoh et al. [28] investigated effects of gaseous ozone in papaya fruit. They found that exposure to the gas produced increase in polyphenol contents and in antioxidant potential of the plant material.

3.4. Chemical Composition of HS-SPME

One of the determinants of the quality of rhubarb petioles is their smell. The instrumental method of determining the composition of aromatic compounds is the HS-SPME method [17]. This method is considered an "electronic nose" that can replace the subjective organoleptic evaluation [29]. Most plants produce fragrances as secondary metabolites. These compounds mainly belong to the group of terpenes and esters. The composition of rhubarb aroma compounds has not been studied so far. The analysis of the control sample (without ozonation) showed that the aroma of rhubarb is mainly caused by the bundles of sesquiterpene hydrocarbons and their oxygen derivatives. In this group, α-bergamotene, (E)-β-ionone, α-farnesene and neryl acetone were identified (Table 1). These compounds have relatively high molecular weights, and therefore low volatility. This fact is confirmed by the low level of recorded signals during the chromatographic analysis. It should be noted that the analysis was carried out on the petioles without shredding them, which had a relative impact on the emission surfaces of volatile compounds. This surface is incomparably lower than that of other plants, e.g., herbal plants. The fact that the emission of aromatic compounds is carried out from the plant surface directly affects the observations made during the ozonation process. The analysis of Table 1 and Figure 9 show that significant differences were observed in the composition of the volatile fraction depending on the ozonation conditions used. Most of the fragrance compounds have unsaturated bonds in their structure, which can undergo 1–3 dipolar ozone cycloaddition. As a result of this process, ozonides were formed from alkenes with aldehydes as intermediates. The oxidative breakdown of ozone likely resulted in the formation of highly volatile carbonyl compounds that were quickly removed from the plant surface [30]. Presumably, a similar distribution occurred in the case of the identified unsaturated alcohol cis-9-cktadecen-1-ol. A phthalic acid aster has also been identified in the subsurface phase. This compound presumably came from environmental pollutants or pesticides used to chemically protect rhubarb plants during the production process. These compounds are considered to be extremely resistant compounds [31], which is also confirmed by our results. It can be seen in the table that with the increasing dose of ozone, the content of this compound in the headspace increased to over 60% after using the highest ozone concentrations. This proves that this compound has not been ozonized. The results of the analysis of the head space indicate the influence of the ozonation process on the petioles of rhubarb. It should be noted, that this effect is only surface and does not significantly change the quality of the raw material, the main components of which are stored in internal tissues.

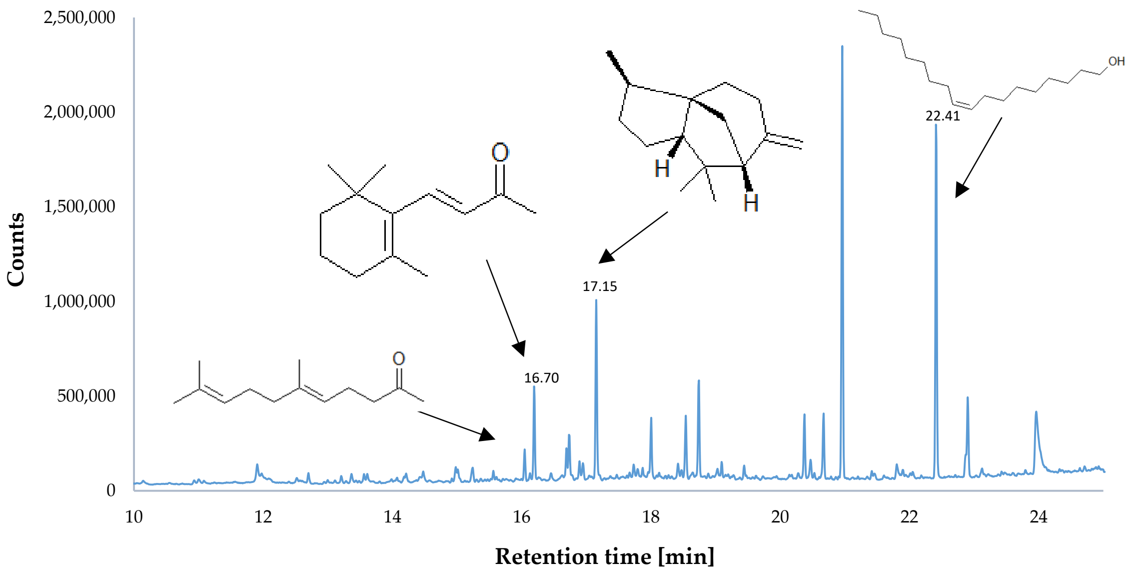

Figure 9. Chromatograms SPME-GC for the volatile fraction of rhubarb petioles (100 ppm 30 min).

Table 1. Chemical composition of headspace fractions of rhubarb petioles.

No.	RT [1] (min)	Peak Share in the Chromatogram (%)							Ordinary Substance Name	Systematic Substance Name	No CAS
		Control	10 ppm 5 min	10 ppm 15 min	10 ppm 30 min	100 ppm 5 min	100 ppm 15 min	100 ppm 30 min			
1	11.90	trace	trace	11.88	trace	trace	trace	trace	(±)-menthone	5-methyl-2-propan-2-ylcyclohexan-1-one	89-80-5
1	16.05	trace	trace	trace	1.33	trace	trace	trace	α-bergamotene	4,7-dimethyl-7-(4-methylpent-3-enyl)bicyclo[3.1.1] ept-3-ene	17699-05-7
2	16.20	11.09 ^{c}	6.13 ^{a}	8.74 ^{b}	6.34 ^{a}	12.33 ^{c}	14.86 ^{d}	trace	neryl acetone	(5Z)-6,10- dimethylundeca-5,9-dien-2-one	3879-26-3
3	16.70	trace	14.43 ^{c}	trace	2.20 ^{a}	trace	trace	7.66 ^{b}	(E)-β-ionone	(E)-4-[(5R)-5,6,6-trimethylcyclohexen-1-yl], but-3-en-2-one	79-77-6
4	16.88	trace	trace	trace	trace	trace	trace	trace	α-farnesene	(3E,6E)-3,7,11-trimethyldodeca-1,3,6,10-tetraene	502-61-4
5	17.15	30.15 ^{d}	4.87 ^{a}	8.16 ^{b}	trace	trace	trace	16.17 ^{c}	(+)-β-funebrene	3,8,8-trimethyl-6-methyleneoctahydro-1H-3a,7-methanoazulene	79120-98-2
6	17.99	trace	12.51 ^{b}	trace	9.21 ^{a}	13.52 ^{b}	trace	trace	-	propanoic acid, 2-methyl-, 1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester	74381-40-1
7	18.53	trace	5.81 ^{b}	5.89 ^{b}	4.85 ^{a}	trace	10.57 ^{d}	8.51 ^{c}	-	1,2,3,4,4a,5,8,9,12,12a-decahydro-1,4-methanobenzocyclodecene	74708-73-9
8	20.36	trace	trace	trace	2.58	trace	trace	trace	-	(Z)6-pentadecen-1-ol	68797-95-5
9	20.67	trace	trace	trace	4.20	trace	trace	trace	hexahydrofarnesyl acetone	6,10,14-trimethylpentadecan-2-one	502-69-2
10	20.96	28.14 ^{a}	49.43 ^{c}	36.95 ^{b}	36.60 ^{b}	61.23 ^{d}	49.81 ^{c}	36.12 ^{b}	-	butyl isobutyl phthalate	17851-53-5
11	22.41	26.60 ^{c}	4.55 ^{a}	21.87 ^{c}	32.90 ^{d}	9.58 ^{b}	20.20 ^{c}	31.53 ^{d}	oleyl alcohol	cis-9-cktadecen-1-ol	143-28-2
TOTAL		95.98 ^{a}	97.73 ^{c}	93.49 ^{a}	99.94 ^{d}	96.66 ^{b}	95.44 ^{a}	99.99 ^{d}			

The difference in the value of the chemical composition of the volatile fraction for the tested variants is marked with small letters, $p < 0.05$.

3.5. Microbiological Analysis

Ozonation of rhubarb petioles caused a change in the number of mesophilic lactic acid bacteria per 1 g (cfu—colony-forming units g^{-1}) of the tested material. The use of all proposed combinations of dose and ozonation time resulted in a significant reduction in cfu of tested bacteria by at least 1 log cfu g^{-1}. The dose of ozone with a concentration of 100 ppm for 30 min had the most beneficial effect on reducing the number of mesophilic lactic acid bacteria in rhubarb petioles (Figure 10). Limiting the number of mesophilic lactic acid bacteria directly affects the storage stability and mechanical properties of rhubarb petioles. By metabolizing simple sugars, these bacteria produce polysaccharide-hydrolyzing enzymes, which are able to hydrolyze polysaccharides, the main constructing components of cell walls. The breakdown of these polysaccharides during the fermentation leads directly to the loss of turgor petioles of rhubarb and, in a further stage, to an increased loss of water [32].

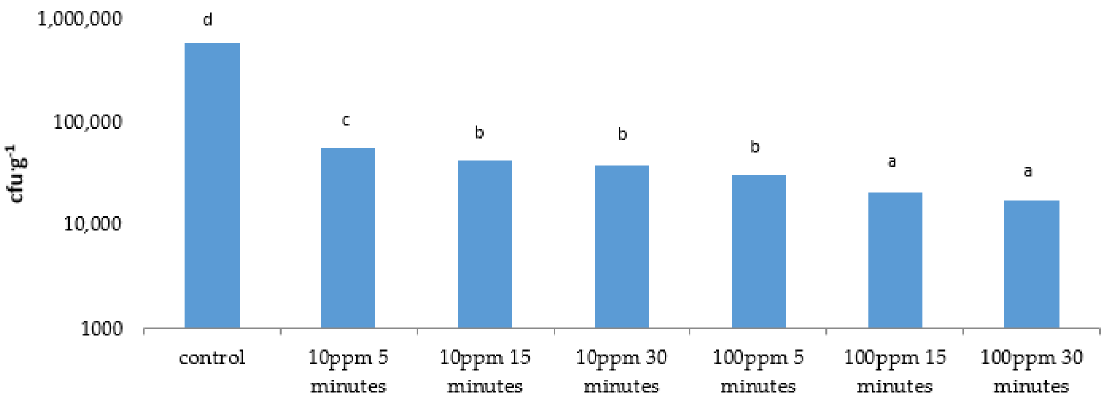

Figure 10. Number of mesophilic lactic acid bacteria in 1 g in rhubarb petioles. The significance of differences in cfu g^{-1} of mesophilic lactic acid bacteria is marked with different letters, $p < 0.05$.

The results of the microbiological analysis correspond with the analysis of the composition of volatile compounds. The reduction of the number of microorganisms on the surface of petioles of rhubarb coexist with a reduction of the content of volatile compounds detected by the HS-PME method.

The yeasts and molds, mesophilic aerobic bacteria and presence of anaerobic spore-forming bacteria were not detected in plant material. Aguayo et al. [33] also showed the possibilities of using ozone to extend the storage life of tomatoes. The investigators achieved a statistically significant reduction in the total mesophilic count of up to 1.1 log cfu ($p < 0.05$) compared to controls. Horvitz et al. [34] ozonated freshly harvested red peppers. Ozonation reduced the number of mesophilic bacteria by up to 2.6 log cfu compared to the control.

4. Conclusions

The paper describes the effects of ozone treatment on quality during shelf life, as well as the mechanical and chemical properties of rhubarb petioles. The study shows that the application of gaseous ozone leads to reduced microbial load, loss of water and improved mechanical properties in the plant material subjected to tests. Furthermore, the findings show increased contents of vitamin C after ozone treatment was applied at a rate of 100 ppm for 30 min as well as higher total contents of polyphenols in the rhubarb. Moreover, the observed increase was depended on the applied dose of ozone. A similar tendency was observed in the case of antioxidant potential, with the highest increase resulting from the treatment applied at a rate of 100 ppm for 5 min. The current findings show that ozone treatment may effectively be used to extend the shelf life of rhubarb petioles. Taking into account the economic aspect, the best effect was achieved for ozonation with a dose of 100 ppm within 30 min because the loss of mass of the stored raw material for this dose was the lowest. Future research should concern the explanation of the observed changes in the chemical composition of rhubarb petioles by analyzing the accompanying biochemical processes, especially the activity of selected enzymes, markers of oxidative stress.

5. Patents

A method of extending the shelf life of rhubarb or celery in non-refrigerated conditions, the use of ozone to extend the non-refrigerated storage life of these vegetables and a means of transport for ozonating vegetables. Patent application: P.435808.

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