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MODIFICATION OF THE INTENSITY OF COMPOUNDS BIOSYNTHESIS IN THE PETIOLES OF RHUBARB (*Rheum rhaponticum* L.) INDUCED BY THE OZONATION PROCESS

– Research paper –

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Abstract: Rhubarb (*Rheum rhaponticum* L.) is a vegetable that, despite many health-promoting properties, contains large amounts of oxalic acid, which has a negative impact on human health. Treatment of rhubarb petioles with gaseous ozone is one way to improve their quality by changing their chemical properties. As part of the research, an analysis of the mechanical and chemical properties of rhubarb petioles was performed. The analyzes showed that the applied doses of ozone of 10 ppm during 1, 3 and 5 minutes lowered the concentration of oxalic acid during 5 days of tests performed after the ozonation treatment. The most favorable effect on the reduction of oxalic acid, amounting to 36%, was caused by gaseous ozone at a concentration of 10 ppm during 5 minutes compared to the control. In addition, it was noted that on the 1st and 3rd day after using ozone gas, the antioxidant activity of the tested raw material increased significantly. Also on the same days of testing, the total amount of polyphenols increased as a result of the use of ozone fumigation. The largest increase in the total amount of polyphenols, amounting to 12%, was observed for a dose of 10 ppm 5 minutes on the 1st day after the ozonation treatment compared to the control variant. In turn, no positive effect of the ozonation process on the content of vitamin C and the value of destructive force during three-point bending of rhubarb leaf petioles was noted.

Key words: rhubarb petioles, ozone, oxalic acid

INTRODUCTION

Rhubarb is a perennial vegetable belonging to the *Polygonaceae* family, with about sixty species, including *Rheum rhabarbarum.* The main reason for the popularity of growing rhubarb is its easeand low-input cultivation technology (Clapa et al., 2020). Rhubarb (*Rheum rhaponticum* L.) has been cultivated in China for several thousand years, mainly for medicinal purposes. Initially imported to Europe in the form of dried rhizomes as a medicine. Currently, it is cultivated in almost all climatic zones (Anyszka et al., 2017).

Numerous studies of rhubarb have shown that it is a plant with many health-promoting properties. The main therapeutic effect of rhubarb is: analgesic effect, protection of the intestinal mucosa and bacteria present there, regulation of the flora of the digestive system. In addition, it reduces fibrosis, but also has anti-inflammatory properties. Rhubarb has an antibacterial effect against bacteria such as *Lactobacillus*, *Staphylococcus aureus*, *Escherichia coli*, *Helicobacter pylori*; rhubarb methanol extract is more effective against *E. coli*, *Klebsiella pneumoniae*, *Bacillus subtillis* compared to standard antibiotics (Zheng et al., 2013; Cao et al. 2017; Xiang et al., 2020).

Rhubarb is a source of biologically active ingredients responsible for laxative, diuretic, antibacterial, anti-inflammatory and anticancer properties (Huang et al., 2019; Jintao et al., 2018). In rhubarb petioles, despite the presence of several valuable natural active ingredients (anthraquinones, dianthrones, stilbenes and flavonoids), which contribute to the improvement of human and animal health (Zheng et al., 2013; Krafczyk et al., 2008)

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contain large amounts of organic acids, including oxalic acid, which has a negative impact on consumer health (Oszmiański and Wojdyło, 2008). Oxalates occur in food of plant and animal origin in the form of soluble sodium and potassium salts and insoluble - calcium oxalate (Judprasong et al. 2006; Rahman et al. 2013).

There are ways to reduce the oxalic acid content in rhubarb petioles. One of them is boiling in water and steaming rhubarb petioles. Cooking is a method of reducing soluble oxalates, especially when water is not part of the food after the petioles have been cooked. Depending on the cooking method used, the amount of soluble oxalates relative to the total amount of remaining oxalates in cooked rhubarb may vary (Savage et al. 2011). In addition, oxalates can be reduced by blanching and by a fermentation process (Van Boekel et al. 2010). Another way to reduce the oxalic acid content in rhubarb petioles is to use calcium, which has the ability to bind with soluble oxalate to form insoluble calcium oxalate. The availability of oxalate in food is altered by the addition of additional calcium. For example, the addition of cow's milk and/or coconut milk significantly reduces the soluble oxalate content of heat-treated rhubarb petioles (Savage et al. 2009). However, these methods generate insoluble oxalic acid compounds in products, mainly calcium oxalate, which, in contact with gastric juices with a low pH, are converted to easily absorbable oxalic acid.

The methods of reducing the content of oxalic acid in rhubarb petioles described above do not allow for the production of petioles with a reduced content of oxalic acid in a form useful for the food industry, i.e. fresh, unprocessed.

The aim of the work is to determine the effect of gaseous ozone used during the growing season on the mechanical and chemical properties of rhubarb petioles, with particular emphasis on changes in the concentration of oxalic acid in the tested raw material.

MATERIAL AND METHODS

Plant material

The research material consisted of rhubarb petioles (Rheum rhaponticum L.) of Viktoria variety. The rhubarb plants to be tested had petioles exceeding 20 cm in length and were at least 3 cm wide in the middle part of the petiole.

Ozonation of plant material

Rheum rhaponticum L. plants during the vegetation period in field conditions were placed in an ozonation chamber, which was connected to the Korona L5 ozone generator (Science and Implementation Laboratory "Korona" Piotrków Trybunalski). Gaseous ozone concentration was measured using the 106 M 2B Technologies ozone detector in the measurement range of 0–1000 ppm. Ozonation was carried out at 20°C in three repetitions. 10 ppm ozone gas was applied to the plants for 1, 3 and 5 minutes. Then, on the 1st, 3rd and 5th day after ozonation, samples of petioles were collected for testing and mechanical tests and chemical analyzes were performed.

Mechanical properties

Three-point bending test

Rhubarb leaf petioles were subjected to strength tests using Zwick/Roell Z010 machine. The research was carried out on freshly harvested petioles. The bending test was carried out in the middle part of the petiole, in 36 repetitions for each series of tests.

The bending process on the Zwick/Roell Z010 testing machine was carried out with the set work parameters: initial force to strain the sample F= 5N, module speed in the bend test 0.5 $mm·s^{-1}$. The anvil spacing was 100 mm for anvil and die radii of 5 mm and an anvil width of 40 mm.

Chemical properties

Antioxidant potential, polyphenol content, vitamin C, oxalic acid

The antioxidant potential was determined by the method (ABTS-2,2′-azino-bis(3-ethylbenzothiazolin-6-sulfonic acid) and DPPH (2,2-diphenyl-1-picrylhydrazyl), total polyphenol content by the method described by Matłok et al. (2020b). Vitamin C was determined by the titration method according to the methodology presented by Matłok et al. (2020a).

The amount of oxalic acid was determined by the titration method. 4 g of rhubarb petioles, which had previously been homogenized, were weighed on a technical scale. Then 100 ml of boiling distilled water was added. Waited 5 minutes then the solution was filtered. 10 ml of the filtrate was transferred to a centrifuge tube. 5 ml of 5% $CaCl_2$ solution and 5 ml of acetone were added and mixed. Put in the fridge for 30 minutes. The resulting calcium oxalate precipitate was centrifuged in a centrifuge at 3000 rpm for 10 minutes. The supernatant was decanted and the precipitate was quantitatively transferred to a 100 ml conical flask with 5 ml of 10% sulfuric acid and dissolved hot in a water bath. It was titrated immediately (hot) with 0.02 N potassium permanganate solution until a pink color was obtained, which persisted for about 1 minute. Based on the obtained results, the amount of soluble oxalic acid in 100 g of the product was calculated, assuming that 1 ml of 0.02 N $KMnO_4$ corresponds to 0.90 mg $(COOH)_2$. The concentration of oxalic acid was tested within 5 days of the ozonation process.

Statistical analysis

In order to verify the significance of the influence of ozonation time on the quality parameters for each analysis period, a one-way ANOVA (analysis of variance) was used at 0.05. These analyzes were carried out using the STATISTI-CA12.5 PL program by StatSoft (Matłok et al. 2020b).

RESULTS AND DISCUSSION

Measurement of mechanical properties

Figure 1 shows the average force values during three-point bending of rhubarb leaf petioles after ozonation in various combinations of dose and exposure time compared to the control. Taking into account the different variants of ozonation, the petioles of plants ozonated with a dose of 10 ppm for 1 minute were characterized by the highest resistance to three-point bending compared to other variants of ozonation. After application of the dose of 10 ppm for 3 and 5 minutes, reduced values of the tested parameter were noted in comparison to the control sample and the ozonated sample with the dose of 10 ppm for 1 minute. The average value of the three-point bending force within 5 days from the start of the experiment was 96.22 N for the control sample, and 95.48 N for the 10 ppm ozonated sample for 1 minute. For the remaining variants, i.e. 10 ppm, a reduction in the destructive force was observed in 3 and 5 minutes compared to the other variants. The average force value for the 10 ppm variant for 3 minutes was 85.91 N, and for the 10 ppm variant for 5 minutes it was 84.66 N.

Antos et al. (2018) reported an increase in the damage resistance of apple fruit after ozonation. Horvitz and Cantalejo (2012) conducted experiments with red pepper fruits cut into slices and treated with ozone gas at a dose of 0.7 $\mu L \cdot L^{-1}$ of gaseous ozone for 1, 3 and 5 min. They found that the firmness of non-ozonated peppers decreased faster, the ozonated fruits had slightly higher firmness than the control after 14 days of storage of the samples. Zardzewiały et al. (2020) found increased values of destructive force compared to the control for rhubarb petioles subjected to ozonation immediately after harvest. The highest average values of destructive force for rhubarb petioles were found after applying a dose of 100 ppm for 30 minutes. The process of ozonation with a dose of 10 ppm during 5 minutes did not have a positive effect on the destructive force values compared to the control sample.

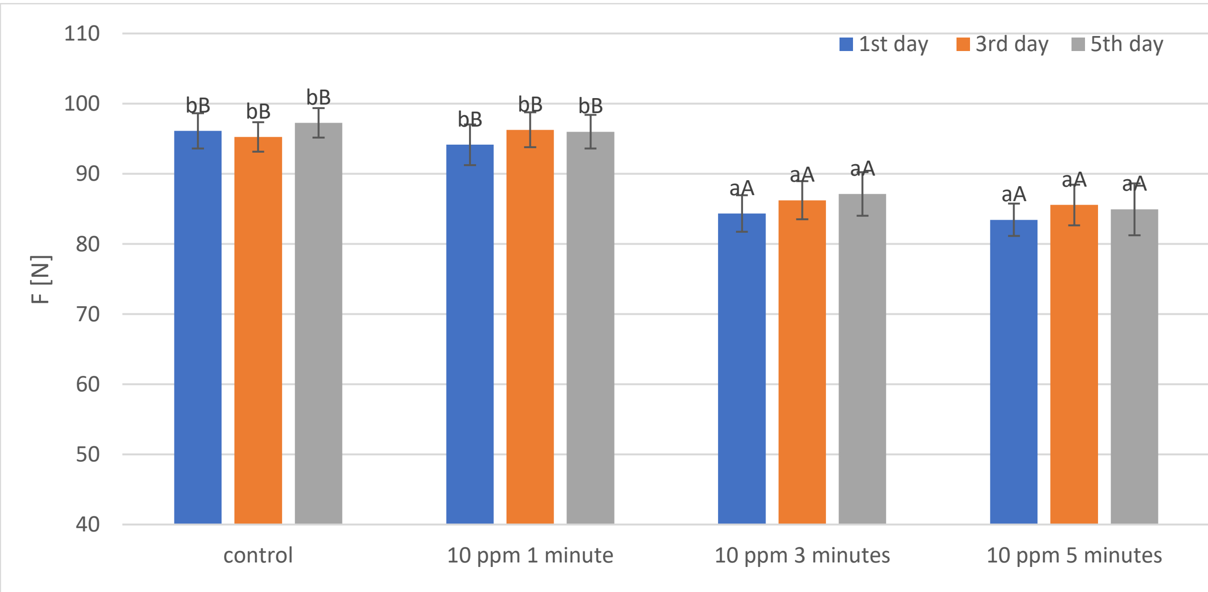

Figure 1. Average values of destructive force during three-point bending of rhubarb petioles (n = 3). Statistically significant differences between marked by different small and big letters ($p \leq 0.05$).

Oxalic acid content

The use of ozone gas at specific doses causes oxidative stress in plant cells. Ozone as a strong oxidant can affect oxalic acid levels by directly oxidizing it. In addition, gaseous ozone affects the modification of plant metabolism, which generates changes in the amount of secondary metabolites determining the quality of rhubarb petioles. The amount of oxalic acid was tested within 5 days of using ozone gas and the resulst are evidenced in Figure 2. For the control sample (non-ozonated plants), the average content of oxalic acid during the 5 days of the experiment was 366 mg·100 g^{-1}. As a result of the application of each dose of gaseous ozone, a decrease in the concentration of the tested acid was noted compared to the control. Already on the first day, a significant reduction in the amount of oxalic acid was observed, taking into account the values of the tested parameter for the control variant. The applied dose of gaseous ozone of 10 ppm during 1 minute reduced the amount of oxalic acid by over 8% on average over 5 days of testing compared to the control sample. Gaseous ozone at a dose of 10 ppm for 3 minutes decrease the amount of oxalic acid by over 29% on average compared to the control variant. However, the dose of ozone of 10 ppm for 5 minutes was the most effective, reducing the tested acid by more than 36% compared to the control variant.

Oxalic acid is an organic compound found in various parts of plants, both wild and cultivated. It is a by-product of many metabolic processes and can perform important functions in plants, such as participation in defense against pathogens, regulation of calcium ion concentration and participation in antioxidant reactions (Cai et al., 2018). However, the excessive presence of oxalic acid in plant tissues can lead to toxic effects, e.g. to inhibit cell growth and damage. Therefore, plants have developed mechanisms that allow the control and regulation of oxalic acid levels in cells. One of the main regulatory mechanisms is the inhibition of metabolic pathways that participate in the synthesis and accumulation of oxalic acid. Oxalic acid biosynthesis in plants can occur through various metabolic pathways, but the most important of them is the pathway of oxalic acid origin from oxaloacetate. This pathway is catalyzed by enzymes such as oxaloacetate hydrolase and oxaloacetate reductase, which are involved in the conversion of oxaloacetic acid to oxalic acid. When the concentration of oxalic acid in plant cells increases to an unfavorable level, there are regulatory mechanisms that limit the synthesis and accumulation of this compound. Inhibition of oxalic acid metabolism pathways is critical to ensure an optimal balance between oxalic acid levels and plant cell function. Proper regulation of oxalic acid accumulation allows plants to effectively defend against pathogens, maintain an appropriate concentration of calcium ions and protect against oxidative stress (occurring when ozone is used).

A competing (or coexisting) mechanism of reducing the content of oxalic acid may be the activation of metabolic pathways leading to its decomposition. Reducing the concentration of oxalic acid in the event of its excessive accumulation, which may be the result of abiotic stress (e.g. drought, extreme temperatures), pathogen infection or the action of other irritants, including ozone, is an important plant defense mechanism.

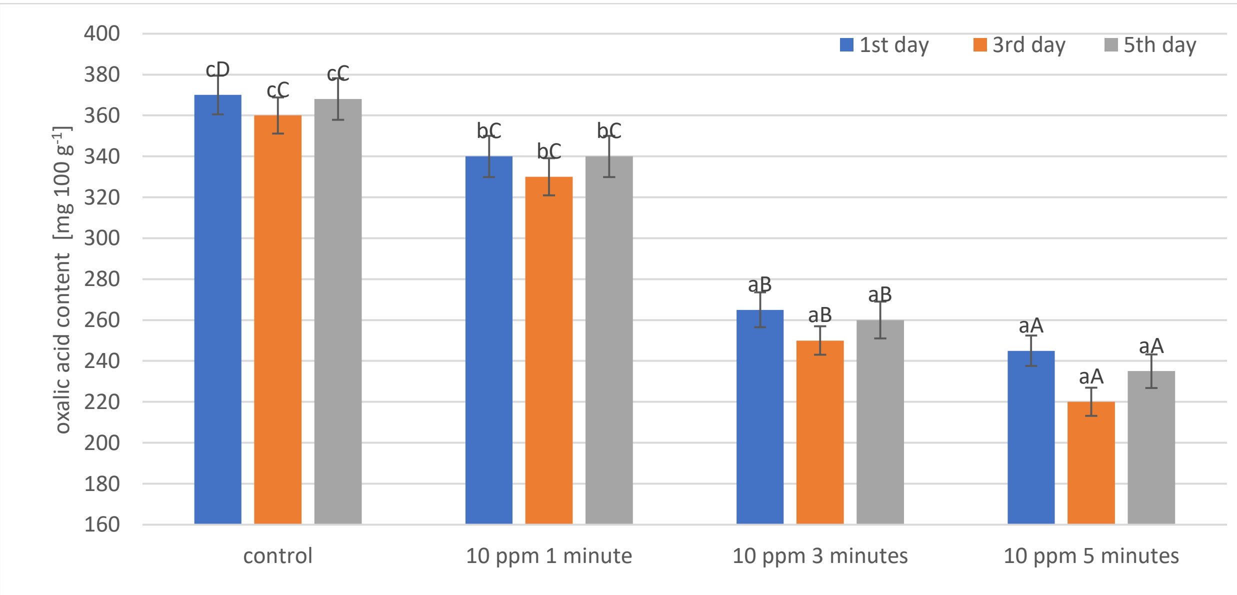

Figure 2. Oxalic acid content in rhubarb petioles, depending on the duration of ozonation compared to the control sample (n = 3). Statistically significant differences between marked by different small and big letters ($p \leq 0.05$).

The process of activation of oxalic acid degradation pathways may involve a number of regulatory mechanisms, such as induction of biosynthesis of oxalic acid degrading enzymes. This induction is usually caused by the expression of genes encoding enzymes responsible for the breakdown of this compound. These enzymes include oxalate-oxidase and oxalate-dehydrogenase, which catalyze the decomposition of oxalic acid into other compounds less harmful to cells. Plants also have specific proteins that transport oxalic acid to various cellular compartments. Activation of these proteins can affect the rate of removal of oxalic acid from the cell or direct it to places where its toxicity is minimized (Kumar et al. 2019). However, in the case of ozone application, it seems that this is the plant's response to oxidative stress and it is an adaptation that increases the plant's resistance to this factor.

Antioxidant potential, polyphenol content, vitamin C

Gaseous ozone used under controlled conditions causes oxidative stress in plants. The effect of this action is the activation of enzymes, causing quantitative changes in secondary metabolites and thus affecting the quality of plant raw materials (Piechowiak i in. 2019b).

Ozonation of rhubarb plants affected the content of bioactive compounds. In the case of the ABTS test, an increase in the content of the tested parameter was observed for each ozone dose applied one, three and five days after ozonation. Based on the results, it was found that samples exposed to ozone at a concentration of 10 ppm for 5 minutes had the highest antioxidant potential at each test date (Figure 3). In the case of the DPPH method, on the 1st and 3rd day after the process of using ozone gas, an increase in the tested parameter was noted compared to the control. However, on the 5th day, a downward trend was observed for the analyzed indicator of antioxidant potential compared to the control sample (Figure 4).

The assessment based on the ABTS method (Figure 3) showed an average increase of the examined parameter by 7.4% for all tested variants in comparison with the antioxidant potential of control samples (non-ozonated rhubarb plants). In the DPPH test, a similar trend was observed, but only for samples tested on the 1st and 3rd day after the ozonation process. The increase in the tested parameter was samples 3.1% compared to controls. On the other hand, for the analyzes performed on the 5th day for all tested ozonized variants, a decrease in the value of the tested parameter by 2.6% was recorded compared to the control sample. Changes in the antioxidant potential were probably caused by the effect of an oxidative explosion in the apoplastic space of plant cells treated with ozone. The result of this action was the activation of mechanisms involved in the biosynthesis of low-molecular antioxidants that easily react with free radicals (Waszczak et al. 2018). Gaseous ozone affects the antioxidant potential of plant materials, which was also observed by other scientists conducting research on raspberries, blueberries or papaya (Piechowiak et al. 2019a,b; Szymanowska et al. 2015; Onopiuk et al. 2017; Yeoh et al. 2014). Taking into account the different doses of ozone (used in relatively high concentrations and short times), a positive effect of O3 on the antioxidant potential of the tested plants was noted in most studies, which produce higher amounts of antioxidants as a result of the ozonation process (Matłok et al. 2020b; Matłok et al. 2022).

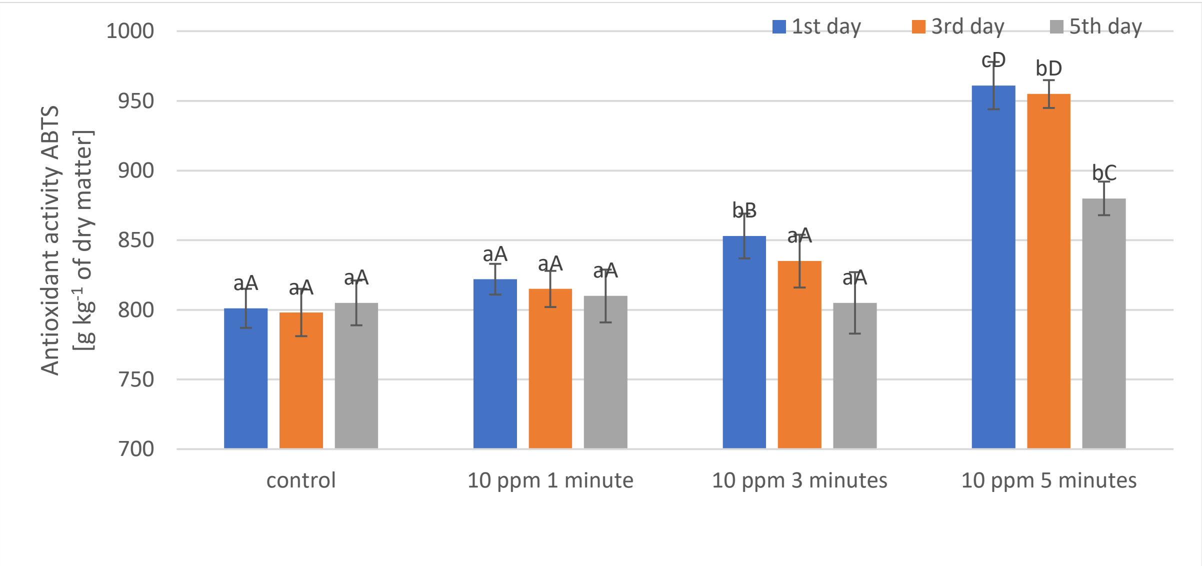

Figure 3. Antioxidant activity of the ABTS test of rhubarb petioles depending on the ozonation dose (n = 3). Statistically significant differences between marked by different small and big letters ($p \leq 0.05$).

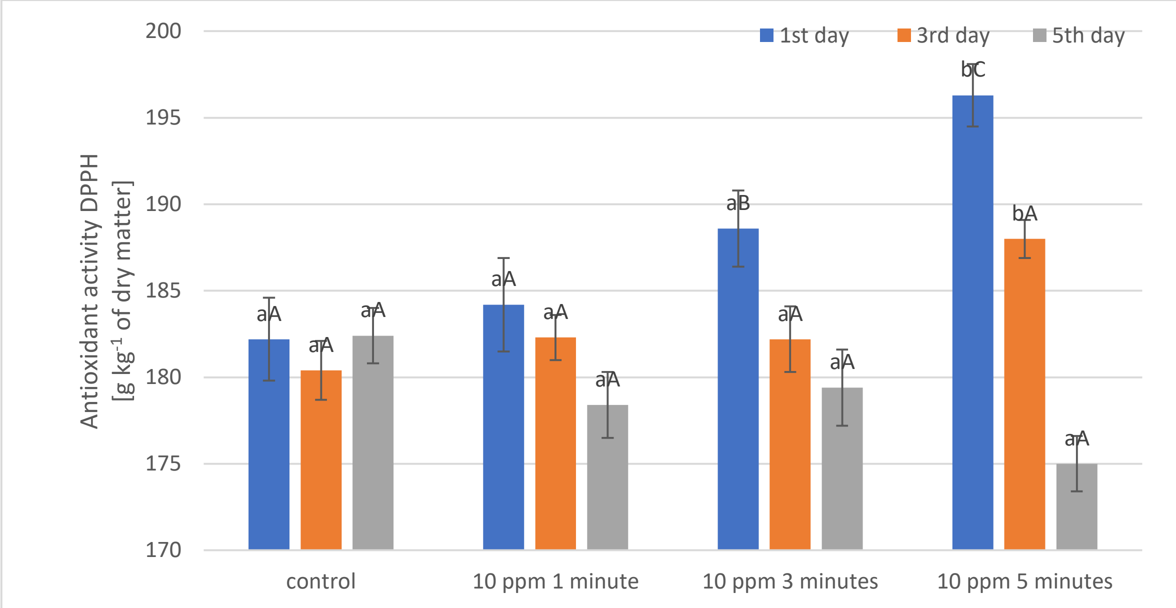

Figure 4. Antioxidant activity of the DPPH test of garden petioles depending on the ozonation dose (n = 3). Statistically significant differences between marked by different small and big letters ($p \leq 0.05$).

Ozonation of rhubarb leaf petioles with an ozone dose of 100 ppm for 15 and 30 minutes resulted in favorable changes in the antioxidant potential of the tested material stored in controlled conditions. Ozone concentration at the level of 10 ppm also increased the antioxidant potential of rhubarb leaf petioles, but this increase was definitely smaller than in the case of high doses of ozone (Zardzewiały et al. 2020).

Based on the results of the study, it was shown that ozonation of rhubarb plants during vegetation affected the total content of polyphenols in the examined rhubarb leaf petioles. Based on the research results, it was shown that the total content of polyphenols changes as a result of ozonation of rhubarb plants during the growing season (Figure 5). As a result of the use of gaseous ozone in the analyzed doses, the highest total content of polyphenols was recorded on the first day of the analysis compared to the control variant. In addition, the total polyphenol content for ozonated variants decreases within 5 days of testing. On the fifth day of the study, no beneficial effect of the dose of 10 ppm for 1 and 3 minutes on the concentration of polyphenols in the tested plant material was found. In the case of an ozone dose of 10 ppm for 5 minutes, the most favorable effects of ozone fumigation were observed compared to the control variant. It was found that the dose of 10 ppm during 5 minutes increased the total content of polyphenols during the 5-day period of laboratory analyzes by an average of 8% compared to the control sample.

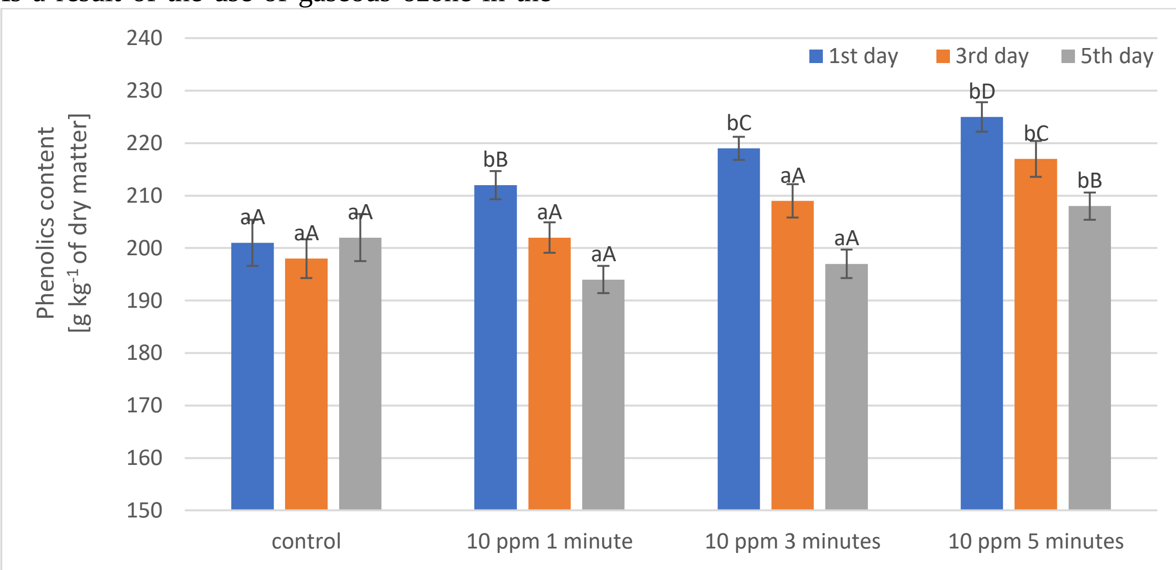

Figure 5. Total content of polyphenols in rhubarb petioles depending on the dose of ozonation (n = 3). Statistically significant differences between marked by different small and big letters ($p \leq 0.05$).

The storage process affects the changes in the content of polyphenols in fruits and vegetables. In most cases, a decrease in the content of polyphenols is noted, which depends on the conditions of the storage process. The use of gaseous ozone allows to reduce polyphenol losses during this process (Piechowiak et al. 2019a). In the literature, there have been many cases of an increase in the amount of these compounds after the use of gaseous ozone in the storage of plant raw materials (Rudolphi-Skórska et al. 2018; Li et al. 2022; Chen et al. 2016; Zhand et al. 2020). As a result of the use of stress factors such as ozone, secondary metabolites are formed, which are produced by the enzyme L-phenylalanine ammonia lyase (PAL) (Gałązka 2013).

Figure 6 shows the effect of ozonation on the vitamin C content in rhubarb petioles during storage. Natural metabolic processes responsible for the decrease in the amount of vitamin C in rhubarb petioles were accelerated as a result of the use of ozone gas. As a result of the use of gaseous ozone, a decrease in vitamin C content was observed during storage of the examined garden rhubarb petioles. The lowest values of vitamin C were recorded on the 5th day after applying an ozone dose of 10 ppm for 5 minutes. In turn, the highest concentration of vitamin C was tested in the control sample, regardless of the day of storage, compared to the ozonized variants.

Research by scientists indicates that gaseous ozone affects changes in the content of vitamin C in raspberries (Matłok et al. 2022), garlic (Hong et al. 2021), strawberries (Zhang et al. 2011) or bananas and pineapples (Alothman et al. 2010).

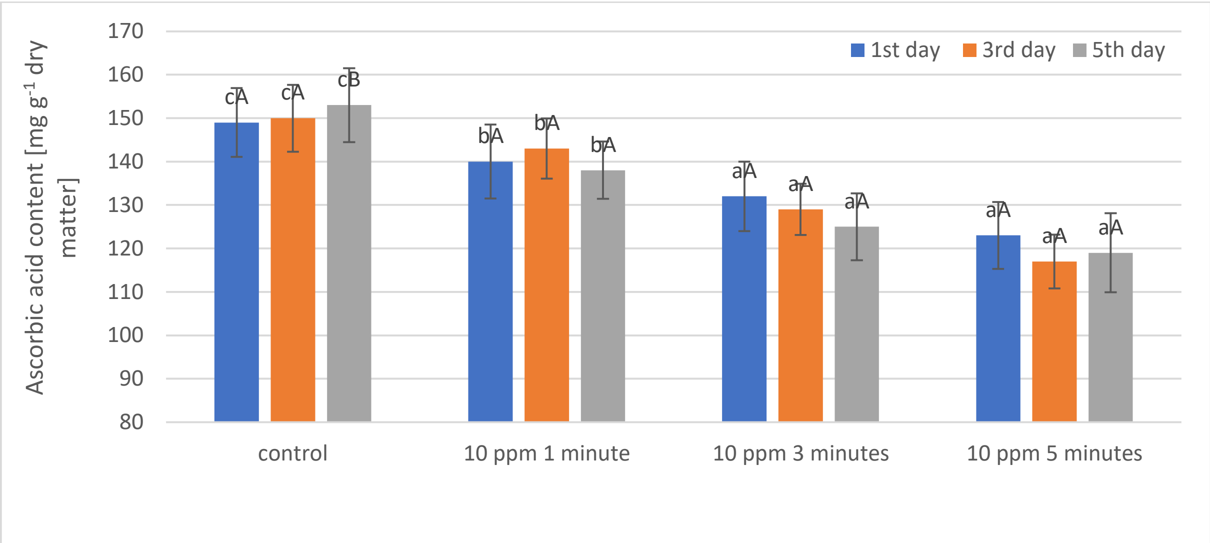

Figure 6. Total content of vitamin C in rhubarb petioles depending on the dose of ozonation (n = 3). Statistically significant differences between marked by different small and big letters ($p \leq 0.05$).

CONCLUSION

The conducted research on the possibility of using gaseous ozone during the growing season of rhubarb to improve the quality of petioles of this plant showed that a properly selected dose of ozone has a positive effect on selected parameters of the tested plant material. As a result of fumigation with this gas, regardless of the selected ozonation dose, petioles with a significantly reduced content of oxalic acid were obtained. Analyzing the results of antioxidant activity (ABTS test), it should be stated that gaseous ozone improved the quality of the tested petioles. The results of antioxidant activity - the DPPH test indicate that on the 1st and 3rd day after the ozonation process, an increase in the value of the tested parameter was obtained for each of the ozone doses used. On the other hand, on the 5th day after ozonation, a decrease in the tested parameter was noted compared to the control sample. A similar tendency as for the results of the DPPH test was observed for the total content of polyphenols. However, the content of ascorbic acid as a result of the ozonation process, regardless of the dose, is reduced compared to the control sample. The mechanical properties as a result of the ozonation process were also subject to changes. The applied doses of ozone did not improve the results of the destructive force during three-point bending of rhubarb petioles. On the other hand, the dose of 10 ppm during 3 and 5 minutes decreased the value of the tested parameter in comparison to the control sample.

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