

THE IMPACT OF THE OZONATION PROCESS ON THE CONTENT OF BIOACTIVE COMPOUNDS IN ZUCCHINI FRUITS AND THE CONCENTRATION OF SELECTED GASES DURING THEIR STORAGE

– Research paper –

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Abstract: The article presents the impact of ozone gas on the mechanical, chemical and microbiological parameters of zucchini fruit. Moreover, the influence of gaseous ozone on the concentration of gases in storage chambers was determined. Immediately after harvesting, zucchini fruits were treated with ozone at the following doses: 10 ppm -15 and 30 min; 50 ppm -15 and 30 min) in order to modify selected bioactive compounds. Then the ozonation process was repeated every 48 hours. Zucchini fruits were stored in a climatic chamber at 7°C and 90% humidity. Laboratory analyses were performed on the 1st and 10th day of storage. As a result of the use of the tested variable factor, no significant changes in the mechanical properties were noted. However, a significant effect of selected doses of ozone gas, i.e. 10 ppm 30 minutes and 50 ppm 15 minutes on the 10th day of storage, on the increase in vitamin C concentration compared to the control was demonstrated. In the case of the total polyphenol content, there was also a significant effect of the ozone dose of 50 ppm applied for 15 and 30 minutes on the 1st day of storage on the increase in the value of this parameter compared to the control. The antioxidant potential in the ABTS test increased significantly on the 1st day of storage after applying an ozone dose of 50 ppm for 15 and 30 minutes. In turn, in the DPPH test, only for an ozone dose of 10 ppm for 15 and 30 minutes on the 10th day of testing, a significant increase in the tested parameter was recorded. The positive effects of ozone use were observed during the storage of zucchini fruits in terms of reducing the microbiological load. The 50 ppm dose applied for 15 and 30 minutes had the most beneficial effect on reducing the number of aerobic mesophilic bacteria and the number of yeasts and molds. Ozone gas is an important factor inhibiting the production of ethylene by zucchini fruit during storage, thus extending the storage life of the tested raw material.

Keywords: *ozone gas, zucchini, ethylene, bioactive compounds*

INTRODUCTION

Zucchini (*Cucurbita pepo* var. *giromontiina*) belongs to the Cucurbitaceae family and is considered a culinary valuable vegetable. It is cultivated primarily in tropical and subtropical conditions [Kathiravan et al., 2006], and can also be grown in temperate regions. Zucchini cultivation is of great economic importance among vegetable crops around the world [Parris et al., 1996]. It is an annual plant of great importance for the processing industry [Mancini et al., 1999].

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Zucchini, being a seasonal vegetable, contains a number of beneficial microelements, carotenoids, vitamins and phenolic compounds [Blacko-Diaz et al. 2014; Martínez-Valdivieso et al., 2014 and 2015]. Zucchini fruit was used in traditional folk medicine to treat colds and relieve pain. Moreover, it has anticancer, anti-inflammatory, antiviral and antimicrobial properties [Menéndez et al., 2006, Møller and Loft, 2004; Oloyede et al., 2012; Shokrzadeh et al., 2010; Wang et al., 2007]. Zucchini is a physiologically immature fruit. During post-harvest storage, it produces low levels of ethylene [Baladrán-Quintana et al. 2003]. Zucchini fruits are very sensitive to physiological disorders

caused by cooling, which occurs when the fruits are exposed to low temperatures [Sevillano et al., 2009]. Balandrán-Quintana et al. [2003] reported that storing zucchini fruit at a temperature of 2.5°C stimulates the production of CO₂ and ethylene.

It is estimated that up to 40-50% of plant raw materials spoil before they are consumed, primarily due to mechanical damage and water loss [Kitinoja and Kader 2002]. Another important aspect is nutritional losses, i.e. loss of vitamins, antioxidants and health-promoting substances [Siddiqui et al., 2015; Barman et al., 2015; Nayyer et al., 2015]. The main causes of post-harvest losses: inappropriate temperatures during storage and transport, improper packaging and lack of knowledge about optimal conditions for transport and storage of plant raw materials [Kitinoja et al., 2011].

The final quality of the product before harvest depends on the cultivation technology used by the manufacturer. Factors affecting the quality of crops include the frequency of irrigation, the use of fertilization, pest and disease control, and the ability to use growth regulators. Moreover, weather conditions such as the amount and intensity of rainfall, wind strength, hailstorms affect the overall quality of fruit and their suitability for storage [Bekele, 2018]. It should be noted that fruits have a high water content when harvested, which creates an ideal environment for the development of microorganisms. Harvesting, i.e. separating the fruit from the parent plants, does not stop the biochemical and metabolic processes that lead to the degradation of the collected raw materials [Parmar et al., 2023].

There are many methods that have a beneficial effect on the storage life of fruit and improve their quality. The durability of fruit is influenced by many variables, such as temperature, variety, storage method and ethylene concentration [Khera et al., 2024]. When storing fruit, producers use many methods to extend the storage life, such as: ozone gas [Głowacz et al., 2015], chemical spraying [Sumedrea et al., 2018], ultrasound [Sridhar et al., 2021], active packaging [Ghodki et al., 2021] or

electromagnetic waves [Sadaf et al., 2023]. In addition, heat treatment methods are used, which effectively inactivate microorganisms, thus reducing the microbiological load [Kshirod et al., 2022]. The negative effect of heat treatment is the impact on some quality parameters, i.e. texture, color, aroma, taste and nutritional value [Tănase et al., 2022]. This is where new technologies come in handy and reduce this impact. The use of methods based on the action of gaseous or water ozone, ultrasound and UV-C radiation are examples of non-thermal methods that may potentially be used in the process of extending storage shelf life and improving the quality of fruit or vegetables [Parnar et al., 2023]. These methods increase microbiological safety, durability of products and do not affect quality features [Ekonomou et al., 2021, Chacha et al., 2021].

The ozonation is a valuable way to extend the storage life of fruit in the supply chain [Ozkan et al., 2011] Ozone gas is a disinfectant for plant materials by reducing the microbiological load [Rivas-Arancibia et al., 2022]. In addition, this technology is of great importance because ozone does not leave any residue in products. Additionally, the use of gaseous ozone affects the modification of biochemical processes in fresh and stored fruits and vegetables [Botondi et al., 2021]. Liu et al. [2021] found that ozone significantly inhibited the growth rate of aerobic bacteria, coliform bacteria, and yeast. Dilmaçunal et al. [2014] tested melons and found that ozone gas extended the storage life of the tested fruits. Chauhan et al. [2011] observed that ozone gas reduces the respiration rate and ethylene production in carrots during storage

The research results provide an assessment of the impact of the ozone on selected microbiological, mechanical and chemical properties of zucchini fruit after harvest and during storage. The fruits obtained from cultivation were analyzed for selected bioactive compounds (polyphenol content, vitamin C content, antioxidant potential) and selected gases were measured during their storage.

MATERIALS AND METHODS

Plant materials

The research material consisted of zucchini fruits of the "Astra Polka" variety. The fruit was obtained from our own cultivation (50°1'49"N 22°21'57"E, Gać, Podkarpackie Voivodeship, Poland). Fruit samples were collected in the second half of July 2023. Zucchini fruits of each variant were stored for 10 days in a climatic chamber at a temperature of 7°C and humidity of 90%.

Ozonation of fruit

Immediately after harvesting, the fruit was divided randomly into five batches, each weighing 10,000 g. The first batch was not treated with ozone (control sample). The remaining four batches were ozonated in a chamber with dimensions length × width × height 0.6 m × 0.5 m × 0.5 m. The Korona L5 ozone generator (Scientific and Implementation Laboratory "Korona", Piotrków Trybunalski,

Poland) was used to carry out the ozonation process. In the fumigation process of zucchini fruit, the gas concentration was measured with an ozone detector 106 M 2B Technologies (Broomfield, CO, USA) in the measurement range 0–1000 ppm. The experiment for each variant was performed in three independent repetitions. The ozonation process was carried out every 48 hours. The following ozone doses were used in the experiment: 0 ppm 0 minutes – control; 10 ppm for 15 minutes; 10 ppm for 30 minutes; 50 ppm for 15 minutes; 50 ppm for 30 minutes.

Before starting the experiment, preliminary studies were conducted to determine the tolerance threshold of zucchini fruit of the “Astra Polka” variety to gaseous ozone. For this purpose, zucchini fruit were exposed to gaseous ozone at concentrations of 2, 5, 10, 20, 50, 100 and 200 ppm for 5, 15, 30 and 45 minutes. Based on the results of pre-liminary studies, possible ozone doses were selected.

Mechanical properties - compression test

Selected mechanical parameters of zucchini fruit were measured in a compression test between two flat horizontal plates using a Zwick/Roell Z010 testing machine (Zwick Roell Polska Sp. z o.o. Sp. K., Wrocław, Poland). The tests on the resistance of zucchini tissues to mechanical damage in the process of uniaxial compression were carried out on cut cylindrical samples with a diameter of 12.00 mm and a sample height of $18.59 \text{ mm} \pm 0.17$. The tests were carried out on fresh fruit of similar size and material exposed to various doses of ozone and stored for 10 days. The uniaxial compression process was carried out with the following operating parameters: initial force when compressing the sample $F=0.5 \text{ N}$, module speed during the compression test $0.5 \text{ mm}\cdot\text{s}^{-1}$.

Measurement of Water Content

The water content of tested zucchini fruits was determined using drying method ($105 \text{ }^\circ\text{C}$), in accordance with PN-90/A-75101-03: 1990., using a laboratory moisture analyser (Radwag, Poland). Measurements were made on the 1st and 10th day of storage using a laboratory dryer-SLW 115 SMART (POL-EKOWódziszław Śląski, Poland). For the analysis of water content of zucchini cylindrical samples with a diameter of 12.00 mm and a height of $18.59 \text{ mm} \pm 0.17$ were used.

Determination of pH and Acidity

The titratable acidity and the pH of the zucchini were analysed through the potentiometric titration of the sample for analysis with a standard 0.1 M

NaOH solution at $\text{pH} = 8.1$ using TitroLine 5000 (SI Analytics, Weiheim, Germany) according to methodology of PN-EN 12147:2000. The obtained results are expressed as g of citric acid per 100 g of fruit. Each fruit was analysed 24 h after ozonation. The analyses were performed in triplicate.

Determination of the amount of bioactive components

The determination of the ascorbic acid concentration in zucchini fruit was carried out in accordance with the PN-A-04019:1998 standard [Food Products, 1998]. The total content of polyphenols in zucchini fruits was determined using the Folin–Ciocalteu method according to the methodology developed by Piechowiak et al. [2019]. Antioxidant activity: DPPH test was determined according to the methodology described by Djordjević et al. [2010], the ABTS test was determined according to the methodology described by Jakobek et al. [2007]. In turn, the FRAP test was determined according to the methodology described by Chiabrande and Giacalone [2015].

Microbiological Analysis of Zucchini Fruit

The microbiological load of zucchini fruit was analyzed on the 1st and 10th day of storage. The determination of the number of yeasts and molds in 1 g of zucchini fruit was carried out in accordance with the standard PN-EN ISO 21527-1:2009. The counting of mesophilic aerobic bacteria in a given raw material was carried out using the plate counting method (Ae) (O) PB-77/LM, edition 4 of December 7, 2015.

Measurements of gas concentrations

In climatic chambers where the zucchini fruits of the analyzed variants were stored, measurements of the concentration of O_2 (%), CO_2 (%) and C_2H_4 (ppm) were carried out. Measurements were performed 20 times for each analyzed variant. Gas concentrations were determined using an F-950 gas analyzer (STEP Systems GmbH, Nuremberg, Germany). The air flow from the storage chambers during the measurements was $33 \text{ ml}\cdot\text{min}^{-1}$. The measurements were carried out 60 minutes after the end of the ozonation process.

Statistical Analysis

To check the significance of the effect of ozone on the tested parameters of zucchini fruit during storage, an analysis of variance was used with a significance level of $\alpha=0.05$. These analyzes were performed using the STATISTICA 12.0 package.

RESULTS AND DISCUSSION

Immediately after harvesting, the zucchini fruit was randomly divided into five batches. The first batch was not treated with ozone (control test). The remaining four batches were ozonated in a specially prepared chamber for this treatment. Ozonation was performed cyclically every 48 hours. Different doses of ozone were used in the experiment.

Ascorbic Acid Content in zucchini fruits

Ascorbic acid is a chemical compound commonly known as vitamin C. It has antioxidant properties and has a positive effect on the human body [Moss et al., 1989]. Ascorbate is an antioxidant and free radical scavenger. Moreover, it accelerates the course of key enzymatic reactions [Ryan et al., 2010]. Vitamin C slows down the formation of free radicals [Burger et al., 2017] and prevents diseases related to oxidative stress [Padayatty et al., 2003]. The content of ascorbic acid 1 day after the ozonation process significantly increased only after applying an ozone dose of 50 ppm for 30 minutes. In the remaining cases, no positive impact of the ozonation process on the tested parameter was noted. However, on the 10th day of storage, for the ozone doses used, i.e. 10 ppm 30 minutes and 50 ppm 15 minutes, the concentration of ascorbic acid increased significantly compared to the control. Moreover, it was noticed that the ozone dose of 10 ppm for 15 minutes had a negative impact on the concentration of the tested acid compared to the control sample during 10 days of storage of the tested fruits (Figure 1). The use of low concentrations of ozone can affect the ability of fruits and vegetables to synthesize vitamin C. In addition, ozone gas can also slow down the loss of vitamin C content during storage. However, it

should be noted that ozone used in higher concentrations may contribute to the oxidation of vitamin C [Perez et al., 1999]. Liu et al. [2021] examining garlic found that the content of ascorbic acid in this raw material increased significantly during storage as a result of the use of ozone gas at low concentrations. Based on their research, Pérez et al. [1999] and Shah et al. [2004] suggested that ozone stress may lead to the biosynthesis of ascorbic acid from carbohydrate reserves.

El Moniem et al. [2022] examining the effect of gaseous ozone on the concentration of vitamin C in avocado fruits, found a beneficial effect of gaseous ozone on the concentration of the analyzed vitamin. Zhang et al. [2011] treated strawberry fruit after harvest with ozone gas at a concentration ranging from 0 ppm to 8 ppm. The test results showed that ozone gas at a concentration of 4 ppm inhibited the decrease in ascorbic acid in the tested fruits. Li et al. [2022] examined pitaya fruits and found that the ascorbic acid content decreased with increasing storage time. Additionally, they noted that ozone gas delayed the decline in ascorbic acid. Green fruits and vegetables show greater oxidation effects due to ozone gas. However, there are many studies conducted on green plant products that show that ozonation has no negative effect on the ascorbic acid content of lettuce [Ölmez et al., 2009; Hassenber et al., 2007], spinach [Szydłowska-Czerniak et al., 2010] and arugula leaves [Gutiérrez et al., 2018]. At the same time, ozone often exhibits eliciting activity, which leads to increased biosynthesis of selected compounds, most often low-molecular-weight ones with antioxidant properties [Sandermann et al., 1998].

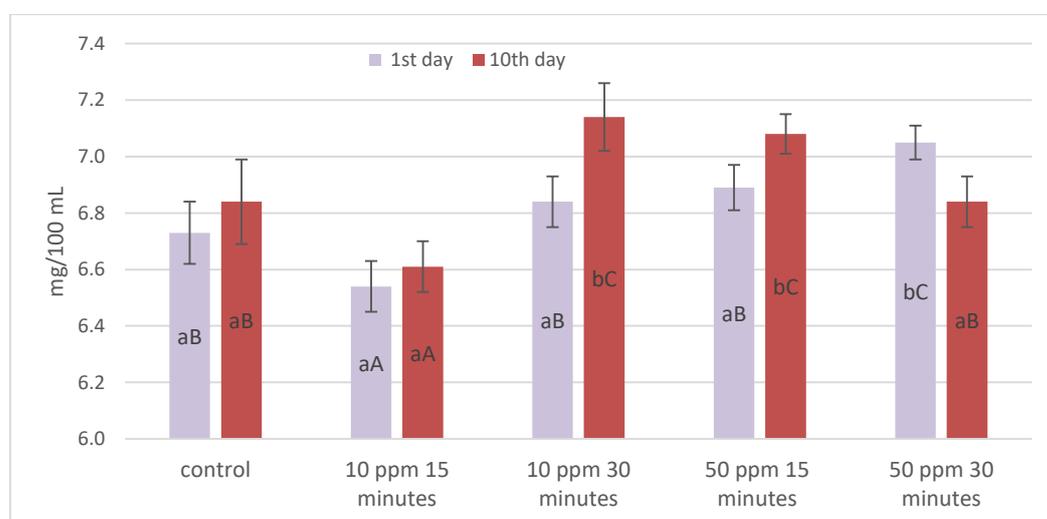


Figure 1. Content of ascorbic acid in zucchini fruit during storage.

NOTE: lowercase letters - differences between test dates; capital letters – differences between ozone doses.

Total phenolic content

The influence of ozone on the chemical properties of plant raw materials is unquestionable. The concentration of polyphenolic compounds changes as a result of the use of ozone gas. Scientists report that quantitative changes in the concentration of polyphenol compounds are the result of the so-called "Oxidative stress". Ozone gas molecules affect the cellular structure and influence the production of phenolic compounds. The ozone gas fumigation process may affect the release of some phenolic compounds bound in cell walls [Alothman et al., 2010]. It is worth noting that various phenolic compounds accumulate in cell walls [Paissoni et al., 2017]. Moreover, changes in phenolic compounds may be caused by ozone-induced enzymatic activity. It was found that enzyme activation causes rapid accumulation of phenolic compounds [Rodoni et al., 2009].

In the control sample, the concentration of the tested parameter decreased during storage. Similar dependencies were found for the ozone dose of 50 ppm for 15 minutes and 50 ppm for 30 minutes. For the remaining ozone doses, an increase in the tested parameter was observed during 10 days of storage. On the 1st day of storage, a significant increase in the tested parameter was found for the ozone dose of 50 ppm for 15 minutes and 30 minutes. In turn, on the 10th day of storage, each ozone dose significantly increased the total polyphenol content compared to the control (Figure 2).

An appropriate dose of gaseous ozone has a beneficial effect on the content of phenolic compounds. The results of research conducted by

Zhang et al. [2011] report that as a result of the use of an appropriate dose of gaseous ozone, the content of phenolic compounds in strawberry fruit increased. Continuous exposure of zucchini fruit to ozone at a concentration of $0.1 \mu\text{mol mol}^{-1}$ had no significant effect on the concentration of polyphenols [Głowacz et al., 2015]. Balawejder et al. [2021] studied the effect of ozone gas and ozone dissolved in water on the chemical properties of apples. Based on the test results, they found that the content of polyphenols increased after using selected doses of the tested variable factors compared to the control variant. Ozone gas causes changes in the structure of the skin and cell wall, which may lead to the release of some conjugated phenolic compounds [Paissoni et al., 2017]. The sensitivity of phenolic compounds to ozone is most likely dependent on the type and location of the compounds in the cell [Karaca et al., 2014]. Ozone stimulates antioxidant systems located mainly in the apoplast. Ranieri et al. [1996] observed that ozone treatment increased the amount of free and glycoside-bound phenolics in the cellular material. Another study showed that ozone treatment reduced the amount of cell wall-bound phenolics [Wiese et al., 2003]. Phenolic compounds probably increase due to changes in enzyme concentration and activity, which are induced by ozone. Phenolic compounds can be rapidly accumulated due to activation of pre-existing enzymes [Rodoni et al., 2010]. Most often, PAL is activated [Sachadyn-Król et al., 2016], which leads to increased biosynthesis of phenolic compounds.

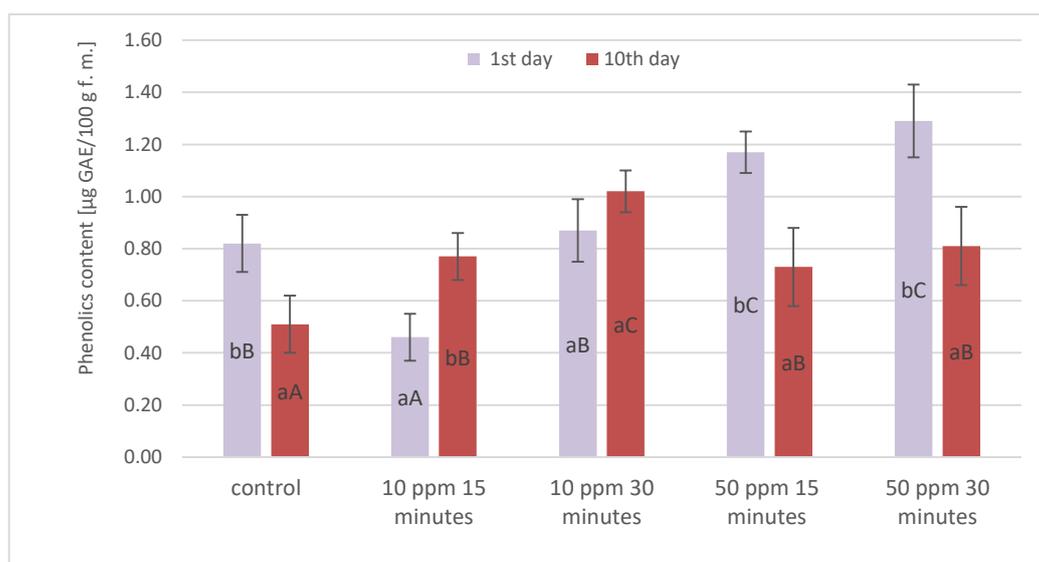


Figure 2. Total polyphenol content in zucchini fruit during storage

NOTE: lowercase letters - differences between test dates; capital letters – differences between ozone doses.

Antioxidant Activity

The antioxidant properties of plant products are an important element of the human diet and have a beneficial effect on health. Products rich in antioxidants counteract the effects of free radicals, which are a threat to cellular structures [Stolarzewicz et al. 2013]. A diet rich in fruit and vegetables is an important way to prevent many diseases, e.g. diabetes, atherosclerosis, Alzheimer's

disease and cancer [Domenico et al., 2012; Dutta, 2015; Ojeda et al., 2016; Moga et al., 2016]. In the case of ABTS, DPPH and FRAP tests regarding antioxidant activity, similar relationships were found. On the first day after the ozonation process, significant dependencies were noted for the ABTS test for the ozone dose of 50 ppm 15 minutes and 30 minutes compared to the control.

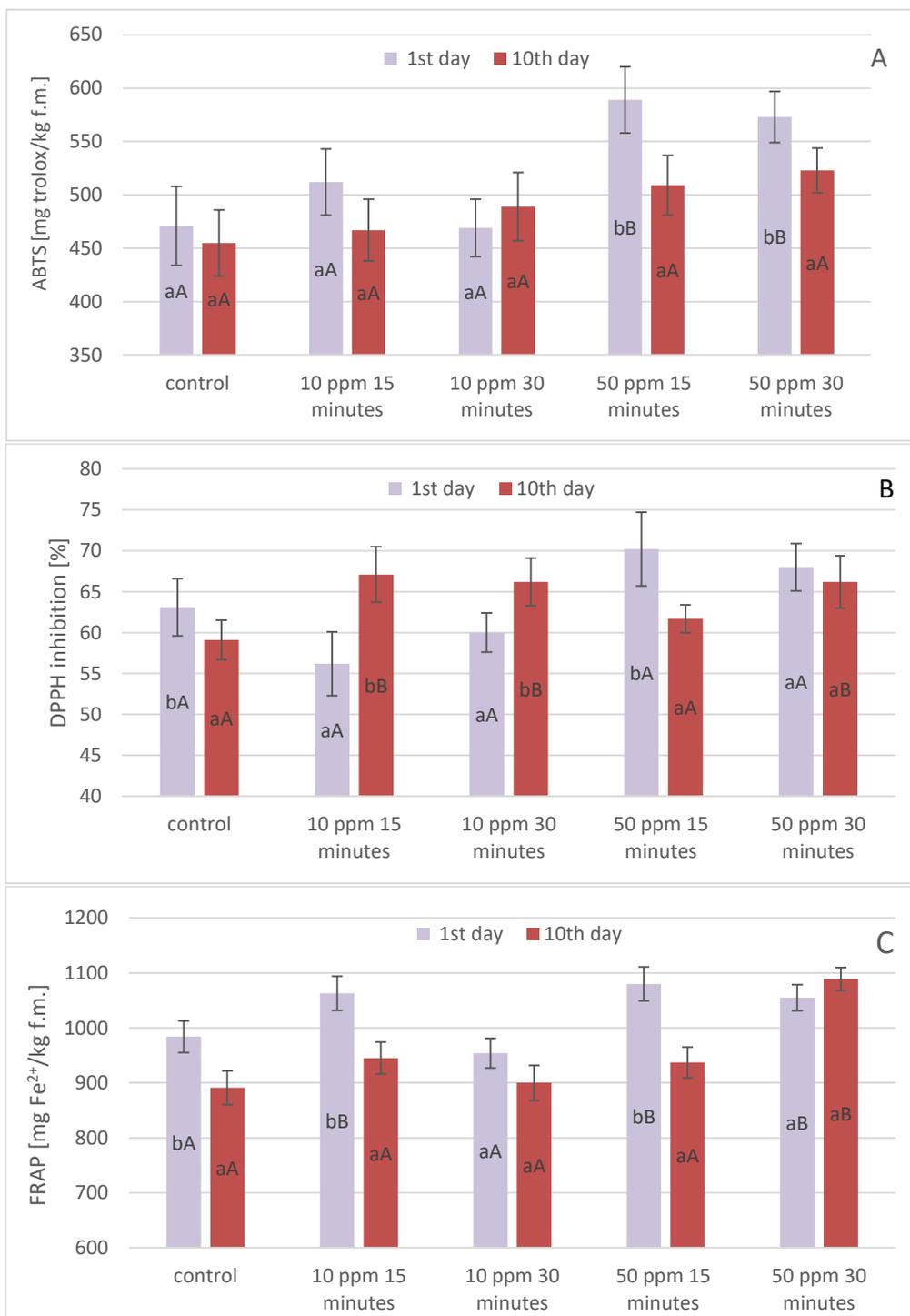


Figure 3. Antioxidant activity. ABTS test (A), DPPH test (B) and FRAP test (C) in zucchini fruit depending on the ozone dose and storage time. NOTE: lowercase letters - differences between test dates; capital letters – differences between ozone doses.

No significant relationships were found for the remaining doses. In turn, on the last, 10th day of storage, no significant differences were observed for the tested parameter for ozonated fruit compared to the control sample (Figure 3a). In the case of the DPPH test, on the first day of storage, no significant effect of the applied variable factors was found. On the 10th day, a significant increase in the tested parameter was noted for the ozone dose of 10 ppm for 15 minutes and 30 minutes and 50 ppm for 30 minutes compared to the control variant (Figure 3b). Analyzing the results for the FRAP test, a significant increase in the tested parameter was noted for the ozone dose of 10 ppm 15 minutes and 50 ppm 15 and 30 minutes compared to the control. On the 10th day of analysis, an increase in the tested indicator was found for an ozone dose of 50 ppm in 30 minutes compared to the control variant. The remaining analyzed samples were characterized by similar values of the tested indicator (Figure 3c). Comparing the research results of this article with the results of other analyzes (FRAP test), it should be stated that zucchini is a raw material with high antioxidant properties comparable to red cabbage, broccoli, strawberry or raspberry [Šapčanin et al., 2017]. In turn, in the FRAP test, the tested zucchini fruits had a value of the tested parameter in the range of 1000-1500 Fe_2SO_4 kg of fresh weight [Castro-Cegrí et al., 2023]. Ong et al. [2014] found that antioxidant activity measured by FRAP tests increased after the application of ozone gas on papaya fruit. Castro-Cegrí et al. [2023], examining the antioxidant properties of zucchini, found that these fruits have antioxidant activity of approximately 700 mg of trolox/kg of fresh weight in the ABTS test. Other studies [Brodowska et al., 2015] confirm the beneficial effect of gaseous ozone on the antioxidant properties of ozonated berries in test

ABTS. Similar results were observed in test ABTS for raspberries treated with ozone gas [Onopiuk et al., 2017]. Zhu et al. [2019] applied ozone gas to mandarin fruits. They found that the antioxidant activity of the tested fruits in ABTS test increased after the application of ozone gas.

Ong et al. [2014] found that antioxidant activity measured by DPPH in tested fruits were characterized by 70% inhibition. Rodoni et al. [2010] used gaseous ozone on tomato fruits (test DPPH) and did not note any changes in antioxidant activity during their storage. Minas et al. [Minas et al., 2012] applied ozone gas to kiwi fruits. They found that the antioxidant activity of the tested fruits in DPPH test increased after the application of ozone gas.

Changes in acidity and pH of zucchini fruit depending on storage time and ozonation dose.

Organic acids are a natural component of fruits and vegetables. The amount of organic acids in plant materials is variable and depends on biotic factors, e.g. species and variety, and abiotic factors such as soil and climate conditions. The concentration of organic acids decreases during vegetation [Saradhulhat et al., 2007]. On the first day of storage, it was noted that the application of each dose of ozonation significantly increased the acidity of the tested fruits.

The highest acidity value for the tested fruits was noted after the application of an ozone dose of 10 ppm for 30 minutes compared to the control sample. In the case of the control sample and the sample ozonated with a dose of 10 ppm for 15 minutes, the highest increases in acidity were noted during fruit storage, comparing the results from the 1st and 10th day of the study. In turn, for the 10 ppm for 30 minutes variant, there was a significant decrease in acidity during storage.

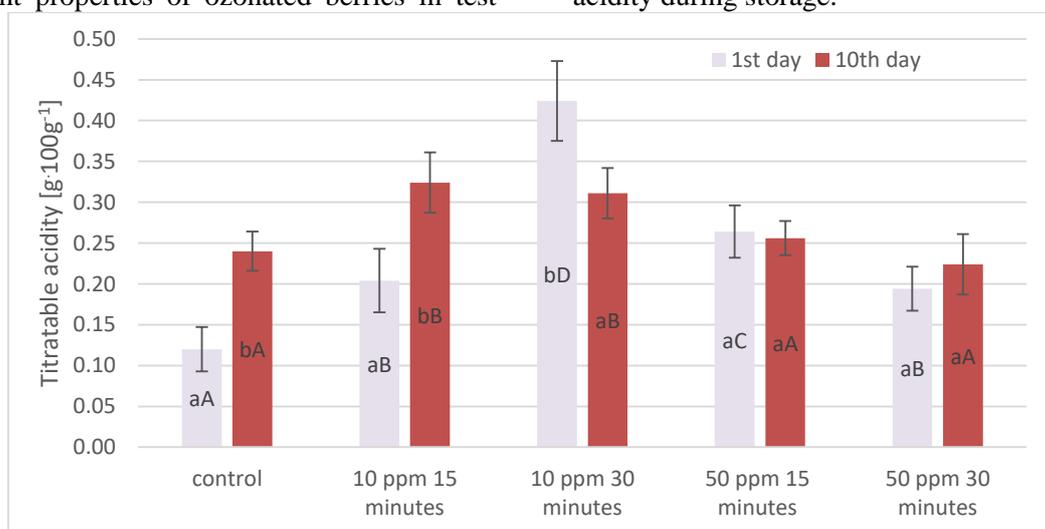


Figure 4. Acidity of zucchini fruit during storage. NOTE: lowercase letters - differences between test dates; capital letters – differences between ozone doses.

On the 10th day of storage, a significant increase in the tested parameter was noted for the 10 ppm for 15 minutes and 30 minutes variants compared to the control sample. For the remaining ozone doses, no significant effect of the tested factor on the increase in acidity was noted on the 10th day of storage. These changes may be due to limited oxidation of organic substances, which may lead to an increased content of organic acids. Most organic acids are weak acids, they have little effect on the quality of the fruit, with noticeable changes in their content (Figure 4) [Zhang et al., 2023].

Organic acids, in addition to their effect on the taste of food, stimulate the growth of microorganisms, coagulate proteins and perform a buffering function. Organic acids in plant materials are mainly malic and citric acids, which alkalize the human body, inhibit the growth of undesirable microflora, and also affect the course of metabolic processes [Silva et al., 2004; Valentão et al., 2005]. In addition, organic acids, due to their antioxidant properties,

play an important protective role against various diseases. Organic acids, with the exception of oxalic acid, improve the absorption of non-heme iron from plant foods [Silva et al., 2004].

Zucchini fruits on the 1st day of storage were characterized by a pH value of 6.89. The ozonation process with a dose of 50 ppm for 15 minutes significantly increased the pH of zucchini fruits by 2.17% compared to the control. No significant changes in the pH of the fruits were observed for the other variable factors used. On the other hand, on the last 10th day of storage, the pH value of the tested fruits changed compared to the 1st day of the study. For the 50 ppm for 15 minutes variant, a significant increase in the pH value was noted compared to the control sample and the other ozonated variants. These results correspond to the titratable acidity, however, it can be concluded that a higher ozone dose produces more acids with lower molar masses and higher pKa (acid dissociation constant) [www1].

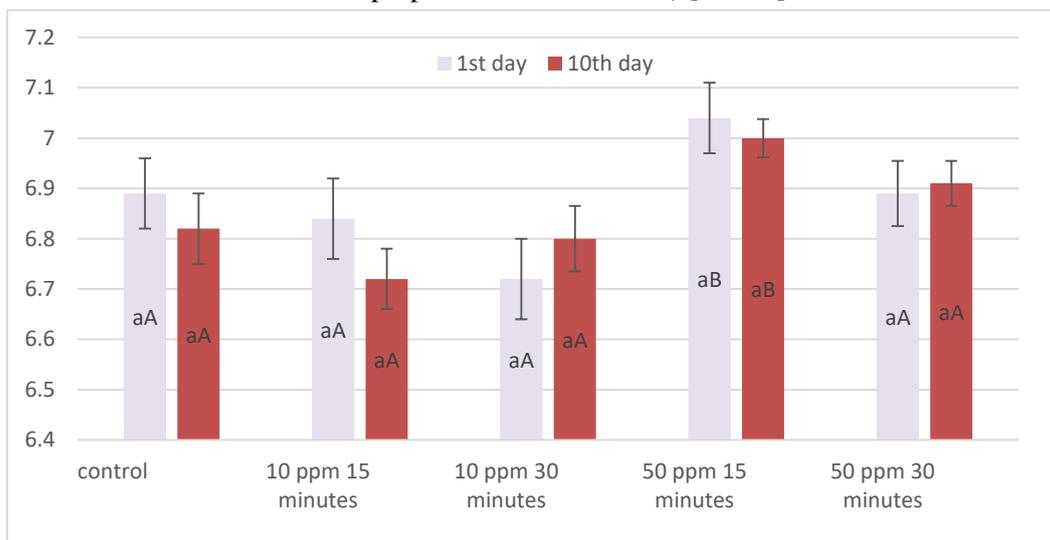


Figure 5. Changes in pH of zucchini fruit during storage. NOTE: lowercase letters - differences between test dates; capital letters – differences between ozone doses.

Microbiological analysis

Currently, there are practically no fungicides or bactericides that, when used after harvesting plant raw materials, are safe and do not leave toxic residues. In order to minimize the problems of residues after using chemicals, other effective disinfectants are being sought that will be effective against microorganisms [Gabriela et al., 2020]. The use of ozone gas as an alternative disinfection technology can successfully replace other disinfectants, e.g. chlorine. Ozone at concentrations of 0.15–5.0 ppm has been shown to inhibit the growth of food spoilage bacteria and yeasts [Jay et al., 2005; Asgar et al., 2014].

The microbiological load of zucchini fruit changed during storage. Ozonation performed cyclically every 48 hours reduced the number of aerobic mesophilic bacteria. On the first day of storage, the number of tested microorganisms decreased after applying an ozone dose of 50 ppm for 15 and 30 minutes; these differences were not statistically significant. The ozonation dose of 10 ppm for 15 and 30 minutes did not reduce the microbiological load. In turn, in the last 10 days of storage, a significant decrease in the tested bacteria was found for the ozone dose of 50 ppm for 15 and 30 minutes compared to the control sample. The ozone dose of 10 ppm for 15 and 30 minutes did not reduce the microbiological load on zucchini fruit (Figure 6).

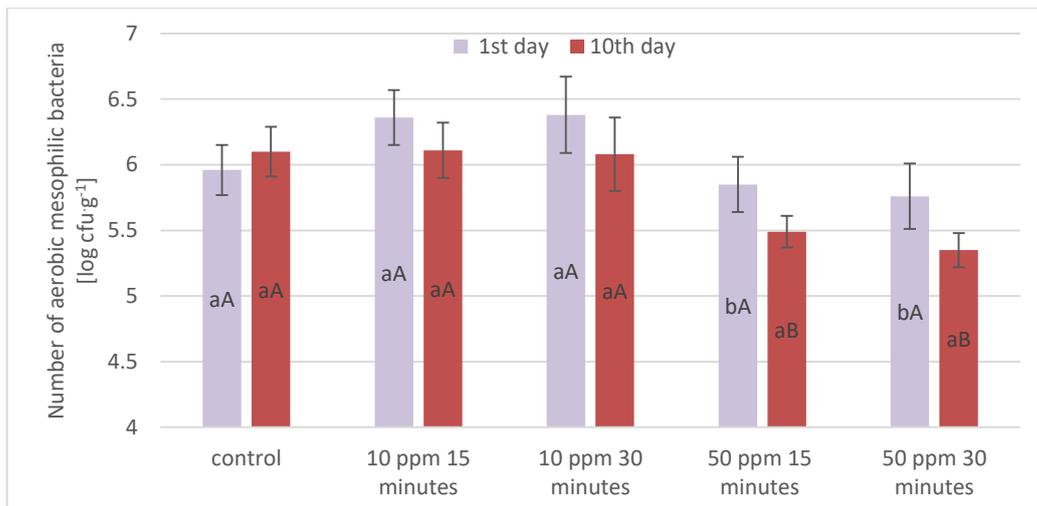


Figure 6. Microbiological load. The number of aerobic mesophilic bacteria in zucchini fruits depending on the ozone dose and storage time. NOTE: lowercase letters - differences between test dates; capital letters – differences between ozone doses

The number of yeasts and molds decreased during 10 days of storage as a result of cyclic ozonation. On the first day, there was no significant impact of the applied ozonation doses on the tested microbiological load parameter. On the 10th day, a significant decrease in the number of yeasts and molds was observed for ozone doses of 50 ppm 15 minutes and 50 ppm 30 minutes. The reduction of the tested parameter was 16.91% and 20.24%, respectively, for the above-mentioned ozonation doses compared to the control (Figure 7). Ozonation carried out in the form of fumigation has been used as a disinfecting treatment in the process of storing and preserving fruit and vegetables. The main advantage of gaseous ozone is that applying it directly to plant raw materials does not deteriorate their quality and acts as an antimicrobial agent [Patil et al., 2009]. For example, raspberry fruits stored at room temperature after applying ozone gas at a dose of 8–10 ppm for 30 minutes every 12 hours for 3 days limited the growth of aerobic mesophilic

bacteria and fungi [Piechowiak and Balawejder, 2019]. Other studies have shown that ozone gas significantly reduces the microbiological load of kiwi [Beirão Da Costa et al., 2014], strawberries [Chen et al., 2019] and carrots [Souza et al., 2018], thereby maintaining the physical and chemical quality of raw materials. In addition, ozone gas at a concentration of 9 ppm applied to cut pepper fruit for 6 hours resulted in a reduction in the number of *E. Coli* by 2.89 log cfu⁻¹, *S. Typhimurium* by 2.56 log cfu⁻¹ and *L. monocytogenes* by 3.06 log cfu⁻¹ compared to the control [Alwi et al., 2014]. Other scientific reports indicate that the process of fumigation of green beans and cherry tomatoes with ozone at a concentration of 126–136 ppm for 3 and 15 minutes reduces pathogens by 1 and 4 log cfu⁻¹, respectively. Regular exposure to ozone, i.e. 3 minutes a day of the analyzed raw materials, reduces the number of bacteria and molds by over 5 log cfu⁻¹ compared to the control [Roy et al., 2021].

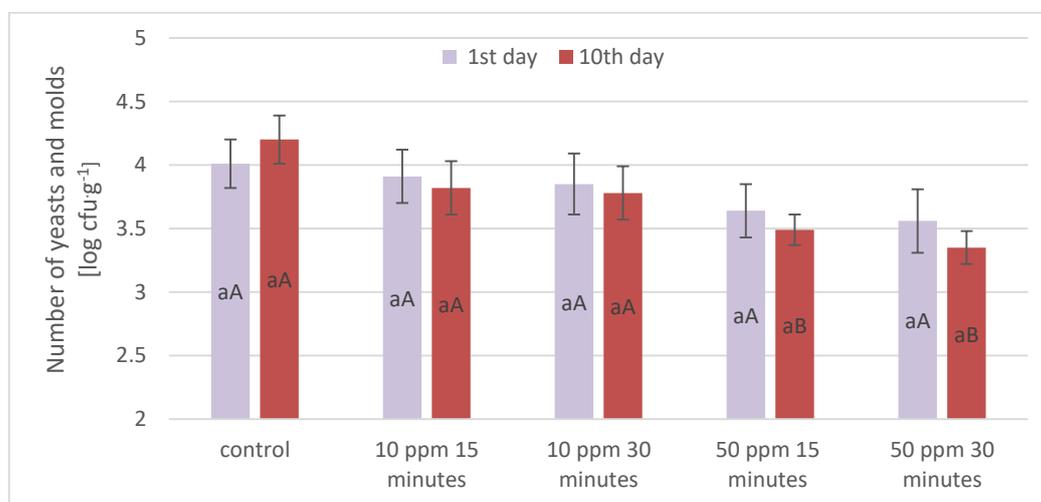


Figure 7. Microbiological load. The number of yeasts and molds in zucchini fruit depending on the ozone dose and storage time. NOTE: lowercase letters - differences between test dates; capital letters – differences between ozone doses.

Changes in ethylene and carbon dioxide concentrations during storage of zucchini fruit

Even though zucchini fruits are classified as non-climacteric, there are scientific studies showing that the ripening of these fruits also depends on the concentration of ethylene [Garcia et al., 2020]. Ethylene concentration increases as ripening progresses, leading to a reduction in fruit firmness and shelf life [Megias et al., 2015]. Ethylene concentrations changed during 10 days of storage. The highest ethylene values were found for the control. On day 10, an ethylene concentration of 4.6 ppm was found for this variant. The use of a cyclic ozonation process resulted in a reduction in the concentration of the tested gas during storage. Already on the first day of storage, a significant decrease in ethylene concentration was observed after the use of gaseous ozone at a dose of 10 ppm 30 minutes and 50 ppm 15 and 30 minutes compared to the control. Similar trends for the tested ozone doses were recorded on the 5th and 10th day

of measurements. There was no significant effect of the ozone dose of 10 ppm for 15 minutes on the reduction of ethylene concentration for zucchini fruit compared to the control sample (Figure 8).

The results of the respiration of zucchini fruit during storage are presented in Figure 9. The carbon dioxide concentration changed significantly during storage. In the case of the control sample, the highest values of the tested gas were recorded during 10 days of storage. On the first day after the ozonation process, there was no significant effect of ozone gas on changes in CO₂ concentration. Cyclical ozonation process with doses of 10 ppm 30 minutes, 50 ppm 15 and 30 minutes resulted in a decrease in carbon dioxide concentration compared to the control variant. The same relationship was also noted on the 10th day of storage (Figure 9). Based on the results, it should be concluded that an appropriately selected ozonation dose slows down the respiration process of zucchini fruit during storage under controlled conditions.

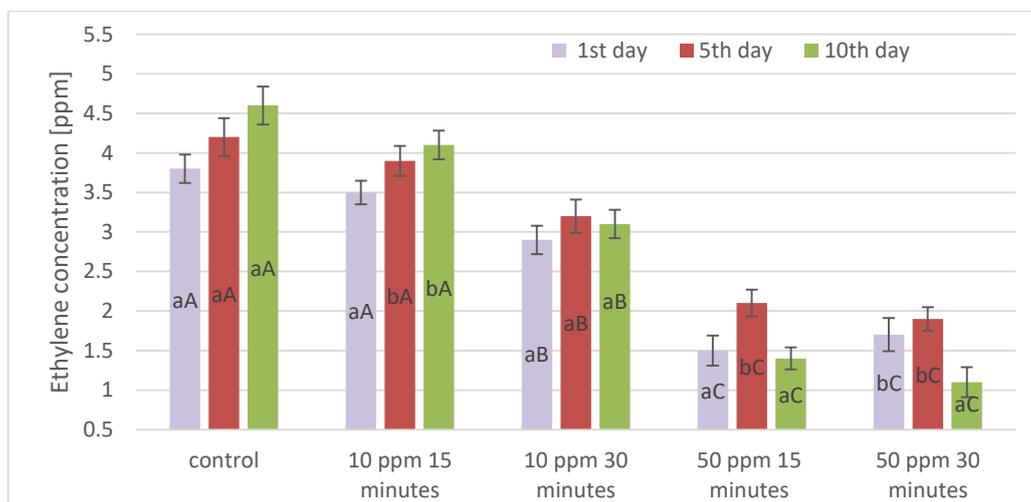


Figure 8. Changes in C₂H₄ concentration [ppm] on days 1, 5 and 10 of storage for different variants of the experiment. NOTE: lowercase letters - differences between test dates; capital letters – differences between ozone doses.

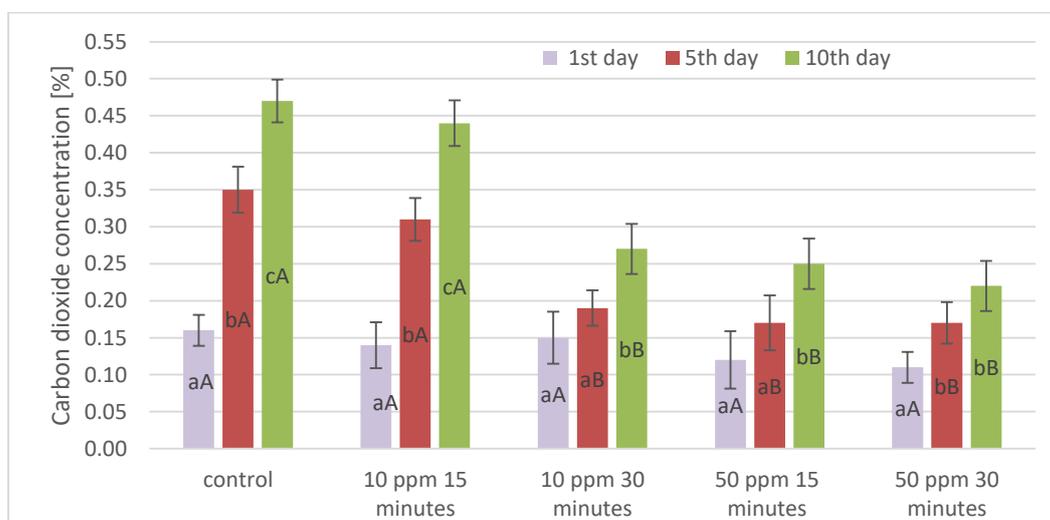


Figure 9. Changes in CO₂ concentration [%] on the 1st, 5th and 10th day of storage for different variants of the experiment. NOTE: lowercase letters - differences between test dates; capital letters – differences between ozone doses.

In order to assess whether storage conditions are appropriate, the respiration rate should be checked. Under normal conditions, as the rate of respiration increases, the consumption of stored nutrients increases [Fang et al., 2021]. Ozone gas inhibits the ethylene production process, thus extending storage life [Minas et al., 2014]. Properly selected ozone concentration helps maintain a low respiration rhythm and ethylene production rate [Zambre et al., 2010]. High concentration of ethylene in storage chambers may lead to faster ripening and spoilage of stored fruit. Regulation of ethylene concentration and its control during storage may extend the shelf life of stored plant raw materials [Embrahimi et al., 2022]. Ozone gas at a concentration of $0.04 \mu\text{L}\cdot\text{L}^{-1}$ reduced the ethylene level in vegetable storage rooms to an undetectable level. Even an ozone concentration of $0.4 \mu\text{L}\cdot\text{L}^{-1}$ was effective in removing ethylene from the atmosphere of fruit storage rooms [Skog et al., 2001].

Measurement of mechanical properties

Mechanical properties are necessary for the design of devices and machines used for harvesting, transporting, processing and packaging fruit and vegetables [Uba et al., 2020]. The average values of the destructive force in the process of compression of fruits of different variants are shown in figure 10. In the case of each variant, a decrease in the destructive force was observed during 10 days of storage. Decreases in destructive force during storage of zucchini fruit were at a similar level for both the control and ozonated variants. Both on the 1st and 10th day of storage, there was no significant impact of the ozone gas on mechanical properties of the tested fruits. Research on red peppers reports that the raw material subjected to the ozonation process was characterized by greater hardness compared to the control (non-ozonated) peppers [Horvitz and Cantalejo, 2012]. Antos et al. [2018] observed an increase in the resistance to damage of apple tissues after the ozonation process.

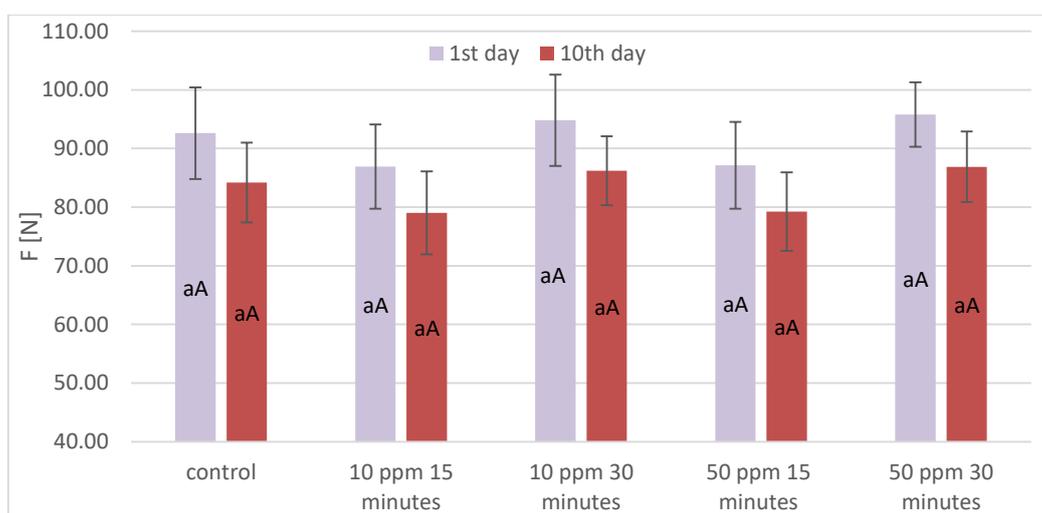


Figure 10. Average values of the destructive power of zucchini fruit depending on the ozone dose used and storage time. NOTE: lowercase letters - differences between test dates; capital letters – differences between ozone doses.

Measurement of Moisture Content

On the 1st day of storage, the highest water content was recorded for zucchini fruits ozonated with a dose of 50 ppm for 15 minutes - 94.01%. In turn, the lowest water concentration was recorded for the control sample - 93.54%. On the 10th day of storage, the water content decreased for each variant compared to the 1st day of storage. The lowest water concentration was characterized by fruits treated with an ozone dose of 50 ppm for 15 minutes - 90.01%, while the highest was for fruits ozonated with a dose of 50 ppm for 30 minutes - 91.52%. No statistically significant differences in water content were found for ozonated fruits compared to the control sample (Figure 11).

Zucchini fruit is made up of 95% water. The high water content makes this fruit a low-calorie food [Health Matters Program 2011]. The water content in plant materials decreases during their storage [Levinsh et al., 2023]. Zapałowska et al. [2021] found that sea buckthorn fruit exposed to ozone at a dose of 100 ppm for 30 minutes showed a smaller decrease in water content during storage compared to the control sample.

Other research results indicate that ozonation reduces mass loss compared to the control sample during storage fennel [Lin et al., 2023], green asparagus [Pretell-Vásquez et al., 2023] or radish tubers [Zardzewiały et al., 2023].

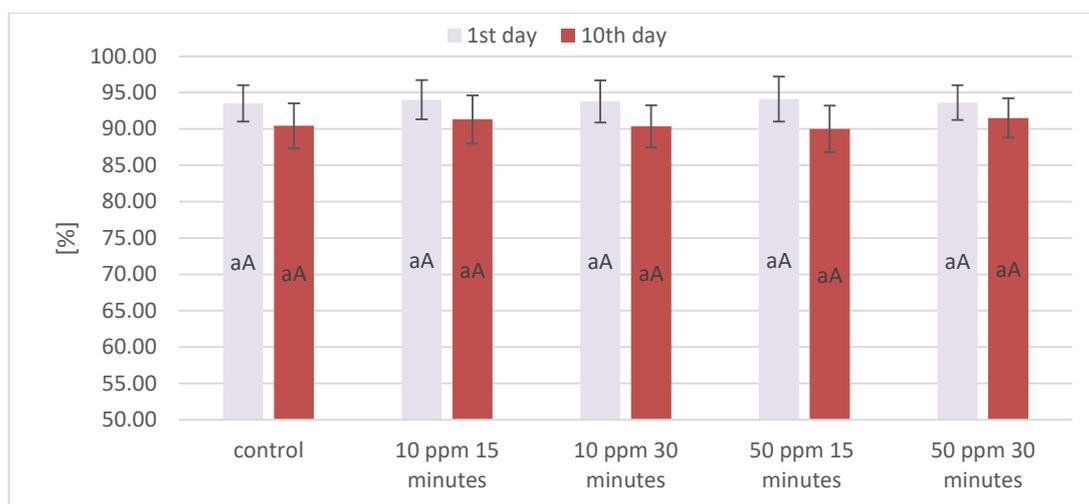


Figure 11. Water content in zucchini fruits treated with ozonation and in the control sample on the 1st and 10th day of storage. NOTE: lowercase letters - differences between test dates; capital letters – differences between ozone doses.

CONCLUSION

The effect of the ozonation process of zucchini fruit, performed cyclically every 48 hours, was determined based on analyses performed during a 10-day storage period. Selected mechanical properties, the content of selected bioactive compounds, microbiological load and the amount of gases in the storage process were determined. The conducted studies have shown that the use of gaseous ozone does not affect the mechanical properties and water content of zucchini fruit. In addition, ozone gas reduced the microbiological

load of the tested fruit. Moreover, the tests indicate that the increase in the content of bioactive compounds in the tested raw material occurs after the application of selected ozonation doses. The analysis results indicate that ozone gas has a beneficial effect on the parameters determining the quality of zucchini fruit and on extending the storage period. Moreover, ozone gas is an important factor influencing the reduction of the amount of ethylene during the storage process of the tested fruit.

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