Impact of ozonisation on pro-health properties and antioxidant capacity of ‘Honeoye’ strawberry fruit

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ABSTRACT

The objective of the research was to determine the impact of concentration and ozonisation time on the selected chemical properties and total antioxidant capacity of ‘Honeoye’ strawberries cultivar. Efficiency of gaseous ozone usage as a method of extending the fruit shelf life was proved. Strawberries were exposed to ozone stream with concentration of 0.3–1.2 mg/L within 60, 120, 150 and 180 min. The scope of research included measurements of the titratable acidity, content of soluble solid substances and the following pro-health characteristics: the total antioxidant capacity, phenolic compounds content and vitamin C content. On the second, fourth and sixth day of storage, an assessment was carried out on the fruit mass changes. Statistically significant differences (p < 0.05) in the titratable acidity changes and soluble solids concentration were reported. The application of ozone allowed reduction of strawberries weight losses during storage without a negative impact on their pro-health properties.

1. Introduction

Strawberries (\textit{Fragaria x ananassa Duch.}) are a valuable source of ascorbic acid and phenolic compounds having one of the highest antioxidant capacities among fruits (Aaby, Mazur, Nes, & Skrede, 2012; Basu, Nguyen, Betts, & Lyons, 2014; Cardeñosa et al., 2015; Giampieri, Alvarez-Suarez, & Battuto, 2014). These compounds inactivate reactive forms of oxygen and free radicals. As a result, their consumption is advantageous for the human health (Alvarez-Suarez et al., 2014; Giampieri, Alvarez-Suarez, Mazzoni, et al., 2014). Strawberries in a diet increase the protection, \textit{inter alia}, against tumours, type 2 diabetes, obesity and cardiovascular diseases (Giampieri et al., 2015; Nuñez-Mancilla, Pérez-Won, Uribe, Vega-Gálvez, & Di Scala, 2013; Zimmer et al., 2012). Strawberry fruits are rich source of many substances with antioxidant properties and in particular, phenolics which constitute one of the main groups of secondary metabolism compounds. Phenolic compounds act as antioxidants and their activity is determined pursuant to the chemical structure, which they posse (Forbes-Hernandez et al., 2015). Vitamin C is one of the most important antioxidants found in fruits and vegetables (Odriozola-Serrano, Hernandez-Jover, & Martin-Bellosa, 2007). Among fruits, strawberries are considered as one of the richest in ascorbic acid (Derossi, Pilli, & Fiore, 2010; Skrovankova, Sumczynski, Mícek, Juríkova, & Sochor, 2015). Vitamin C content in fresh strawberries according to Klopotek, Otto, and Böhm (2005) is 35–104 mg/100 g but its level depends on the cultivar and the fruit maturity degree.

Unfortunately, a seasonal supply of fresh strawberries causes that they must be subjected to preservation and storage processes, \textit{inter alia}, by refrigeration. The freezing process does not fully protect the product against unfavourable changes during storage including reduction of the...
antioxidant properties of this product. Also Skupień (2003) and Kolińska (2008) in their research reported reduction of the vitamin C content and total antioxidant capacity as a result of a refrigeration process. Vitamin C is thermostable (Burdurlu, Koca, & Karadeniz, 2006; Sapei & Hwa, 2014) and very sensitive to light, oxygen (Roig, Rivera, & Kennedy, 1995) and presence of metal ions (Serpen & Gökmek, 2007). Refrigeration of strawberries caused inhibition of biological processes and partial damage to the cell structure. Quality changes of frozen strawberries are a resultant of chemical and biochemical changes and physical changes which take place mainly on the fruit surface. These changes have unidirectional decomposition processes in nature and are accompanied by reduction of the sensory quality and pro-health properties. Physical, chemical and biochemical changes which take place in frozen strawberries affect the nutritive value of strawberries.

The usage of ozone in the food industry as a bactericidal factor which extends the shelf life may limit the usage of traditional chemical compounds which are harmful to natural environment e.g. chloride (Ali, Ong, & Forney, 2014). EPA’s (Environmental Protection Agency) research proved that ozone is safer for environment than chloride and other measures (EPA, 1999). The advantage of using ozone is a fast decomposition of this compound to oxygen but there are no other products of this reaction and only some by-products of disinfection are formed. The use of ozonisation in the food industry establishments proves that this technology is safe and environment-friendly. Many tests and experiments have been carried so far to evaluate the impact of gaseous ozone on the condition of fruit during their storage (Alexandre, Santos-Pedro, Brandão, & Silva, 2011; Skog & Chu, 2001). Ozone is also used in order to limit gathering of volatile substances which cause unwanted pre-ripening of fruit. Freeing the third atom of oxygen, ozone is an effective measure for destruction of microorganisms which occur at the surface of fruits and vegetables. This gas is bactericidal and fungicidal and is also used for elimination of pesticides, herbicides and other organic and non-organic residues (Abdel-Wahhab et al., 2011; Gaou et al., 2005).

The objective of the presented research was to investigate/determine the impact of ozone on pro-health properties of ‘Honeoye’ strawberries during their 6-day storage. During the experiment, the titratable acidity, content of soluble solid substances, total antioxidant capacity, phenolic compounds content and vitamin C content were investigated. Moreover, fruit mass was evaluated, which allowed determination of the percentage mass losses of ozonised and non-ozonised strawberries during storage.

2. Materials and methods

2.1. Plant material

‘Honeoye’ strawberry fruit constituted research material. They were obtained in July 2015. Soon after the harvest, plant material in the form of the whole fruit was sorted on account of the size and degree of fruit maturity and divided into 17 groups of the mass of 1.0 kg each. The first group constituted a control sample (c0), and the remaining were placed in the cooling chamber to which ozone in the gas form was supplied. For enrichment of air in the gaseous ozone, the ozone generator Korona 02/10 was used (Ekotech, Poland) which generated ozone with the capacity of 13 g/h. The level of the generated ozone was measured with the measuring head GDX-70 (Alter S.A., Poland) with the measurement scope 0–5 mg/L. Strawberries were subjected to ozone stream with increasing concentration 0.3 (c1), 0.6 (c2), 0.9 (c3), 1.2 (c4) mg/L and during ozonisation 60 (t1), 120 (t2), 150 (t3), 180 (t4) min.

After the ozonisation process, each group of strawberries was divided into two subgroups. The first one was subjected to direct chemical tests and determined as D0. The scope of research included general acidity measurements, soluble solids content, total antioxidant capacity, phenolic compounds content and vitamin C content. For chemical assay, only healthy fruits without damage signs were collected. The remaining part of ozonised strawberries along with the control sample was refrigerated in the temperature of 4 ± 1°C for 6 days (D6).

On the second, fourth and sixth day of storage, additionally, evaluation of fruit weight losses was carried out, which allowed determination of the percentage weight decrease of strawberries subjected to ozonisation and the non-ozonised control group.

2.2. Titratable acidity and soluble solid concentration

Aliquots (6.00 g) of juice were diluted with 100 mL distilled water and the TA determined by titration with 0.1 N NaOH to an end point of pH = 8.1. The results were converted to percent citric acid [(mL NaOH × 0.1 N × 0.064/6.00 g of juice) × 100] and expressed in fresh weight. The soluble solids content was obtained by a refractometer (Brix 0–32%, Atago, Japan) and expressed in “Brix (Monaco, Costa, Uliana, & Lima, 2014).

2.3. Total phenolic content and total antioxidant activity

The phenolic compounds sum was determined with Folin and Ciocalteu method (Singleton & Rossi, 1965). For this purpose, ca. 20 g of strawberries was homogenised and then extract was filtered threefold through Whatman filter no. 1. To 0.1 mL of the filtered substance, 6.0 mL of distilled water and 0.5 mL Folin’s reagent were added. After 3 min, 1.5 mL of saturated sodium carbonate of 200 mg/mL concentration was added (Sigma Aldrich Inc., USA) and then it was filled with water to 10 mL. Reactive mixture was stored by 30 min in the water bath (WNB 7 Memmert, Germany) in the temperature of 40°C. Absorbance was measured spectrophotometrically at the waves’ length of A = 760 nm (Tecan Spark™ 10M, Männedorf, Switzerland). The phenolic compounds sum was expressed as a equivalent gallic acid (GA) (Avantor Performance Materials, Poland) based on the previously prepared calibration curve. The results were provided as an average from three iterations in mg GA equivalent per kg of sample (fresh weight).

Anti-radical activity of the analysed strawberries was measured as a reductive capacity referred to 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Nuñez-Mancilla et al., 2013; Sogvar, Saba, & Emamifar, 2016). For this purpose, 1 mL of extract was added to 2 mL of ethanol DPPH solution (0.15 mmol/L). Reactive mixture was mixed for 30 s and placed aside for 20 min in a dark place. Absorbance was
measured at the waves length of 517 nm (Tecan Spark™ 10M, Männedorf, Switzerland). As a reference solution, ethanol with 80 g/l concentration was used. A control sample was prepared without extract addition. All solvents and reagents were purchased in Sigma company (Sigma Aldrich Inc., USA). Measurements were carried out in three iterations. The total antioxidant activity (TAA) expressed as percentage of DPPH radical reduction was calculated from the following formula:

$$\text{TAA(\%)} = \left( \frac{\text{Abs}_{\text{sample}} - \text{Abs}_{\text{control}}} {\text{Abs}_{\text{sample}}} \right) \times 100$$

where TAA is the total antioxidant activity and Abs is measured absorbance.

2.4. Vitamin C content determination

The vitamin C content was determined by titration with 2,6-dichlorophenolindophenol (DCIP) (AOAC, 2000). An amount of 20 g of fruit was collected, and after homogenisation and threefold filtering, 10 mL of clear juice was measured. Then, it was quantitatively transferred to a measuring flask of 50 mL volume and after filling with 2% water solution of oxalic acid to the line, it was carefully mixed. The reaction mixture in the amount of 10 mL was collected then to a cornical flask and then titrated with standard solution of DCIP to the moment of obtaining light pink colour which lasted 15 s. The ascorbic acid content was expressed as mg per 1 kg of fresh fruit.

2.5. Assessment of fruit weight losses during storage

Ozonised fruit and control group was subjected to strict mass control which finally allowed calculation of the percentage decrease of weight of ozonised and non-ozonised fruit, stored in the temperature of 4 ± 1°C after the total time of 6 days from the first ozonisation.

2.6. Statistical analysis

The statistical analysis of the obtained results was carried out by means of Statistica 10.0 (Statsoft, Tulsa, USA) based on the analysis of variance for a two-factor experiment (I factor: dose and ozonisation time, II factor: storage period). Significance of differences between the average values was verified with Tukey’s test at the level of significance of p < 0.05 (Tukey, 1977).

3. Results and discussion

3.1. TA and soluble solid concentration

During storage, strawberries are subjected to many unfavourable processes which cause its fermentation. During fermentation, sugars are metabolised and the titratable acidity changes. TA is a fundamental parameter which decides on the fruit quality and its increasing level may indicate incorrect storage conditions. Citric acid (% of citric acid) prevails and on its basis, the TA of strawberries is expressed. Main components of solid substances soluble in fruit juice are sugars, which may be measured refractometrically in the °Bx scale. The titratable acidity results and soluble solids concentration of particular groups of strawberries on the 0 and 6th day of storage were presented in Table 1.

The titratable acidity in investigated fruit was 0.78–1.14% of citric acid at the beginning of the experiment (D0). The titratable acidity values in majority of groups differed statistically significantly (p < 0.05). When comparing changes of acidity during a 6-day storage, a noticeable decrease in each group was reported. After the 6-day, storage acidity dropped to 0.65–0.82% per citric acid. Average values of TA between storage periods decreased by 0.23 ± 0.09% per citric acid.

Table 1. Impact of concentration and ozonisation time on the titratable acidity and content of soluble solid substances measured on the 0 and 6th day of storage in the temperature of 4 ± 1°C, where c1–c4 ozone concentration (0.3, 0.6, 0.9 and 1.2 mg/L) and t1–t6 ozonisation time (60, 120, 150 and 180 min).

| Concentration (mg/L) and ozonisation time (min) | Titratable acidity (%) | Storage period (days) | Soluble solids concentration (°Bx) |
|-----------------------------------------------|------------------------|-----------------------|-----------------------------------|
|                                               | 0                      | 6                     | 0                                 | 6                                |
| C0   | 1.01 ± 0.00e1           | 0.81 ± 0.00d1         | 8.26 ± 0.04c1         | 10.17 ± 0.02b1                   |
| C1   | 0.95 ± 0.01d1          | 0.77 ± 0.00c1        | 8.59 ± 0.02b1         | 9.14 ± 0.07ab1                   |
| C2   | 0.92 ± 0.01b1          | 0.78 ± 0.00b1        | 8.64 ± 0.06a1         | 9.06 ± 0.08a1                    |
| C3   | 0.93 ± 0.01a1          | 0.70 ± 0.01a1        | 8.44 ± 0.07b1         | 9.20 ± 0.07c1                    |
| C4   | 0.88 ± 0.01a1          | 0.75 ± 0.06b1        | 8.75 ± 0.08c1         | 9.33 ± 0.09c1                    |
| C5   | 1.09 ± 0.01b1          | 0.66 ± 0.01a1        | 8.86 ± 0.06d1         | 9.39 ± 0.07cd1                   |
| C6   | 0.78 ± 0.01a1          | 0.68 ± 0.01ab        | 8.97 ± 0.07cd1        | 9.56 ± 0.11e1                    |
| C7   | 0.99 ± 0.01d1          | 0.66 ± 0.00a1       | 9.13 ± 0.09ad1        | 9.59 ± 0.02b1                    |
| C8   | 0.95 ± 0.01c1          | 0.74 ± 0.00b1        | 9.12 ± 0.09ad1        | 9.91 ± 0.08ace1                  |
| C9   | 1.08 ± 0.01b1          | 0.80 ± 0.01a1        | 9.33 ± 0.06b1         | 9.98 ± 0.08b1                    |
| C10  | 1.01 ± 0.01a1          | 0.78 ± 0.01a1        | 9.13 ± 0.15b1         | 10.01 ± 0.09c1                   |
| C11  | 0.92 ± 0.00d1          | 0.73 ± 0.01ad        | 9.76 ± 0.12b1         | 10.35 ± 0.07be1                  |
| C12  | 0.95 ± 0.01b1          | 0.80 ± 0.00c1        | 9.61 ± 0.08ad1        | 10.15 ± 0.07bf1                  |
| C13  | 1.14 ± 0.01f1          | 0.76 ± 0.00b1        | 8.92 ± 0.08a1         | 9.31 ± 0.04bc1                   |
| C14  | 0.85 ± 0.01g1          | 0.65 ± 0.01a1        | 9.11 ± 0.11b1         | 9.33 ± 0.06b1                    |
| C15  | 0.97 ± 0.00a1          | 0.67 ± 0.00b1        | 9.17 ± 0.03c1         | 9.51 ± 0.03c1                    |
| C16  | 0.97 ± 0.01g1          | 0.82 ± 0.00a1        | 9.41 ± 0.02c1         | 9.71 ± 0.02e1                    |

A–B: The mean values marked with various capital letters in ascending order show significant statistical differences within rows.

a–i: The mean values marked with various lowercase letters in ascending order show significant statistical differences within columns.

A–B – los valores promedio marcados con diferentes letras mayúsculas en orden ascendiente muestran diferencias estadísticas significativas entre las filas.

a–i – los valores promedio marcados con diferentes letras mayúsculas en orden ascendente muestran diferencias estadísticas significativas entre las columnas.
Table 2. Impact of concentration and ozonisation time on the total phenolic content (mg GA equivalent/kg) and total antioxidant activity (%) measured on the 0 and 6th day of storage in the temperature of 4 ± 1°C, where c1–c4 ozone concentration (0.3, 0.6, 0.9 and 1.2 mg/L) and t1–t4 ozonisation time (60, 120, 150 and 180 min).

Table 2. Impacto de la concentración y el tiempo de ozonización en el contenido total fenólico (mg GA equivalente/kg) y actividad antioxidante total (%) calculada el día 0 y 6 de almacenamiento a temperatura de 4 ± 1°C, concentración de ozono c1–c4 (0.3; 0.6; 0.9; 1.2 mg/L) y tiempo de ozonización t1–t4 (60; 120; 150; 180 min).

### Chemical quality parameters

| Concentration (mg/L) and ozonisation time (min) | 0  | 6  | Storage period (days) | Total phenolic content (mg GA eq/kg) | Total antioxidant activity (%) |
|-----------------------------------------------|----|----|-----------------------|--------------------------------------|-------------------------------|
| c1f0                                          | 1651.5 ± 28.5lb | 1593.3 ± 5.1keqg | 40.04 ± 0.69bc | 50.65 ± 0.87bc |
| c1f1                                          | 1957.7 ± 11.8lf | 1762.7 ± 4.9lnj | 51.86 ± 0.68def | 70.39 ± 0.93ghf |
| c1f2                                          | 1785.4 ± 10.6dfe | 1579.1 ± 11.4dke | 48.30 ± 1.56ddef | 76.99 ± 1.04bf |
| c1f3                                          | 1796.3 ± 24.1lf | 1677.5 ± 27.4pgh | 50.69 ± 0.31bdef | 71.52 ± 0.93bc |
| c1f4                                          | 1841.7 ± 36.8he | 1710.9 ± 5.1pgh | 45.45 ± 0.56bhec | 67.67 ± 0.80bhec |
| c1f5                                          | 1691.5 ± 34.1lb | 15520.0 ± 34.6bcd | 54.55 ± 0.72cdef | 63.11 ± 0.92bc |
| c2f0                                          | 1917.2 ± 30.6lf | 1711.7 ± 6.4pgh | 63.29 ± 0.61bde | 59.59 ± 1.34bc |
| c2f1                                          | 1836.9 ± 10.3dfe | 1645.6 ± 23.8qkg | 65.82 ± 0.76qeq | 70.78 ± 0.93bgh |
| c2f2                                          | 1790.0 ± 16.3gd | 1580.9 ± 32.3dhe | 48.57 ± 1.01ddef | 56.49 ± 0.65bc |
| c2f3                                          | 1759.9 ± 9.7cd | 1674.4 ± 33.7pgh | 46.51 ± 1.36dcd | 64.51 ± 0.73bhef |
| c2f4                                          | 1899.9 ± 5.4bgh | 1716.3 ± 22.1qkg | 55.15 ± 1.35def | 62.16 ± 1.3bdeef |
| c3f0                                          | 1922.6 ± 8.0sgh | 1722.4 ± 20.5qgh | 53.86 ± 1.13ddef | 69.50 ± 1.19bgh |
| c3f1                                          | 1900.9 ± 23.1dfe | 1580.3 ± 19.8ddef | 45.08 ± 0.77bdef | 67.63 ± 0.61bhef |
| c3f2                                          | 1501.5 ± 49.4he | 1454.9 ± 33.7pgh | 43.86 ± 1.24cde | 64.22 ± 0.96bc |
| c3f3                                          | 1472.2 ± 39.3he | 1440.8 ± 34.9pgh | 38.10 ± 0.77bgh | 45.36 ± 3.2pgh |
| c3f4                                          | 1662.5 ± 8.8sgh | 1476.8 ± 33.7abc | 42.36 ± 1.95bc | 51.74 ± 0.96bc |
| c4f0                                          | 1468.9 ± 41.5f | 1413.3 ± 23.7bf | 45.90 ± 0.91bc | 51.47 ± 0.84bgh |

A–B: The mean values marked with various capital letters in ascending order show significant statistical differences within rows.

a–j: The mean values marked with various lowercase letters in ascending order show significant statistical differences within columns.

A–B – los valores promedio marcados con diferentes letras mayúsculas en orden ascendente muestran diferencias estadísticas significativas entre las filas.

a–j – los valores promedio marcados con diferentes letras minúsculas en orden ascendente muestran diferencias estadísticas significativas entre las columnas.

Both the ozone concentration and ozonisation time caused statistically significant differences of acidity in the investigated groups of strawberries.

During the 6-day storage, the increase of soluble solid substances content was reported both in the control group as well as in each of the ozonised groups of strawberries, which results from life processes which take place in fruit. Ozonised strawberries had higher soluble solid concentration (SSC) in comparison to the non-ozonised fruit, which Tzortzakis, Borland, Singleton and Barnes (2007) explained in his research with the increase of the level of prevailing carbohydrates (glucose and fructose) caused under the influence of ozone. The highest change of SSC was reported in the control sample c0f0 because during the 6-day storage, the increase by 1.91°Bx took place. In case of ozonised strawberries samples, the change in the sugars content was lower, which is related probably to the impact of ozone on delaying fruit ripening. The highest content of sugars was in case of a sample which was subjected to 150 min exposition to ozone with 0.6 mg/L concentration. SSCs were initially 9.76 ± 0.12, and after 6 days, it increased to 10.35 ± 0.07°Bx. When this dose and ozonisation time was exceeded, sugar content dropped. Similar tendencies of the titratable acidity drop and increase of soluble solid substances was presented by Ali et al. (2014) during the research on the ozonisation impact on the antioxidant capacity of papaya fruit and by Sogvar et al. (2016) who carried out research on strawberries covered with natural layers. Ozonisation and the 6-day storage of strawberries caused statistically significant (p < 0.05) changes in soluble solids concentration.

3.2. Total phenolic content and total antioxidant activity

In relation to the phenolic compounds structure, their impact on the oxidation process course may be completely different. However, it was proved that these compounds may act as compounds which block free radicals and prevent reactions caused by a single active oxygen atom. Impact of ozone on phenolic compounds content and antioxidant capacity was presented in Table 2. Storing strawberries in the atmosphere which contains ozone with the concentration from 0.3 to 1.2 mg/L and within 60–180 min caused statistically significant (p < 0.05) changes of phenolic compounds content in comparison to the non-ozonised control group. Similar total phenolic content (TPC) rising trends as a result of ozonisation were reported by Alothman, Kaur, Fazilah, Bhat and Karim (2014) in his research, where the content of polyphenols in pineapple and bananas increased considerably by 15.7% and 8.2%, respectively, after 20-min exposition of ozone in the 0.72–mmol dose. An upwards tendency may be caused by activation of phenylalanine ammonia lyase (PAL) resulting from the presence of ozone. PAL is one of the key enzymes which participate in the phenolic compounds synthesis in plant tissues (González-Aguilar, Zavaleta-Gatica, & Tiznado-Hernández, 2007). Moreover, numerous studies suggest that presence of ozone may cause inhibition of such enzymes as polyphenol oxidase (PPO) and peroxidase (POD), which often cause oxidation of phenolic compounds in fruit and vegetables (Yeoh, Ali, & Forney, 2014). For instance, inhibition of PPO and POD activity was discovered during ozonisation of carrot (Chauhan, Raju, Ravi, Singh, & Bawa, 2011). According to Tiwari, O’Donnell, Patras, Brunton and Cullen (2009) research, 1.6–7.8% w/w ozone treatment for 0–10 min has an influence on the content of anthocyanins in strawberry juice. Anthocyanins are subject to significant degradation to their constituent phenolic acids which, among other things, may affect the TPC, whose level is not reduced in strawberries stored (Dragišić Maksimović et al., 2015).
The highest phenolic compounds content was in case of strawberries from group C2 (189.9 ± 5.4 mg GA equivalent/kg) and the lowest from group C4 (1468.9 ± 41.5 mg GA equivalent/kg). TPC decrease could have been caused by too high dose of ozone (1.2 mg/L) or a long ozonisation time (180 min) of strawberries. After a long exposition time of strawberries from group C4 to ozone, a spontaneous decomposition of this gas could have taken place which would lead to formation of free radicals such as hydroperoxyl (\(\text{HO}_2\)), hydroxyl (\(\text{OH}\)) and superoxide anion (\(\text{O}^-\)) radical. According to Hoigné and Bader (1983), by-products of ozone decomposition may thus lead to the reduction of polyphenols content in fruit. TPC of strawberries ozonised at the highest concentration decreased by 11.06% in relation to the non-ozonised control group. Moreover, after 6 days of storing, TPC level was the lowest in these samples (1413.3 ± 23.7 mg GA equivalent/kg).

In the research on the pro-health properties of strawberries, their capacity to deactivate of free radicals was used with DPPH reagent. At the beginning of the experiment (D0), a control sample of strawberries proved total antioxidant activity (TTA) at the level of 43.04 ± 0.69%; during the 6-day storage, it increased by 7.61%. Strawberries from the group C2 (0.6 mg/L) and those subjected to ozonisation within t2 and t3 (120 and 150 min) had a high capacity to neutralise DPPH-free radical. Their TTA was at the average of 64.56 ± 0.58%. The lowest TTA was in the group C4 (38.10 ± 0.77%). Also, after 6 days of storage, the capacity to deactivate free radicals in this group was the lowest (45.36 ± 3.27%). Along with the storage time, anti-oxidant capacity statistically significantly increased (\(p < 0.05\)). Only in the ozonised group C2, no statistically significant TAA increase was proved during the 6-day storage (\(p = 0.061\)).

Phenolic compounds are secondary plant metabolites which have a high capacity to deactivate free radicals and they influence greatly on the TTA value. In the research on the relation between the phenolics content and antioxidant properties of berries, Kalt, Forney, Martin, and Prior (1999) reported high correlation between the phenolics sum (\(r = 0.83\)) and the total antioxidant capacity. However, correlation coefficient indicates that not all of phenolic’s activities could be directly related to the molecular function as antioxidants exclusively.

### 3.3. Vitamin C content determination

Average values of vitamin C obtained as a result of research are within 658.2–1059.7 mg/kg. It was proved that ozonisation may constitute a factor which limits vitamin C loss. Strawberries subjected to gaseous ozone activity had a higher content of ascorbic acid in comparison to the control sample which was presented in Table 3. During storage, vitamin C decreased successively as a result of oxidizing of \(\alpha\)-ascorbic acid to dehydroascorbic acid, which is further oxidised with the loss of biological activity. On the sixth day of storage, the ascorbic acid loss in ozone samples was at the average of 96.1 mg/kg of vitamin C when in the control sample, the content of this vitamin dropped by 135.6 mg/kg. The observed decrease of ascorbic acid losses after ozonisation was probably caused by the inhibition effect of \(O_3\) for ascorbic POD and ascorbic oxidase enzymes activity, which are responsible for ascorbic acid degradation. These changes may also be due to oxidative processes occurring during storage. A similar oxidative degradation of ascorbic acid in the presence of oxygen has been reported by Tiwari et al. (2009). The highest stability of vitamin C was reported in group C4, where its content decreased by 14.7 mg/kg in comparison to D0 group.

### 3.4. Assessment of fruit weight loss during storage

The fruit storage losses are related to ripening processes and storage conditions. Strawberries weight loss during storage takes place mainly as a result of water loss through vapouring and losses of carbon reserves as a result of breathing. Speed with which water is lost depends mainly on the pressure gradient between fruit tissues and surrounding atmosphere (Duarte-Molina, Gómez, Agueda Castro, & Alzamora, 2016). Changes of strawberries weight during storage were presented in Figure 1, but the results from the ozonised groups were assumed on the plot as an average from all 16 groups and varied groups on account of the dose and ozonisation time were not presented. Systematically carried out mass balance on the 2nd, 4th and 6th day proved that ozone may efficiently reduce mass losses during storage of strawberries in comparison to the control group. The most favourable on account of limitation of weight losses was ozonisation of strawberries for 120 min and at the ozone concentration of 0.6 mg/L. The weight loss was 6.03% in comparison to the initial weight (data not shown). Strawberries weight losses during storage were reported both in the control group as well as in the ozonised group but no statistical significance between the control and

| Vitamin C (mg/kg) | Concentration (mg/L) and ozonisation time (min) |
|------------------|-----------------------------------------------|
|                  | 0                                              | 6                                              |
| C1F2             | 1488.8 ± 13.8                                  | 713.2 ± 22.2                                   |
| C1F3             | 1007.5 ± 11.4                                  | 907.5 ± 30.6                                   |
| C2F2             | 1032.2 ± 27.1                                  | 955.2 ± 39.8                                   |
| C2F3             | 1059.7 ± 33.6                                  | 927.7 ± 28.2                                   |
| C3F2             | 1054.2 ± 20.8                                  | 916.7 ± 41.3                                   |
| C3F3             | 962.7 ± 36.6                                   | 802.0 ± 27.5                                   |
| C4F2             | 971.7 ± 35.8                                   | 942.3 ± 44.1                                   |
| C4F3             | 964.3 ± 31.3                                   | 737.0 ± 38.1                                   |
| C4F4             | 922.2 ± 46.8                                   | 801.2 ± 30.3                                   |
| C4F5             | 817.7 ± 36.6                                   | 847.0 ± 29.1                                   |
| C4F6             | 850.7 ± 28.2                                   | 801.2 ± 36.6                                   |
| C4F7             | 787.4 ± 19.3                                   | 744.3 ± 46.1                                   |
| C4F8             | 788.3 ± 24.8                                   | 658.2 ± 31.3                                   |
| C4F9             | 819.5 ± 36.1                                   | 804.8 ± 35.4                                   |
| C4F10            | 801.2 ± 20.8                                   | 663.7 ± 24.8                                   |
| C4F11            | 733.3 ± 22.9                                   | 597.7 ± 22.2                                   |
| C4F12            | 804.8 ± 20.8                                   | 720.5 ± 24.0                                   |

- A-B: The mean values marked with various capital letters in ascending order show significant statistical differences within rows.
- a-g: The mean values marked with various lowercase letters in ascending order show significant statistical differences within columns.
- A-B – los valores promedio marcados con diferentes letras mayúsculas en orden ascendente muestran diferencias estadísticas significativas entre las filas.
- a-g – los valores promedio marcados con diferentes letras minúsculas en orden ascendente muestran diferencias estadísticas significativas entre las columnas.
of ozone allowed reduction of mass losses during a 6-day storage with negative impact on their pro-health quality. The highest phenolic compounds content was in case of strawberries from group C1 (0.9 mg/L, 120 min). Strawberries from the group C2 (0.6 mg/L) and those subjected to ozonisation with T2 and T3 (120 and 150 min) had a high capacity to neutralise DPPH-free radical. The most favourable on account of limitation of weight losses was ozonisation of strawberries for 120 min and at the ozone concentration of 0.6 mg/L. The results obtained in this study indicate that the most favourable for strawberries quality is the ozonisation for 120 min at 0.6 mg/L ozone concentration.

Disclosure statement
No potential conflict of interest was reported by the authors.

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References
Aaby, K., Mazur, S., Nes, A., & Skrede, G. (2012). Phenolic compounds in strawberry (Fragaria × ananassa Duch.) fruits: Composition in 27 cultivars and changes during ripening. Food Chemistry, 132, 86–97. doi:10.1016/j.foodchem.2011.10.037
Abdel-Wahhab, M.A., Sehab, A.F., Hassanien, F.R., El-Nemr, Sh.E., Amra, H. A., & Abdel-Alim, H.A. (2011). Efficacy of ozone to reduce fungal spoilage and aflatoxin contamination in peanuts. Journal of Nuts and Related Sciences, 2, 01–14.
Alexandre, E.M.C., Santos-Pedro, D.M., Brandão, T.R.S., & Silva, C.L.M. (2011). Influence of aqueous ozone, blanching and combined treatments on microbial load of red bell peppers, strawberries and watercress. Journal of Food Engineering, 105, 277–282. doi:10.1016/j.jfoodeng.2011.02.032
Ali, A., Ong, M.K., & Forney, C.F. (2014). Effect of ozone pre-conditioning on quality and antioxidant capacity of papaya fruit during ambient storage. Food Chemistry, 142, 19–26. doi:10.1016/j.foodchem.2013.07.039
Allothman, M., Kaur, B., Fazliah, A., Bhat, R., & Karim, A.A. (2010). Ozone-induced changes of antioxidant capacity of fresh-cut tropical fruits. Innovative Food Science & Emerging Technologies, 11, 666–671. doi:10.1016/j.ifset.2010.08.008
Alvarez-Suarez, J.M., Giampieri, F., Tulipani, S., Casoli, T., Di Stefano, G., Gonzalez-Paramas, A.M., … Battino, M. (2014). One-month strawberry-rich anthocyanin supplementation ameliorates cardiovascular risk, oxidative stress markers and platelet activation in humans. The Journal of Nutritional Biochemistry, 25, 289–294. doi:10.1016/j.jnutbio.2013.11.002
AOAC. (2000). Official methods of analysis (17th ed., pp. 27). Gaithersburg, MD: Association of Official Analytical Chemists.
Basu, A., Nguyen, A., Betts, N.M., & Lyons, T.J. (2014). Strawberry as a functional food: An evidence-based review. Critical Reviews in Food Science and Nutrition, 54, 790–806. doi:10.1080/10408398.2011.608174
Burdurlu, H.S., Koca, N., & Karadeniz, F. (2006). Degradation of vitamin C in citrus juice concentrates during storage. Journal of Food Engineering, 74, 211–216. doi:10.1016/j.jfoodeng.2005.03.026
Cardehosa, V., Medrano, E., Lorenzo, P., Sanchez-Guerrero, M.C., Cuevas, F., Pradas, I., & Moreno-Rojas, J.M. (2015). Effects of salinity and nitrogen supply on the quality and health-related compounds of strawberry fruits (Fragaria × ananassa cv. Primorios). Journal of the Science of Food and Agriculture, 95, 2924–2930. doi:10.1002/jsfa.7034
Chauhan, O.P., Raju, P.S., Ravi, N., Singh, A., & Bawa, A.S. (2011). Effectiveness of ozone in combination with controlled atmosphere on quality characteristics including lignification of carrot sticks. Journal of Food Engineering, 102, 43–48. doi:10.1016/j.jfoodeng.2010.07.033
Derossi, A., De Pili, T., & Fiore, A.G. (2010). Vitamin C kinetic degradation of strawberry juice stored under non-isothermal conditions. LWT- Food Science and Technology, 43, 590–595. doi:10.1016/j.lwt.2009.10.006

4. Conclusion
To sum up, gaseous ozone acts superficially on the bacterial flora which occurs naturally on strawberries as well as considerably influences their chemical composition. The presented results indicate occurrence of the relation between stability of antioxidant components and the ozone dose and ozonisation time of strawberries. Fruit treated with ozone had higher SSC, significantly statistically higher level of phenolic compounds and showed higher total antioxidant capacity in comparison to the non-ozonised fruit. The application

Figure 1. Assessment of the fruit weight loss during storage of control group and the group subjected to ozonisation.

Figure 1. Estudio de la pérdida de peso del fruto durante el almacenamiento del grupo control y el grupo sujeto a la ozonización.

a–d – The mean values marked with various letters show significant statistical differences at the level of significance p < 0.05.
a–d – los valores promedio marcados con diferentes letras muestran diferencias estadísticas significativas a un nivel de significación p < 0.05.

ozon group was reported (p > 0.05). On the other hand, great differences (statistically significant at the level of p < 0.05) in the strawberries weight on the 2nd, 4th and 6th day of storage were respectively obtained. The biggest difference in weight loss was reported on the 6th day of storage, where the weight of the control group decreased at the average by 9.22 ± 0.51% when in the ozonised group, the weight loss was at the level of 7.31 ± 1.29%. Weight loss was at the average of 1.91%.

Results show a reverse tendency in comparison to the research carried out by Palou, Crisosto, Smilanick, Adaskaveg, and Zoffoli (2002) where the increased pace of water loss of ozonised peach fruit ‘Zee Lady’ took place. It was reported that higher concentration of ozone may lead to damage of peel as a result of which weight is lost in a shorter time. Incorrect selection of a dose and a long exposition time of fruit to gas ozone may not only influence superficially the bacterial flora which occurs naturally on strawberries but also destroy their natural protective barrier. In the research of Dragić Maksimović et al. (2015), ionisation chamber caused an important weight loss reduction due to the prevention of excessive moisture loss.

It was reported that the use of the ozone dose in the research (C1–C4) and the ozonisation time (T1–T4) did not cause excessive water loss and did not cause the strawberries weight reduction during the 6-day storage.
Dragičič Maksimović, J., Poledica, M., Mutavdžić, D., Mojović, M., Radićovijević, D., & Milivojević, J. (2015). Variation in nutritional quality and chemical composition of fresh strawberry fruit: Combined effect of cultivar and storage. *Combined Effect of Cultivar and Storage. Plant Foods for Human Nutrition*, 70, 77–84. doi:10.1007/s11130-014-0464-3

Duarte-Molina, F., Gómez, P.L., Aguila Castro, M., & Alzamora, S.M. (2016). Storage quality of strawberry fruit treated by pulsed light: Fungal decay, water loss and mechanical properties. *Innovative Food Science & Emerging Technologies*, 34, 267–274. doi:10.1016/j.ifset.2016.01.019

EPA. (1999). *United States Environmental Protection Agency: Alternative disinfectants and oxidants guidance manual*, 815 R 99014. [https://www.discountphd.com/course/disinfecting_the_water_using_common_disinfectants.pdf](https://www.discountphd.com/course/disinfecting_the_water_using_common_disinfectants.pdf)

Forbes-Hernández, T.Y., Gasparinini, M., Afrin, S., Bombpadre, S., Mezzetti, B., Quiles, J.L., … Battino, M. (2015). The healthy effects of strawberry polyphenols: Which strategy behind antioxidant capacity? *Critical Reviews in Food Science and Nutrition*. doi:10.1080/10408398.2015.1051919

Gaou, I., Dubois, M., Pfohl-Leszkowicz, A., Coste, C., De Jouffrey, S., & Parent-Massin, D. (2005). Safety of Oxygreen®, an ozone treatment on wheat grains. Part 1. A four-week toxicity study in rats by dietary administration of treated wheat. *Food Additives and Contaminants*, 22, 1113–1119. doi:10.1080/02652030500307156

Giampieri, F., Alvarez-Suárez, J.M., & Battino, M. (2014). Strawberry and human health: Effects beyond antioxidant activity. *Journal of Agricultural and Food Chemistry*, 62, 3867–3876. doi:10.1021/jf405455n

Giampieri, F., Alvarez-Suárez, J.M., Mazzoni, L., Forbes-Hernandez, T.Y., Gasparinini, M., González-Paramás, A.M., … Battino, M. (2014). An anthocyanin-rich strawberry extract protects against oxidative stress damage and improves mitochondrial functionality in human dermal fibroblasts exposed to an oxidizing agent. *Food & Function*, 5, 1939–1948. doi:10.1039/C4FO00048B

Giampieri, F., Bombardelli, E., & Battino, M. (2014). Strawberry as a health promoter: An evidence based review. *Food & Function*, 6, 1386–1398. doi:10.1039/C3FO00147A

González-Aguilar, G.A., Zavaleta-Gatica, R., & Tiznado-Hernández, M.E. (2007). Improving postharvest quality of mango ‘Haden’ by UV-C treatment. *Postharvest Biology and Technology*, 45, 108–116. doi:10.1016/j.postharvbio.2007.01.012

Hoigné, J., & Bader, H. (1983). Rate constants of reactions of ozone with organic and inorganic compounds in water. I. Non-dissociating organic compounds. *Water Research*, 17, 173–183. doi:10.1016/0043-135X(83)90098-2

Kalt, W., Forney, C.F., Martin, A., & Prior, R.L. (1999). Antioxidant capacity, vitamin C, phenolics, and anthocyanins after fresh storage of small fruits. *Journal of Agricultural and Food Chemistry*, 47, 4386–4384. doi:10.1021/jf990266h

Klopotek, Y., Otto, K., & Böhm, V. (2005). Processing strawberries to different products alters contents of vitamin C, total phenolics, total anthocyanins, and antioxidant capacity. *Journal of Agricultural and Food Chemistry*, 53, 5640–5646. doi:10.1021/jf047947v

Kolniak, J. (2008). Effect of freezing and thawing methods and cryoprotective supplements addend on the content of total polyphenols, anthocyanins and antioxidant capacity in frozen strawberries. *Food. Science Technology*. Quality, 5, 135–148.

Monaco, K.A., Costa, S., Ullana, M., & Lima, G. (2014). Sanitizers effect in mango pulp and peels antioxidant compounds. *Food and Nutrition Sciences*, 5, 929–935. doi:10.4236/pons.2014.510103

Núñez-Mancilla, Y., Pérez-Won, M., Uribe, E., Vega-Gálvez, A., & Di Scala, K. (2013). Osmotic dehydration under high hydrostatic pressure: Effects on antioxidant activity, total phenolics compounds, vitamin C and colour of strawberry (Fragaria vesca). *LWT- Food Science and Technology*, 52, 151–156. doi:10.1016/j.lwt.2012.02.027

Odrziol-Belloso, I., Hernandez-Jover, T., & Martin-Belloso, O. (2007). Comparative evaluation of UV–HPLC methods and reducing agents to determine vitamin C in fruits. *Food Chemistry*, 105, 1151–1158. doi:10.1016/j.foodchem.2007.02.037

Palou, L., Crisosto, C.H., Smailanick, J.L., Adaskaveg, J.E., & Zoffoli, J.P. (2002). Effects of continuous 0.3 ppm ozone exposure on decay development and physiological responses of peaches and table grapes in cold storage. *Postharvest Biology and Technology*, 24, 39–48. doi:10.1016/S0925-5210(01)00181-1

Roig, M.G., Rivera, Z.S., & Kennedy, J.F. (1995). A model study on rate of degradation of L-ascorbic acid during processing using home-produced juice concentrates. *International Journal of Food Sciences and Nutrition*, 46, 107–115. doi:10.3109/09637489509012538

Sapei, L., & Hwa, L. (2014). Study on the kinetics of vitamin C degradation in fresh strawberry juices. *Procedia Chemistry*, 9, 62–68. doi:10.1016/j.proche.2014.05.008

Serpen, A., & Gökmem, V. (2007). Reverse degradation kinetics of ascorbic acid under reducing and oxidizing conditions. *Food Chemistry*, 104, 721–725. doi:10.1016/j.foodchem.2006.11.073

Singleton, V.L., & Rossi, J.A. (1965). Colorimetry of total phenolics with phosphomolybdic and phosphovanadomolybdic acid reagents. *American Journal of Enology and Viticulture*, 16, 144–147.

Skog, J.L., & Chu, C.K. (2001). Effect of ozone on qualities of fruits and vegetables in cold storage. *Canadian Journal of Plant Science*, 81, 773–778. doi:10.4141/P00-110

Skrovankova, S., Sumczynski, D., Milce, J., Jurikova, T., & Sochor, J. (2015). Bioactive compounds and antioxidant activity in different types of berries. *International Journal of Molecular Sciences*, 16, 24673–24706. doi:10.3390/ijms161024673

Skupień, K. (2003). Estimation of chosen quality traits of fresh and frozen fruit of six strawberry cultivars. *ActaScientiarum Polonorum Hortorum Cultus*, 2, 115–123.

Sogvar, O.B., Saba, M.K., & Emamifar, A. (2016). Aloe vera and ascorbic acid coatings maintain postharvest quality and reduce microbial load of strawberry fruit. *Postharvest Biology and Technology*, 114, 29–35. doi:10.1016/j.postharvbio.2015.11.019

Tiwari, B.K., O’Donnell, C.P., Patras, A., Brunton, N.P., & Cullen, P.J. (2009). Effect of ozone processing on anthocyanins and ascorbic acid degradation of strawberry juice. *Food Chemistry*, 113, 1119–1126. doi:10.1016/j.foodchem.2008.08.085

Tukey, J.W. (1977). Some thoughts on clinical trials, especially problems of multiplicity. *Science*, 198, 679–684. doi:10.1126/science.333584

Tzortzakis, N., Borland, A., Singleton, I., & Barnes, J. (2007). Impact of atmospheric ozone-enrichment on quality-related attributes of tomato fruit. *Postharvest Biology and Technology*, 45, 317–325. doi:10.1016/j.postharvbio.2007.03.004

Yeoh, W.K., Ali, A., & Forney, C.F. (2014). Effects of ozone on major antioxidants and microbial populations of fresh-cut papaya. *Postharvest Biology and Technology*, 89, 56–58. doi:10.1016/j.postharvbio.2013.11.006

Zimmer, A.R., Londer, B., Miron, D., Schapoval, E., Oliveira, J., & Gossman, G. (2012). Antioxidant and anti-inflammatory properties of *Capsicum baccatum*: From traditional use to scientific approach. *Journal of Ethnopharmacology*, 139, 228–233. doi:10.1016/j.jep.2011.11.005