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To cite this article: Anna Onopiuk, Andrzej Póltorak, Małgorzata Moczowska, Arkadiusz Szpicer & Agnieszka Wierzbicka (2017) The impact of ozone on health-promoting, microbiological, and colour properties of *Rubus ideaus* raspberries, *CyTA - Journal of Food*, 15:4, 563-573, DOI: [10.1080/19476337.2017.1317669](https://doi.org/10.1080/19476337.2017.1317669)

To link to this article: <https://doi.org/10.1080/19476337.2017.1317669>



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Published online: 13 Jun 2017.



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The impact of ozone on health-promoting, microbiological, and colour properties of *Rubus ideaus* raspberries

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ABSTRACT

The purpose of the study was to define the impact of ozonisation duration and concentration on selected chemical, physical, and microbiological properties of *Rubus ideaus* red-fruited raspberries of the Polka variety. Raspberry fruit was exposed to a stream of ozone in concentration of 0.3 and 0.9 mg/L over a period of 60 and 120 min. The scope of the study covered the measurements of titratable acidity, soluble solids concentration, and such health-promoting indicators as content of phenol compounds, flavonoids, anthocyanins and vitamins C, total antioxidant activity (TAA) on 0, 4th, and 8th day of storage. The ozone treated fruit had higher level of phenol compounds and demonstrated higher TAA compared to untreated raspberries. The use of ozone made it possible to curb the development of yeast and moulds on the surface of raspberries, in which the dosage of 0.9 mg/L and treatment time of 60 min proved most efficient.

Impacto del ozono en las propiedades promotoras de la salud, microbiológicas y de color de la frambuesa *Rubus ideaus*

RESUMEN

El presente estudio se propuso definir el impacto que la duración y la concentración de la ozonización tienen sobre determinadas propiedades químicas, físicas y microbiológicas de frambuesas rojas, *Rubus ideaus*, de la variedad polka. Con este objetivo, la fruta de la frambuesa fue expuesta a un chorro de ozono en concentraciones de 0,3 y 0,9 mg/L durante 60 y 120 minutos. El alcance del estudio permitió medir la acidez valorable, la concentración de sólidos solubles y los indicadores de las propiedades promotoras de la salud, que incluyen el contenido de compuestos fenólicos, flavonoides, antocianinas y vitamina C, así como el total de actividad antioxidante en los días 0, 4 y 8 de almacenamiento. La fruta sometida al tratamiento con ozono presentó niveles más elevados de compuestos fenólicos y de actividad antioxidante en comparación con los mostrados por las frambuesas no tratadas. Además, el uso de ozono ayudó a limitar el crecimiento de levadura y moho en la superficie de las frambuesas, constatándose que el tratamiento con una dosis de ozono de 0.9 mg/L durante 60 minutos fue el más eficiente.

ARTICLE HISTORY

Received 19 November 2016
Accepted 3 April 2017

KEYWORDS

Ozonisation; total antioxidant activity; microbiological safety; raspberry fruit

PALABRAS CLAVES

ozonización; actividad antioxidante total; seguridad microbiológica; fruta de frambuesa

1. Introduction

European raspberry (*Rubus ideaus*) is one of the first berries to have been collected by the human and is cultivated in many varieties differing in the size, colour, aroma, and fragrance of the fruit. Raspberry is today highly esteemed and desired by consumers mostly for taste value, yet also for the proven health-promoting properties (Paredes-López, Cervantes-Ceja, Vigna-Pérez, & Hernández-Pérez, 2010). It is so as the berries are a rich source of antioxidants, especially anthocyanins (Chen, Xin, Zhang, & Yuan, 2015), ascorbic acid (Miret & Munné-Bosch, 2016), phenolic acids (Mazur, Nes, Wold, Remberg, & Aaby, 2014), and flavonoids (Giampieri et al., 2015; Ludwig et al., 2015; Mullen et al., 2002; Skrovankova, Sumczynski, Mlcek, Jurikova, & Sochor, 2015). Studies confirm that extracts from various varieties of cultivated raspberries prove high antioxidant activity, among others, by scavenging hydroxyl radicals, singlet oxygen, hydrogen supraoxide, and hyperoxide radicals (Bobinaitė, Viškelis, & Venskutonis, 2016; Wang & Jiao, 2000). Antioxidants contained in raspberries inactivate the reactive forms of oxygen and free radicals, which makes their consumption beneficial for human

health (Bobinaitė et al., 2016). Inclusion of raspberries in diet increases protection against among others various forms of cancer (Carvalho et al., 2013; Del Rio et al., 2013; Hassani, Shariatpanahi, Tavakoli, Nili-Ahmadabadi, & Abdollahi, 2015) and has anti-inflammatory properties (Flores & Ruiz Del Castillo, 2014). Raspberries reinforce and stimulate proper activity of the heart and circulatory system (Anttonen & Karjalainen, 2005) and could also be helpful in diets targeted on managing early stages of type II diabetes and hypertension (Cheplick, Kwon, Bhowmik, & Shetty, 2007).

Raspberries belong to fruit rich in vitamin, phenol compounds, and other nutrients. They are nonetheless highly sensitive to the loss of water and vulnerable to spoilage, which shortens their period of distribution (Ali, Svensson, Alsanius, & Olsson, 2011; Haffner, Rosenfeld, Skrede, & Wang, 2002; Stavang et al., 2015). Seasonal sales of fresh raspberries require subjecting the fruit to special processes of extending shelf life and storage, among others by the application of edible coatings (Guerreiro, Gago, Miguel, Faleiro, & Antunes, 2016), modified and controlled atmospheres packaging (Giovannelli, Limbo, &

Buratti, 2014; Giuggioli, Briano, Girgenti, & Peano, 2015; Van Der Steen, Jacxsens, Devlieghere, & Debevere, 2002), and use of pulsed light (PL) (Xu & Wu, 2016). A promising solution in extending the shelf life of raspberries could be the use of gaseous ozone (O_3). Ozone, a triatomic allotrope of oxygen, is characterised by an oxidation potential of 2.07 V, which is high compared to chlorine (1.36 V) and oxygen (1.23 V), and has bactericidal and viricidal properties (Analia, Loreda, Guerrero, & Alzamora, 2015).

The use of ozone as a bactericide and factor for extending shelf life in food industry may furthermore reduce the use of chemical compounds traditionally used for the purpose, which furthermore are noxious for the natural environment. Excess ozone auto-decomposes rapidly to produce oxygen, leaving no residues in food (Analia et al., 2015). Studies of the Environmental Protection Agency confirmed that ozone is safer for the environment than chlorine and other disinfectants (EPA, 1999). An advantage of ozone is its rapid decomposition to oxygen, which results in disinfection producing only few non-toxic by-products. Freeing the third atom of oxygen, ozone is thus an efficient means for destroying microorganisms found on the surface of fruit (Ali, Ong, & Forney, 2014; Tiwari, O' Donnell, Brunton, & Cullen, 2009). The gas is a bactericide and fungicide, used also to remove pesticides, herbicides, and other organic and inorganic residues (Abdel-Wahhab et al., 2011). The studies conducted by Balawejder et al. (2014) proved the vulnerability of boscalid and tetramethylthiuram disulfide to degradation on impact of gaseous ozone. Residues of these compounds were reduced by nearly 38% on raspberry fruit and by 58% on blackcurrant.

Decreasing the microbiological burden on berries is as important as maintenance of their appropriate quality, structure, colour, and aromatic values (Priyanka, Rastogi, & Tiwari, 2014). The goal of the presented studies was to test/define the impact of ozone on health-promoting properties of raspberries of Polka variety during their 8-day storage. The experiment included studying total acidity, soluble solids concentration, content of phenol compounds, anthocyanins, flavonoids, vitamins C, and total antioxidant activity (TAA). A microbiological analysis was conducted to determine the total count of yeasts and moulds, and the raspberry colour was measured in CIE $L^*a^*b^*$ system.

2. Materials and methods

2.1. Plant material

The plant material used for testing was the fruit of Polka variety raspberry harvested in July 2016. All raspberries were of eating quality and were carefully selected to be identical in terms of shape, size, colour, and ripening stage and did not have blemishes or damage. Fruits were collected in small polyethylene terephthalate containers with a capacity of 125 g and placed in boxes for transporting. They were transported isothermal transport equipped with a refrigeration unit. Raspberries were transported in temperature $4 \pm 1^\circ\text{C}$ for 1 h. The first was the control sample (c_0t_0), and the remaining ones were placed in a cooling chamber to which gaseous ozone was supplied. To enrich the air in the ozone gas, ozone generator Korona 02/10 (Ekotech, Poland) has been used. The generator produces the ozone with a yield of 13 g/h. The generator was equipped with a glass-metal corona discharge lamp coated with a dusted alloy of platinum–iridium. The level of generated ozone was measured using a measuring head GDX-70 (Alter SA, Poland) with a

measuring range from 0 to 5 mg/L. Determination of the generated ozone concentration was possible by the application of the electrochemical sensors. The principle of electrochemical ozone sensors was based on changes of electrolyte under the influence of changing concentrations of ozone. During these changes, electrons went through from the one phase to the another and the gas atoms which directly were involved in the process have changed the oxidation potential. The raspberry fruit was exposed to the impact of air streams with ozone concentration ranging from 0.3 (c_1) to 0.9 (c_2) mg/L for the period of 60 (t_1) and 120 (t_2) min.

After the process of ozone treatment, each batch of the raspberries was divided into three sub-batches. The first (D0) was subjected directly to chemical studies. The scope of tests covered measurements of total acidity, soluble solids concentration, as well as content of phenol compounds, flavonoids, anthocyanins and vitamins C, and total antioxidant capacity. Fruit colour was measured, and the fruit was subjected to microbiological analysis for the total enumeration of yeasts and moulds. The remaining ozone-treated raspberries together with the control sample were cold stored at $5 \pm 1^\circ\text{C}$ for 8 days. The analyses were performed at 0, 4th, and 8th day, respectively.

2.2. Titratable acidity and soluble solid concentration

Aliquots (5.00 g) of juice were diluted with 100 mL of distilled water and the titratable acidity (TA) was determined by titration with 0.1 N NaOH to an end point of pH = 8.1. The results were converted to per cent citric acid [(mL NaOH \times 0.1 N \times 0.064/5.00 g of juice) \times 100] and expressed in fresh weight. The soluble solids concentration was obtained by refractometre (Brix 0–32%, Atago, Japan) and expressed in Brix (Giuggioli et al., 2015; Monaco, Costa, Uliana, & Lima, 2014).

2.3. Total phenolic content

For the determination of the total phenolic content (TPC), Folin and Ciocalteu method was used (Singleton & Rossi, 1965). To achieve this, approximately 30 g of raspberry was homogenised in an Ultra Turrax homogeniser (IKA T18 basic, Germany) and later filtered three times through a Whatman filter No. 1. Then, 0.1 mL of the filtrate was blended with 6.0 mL of distilled water and 0.5 mL of Folin and Ciocalteu phenol reagent (Sigma Aldrich Inc., US). After 3 min, 1.5 mL of sodium carbonate (7.5% w/v; Sigma Aldrich Inc., US) was added, and the whole was topped up with water to 10 mL. The reaction mixture was stored in water bath (WNB 7 Memmert, Germany) for 40 min at the temperature of 40°C . Absorbance was measured spectrophotometrically at wavelength of $\lambda = 760$ nm (Tecan Spark™ 10 M, Männedorf, Switzerland). The TPC was expressed as gallic acid (GA) equivalent (Avantor Performance Materials, Poland) based on the previously designed calibration curve. The results were expressed as the average of three iterations, in milligrams of GA per 100 g of raspberry.

2.4. Total flavonoid content

The determination of total flavonoid content (TFC) was conducted with a spectrophotometer and made used of hydrated aluminium chloride (Monaco et al., 2014); 1 mL of ethanol extract was blended with 1 mL of 2% water solution of $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$, and the mixture was intensively stirred before incubation at room temperature for 10 min. A blind sample without

the extract was also prepared. Absorbance was measured at 430 nm wavelength. TFC values were read from the model curve, and the result was quoted in mg of quercetin per 100 g of fruit weight. The arithmetic mean of three independent trials was quoted as the final result.

2.5. Total anthocyanin content

Total anthocyanin content (TAC) of raspberries was measured using pH differential method described by Li and Wu (2013) with slight modification. Raspberries were extracted in a 0.1-M ethanol solution of HCl. It was first stirred and later centrifuged at 18,000 rpm for 5 min (MPW-251, MPW Med. Instruments, Poland). The supernatant (50 mL) was decanted to volumetric flasks and repeatedly topped up with 15 mL of the extraction solution. The sequence was repeated three times. Then, 2 mL of the supernatant was topped up with 3 mL of pH = 1.0 buffer (0.2 M KCl in 0.2 M HCl solution). Another 2 mL of the supernatant was topped up with 3 mL of pH = 4.5 buffer (1.0 M of CH₃COONa in 1.0 M HCl solution). Samples were stored at room temperature in a dark place for 30 min. The absorbance of each mixture was measured at 510 and 700 nm. The absorbance (A) of anthocyanin extract was calculated from the following formula:

$$A = (A_{510} - A_{700})_{\text{pH}1.0} - (A_{510} - A_{700})_{\text{pH}4.5}$$

where A denoted the absorbance of the anthocyanin extract, A₅₃₅ is the absorbance measured at 535 nm, and A₇₀₀ is the absorbance measured at 700 nm.

The anthocyanin concentration in the raspberry sample was expressed in equivalence of cyanidin-3-glucoside, using the following formula:

$$\text{anthocyanin content} = \frac{(A \times \text{MW} \times \text{DF} \times 1000)}{(\epsilon \times 1)}$$

where MW (449.2 g/mol) is the molecular weight of cyanidin-3-glucoside, DF is the dilution factor, and ϵ is molar absorptivity, which for cyanidin-3-glucoside equals 26.900 L/mol cm.

2.6. Total antioxidant activity

Antioxidant activity of the raspberry sample was measured as the capacity for reduction of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical (De Souza et al., 2014). For that purpose, 3.9 mL of DPPH ethanol solution (600 μ M) was added to 100 μ L of the extract. The reaction mixture was blended for 30 s and set off for 20 min in a dark place. Absorbance was measured at wavelength of 517 nm (Tecan Spark™ 10 M, Männedorf, Switzerland), and the reference solution was 80 g/L ethanol. The sample was prepared without admixture of the extract. All the solvents and reactors were purchased from Sigma (Sigma Aldrich Inc., US). Measurements were conducted in three iterations. The TAA expressed as the percentage of the DPPH radical was calculated from the following formula:

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

where TAA stands for total antioxidant activity and Abs for absorbance.

2.7. Vitamin C content determination

Vitamin C content was determined by titration with 2,6-dichlorophenolindophenol (DCIP) (AOAC, 2000). A sample of around 20 g of fruit was taken, of which 10 mL of clear juice was measured after homogenisation and three filtering rounds. The juice was transferred to a 50-mL volumetric flask, and after replenishing precisely to the line with a 2% water solution of oxalic acid, carefully stirred. The 10 mL of the reaction mixture was poured into a conical flask and immediately titrated with standard solution of DCIP until obtaining light-pink colour retained for 15 s. The ascorbic acid content was expressed in milligrams per 100 g of fresh fruit (Onopiuk et al., 2016).

2.8. Microbiological analysis – enumeration of yeasts and moulds

The microbiological analysis began with preparation of the input suspension. Samples of 10 g (taken from five different places inside the packaging) were removed into a sterile bag and blended with nine times greater volume of solvent (solvent: saline peptone, Sigma Aldrich Inc., US) and stirred at 260 rpm in a stomacher (400 Circulator, Seward Co., West Sussex, UK) for 1 min. Thus prepared, the input suspension accounted for 10⁻¹ dilution.

The enumeration of yeasts and moulds was conducted compliant to Polish Norm PN-ISO 7954:1999. The appropriately prepared input suspension and its successive dilutions (1 mL each) were inoculated on two parallel Petri dishes and inundated with approx. 18–20 mL of agar medium with extract, glucose, and chloramphenicol chilled to 45°C (\pm 2°C). Subsequently, the medium was blended with the inoculum so as to obtain equal distribution of microorganisms. The Petri dishes were left on cold, level surface for cooling and congealing. Later, they were turned over and incubated at 25°C (\pm 1°C) for 5 days. All the colonies on dishes with <150 colonies were counted (Williamson, McNicol, & Dolan, 1987), and the results were quoted in colony-forming units per gram (cfu/g).

2.9. Colour measurements

The surface colour of fresh raspberries was assessed with Minolta CR-400 colour intensity meter (Konica Minolta, Inc., Tokyo, Japan). The colour values were expressed according to the Commission International de l'Eclairage system and reported as CIE L* (lightness), CIE a* (redness), and CIE b* (yellowness). The result for the sample was calculated as the arithmetic mean from 10 measurements (Xu & Wu, 2016). For colour measurements, 10 units of raspberries from each group were used.

2.10. Consumer evaluation

The consumer evaluation of strawberries was performed by 30 untrained panellists recruited among students and faculty members from the Warsaw University of Life Sciences campus. Panellists ages ranged from 21 to 53 years. Consumers evaluation sessions were conducted in isolated rooms with white light at 23°C. Strawberries were delivered completely randomly in coded (3-digit) plastic containers. All information about degree of likeness for the samples were recorded on a questionnaire. Panellists were asked to express their opinion on a 10-cm unstructured scale by placing a vertical line on the scale. Six sensory attributes were evaluated: colour, external appearance,

taste, aroma, texture, and overall acceptability. Consumers were instructed to eat crackers and rinse mouth with water during the forced 30-s break between samples. Quantification of the results was obtained by measuring the distance from zero to the vertical line. The results from the unstructured scale were converted to numerical values (from 0 to 10 units) where 0 point meant the lowest level of acceptance and 10 points meant the highest one (Villamor, Daniels, Moore, & Ross, 2013).

2.11. Statistical analysis

The statistical analysis of the results obtained was conducted in Statistica 10.0 software (Statsoft, Tulsa, US) using variance analysis for a two-factor experiment (first factor: ozone dosage and treatment duration, second factor: storage period). The significance of differences between the mean values was verified with Tukey's range test at the level of significance of $p < 0.05$. The effect of ozone doze (A), ozonisation time (B), storage period (C), and its' interactions with each other was tested using MANOVA for significance level of $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$.

3. Results and discussion

3.1. TA and soluble solid concentration

In case of fresh raspberries, the main reasons for the brevity of storage period are physiological processes and biochemical reactions that take place in the tissues, and the development of microorganisms on their surface. One of the processes is respiration, which involves metabolising of sugars and a change in TA. TA is among the basic parameters that decide about fruit quality, and its enhanced levels may suggest inappropriate storage conditions. The dominant acid used for the expression of the TA in raspberries is the citric acid (% of citric acid). The main components of solids soluble in fruit juice are sugars that were expressed on °Bx scale. The results of total TA and soluble solids concentration of individual groups of stored-fruited raspberries in days 0, 4, and 8 are presented in Table 1.

TA in the fruit batches ranged from 1.15% to 1.31% of citric acid at the beginning of the experiment (D0). Comparing the acidity changes on 0, 4th, and 8th days of storage, a visible increase in each group has been noticed. On 8th day, acidity decreased to 0.81–1.10% recalculated into citric acid. The largest difference was observed in c_1t_1 sample (0.3 mg/L, 60 min), where a drop of approximately

30% was recorded on the 4th day and of approximately 36% on the 8th day. In this batch, the process of respiration was the slowest. Respiratory rate is strictly dependent on the presence of microorganisms present on the surface of raspberries. The dose of 0.3 mg/L and a period of 120 min gave better results than the control because ozone treatment on the surface of the fruit has caused inactivation of microbial cells, respiration proceeded slower. The total acidity is also the result of chemical reaction under the influence of ozone, which easily binds to the double bonds present in organic compounds. Not only the period of storage but also the concentration of ozone and time of ozone treatment resulted in statistically significant differences in acidity in the batches of raspberries used in the study. TA decline during storage might be due to the metabolic changes in fruit resulting from the use of organic acids in respiratory process (Echeverria & Valich, 1989).

On 8th day of storage, increase of soluble solids content in control and in the majority ozonised groups of raspberries has been observed. This fact is a consequence of a life processes taking place in the fruits. The exception were raspberries from the c_2t_2 group (0.9 mg/L, 120 min). On D8, soluble solids concentration was reduced in relation to D4 and it was $9.23 \pm 0.06^\circ\text{Bx}$. Ozone-treated raspberry had higher soluble solid concentration (SSC) content compared to the control batch, which may result from the increase in the level of dominant carbohydrates (glucose and fructose): a resulting of ozone impact. However, factors causing the highest increase of SSC were water losses that occurred during the storage (Tzortzakis, Borland, Singleton, & Barnes, 2007). Additionally, the thin skin of raspberry fruit makes it susceptible to quick loss of water in storage, which also results in elevated SSC (Giuggioli et al., 2015). The results obtained are coherent with literature quoted by De Souza et al. (2014), and Mazur et al. (2014) and lie within the range of $8.83\text{--}10.23^\circ\text{Bx}$. The lowest SSC value was present in control batch raspberries on D0 ($8.83 \pm 0.55^\circ\text{Bx}$), and the highest was in the raspberries from the c_2t_2 batch on D8. Similar SSC growth tendencies in storage were demonstrated by Guerreiro et al. (2016), and by Sogvar, Saba, and Emamifar (2016), who conducted studies on raspberries and strawberries covered with natural coatings. Ozonisation and storage time of raspberries resulted in statistically significant changes in soluble solids concentrations.

Table 1. Impact of concentration and ozonation time on the titratable acidity and content of soluble solid substances measured on the 0, 4, and 8th day of storage in the temperature of $5 \pm 1^\circ\text{C}$, ozone concentration 0.3, 0.9 mg/L and ozonation time 60, 120 min.

Tabla 1. Impacto de la concentración y la duración de la ozonización en la acidez valorable y el contenido de sustancias sólidas solubles, medidos en los días 0, 4 y 8 de almacenamiento a una temperatura de $5 \pm 1^\circ\text{C}$, con concentración de ozono de 0,3 y 0,9 mg/l y duración de la ozonización de 60 y 120 min.

Concentration (mg/L) and ozonation time (min)		Chemical quality parameters					
		Titratable acidity (%)			Soluble solids concentration ($^\circ\text{Bx}$)		
		Storage period (days)					
		0	4	8	0	4	8
Control		1.25 ^{abB} ± 0.01	1.20 ^{bcAB} ± 0.02	1.11 ^{bcA} ± 0.03	8.83 ^{aA} ± 0.55	9.60 ^{abB} ± 0.10	9.87 ^{bB} ± 0.15
0.3 mg/L	60 min	1.27 ^{bb} ± 0.01	0.89 ^{aA} ± 0.02	0.81 ^{aA} ± 0.02	9.00 ^{ab} ± 0.17	9.20 ^a ± 0.10	9.50 ^{ab} ± 0.10
	120 min	1.15 ^{ab} ± 0.06	1.10 ^{bAB} ± 0.06	1.03 ^{bA} ± 0.04	9.47 ^{bA} ± 0.15	9.93 ^{bAB} ± 0.15	10.03 ^{bB} ± 0.21
0.9 mg/L	60 min	1.24 ^{abB} ± 0.02	0.97 ^{aA} ± 0.01	0.88 ^{aA} ± 0.02	9.50 ^b ± 0.10	9.73 ^{ab} ± 0.12	10.00 ^b ± 0.10
	120 min	1.31 ^{bb} ± 0.04	1.28 ^{cb} ± 0.04	1.15 ^{ca} ± 0.05	9.23 ^{ab} ± 0.06	9.43 ^{ab} ± 0.06	9.23 ^a ± 0.06

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

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

a–c: Los valores medios señalados con varias letras minúsculas en orden ascendente indican diferencias estadísticas significativas en las columnas.

A–B: Los valores medios señalados con varias letras mayúsculas en orden ascendente indican diferencias estadísticas significativas en las filas.

3.2. TPC, TFC, TAC, TAA, and vitamin C content determination

Fresh raspberries are a rich source of multiple antioxidant substances, especially phenols, flavonoids, and anthocyanins. They are also a precious source of vitamin C. Environmental conditions including insulation, temperature during fruit growth, type of crop culture, and plant variety all have impact of the concentration of flavonoids and phenol compounds, and subsequently also on the antioxidant activity. The impact of ozone on the phenols (TPC), flavonoid (TFC), anthocyanin (TAC) and vitamin C content and on the capacity for trapping (scavenging) the DPPH radical (TAA) in 0, 4th, and 8th day of storage is presented in Table 2.

Depending of their structure, the impact of phenolic compounds on the process of oxidation may be different. It has nonetheless been demonstrated that these compounds may block free radicals and are capable of preventing reactions caused by the single active atoms of oxygen. Phenolic compounds act as antioxidants, and their activity is defined in line with their chemical structure (Zorița, Florica, Rugină, Lucian, & Socaciu, 2014). Storage of raspberries in atmospheres containing ozone in concentration ranging from 0.3 to 0.9 mg/L in time ranging from 60 to 120 min contributed to a statistically significant ($p < 0.05$) change of phenolic compound content as compared to the control batch untreated with ozone. On D0, the TPC content in the control batch was in the range of 344.77 ± 2.48 mg GA/100 g. Similar TPC in Polka variety of raspberry was also demonstrated by Dragišić and Maksimović et al. (2013)

(314 ± 11 mg GA/100 g of fruit weight) and Bobinaitė, Viškelis, and Venskutonis (2012) (309.4 ± 9.7 mg GA/100 g fruit weight). Raspberry batches subjected to ozone treatment had higher TPC on D0 compared to control ones. A higher level of TPC due to ozone treatment was also confirmed in the studies of Alothman, Kaur, Fazilah, Bhat, and Karim (2010), where the polyphenolic content in pineapples and bananas increased significantly (by 15.7% and 8.2%, respectively) after 20-min exposition to 0.72 mmol of ozone. This growth tendency may be caused by the activation of phenylalanine ammonia-lyase (PAL) resulting from the presence of the gas. PAL is one of the key enzymes participating in the synthesis of phenolic compounds in plant tissues (Dragišić Maksimović et al., 2013). The presence of ozone may contribute to the inhibition of such enzymes as polyphenolic oxidase (EC 1.10.3.1) and peroxidase (POD, EC 1.11.1.7), which often cause oxidation of phenolic compounds in fruit and vegetables (Yeoh, Ali, & Forney, 2014). PAL can be stimulated by different stimulants rather than wounding. The increase in the phenolic contents of the fruits also might have been caused by cell wall modification that occurred during ozone exposure. This modification may have increased the extractability and freed some of the conjugated phenolic compounds in the cell wall (Alothman et al., 2010). Moreover, phenolic compounds have a different propensity to be retained by the cell wall depending on their structure (Paissoni et al., 2016). Rodoni, Casadei, Concellón, Chaves Alicia, and Vicente (2010) studied the effects of short-term treatment with gaseous ozone on the quality of tomato fruits. They demonstrated an increased accumulation in total

Table 2. Impact of concentration and ozonation time on the TPC (mg GA equivalent/100 g), TFC, TAC (mg of cyanidin-3-glucoside/100 g), TAA (%DPPH), and vitamin C content (mg/100 g) measured on the 0, 4, and 8th day of storage in the temperature of $5 \pm 1^\circ\text{C}$, ozone concentration 0.3, 0.9 mg/L and ozonation time 60, 120 min.

Table 2. Impacto de la concentración y la duración de la ozonización en TPC (mg GA equivalente/100g), TFC, TAC (mg de cianidina -3- glucósido/100g), TAA (% DPPH) y contenido de vitamina C (mg/100 g) medidos en los días 0, 4 y 8 de almacenamiento a una temperatura de $5 \pm 1^\circ\text{C}$, con concentración de ozono de 0,3 y 0,9 mg/L y duración de ozonización de 60 y 120 min.

Chemical quality parameters	Concentration and ozonation time	Storage period (days)			
		0	4	8	
TPC mg GA equivalent/100 g of f.w.	Control	60 min	334.77 ^{aC} \pm 2.48	301.87 ^{aBB} \pm 5.51	261.95 ^{aA} \pm 7.33
		120 min	359.43 ^{bCC} \pm 4.51	318.20 ^{bCB} \pm 2.45	276.30 ^{aBA} \pm 6.11
	0.3 mg/L	60 min	358.50 ^{bC} \pm 6.86	338.69 ^{dB} \pm 6.21	305.55 ^{CA} \pm 5.11
		120 min	375.78 ^{CB} \pm 3.83	323.68 ^{cdA} \pm 7.50	321.52 ^{CA} \pm 5.81
	0.9 mg/L	60 min	370.47 ^{bCB} \pm 5.64	298.43 ^{aA} \pm 7.64	284.61 ^{bA} \pm 4.94
		120 min	73.70 ^{cC} \pm 1.80	64.17 ^{bCB} \pm 1.39	51.14 ^{aA} \pm 2.47
TFC mg quercetin equivalent/100 g of f.w.	Control	60 min	61.66 ^{aB} \pm 1.37	58.15 ^{aB} \pm 1.57	49.56 ^{aA} \pm 1.51
		120 min	63.41 ^{abB} \pm 0.19	57.17 ^{aA} \pm 2.52	54.22 ^{abA} \pm 1.28
	0.3 mg/L	60 min	73.44 ^{CB} \pm 0.86	69.23 ^{CB} \pm 3.73	58.21 ^{bA} \pm 3.22
		120 min	68.06 ^{bCC} \pm 0.53	62.99 ^{abB} \pm 1.94	54.89 ^{abA} \pm 1.83
	0.9 mg/L	60 min	56.12 ^{abA} \pm 1.42	73.64 ^{abB} \pm 1.62	80.94 ^{aC} \pm 2.43
		120 min	58.65 ^{abA} \pm 2.68	69.10 ^{abB} \pm 1.65	83.67 ^{aC} \pm 1.12
TAC mg of cyanidin-3-glucoside/100 g of f.w.	control	60 min	60.19 ^{bA} \pm 3.18	76.42 ^{bb} \pm 0.49	82.93 ^{aC} \pm 1.95
		120 min	54.40 ^{abA} \pm 1.35	75.79 ^{bb} \pm 1.80	83.28 ^{aC} \pm 1.14
	0.3 mg/L	60 min	53.05 ^{aA} \pm 1.85	68.35 ^{ab} \pm 1.92	81.00 ^{aC} \pm 2.58
		120 min	54.47 ^{aA} \pm 5.72	62.67 ^{aA} \pm 5.13	57.97 ^{aA} \pm 2.09
	0.9 mg/L	60 min	55.89 ^{abA} \pm 5.61	65.12 ^{aA} \pm 5.86	68.64 ^{abA} \pm 6.41
		120 min	68.21 ^{bA} \pm 1.12	72.44 ^{aA} \pm 4.71	74.71 ^{bA} \pm 3.91
TAA % DPPH	Control	60 min	67.59 ^{abA} \pm 5.86	70.47 ^{aA} \pm 0.46	77.14 ^{bA} \pm 4.08
		120 min	62.71 ^{abA} \pm 4.92	67.86 ^{abB} \pm 5.35	73.99 ^{bbB} \pm 2.53
	0.3 mg/L	60 min	18.02 ^{aB} \pm 0.33	16.25 ^{aAB} \pm 0.74	14.48 ^{aA} \pm 0.69
		120 min	20.03 ^{bCB} \pm 0.64	18.57 ^{bcAB} \pm 0.32	17.78 ^{bCA} \pm 0.80
	0.9 mg/L	60 min	22.05 ^{dB} \pm 0.55	20.77 ^{dAB} \pm 0.91	18.87 ^{CA} \pm 0.03
		120 min	18.78 ^{abB} \pm 0.60	17.18 ^{abAB} \pm 0.55	16.50 ^{bA} \pm 0.53
Vitamin C mg/100 g of f.w.	Control	60 min	21.87 ^{cdC} \pm 1.14	19.77 ^{cdB} \pm 0.97	17.97 ^{bcA} \pm 0.21
		120 min	21.87 ^{cdC} \pm 1.14	19.77 ^{cdB} \pm 0.97	17.97 ^{bcA} \pm 0.21

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

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

TPC: Total phenolic content; GA: gallic acid; TFC: total flavonoid content; TAC: total anthocyanin content; TAA: total antioxidant activity.

a–c: Los valores medios señalados con varias letras minúsculas en orden ascendente indican diferencias estadísticas significativas en las columnas.

A–B: Los valores medios señalados con varias letras mayúsculas en orden ascendente indican diferencias estadísticas significativas en las filas.

TPC: Contenido fenólico total; GA: ácido gálico; TFC: contenido de flavonoides total; TAC: contenido de antocianina total; TAA: actividad antioxidante total.

phenolic compounds by 50% compared to the control after 6 days of fruit storage at a temperature of 20°C.

The highest content of phenolic compounds on D0 was recorded in raspberries from c_2t_1 sample (375.78 ± 3.83 mg GA/100 g), and the lowest was in the control group. TPC content did not differ statistically between the ozone-treated groups on D0. The dose and time of ozonisation have not affected on TPC as significant as day of storage (D0, 4, 8). With storage, TPC dropped in all batches storage. Phenolic compounds were most severely depleted in the control group, with the losses at the level of 21.75% of TPC, and in the c_2t_2 group where the loss was at the level of 23.17%. In the second case, the loss of TPC might have resulted from extensive dosing of ozone (0.9 mg/L) or prolonged treatment (120 min) of raspberries. The potency of ozone depends on its concentration, time, and form of exposure. After longer exposition of raspberries from c_2t_2 batch to ozone, the gas might have decomposed, which led to the development of such free radicals as hydroperoxyl (or perhydroxyl, H_2O_2), hydroxyl ($\cdot OH$), and superoxide ($\cdot O_2^-$). According to Hoigné and Bader (1983), by-products of ozone decomposition may lead to a drop of polyphenolic content in fruit. Another significant factor determining the level of TPC was storage temperature ($5 \pm 1^\circ C$). According to Skrovankov et al. (2015), TPC level in the fruit of red-fruited raspberry may increase even by the factor of 1.5 during a week's storage at 20°C.

Like phenols, flavonoids show antioxidant activity. The main flavonoid present in raspberry is quercetin, which belongs to the class of flavonols. Flavonoid content in the studied raspberries was in the range of 73.70–51.14 mg of quercetin equivalent per 100 g of f.w. in storage, the flavonoids yielded to partial degradation. The largest drop was observed in the control group, where the loss in D8 amounted to 22.56 mg (30.61% as compared to D0). The largest stability was demonstrated by the flavonoids contained in raspberries from the group c_1t_2 (0.3 mg/L, 120 min), where a drop by 14.49% was observed in D8. Ozonation reduced losses in the content of flavonoids. The increase in the content of flavonoids in raspberries could possibly related to modifications in cell walls during the ozonation. This fact has contributed to increasing the efficiency of the extraction process (Allothman et al., 2010). A higher concentration of ozone and longer time of ozonisation (0.9 mg/L, 120 min) did not affect positively on the content of flavonoids in raspberries. Probably occurred excessive oxidation process by direct action of ozone or by indirect reactions of radical formation (Tiwari et al., 2009). Both concentration of ozone, time of treatment, and day of storage had statistically significant impact on the level of flavonoids in the raspberries used in the study.

The TAC in red-fruited raspberries is typically between 20 and 100 mg in 100 g of fresh fruit (Rao & Snyder, 2010). Major anthocyanins found in raspberries and blackberries are derivatives of cyanidins, which commonly exist in nonacylated forms. Red raspberries contain a wide spectrum of anthocyanins, with the major constituents being cyanidin-3-glucoside, cyanidin-3-sophoroside, and cyanidin-3-glucosylrutinoside. During raspberries storage, the content of anthocyanins increased. According to the latest reports of Skrovankova et al. (2015) on bioactive compounds demonstrating anti-oxidative properties in berries, the level of anthocyanins in raspberries can even increase by the factor of 2.5 during a week's storage. While analysing the results obtained, the largest increase of anthocyanin content recalculated into cyanidin-3-glucoside was observed in the c_2t_1 batch of raspberries (0.9 mg/L, 60 min). On 8th day of storage at $5 \pm 1^\circ C$, the level of anthocyanins in

fresh fruit increased by 53.08% compared to D0. Neither ozone dosage nor time of treatment was among factors that had significant statistic influence on the level of anthocyanins. Anthocyanins are located in the outer layers of hipodermis and in the cells are located in the vacuoles, whereas the cell walls and tissue parenchyma which have a direct contact with gaseous ozone do not contain these pigments.

Studies on health-promoting properties of raspberries fall back on the use of the DPPH reagent to assess their capacity to deactivate free radicals. At the beginning of the experiment (D0), the control group of raspberries demonstrated TAA at the level of $54.47 \pm 5.72\%$, on the 8th day of storage increased by 6.43%. Raspberries from batch c_2t_1 (0.9 mg/L, 60 min) featured high capacity for neutralising the free radical DPPH, as in this sample, TAA was on average equal to $67.59 \pm 5.86\%$. The lowest TAA value was recorded in the control batch ($54.47 \pm 5.72\%$) and the sample subjected to treatment with low ozone concentration, i.e. 0.3 mg/L for 60 min ($55.89 \pm 5.61\%$). On the 8th day of storage, the capacity to deactivate free radicals was also lowest in these groups. Taking into account raspberry storage time, it was discovered that the antioxidant capacity increased to a statistically significant degree ($p < 0.05$) with storage time, which could be dictated by the increase in TPC and TAC in raspberry fruit (Table 3). The increase of secondary metabolites concentration in the stored fruits can be explained by the metabolic changes occurring after harvest. During the storage, an increase of antioxidant activity of endogenous enzymes such as catalase, POD, ascorbate POD, or adequate POD has been noticed. These enzymes catalyse the synthesis of protective metabolites (antioxidants), what can be observed as increased antiradical activity of the fruits extracts (Sachadyn-Król et al., 2016). No statistically significant increase in TAA during the 8-day storage was demonstrated only in the control group. The capacity to scavenge the DPPH radical by batches of raspberries subjected to ozone-treatment proved satisfactory. The studies conducted by Giuggioli et al. (2015) corroborate that presence of ozone results in no losses of bioactive substances during raspberries storage.

Of all fruit, raspberries are considered to be among those richest in ascorbic acid. The content of vitamin C in fresh raspberries according to De Souza et al. (2014) ranges between 15 and 38 mg/100 g, with the actual level depending on fruit variety and ripeness. Studying Polka variation raspberries, Bobinaitė et al. (2012) proved vitamin C content at the level of 17.2 ± 0.6 mg/100 g of f.w.

The average content of vitamin C obtained in the studies lies within the range from 18.02 to 22.05 mg/100 g on D0. It was proven that ozone treatment may provide a factor limiting the degradation of vitamin C. Raspberries subjected to the activity of gaseous ozone featured higher ascorbic acid content when compared to the control batch, as presented in Table 2. A similar tendency was demonstrated in the studies of Perez, Sanz, Rios, Olias, and Olias (1999), who reported that at the end of day 3 of cold storage, vitamin C content of ozone treated (0.35 ml/L) strawberries was three times that of control fruit.

Vitamin C content decreased during storage as result of oxidation of L-ascorbic acid to dehydroascorbic acid, which oxidises further and loses biological activity. On the 8th day of storage, the drop in ascorbic acid in ozone-treated samples amounted on average to 2.90 mg of vitamin C per 100 g of fruit weight, while in the control batch, the content of the same vitamin dropped by 3.54 mg/100 g. The observed reduction of ascorbic acid losses after ozone treatment was probably

Table 3. Effect of ozone doze (A), ozonation time (B), storage period (C), and their interactions on titratable acidity (%), soluble solids concentration ($^{\circ}\text{Bx}$), total phenolic content (TPC), total flavonoid content (TFC), total anthocyanin content (TAC), total antioxidant activity (TAA), vitamin C and colour parameters (CIE L^* , a^* , b^*).

Tabla 3. Efecto de la dosis de ozono (A), duración de la ozonización (B), periodo de almacenamiento (C) y sus interacciones en la acidez valorable (%), la concentración de sólidos solubles ($^{\circ}\text{Bx}$), el contenido fenólico total (TPC), el contenido de flavonoides total (TFC), el contenido de antocianina total (TAC), la actividad antioxidante total (TAA), la vitamina C y los parámetros de color (CIE L^* , a^* , b^*).

Parameter	Effect							SEM
	A	B	C	A × B	A × C	B × C	A × B × C	
Titratable acidity (%)	***	***	***	***	NS	***	NS	0.001
Soluble solids concentration ($^{\circ}\text{Bx}$)	NS	NS	***	***	*	**	*	0.015
TPC	NS	NS	***	***	***	NS	***	33
TFC	***	*	***	***	*	*	NS	3.9
TAC	***	NS	***	***	**	NS	***	3.8
TAA	NS	NS	***	***	NS	NS	NS	21.3
Vitamin C	***	***	***	NS	NS	NS	NS	0.46
L^*	NS	NS	**	NS	NS	NS	NS	4.1
a^*	NS	NS	NS	NS	NS	NS	NS	8.56
b^*	NS	NS	*	NS	NS	NS	*	3.55

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; NS: not significant.

TPC: Total phenolic content; TFC: total flavonoid content; TAC: total anthocyanin content; TAA: total antioxidant activity.

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; NS: No significativo.

TPC: Contenido fenólico total; TFC: contenido de flavonoides total; TAC: contenido de antocianina total; TAA: actividad antioxidante total.

caused by the effect of inhibition of enzymatic activity of ascorbate POD and ascorbate oxidase, responsible for the degradation of ascorbic acid. The highest stability of vitamin C in D8 was confirmed in groups c_1t_1 and c_2t_1 , where their content decreased by 2.25 and 2.28 mg/100 g, respectively, as compared to D0 batches. In respect to vitamin stability, 60-min duration of treatment proved most optimal. Interesting conclusions were presented in an article by Alothman et al. (2010), who stated that extension of ozone treatment time contributed to statistically significant reduction of vitamin C content in bananas and pineapples. The vitamin C content decreased in the fruits due to its scavenging of the free radicals formed during the decomposition of ozone.

Literature corroborates that ozone treatment of cold-stored fruit (on the example of strawberry) for 10 days, with supply of ozone dosed at 2, 4, and 8 mg/L to the cooling house, did not result in lowering vitamin C content

in fruit. Moreover, a gradual decay of vitamin C during storage was recorded; yet, the process was far more rapid in non-ozonised fruit (Zhang, Zhang, Wang, & Zhao, 2011).

No dependencies were proved between the content of anthocyanins and ascorbic acid on the one hand and the increase of DPPH radical scavenging capacity. Similar results were obtained in the studies by Benvenuti, Pellati, Malegari, and Bertelli (2004), where the concentration of polyphenols, anthocyanins and reduced form of ascorbic acid were determined in the fruit of raspberry, blackberry, and red and black currant.

3.3. Colour measurements

Results of CIE $L^*a^*b^*$ colour components assessed with a Minolta Chroma Meter CR-400 trichromatic monochromator are presented in Table 4. No impact of ozone on colour parameters in

Table 4. Impact of concentration and ozonation time on the colour parameters CIE $L^*a^*b^*$ measured on the 0, 4, and 8th day of storage in the temperature of $5 \pm 1^{\circ}\text{C}$, ozone concentration 0.3, 0.9 mg/L and ozonation time 60, 120 min.

Tabla 4. Impacto de la concentración y la duración de la ozonización en los parámetros de color CIE $L^*a^*b^*$ medidos en los días 0, 4 y 8 de almacenamiento a una temperatura de $5 \pm 1^{\circ}\text{C}$, con concentración de ozono de 0,3 y 0,9 mg/L y duración de ozonización de 60 y 120 min.

Colour parameters	Concentration and ozonation time		Storage period (days)		
			0	4	8
Lightness (L^*)	Control	60 min	$32.84^{aA} \pm 1.85$	$31.42^{aA} \pm 3.09$	$32.22^{aA} \pm 2.13$
		120 min	$34.59^{bB} \pm 2.01$	$33.71^{aB} \pm 1.24$	$31.16^{aA} \pm 1.96$
	0.3 mg/L	60 min	$33.15^{aA} \pm 2.10$	$33.06^{aA} \pm 1.76$	$32.57^{aA} \pm 2.12$
		120 min	$34.28^{bB} \pm 2.28$	$32.04^{aA} \pm 1.96$	$33.11^{aAB} \pm 1.75$
		60 min	$34.48^{aA} \pm 1.86$	$33.71^{aA} \pm 2.51$	$33.48^{aA} \pm 2.43$
		120 min	$24.29^{aA} \pm 2.21$	$22.02^{aA} \pm 4.30$	$24.24^{aA} \pm 2.58$
Redness (a^*)	Control	60 min	$25.68^{aAB} \pm 2.45$	$26.16^{bB} \pm 1.75$	$23.08^{aA} \pm 2.94$
		120 min	$25.19^{aA} \pm 2.30$	$23.20^{aB} \pm 2.27$	$24.82^{aA} \pm 3.82$
	0.9 mg/L	60 min	$25.40^{aA} \pm 3.13$	$23.95^{aB} \pm 2.28$	$25.35^{aA} \pm 2.77$
		120 min	$24.60^{aA} \pm 2.74$	$25.14^{aB} \pm 4.04$	$25.50^{aA} \pm 3.67$
		60 min	$9.73^{aA} \pm 1.19$	$8.13^{aA} \pm 2.28$	$9.24^{aA} \pm 1.52$
		120 min	$10.99^{bB} \pm 2.40$	$10.82^{bB} \pm 1.31$	$8.27^{aA} \pm 1.17$
Yellowness (b^*)	Control	60 min	$10.08^{aA} \pm 1.79$	$8.68^{aB} \pm 1.28$	$9.51^{aA} \pm 2.05$
		120 min	$10.92^{bB} \pm 2.22$	$8.82^{aB} \pm 1.48$	$9.54^{aAB} \pm 1.57$
	0.9 mg/L	60 min	$9.83^{aA} \pm 1.83$	$9.93^{aB} \pm 2.42$	$10.08^{aA} \pm 2.43$

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

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

a–b: Los valores medios señalados con varias letras minúsculas en orden ascendente indican diferencias estadísticas significativas en las columnas.

A–B: Los valores medios señalados con varias letras mayúsculas en orden ascendente indican diferencias estadísticas significativas en las filas.

studied raspberry fruit was observed. Statistical analysis has proved that the results obtained from the control batch are not different in a statistically significant way (significance level $p < 0.05$) from samples ozonised in the experiment. It is believed that the duration and dosage of ozone treatment applied (0.3–0.9 mg/L, 60–120 min) had no impact on the change of fruit surface colour. The only statistically significant difference was demonstrated in batch c_1t_1 , where the L^* parameter decreased from 34.59 ± 2.01 (D0) to 31.16 ± 1.96 (D8). During the experiment (D0, 4, and 8), the colour of raspberries surface has not been changed significantly, which is a proof of satisfactory quality of the fruit. It is so as too rapid falls in the L^* parameter during storage may suggest darkening of fruit and quick process of decay. An additional change of colour from pink to dark red also leads to the loss of attractiveness to consumers according to Giuggioli et al. (2015). Unfortunately, like in the studies conducted by Haffner et al. (2002), it was impossible to prove that the colour of the surface of the raspberry can become a good marker of the level of anthocyanins in this fruit.

3.4. Microbiological analysis – enumeration of yeasts and moulds

An analysis based on the enumeration of yeasts and moulds in line with PN-ISO 7954:1999 norm made it possible to study the impact of ozone treatment on microbiological safety of raspberry fruit. The results are presented in Table 5. The total count of yeast and moulds in the control sample amounted to $17.83 \pm 0.29 \times 10^3$ cfu/g, while in the ozone-treated groups, a small drop in their number was observed already in D0. The lowest total number of yeasts and moulds was present in raspberry from the batch c_2t_2 ($13.53 \pm 0.47 \times 10^3$ cfu/g). A statistically significant increase in the number of microorganisms was noticed during storage in all batches, while the raspberry from the control sample was infected with the largest number of colony building moulds and yeast. Ozone contributed to the positive lowering of the yeast and mould count. The dose of 0.9 mg/L in 60 min proved most efficient. Extension of the exposition time to 120 min did not contribute to a noticeable reduction of the microorganisms count. On the other hand, the dose of 0.9 mg/L proved more efficient than 0.3 mg/L. In ozonised raspberry, the degree of infection increased at a slower pace. The data confirm efficiency of application of ozone as a factor increasing the storage time of raspberries thanks to the likely bactericide and fungicide activity. By

reacting with organic compounds causes its oxidation. In the first stage of ozone activity occurs rapidly interrupt of bacteria cell wall. Then, the residues of polyunsaturated fatty acids included in the phospholipids of the cytoplasmic membrane undergo peroxidation what lead to formation of peroxide compounds. Then, the residues of polyunsaturated fatty acids included in the phospholipids of the cytoplasmic membrane undergo peroxidation what lead to formation of peroxides of these compounds. These products cause depolarisation, inhibit the activity of membrane enzymes, and transport proteins.

3.5. Consumer evaluation

Principal component analysis on sensory data for the 15 samples resulted in two principal factors that accounted for 96.94% of the variance (Figure 1). Factor 1 (85.65%) was associated with acceptance of taste, texture, aroma, and overall acceptance. Factor 2 (11.29%) was associated with colour and external appearance. Consumers were able to detect differences between samples, grouping them into distinctive clusters. This is associated with changes of sensory attributes during storage of samples. The dominating descriptors responsible for samples discrimination were colour and external appearance. Moreover, taste, texture aroma, and overall acceptance were the distinguishing parameters that strongly differentiate fresh samples and after storage periods. There was no statistically significant effect of concentration and ozonation time on sensory attributes (colour, taste, texture, aroma, external appearance, overall acceptance). The storage time had a statistically significant effect on the acceptance decline of most of the sensory attributes (taste, texture, aroma, external appearance, overall acceptance) regardless of concentration and ozonation time.

4. Conclusion

The impact of concentration and time of ozone treatment on selected chemical, physical, and microbiological properties of red-fruited raspberries of Polka variety was determined. Efficiency of treatment with gaseous ozone as a method allowing to extend microbiological safety while storing raspberry fruit was demonstrated. Gaseous ozone impacts the bacterial flora present naturally on the surface of raspberries and has a significant impact on their chemical composition. The results presented attest to dependencies present

Table 5. Impact of concentration and ozonation time on the total count of yeast and moulds measured on the 0, 4, and 8th day of storage in the temperature of $5 \pm 1^\circ\text{C}$, ozone concentration 0.3, 0.9 mg/L and ozonation time 60, 120 min.

Tabla 5. Impacto de la concentración y la duración de la ozonización en el conteo total de levadura y moho medido en los días 0, 4 y 8 de almacenamiento a una temperatura de $5 \pm 1^\circ\text{C}$, concentración de ozono de 0,3 y 0,9 mg/L y duración de la ozonización de 60 y 120 min.

Microbiological parameters	Concentration and ozonation time	Storage period (days)			
		0	4	8	
total count of yeast and moulds (cfu/g)	Control	$(17.83 \pm 0.29)^{aA}10^3$	$(38.90 \pm 0.75)^{bB}10^3$	$(57.10 \pm 1.05)^{cC}10^3$	
	0.3 mg/L	60 min	$(16.08 \pm 0.38)^{aA}10^3$	$(40.47 \pm 0.50)^{bB}10^3$	$(45.64 \pm 0.54)^{aC}10^3$
		120 min	$(14.67 \pm 0.58)^{aA}10^3$	$(26.00 \pm 1.00)^{aB}10^3$	$(56.83 \pm 0.76)^{bC}10^3$
	0.9 mg/L	60 min	$(13.93 \pm 0.90)^{aA}10^3$	$(22.80 \pm 0.44)^{aB}10^3$	$(38.80 \pm 0.62)^{aC}10^3$
		120 min	$(13.53 \pm 0.47)^{aA}10^3$	$(25.33 \pm 0.67)^{aB}10^3$	$(42.37 \pm 0.91)^{aC}10^3$

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

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

a–b: Los valores medios señalados con varias letras minúsculas en orden ascendente indican diferencias estadísticas significativas en las columnas.

A–C: Los valores medios señalados con varias letras mayúsculas en orden ascendente indican diferencias estadísticas significativas en las filas.

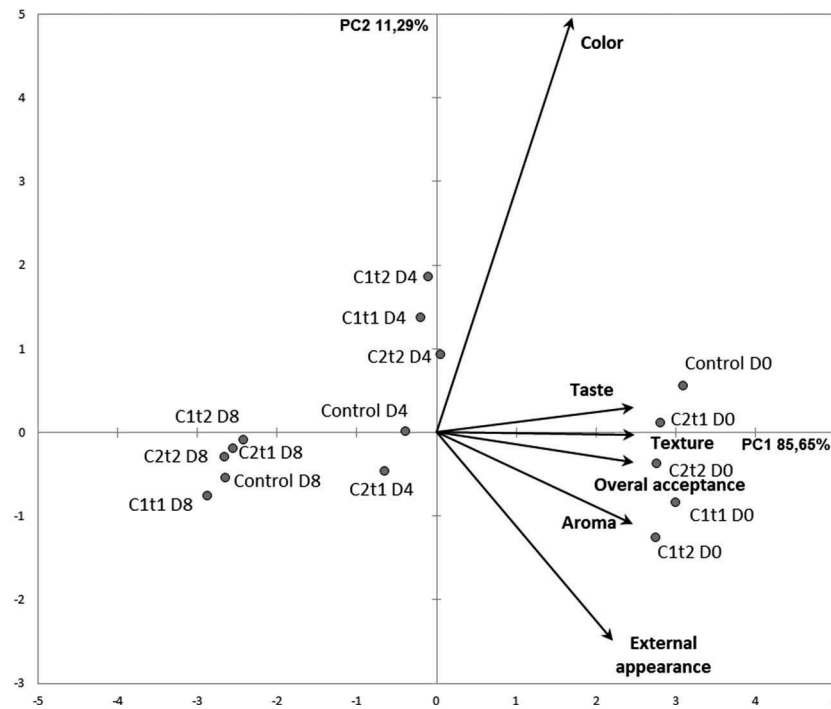


Figure 1. Influence of concentration and ozonation time on sensory attributes measured on the 0, 4th, and 8th day of storage (respectively, D0, D4, D8) in the temperature of $5 \pm 1^\circ\text{C}$, ozone concentration 0.3, 0.9 mg/L (respectively, c_1 , c_2) and ozonation time 60, 120 min (respectively, t_1 , t_2).

Figura 1. Influencia de la concentración y la duración de la ozonización en los atributos sensoriales, medidos en el día 0, 4 y 8 de almacenamiento (D0, D4, D8, respectivamente), a una temperatura de $5 \pm 1^\circ\text{C}$, una concentración de ozono de 0,3 y 0,9 mg/L (C_1 , C_2 , respectivamente) y duración de la ozonización de 60 y 120 min (t_1 , t_2 , respectivamente).

between the stability of antioxidant components on the one hand and the dosage of ozone and the exposition time of the raspberries on the other. Ozone-treated fruit had higher concentration of soluble solids and statistically significantly higher level of phenolic compounds and also demonstrated higher total antioxidant capacity as compared to untreated fruit. The largest increase in anthocyanin content (recalculated into cyanidin-3-glucoside) was observed in the batch c_2t_1 (0.9 mg/L, 60 min), while the most stable flavonoids were present in raspberries from the batch c_1t_2 (0.3 mg/L, 120 min). Ozonisation decreased the loss of vitamin C and did not have negative bearing on the change of fruit surface colour. The use of ozone made it possible to reduce the development of yeast and moulds of the surface of the raspberries and did not make negative impact on the fruit's health-promoting quality. In this case, most efficient was the dose of 0.9 mg/L of ozone and treatment duration of 60 min. Protection of the fruit against the development of microorganisms carries positive effects in the form of increasing health safety of raspberries among others by lowering the risk of development of noxious bacteria and causing the decay of fruit.

Retention of antioxidant properties, stability in storage, and microbiological safety are exceedingly important in the case of berries. The results of the study show that ozone can be applied as an alternative technology for disinfecting in the fresh raspberry fruit sector without spoiling the nutritive value of the fruit.

Disclosure statement

No potential conflict of interest was reported by the authors.

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