



# Effect of Ozonation Process on the Energy Metabolism in Raspberry Fruit During Storage at Room Temperature

Tomasz Piechowiak<sup>1</sup> · Patrycja Sowa<sup>1,2</sup> · Maciej Balawejder<sup>1</sup>

Received: 12 August 2020 / Accepted: 19 January 2021 / Published online: 29 January 2021  
© The Author(s) 2021

## Abstract

The major aim of this research was to investigate the effect of ozone treatment on the energy metabolism in raspberry fruit during storage at room temperature. Raspberries were ozonated with an ozone concentration of 8–10 mg L<sup>-1</sup>, for 30 min, every 12 h of storage at room temperature for 72 h. The results indicated that ozone treatment significantly enhanced the activities of mitochondrial respiratory enzymes, such as succinate dehydrogenase, cytochrome C oxidase, and H<sup>+</sup>-ATPase, which contributed to maintain the high level of ATP and energy charge in fruit during storage. Moreover, the energy metabolism in mitochondria was closely correlated with the antioxidant potential of raspberry fruit. This study has given an experimental evidence that ozonation procedure in proposed process conditions significantly affects the mitochondrial respiratory system leading to maintain the high quality of the fruit over a long period of storage at room temperature.

**Keywords** ATP · Enzymes · Mitochondria · Ozone · Raspberry

## Introduction

Ozonation process is an alternative method for extending the postharvest shelf life of berry fruit. Many recent studies have suggested that ozone treatment inhibits the growth of microorganisms responsible for fruit spoilage as well as reduces the loss of nutritional and sensory value of berry fruit during storage (Alexandre et al. 2011; Carbone and Mencarelli 2015; Chen et al. 2019; Contigiani et al. 2018; Horvitz and Cantalejo 2014; Jaramillo-Sánchez et al. 2019; Piechowiak et al. 2020a, b; Pinto et al. 2020; Yaseen et al. 2015). Our previous study investigated the effectiveness of ozone treatment of raspberry fruit with 8–10 mg L<sup>-1</sup> gas, for 30 min, every 12 h during storage at room temperature (72 h). Ozone treatment significantly decreased the growth of aerobic

bacteria and fungi in raspberry fruit during whole period of storage (Piechowiak et al. 2019). Furthermore, ozone treatment activated the protective mechanism against oxidative stress in raspberry fruit. After ozonation, raspberry fruit featured a higher level of phenolic compounds and total antioxidant capacity than non-ozonated fruit (Piechowiak and Balawejder 2019; Piechowiak et al. 2020a, b).

Numerous scientific reports show that the resistance of the fruit to adverse changes in quality during storage is related to their energy metabolism (Zhou et al. 2014). ATP produced as a result of oxidative phosphorylation is necessary for cells to maintain their proper activity and vitality, the integrity of membranes, and biosynthesis of substances with antibacterial and antioxidative properties (Li et al. 2020). Scientific studies also show that the use of various elicitors, e.g., chlorogenic acid, oxalic acid, 1-methylcyclopropene, or methyl jasmonate, increases the metabolic activity of mitochondria, which corresponds to better fruit quality during storage (Jin et al. 2013, 2014; Li et al. 2020; Shu et al. 2020)

The studies to date on the utilization of ozone treatment in fruit storage principally concerned the effect of ozonation on the level of microbial contamination, biological activity of the fruit, and their sensory characteristics. However, there have been no reports about the mechanism explaining the positive impact of ozone on the quality of fruits. Due to the fact that the activity of mitochondria is responsible for physiological

✉ Tomasz Piechowiak  
tpiechowiak@ur.edu.pl

<sup>1</sup> Department of Chemistry and Food Toxicology, Institute of Food Technology and Nutrition, University of Rzeszow, St. Cwiklinskiej 1a, 35-601 Rzeszow, Poland

<sup>2</sup> Department of Bioenergetics, Food Analysis and Microbiology, Institute of Food Technology and Nutrition, University of Rzeszow, St. Zelwerowicza 4, 35-601 Rzeszow, Poland

response in fruit during storage, the effect of ozonation process on the mitochondrial energy metabolism in raspberry fruit during storage was determined in this study. To achieve this objective, energy charge of fruit and oxidative phosphorylation enzyme activity as well as oxidative stress marker level were analyzed in ozonated and control raspberries. Moreover, the relationship between mitochondria activity and selected quality attributes of ozonated raspberries was explained using multivariate statistical methods (PCA).

## Materials and Methods

### Plant Material

The research material was fresh raspberry (*Rubus idaeus* L.) fruit purchased immediately after harvest from a local fruit producer. Raspberries (1 kg) were stored at room temperature (20–25 °C) for 72 h and ozonated every 12 h using ozone at the concentration of 8–10 mg L<sup>-1</sup> for 30 min using a Korona L5 ozone generator (KoronaOzon, Poland) and ozone analyzer (2B Technologies, USA). A control sample (1 kg) was represented by non-ozonated fruit. The fruit samples (control and ozonated fruit) were collected after 0, 24, 48, and 72 h of storage and kept at -67 °C for a period of 3 months before analysis. A full description of the storage procedure and ozonation is presented in our previous publications (Piechowiak et al. 2019, 2020a, b).

### Determination of ATP, ADP, and AMP Content and Energy Charge

The ATP, ADP, and AMP contents were measured according to the methodology described by Liu et al. (2006) with some modifications. Frozen raspberry tissues (-67 °C) were milled to powder and homogenized (7.50 g) with 10 mL of 0.6 M HClO<sub>4</sub> for 1 min in an ice bath. The homogenate was centrifuged at 7500g (260R-Centrifuge, MPW, Poland) for 15 min at 4 °C. A total of 6 mL of the supernatant was neutralized with 1 M KOH to 6.5–6.8 pH and then diluted to 10 mL. The supernatant was filtered through a nylon filter (0.45 μm) before HPLC analysis.

The HPLC analysis was performed using Young Lin YL 9100 HPLC chromatograph with a UV-Vis detector YL9120 (YL Instrument Co., Ltd., Korea) according to the procedure described by Zhou et al. (2014) with some modifications. The tested compounds were separated on a 4.6 × 250 mm, 2.5 μm Cosmosil C18 MSII column. The mobile phase consisted of 60 mM K<sub>2</sub>HPO<sub>4</sub> and 40 mM KH<sub>2</sub>PO<sub>4</sub> dissolved in deionized and adjusted to pH = 7.0 with 0.1 M KOH (mobile phase A) and 100% methanol (mobile phase B) at a flow rate of 1 mL min<sup>-1</sup>. The gradient separation was performed as follows: 0 min, 100% A; 3.5 min, 95% A, 5% B; 7 min, 80% A, 20% B;

8 min, 75% A, 25% B; and 10 min, 100% B. The chromatograms were recorded at 254 nm and injection volume was 20 μL. The column temperature was set at 25 °C. The identification of ATP, ADP, and AMP was based on the comparison with the retention time of standards using the external standard method. The calibration curves of the analyzed substances were made in triplicate for each individual standard in six concentrations in the range of 1–10 mg L<sup>-1</sup>. The determination coefficient for the calibration curves was higher than  $R^2 \geq 0.9998$ . The limits of detection (LOD) and limits of quantification (LOQ) were determined at a signal-to-noise ratio of 3:1 and 10:1. The obtained LOD was 0.2 mg L<sup>-1</sup> and LOQ 0.6 mg L<sup>-1</sup> for ATP and AMP and for ADP 0.1 mg L<sup>-1</sup> (LOD) and 0.3 mg L<sup>-1</sup> (LOQ). Standards were purchased from Sigma-Aldrich.

To determine the energy charge in fruit, the following formula was used:

$$EC = \frac{ATP + 0.5 ADP}{ATP + ADP + AMP},$$

where ATP, ADP, and AMP mean the contents of these compounds in the fruit sample.

### Extraction of Raspberry Mitochondria

Mitochondria from raspberries were extracted according to the method presented by Zhou et al. (2014). Milled raspberry tissue (50 g) was homogenized (in an ice bath) with 100 mL of 50 mM Tris-HCl buffer (pH 7.5) containing 0.3 M mannitol, 1.0 mM EDTA, 0.1% (w/v) cysteine, 0.1% BSA (w/v), and 0.5% PVP (w/v). The crude homogenate was filtered through a double nylon gauze and centrifuged at 4000g for 10 min at 4 °C. Next, the obtained supernatant was removed to a fresh tube and centrifuged at 12,000g for 10 min at 4 °C. The pellet was resuspended in washing buffer (pH 7.5) containing 10 mM Tris, 0.3 M mannitol, 1.0 mM EDTA, and 0.1% bovine serum albumin (w/v) and again centrifuged at 12,000g for 10 min at 4 °C. The mitochondrial pellet was resuspended in washing buffer.

### Measurement of Enzymatic Activity in Raspberry Mitochondria

The mitochondrial suspension was centrifuged at 12,000g for 10 min and washed two times with PBS buffer (pH 7.4). Next, the mitochondrial pellet was resuspended in PBS buffer containing 0.1% (m/v) triton X-100 and 1 mM phenylmethylsulfonyl fluoride and homogenized with glass beads (30 s, 5×) with cooling in the ice bath (1 min). The homogenate was centrifuged at 15,000g for 15 min. The supernatant obtained was tested for enzymatic activity.

**Succinate Dehydrogenase (SDH) Activity** To plate well, 50  $\mu\text{L}$  of 0.2 M sodium phosphate buffer (pH 7.8), 15  $\mu\text{L}$  of 1% BSA (w/v), 50  $\mu\text{L}$  of 0.3 M succinic acid (Sigma-Aldrich), and 15  $\mu\text{L}$  of 0.03 M  $\text{K}_3[\text{Fe}(\text{CN})_6]$  (Sigma-Aldrich) were added. Enzymatic reaction was initiated by the addition of the enzyme extract. One unit of SDH activity was defined as the amount of enzyme causing a 0.01 decrease in the absorption at 405 nm within 1 min (Zhou et al. 2014).

**Cytochrome C Oxidase (CCO) Activity** The reaction mixture consisted of 25  $\mu\text{L}$  of 1 g  $\text{L}^{-1}$  reduced cytochrome C (Sigma-Aldrich), 125  $\mu\text{L}$  of 75 mM sodium phosphate buffer (pH 7.5), and 25  $\mu\text{L}$  of enzyme extract. The kinetic of the absorbance decrease was measured for 5 min at 550 nm using an EPOCH microplate reader (Biotek). One unit of CCO activity was defined as the amount of enzyme causing a 0.01 decrease in absorbance of the solution.

**Mitochondrial Membrane  $\text{H}^+$ -ATPase activity** The assay medium for  $\text{H}^+$ -ATPase activity measurement consisted of 100  $\mu\text{L}$  of mitochondrial extract and 400  $\mu\text{L}$  of 30 mM Tris-HCl buffer (8.0) containing 0.1 mM  $\text{Na}_3\text{VO}_4$ , 50 mM KCl, 20 mM  $\text{MgSO}_4$ , and 0.1 mM  $\text{NaN}_3$ . Enzymatic reaction was started by adding 100  $\mu\text{L}$  of 30 mM ATP-Tris (pH 8.0). At the same time, a control sample containing no  $\text{NaN}_3$  (mitochondrial membrane  $\text{H}^+$ -ATPase inhibitor) was prepared. Reaction mixture was incubated at 37 °C for 15 min and then terminated by adding 300  $\mu\text{L}$  of 55% TCA. The sample was clarified by centrifugation at 14,000g for 15 min. The level of inorganic phosphorus released after hydrolysis of ATP to ADP catalyzed by  $\text{H}^+$ -ATPase was measured using malachite green method. To plate well, 100  $\mu\text{L}$  of supernatant and 75  $\mu\text{L}$  of malachite green solution containing 120  $\mu\text{M}$  malachite green, 6 mM  $(\text{NH}_4)_2\text{MoO}_4$ , 0.06% polyvinyl alcohol, and 3.4% of sodium citrate were added. After incubation at 37 °C for 15 min, the absorbance at 630 nm was measured. The difference between the absorbance for reaction mixture without  $\text{NaN}_3$  and with  $\text{NaN}_3$  was an expression of mitochondrial membrane  $\text{H}^+$ -ATPase activity. One unit of  $\text{H}^+$ -ATPase activity was defined as the amount of enzyme causing a 0.01 decrease in absorbance within 1 min (Lin et al. 2017).

**Superoxide Dismutase (SOD) Activity** To the 50  $\mu\text{L}$  of 50 mM sodium carbonate buffer (pH 10.2), 15  $\mu\text{L}$  of mitochondrial suspension and 5  $\mu\text{L}$  of 10 mM epinephrine (Sigma-Aldrich) were added. The increase of the absorbance was measured for 10 min at a wavelength of 490 nm using a microplate reader. One unit of SOD activity is the amount of enzyme required to inhibit the initial rate of epinephrine autoxidation by 50% within 1 min.

The Bradford methodology was used for the determination of total protein concentration in the samples (Kruger 1994).

## Determination of ROS Level in Mitochondria

**Superoxide Anion Radical ( $\text{O}_2^{\cdot-}$ ) Level Assay** Briefly, 25  $\mu\text{L}$  of 0.4 mM nitro blue tetrazolium solution (NBT, Sigma-Aldrich) was mixed with 25  $\mu\text{L}$  of mitochondrial suspension and 150  $\mu\text{L}$  of PBS buffer (50 mM, pH = 7.4). A total of 5  $\mu\text{L}$  of superoxide dismutase solution ( $\geq 3000$  U) was added to the control sample. After 30 min of incubation, the absorbance was measured using a microplate reader at 490 nm. In order to determine the level of superoxide anion radical in mitochondria, the following formula was used:

$$\text{O}_2^{\cdot-} [\Delta A \text{ min}^{-1}] = \frac{(A_{30} - A_0) - (A_{S30} - A_{S0})}{30 \text{ min}},$$

where  $A_0$  - initial absorbance of reaction mixture,  $A_{30}$  - absorbance of reaction mixture after 30 min of incubation,  $A_{S0}$  - initial absorbance of reaction mixture with SOD, and  $A_{S30}$  - absorbance of reaction mixture with SOD after 30 min of incubation (Piechowiak and Balawejder 2019).

**Hydrogen Peroxide ( $\text{H}_2\text{O}_2$ ) Generation Assay** Briefly, 2 mL of 50 mM sodium phosphate buffer (pH = 7.4) was mixed with 100  $\mu\text{L}$  of mitochondrial suspension and 25  $\mu\text{L}$  of 4 mM 2',7'-dichlorodihydrofluorescein diacetate (ethanol solution). Catalase (2000–5000 U) solution (10  $\mu\text{L}$ ) was added to the control probe. After 40 min of incubation at 30 °C, the fluorescence of the solution was measured at a wavelength of excitation and emission of 504 nm and 529 nm. In order to determine the level of hydrogen peroxide in mitochondria, the following formula was used:

$$\text{H}_2\text{O}_2, [\Delta F \text{ min}^{-1}] = \frac{(F_{40} - F_0) - (F_{C40} - F_{C0})}{40 \text{ min}},$$

where  $F_0$  - initial fluorescence of reaction mixture,  $F_{40}$  - fluorescence of reaction mixture after 40 min of incubation,  $F_{C0}$  - initial absorbance of reaction mixture with catalase, and  $F_{C40}$  - fluorescence of reaction mixture with catalase after 40 min of incubation (Piechowiak and Balawejder 2019).

## Statistical Analysis

Significance of differences between the mean values was tested using one-way ANOVA and the Tukey test ( $\alpha = 0.05$ ). The relationship between the activity of mitochondria and selected quality attributes of the ozonated and control raspberry fruit was investigated using the principal component analysis (PCA). For this purpose, data contained in our previous publications, i.e., the level of phenolic compounds, glutathione, and antioxidant activity of ozonated and control fruit after 0, 24, 48, and 72 h of storage, were used for PCA (Piechowiak et al. 2019, 2020a, b). PCA results were interpreted on the basis of the scree plots, the correlation coefficients between

the principal components, and analyzed variables as well as the correlation matrix. The statistical analysis was performed with STATISTICA 13.0.

## Results and Discussion

### Changes in Energy Metabolism of Raspberries During Storage in Ozone-Enriched Atmosphere

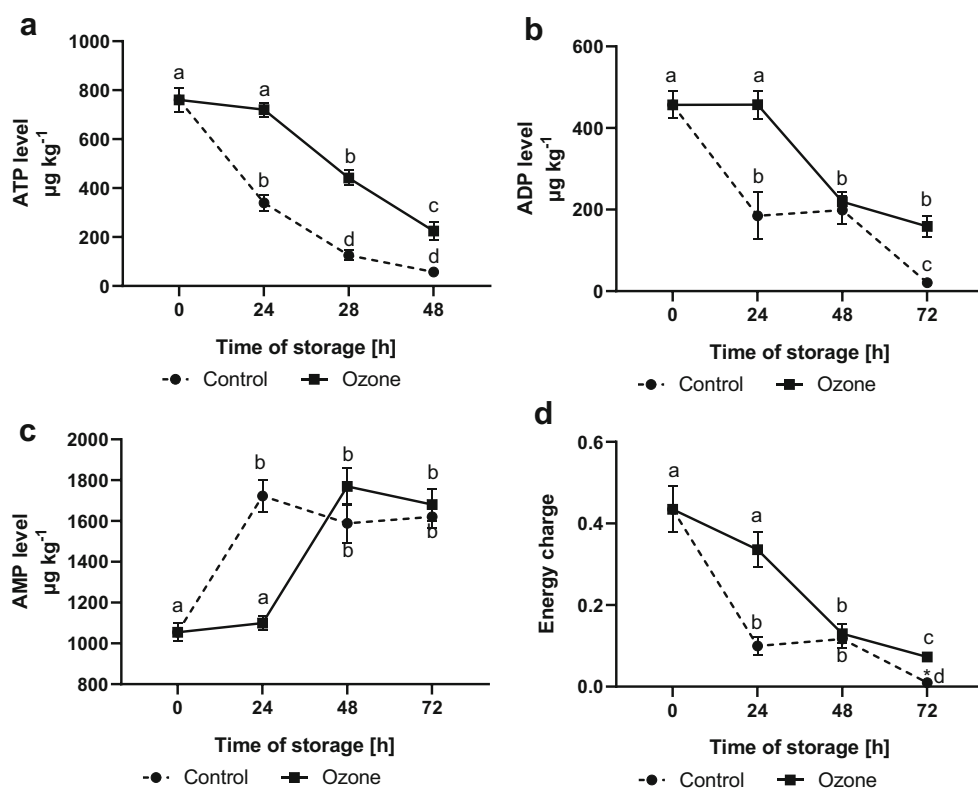
Research showed that ozonation process inhibits the loss of energy status in raspberries during storage at room temperature. As shown in Fig. 1, the contents of ATP and ADP and energy charge (EC) in the ozonated fruit did not change significantly after 24 h of storage, while in the control fruit, this parameters decreased respectively by 56.6, 59.5, and 76.7% compared to the initial value. In the subsequent periods, the levels of ATP, ADP, and EC both in the ozonated and control fruit decreased sharply, but the values of these parameters for the ozonated samples were significantly higher compared to those for the control. However, increased consumption of ATP and ADP during storage led to increase in AMP concentration in the fruit.

ATP level and energy charge of mitochondria are closely associated with the activity of enzymes participating in oxidative phosphorylation, including succinate dehydrogenase, cytochrome C oxidase, and H<sup>+</sup>-ATPase (Jin et al. 2014; Jin et al. 2013; Ge et al. 2019; Li et al. 2020; Zhou et al. 2014).

Succinate dehydrogenase (SDH, EC 1.3.5.1) is the major enzyme of II complex in electron transfer chain (ETC) and catalyzes the dehydrogenation of succinate leading to fumarate generation, while cytochrome C oxidase (CCO, EC 1.9.3.1) catalyzes the reaction between ferricytochrome C and oxygen to yield ferricytochrome C and water (IV complex in ETC). H<sup>+</sup>-ATPase (EC 1.15.1.1) accelerates the hydrolysis of ATP to ADP and a free phosphate ion with the release of energy as well as the formation of the transmembrane electrochemical gradient and the transmembrane proton driving force (Li et al. 2020; Zhou et al. 2014).

Our research showed that the ozonation process improves the activity of enzymes involved in the oxidative phosphorylation in the fruit. SDH activity in ozone-treated fruit reached a maximum level at 24 h of storage (increase by ~ 42%), and then continuously declined until 72 h of storage. In the control fruit, SDH activity showed no significant changes over the first 48 h, and then decreased sharply to a minimum level at 72 h (Fig. 2a). The activity of cytochrome C oxidase and H<sup>+</sup>-ATPase in the control fruit decreased gradually over the first 48 h, and then was relatively stable during the remaining hours. In turn, the activities of these enzymes in the ozonated fruit increased significantly after 24 h and then decreased gradually (Fig. 2b, c). It should therefore be assumed that the higher activity of all analyzed enzymes in the ozonated fruit than the control contributed to the inhibition of the loss of ATP and energy charge in fruit during storage. Probably, improved mitochondrial metabolism in the ozonated fruit could

**Fig. 1** The effect of ozonation process on the level of ATP (a), ADP (b), AMP (c), and energy charge (d) in raspberry fruit during storage at room temperature. Mean values ( $n = 6$ ) with standard deviations (error bars) with the same lowercase letters are not statistically significant according to the *t* Tukey test ( $\alpha = 0.05$ )



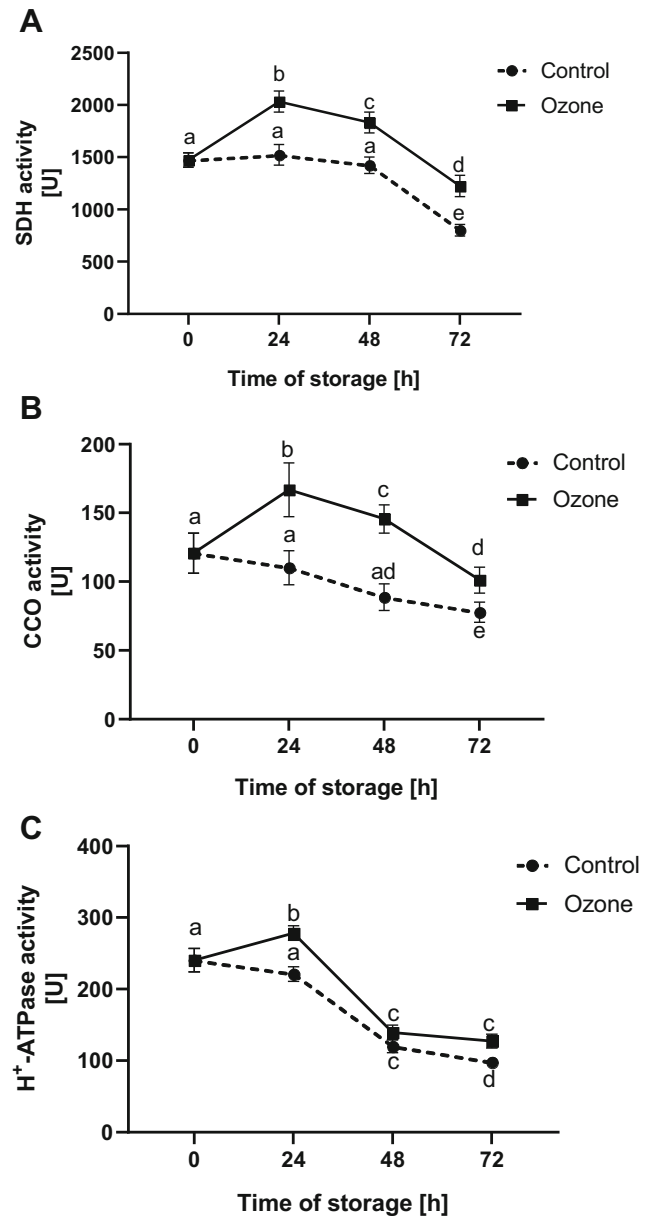
be associated with increased activity of oxygen in the storage atmosphere, because molecular oxygen is the main product of ozone decomposition in the air. This observation is consistent with that of Su et al. (2005). They showed that storage of longan fruit in pure oxygen atmosphere improves the respiration rate leading to maintain high level of ATP and energy charge of fruit.

Many studies have revealed that the use of some elicitors for postharvest fruit treatment induces changes in the activity of enzymes involved in electron transfer chain and the energy metabolism in treated material (Jin et al. 2013; Zhou et al. 2014). For example, Li et al. (2020) proved that benzothiadiazole treatment of apple before storage at room temperature increased the activity of oxidative phosphorylation enzymes leading to increase in ATP level and consequently to improve the fruit resistance to microbial decay. The use of methyl jasmonate elicitation for postharvest treatment of the loquat fruit reduced significantly anthracnose rot caused by *Colletotrichum acutatum* owing to enhancing ATP synthesis (Jin et al. 2013). Benzothiadiazole treatment decreased the lesion diameter of apples exposed to *Penicillium expansum* and it was strongly associated with high energy status in fruit (Li et al. 2020). The positive correlation between the activity of mitochondrial enzymes and the level of ATP, ADP, and energy charge was also noticed in peaches treated with oxalic acid as well as in apples elicited with chlorogenic acid and trisodium phosphate (Ge et al. 2019; Li et al. 2020; Zhou et al. 2014). On the other hand, Lin et al. (2017) investigated the effect of hydrogen peroxide treatment on the level of energy status in longan fruit. Their research indicated that the activities of  $H^+$ -ATPase,  $Ca^{2+}$ -ATPase, and  $Mg^{2+}$ -ATPase in membranes of plasma, mitochondria, and vacuole, as well as the contents of ATP and ADP, were significantly decreased after  $H_2O_2$  treatment, which contributed to the aggravation of the fruit to the browning incidence in the pericarp. Probably,  $H_2O_2$  treatment increased the level of ROS in plant cells, which lead to oxidative stress and consequently oxidative modification of enzyme protein participating in respiratory chain.

### Changes in the Level of Oxidative Stress Markers in Fruit Mitochondria

The reactive oxygen species (ROS) are the by-products of aerobic respiration. Superoxide anion radical is formed through electron reduction of oxygen and the ETC complexes I, II, and III have been recognized as major production sites. In turn, hydrogen peroxide is generated mainly during disproportionation reactions of superoxide anion radical catalyzed by superoxide dismutase. ROS are involved in multiple physiological responses of plants, both to biotic and abiotic stress (Huang et al. 2016). Huang et al. (2016) indicated that, while ROS production in mitochondria occurs during normal action

of the respiratory chain, its rate increases when the respiratory intensity decreases, e.g., due to limited ADP availability or respiratory chain inhibition leading to highly reduced state of ETC components (Huang et al. 2016; Moller et al. 2007). This relationship was also observed in the presented research. As illustrated in Fig. 3, the level of superoxide anion radical ( $O_2^{\cdot-}$ ) and hydrogen peroxide ( $H_2O_2$ ) in fruit increased significantly with the extension of storage time. However, the ozonated fruit exhibited a higher level of  $O_2^{\cdot-}$  and  $H_2O_2$  in mitochondria than the control sample during the whole period of storage.



**Fig. 2** The effect of ozonation process on the activity of succinate dehydrogenase (a), cytochrome C oxidase (b), and  $H^+$ -ATPase (c) in mitochondria from raspberry fruit. Mean values ( $n = 6$ ) with standard deviations (error bars) with the same lowercase letters are not statistically significant according to the  $t$  Tukey test ( $\alpha = 0.05$ )

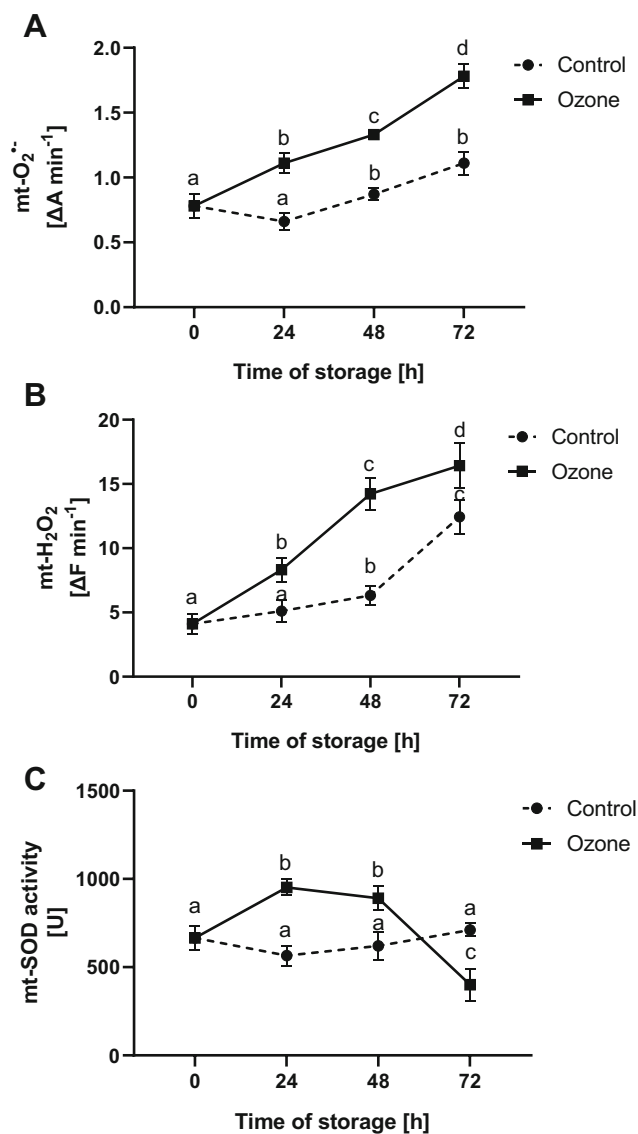
Superoxide dismutase (SOD, EC 1.15.1.1.) plays an important role in mitochondria protecting against the toxic effect of superoxide anion radical. Mitochondrial SOD with the participation of manganese ions catalyzes the scavenging of superoxide anion radical leading to hydrogen peroxide generation (Huang et al. 2016). Our research showed the activity of SOD in mitochondria from the ozonated fruit exhibited an increasing tendency during 48 h of storage. However, after this time, SOD activity markedly decreased, probably due to enzyme protein modification caused by ROS accumulation in mitochondria.

### The Relationship Between the Activity of Energy Metabolism and Antioxidant Potential of Fruit Stored in Ozone-Enriched Atmosphere

The occurrence of oxidative stress in the stored fruit is a major reason for the loss of their quality. Reactive oxygen species cause oxidative damage of cell components, i.e., proteins, lipids, and DNA, leading to the loss of antioxidants, disruption of enzymatic apparatus, and changes in permeability of cell membrane and results in their death. In the context of fruit storage, oxidative stress causes the reduction of biological activity of fruit, the loss of turgor pressure, changes in turgidity, and a higher susceptibility to fruit softening and microbial contamination (Zhou et al. 2014; Cai and Yan 2013). Low molecular weight antioxidants account for a second line of defense (after antioxidant enzymes) in cell protection against oxidative stress. Moreover, they are responsible for shaping the biological and sensory properties of raspberry fruit (Moller et al. 2007). Our previous research showed that ozonated raspberries showed a higher content of glutathione and polyphenols, including anthocyanins, than control, leading to maintain high total antioxidant activity of fruit during the whole period of storage (Piechowiak et al. 2020a, b). Due to the fact that the biosynthesis and transformations of some antioxidants in plants are powered by energy released from ATP decomposition, in the next part of the study, our focus was to investigate whether there is a relationship between the mitochondria metabolism and the antioxidant potential of the fruit. For this purpose, we conducted principal component analysis (PCA). For PCA, we used the results obtained in this research and published in our previous studies (Piechowiak and Balawejder 2019; Piechowiak et al. 2019, 2020a, b).

PCA was applied to reduce the amount of variability in the data while losing only a small amount of information and to present data in two dimensions, concerning the analyzed variables, i.e., ATP level, energy charge, and mitochondrial enzyme activity, as well as phenolic compound level (anthocyanins, ellagitannins, flavanols, proanthocyanidins, total phenolic content), total antioxidant activity, and glutathione (GSH) level in ozonated and control fruit. Based on scree plot (graph not shown) as well as the Kaiser criterion (only the

main components are important, in which eigenvalue exceeds or is close to 1), analyzed variables were reduced to two principal components (PCs). This components explain 88.97% of variance in the case of the ozonated fruit and 92.05% in the control sample (this means that they carry the most information about the studied variables). The first component (PC1) explains 62.49% and 64.96% of variance, while the second (PC2) 26.48% and 27.36%, in ozonated and control samples, respectively (Fig. 4). The differences in PCA visualization resulted from different correlations and relationships between the analyzed variables in the ozonated and non-ozonated fruit (Fig. 4). PCA for the ozonated fruit showed that ATP level,

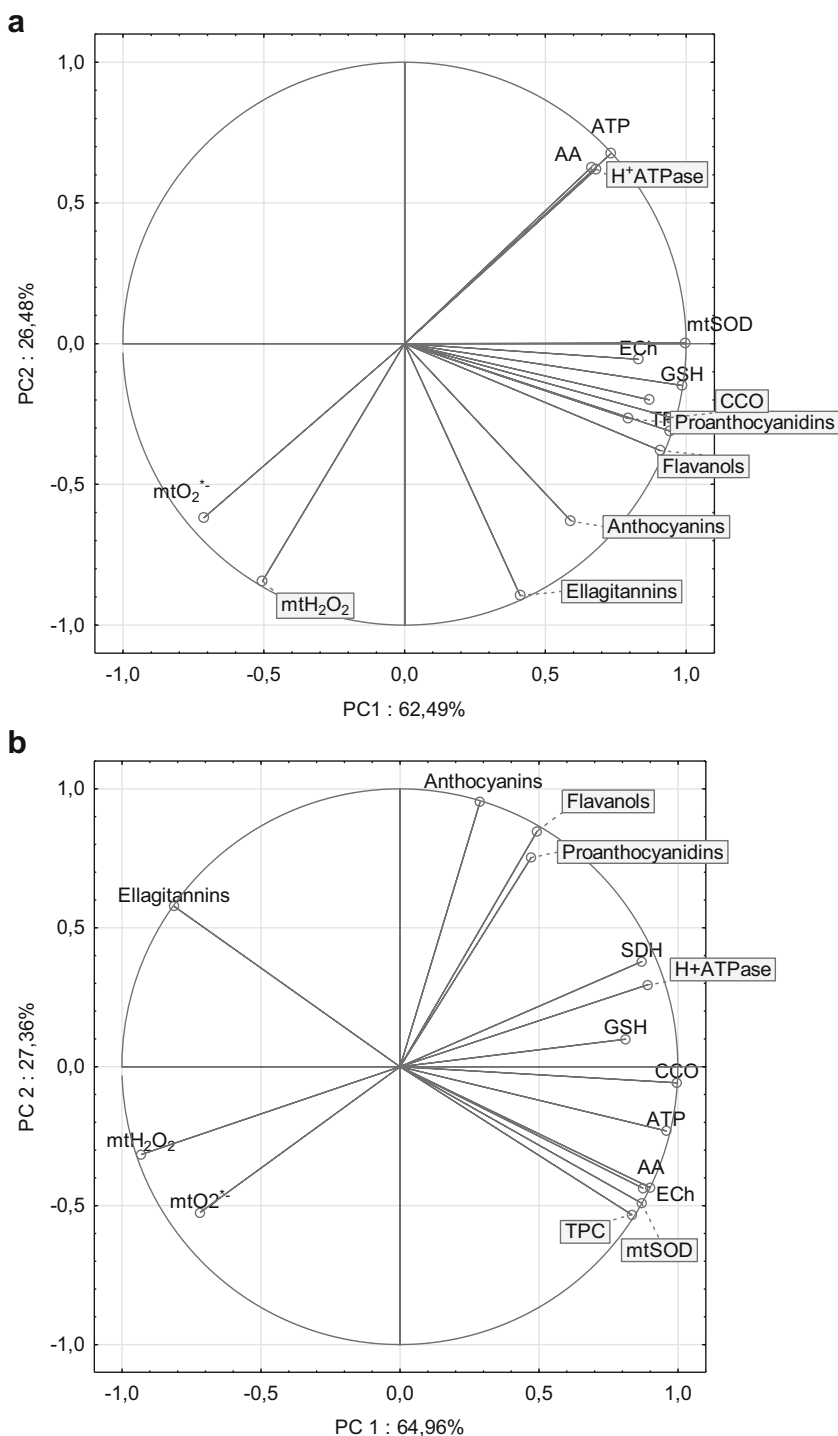


**Fig. 3** Changes in the level of oxidative stress markers in mitochondria from ozonated and control raspberries during storage. The level of superoxide anion radical (a) and hydrogen peroxide (b) and the activity of superoxide dismutase (c) in fruit. Mean values ( $n = 6$ ) with standard deviations (error bars) with the same lowercase letters are not statistically significant according to the  $t$  Tukey test ( $\alpha = 0.05$ )

H<sup>+</sup>-ATPase, and total antioxidant activity (AA) were strongly correlated that was not observed in the non-ozonated sample. Moreover, a stronger correlation between ETC enzymes (SDH, CCO), energy charge, total polyphenol level (TPC), and contents of proanthocyanidins and flavanols as well as glutathione (GSH) level than in the control sample was observed (the vectors are located in close proximity). It should be assumed that the activity of ETC enzymes which improved by

ozonation leads to maintaining a high level of ATP in fruit, which is the source of energy for conducting biochemical reactions in cells, including the biosynthesis of antioxidants. Moreover, an increased level of ROS in mitochondria may activate the expression of genes encoding the enzymes involved in antioxidant system (including phenylpropanoid pathway enzymes). As a consequence, the ozonated raspberries showed higher biological (antioxidant) activity and

**Fig. 4** Projection of analyzed variables as function of PC1 vs PC2 for ozonated (a) and control (b) fruit. One the figure: AA total antioxidant activity, ATP ATP level, CCO cytochrome C oxidase activity, ECh energy charge, GSH glutathione level, SDH succinate dehydrogenase activity, mtO<sub>2</sub><sup>•-</sup> mitochondrial superoxide radical level, mtH<sub>2</sub>O<sub>2</sub> mitochondrial hydrogen peroxide level, mtSOD mitochondrial superoxide dismutase activity, TPC total phenolic content



better resistance to postharvest abiotic stress than the non-ozonated fruit.

## Conclusions

This research showed that ozonation process improves the mitochondrial energy metabolism in raspberry fruit during storage at room temperature. Ozonation increased the activity of enzymes involved in oxidative phosphorylation leading to reduce the loss of ATP and energy charge of fruit. Moreover, the higher activity of mitochondria was closely correlated with the antioxidant potential of the ozonated fruit. Therefore, it should be assumed that ozone, by improving the mitochondrial energy metabolism, activates the mechanisms involved in cell protection against abiotic stress. As a consequence, ozonated fruit shows a good quality over a long period during storage at room temperature. However, it should be emphasized that, in the presented research, the fruit was stored at room temperature, which significantly accelerates all metabolic processes in the fruit. Therefore, the effect of ozone treatment on the energy metabolism in raspberry fruit, stored in cold conditions, may be different.

**Funding** This work was supported by the National Science Centre, Poland (OPUS, 2019/35/B/NZ9/01552).

## Declarations

**Conflict of Interest** The authors declare no competing interests.

**Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

## References

- Alexandre, E. M., Santos-Pedro, D. M., Brandão, T. R., & Silva, C. L. (2011). Influence of aqueous ozone, blanching and combined treatments on microbial load of red bell peppers, strawberries and watercress. *Journal of Food Engineering*, *105*(2), 277–282. <https://doi.org/10.1016/j.jfoodeng.2011.02.032>.
- Cai, Z., & Yan, L. J. (2013). Protein oxidative modifications: Beneficial roles in disease and health. *Journal of Biochemical and Pharmacological Research*, *1*(1), 15–26.
- Carbone, K., & Mencarelli, F. (2015). Influence of short-term postharvest ozone treatments in nitrogen or air atmosphere on the metabolic response of white wine grapes. *Food and Bioprocess Technology*, *8*(8), 1739–1749. <https://doi.org/10.1007/s11947-015-1515-y>.
- Chen, C., Zhang, H., Dong, C., Ji, H., Zhang, X., Li, L., Ban, Z., Zhang, N., & Xue, W. (2019). Effect of ozone treatment on the phenylpropanoid biosynthesis of postharvest strawberries. *RSC Advances*, *9*(44), 25429–25438. <https://doi.org/10.1039/C9RA03988K>.
- Contigiani, E. V., Jaramillo-Sánchez, G., Castro, M. A., Gomez, P. L., & Alzamora, S. M. (2018). Postharvest quality of strawberry fruit (*Fragaria x Ananassa Duch cv. Albion*) as affected by ozone washing: Fungal spoilage, mechanical properties, and structure. *Food and Bioprocess Technology*, *11*(9), 1639–1650. <https://doi.org/10.1007/s11947-018-2127-0>.
- Ge, Y., Chen, Y., Li, C., Wei, M., Li, X., Li, S., Lu, S., & Li, J. (2019). Effect of trisodium phosphate dipping treatment on the quality and energy metabolism of apples. *Food Chemistry*, *274*(15), 324–329. <https://doi.org/10.1016/j.foodchem.2018.08.142>.
- Horvitz, S., & Cantalejo, M. J. (2014). Application of ozone for the postharvest treatment of fruits and vegetables. *Critical Reviews in Food Science and Nutrition*, *54*(3), 312–339.
- Huang, S., Aken, O., Schwarzlander, M., Belt, K., & Millar, A. (2016). The roles of mitochondrial reactive oxygen species in cellular signaling and stress response in plants. *Plant Physiology*, *171*(3), 1551–1559. <https://doi.org/10.1104/pp.16.00166>.
- Jaramillo-Sánchez, G., Contigiani, E. V., Castro, M., Hodara, K., Alzamora, S., Loreda, A., & Nieto, A. (2019). Freshness maintenance of blueberries (*Vaccinium corymbosum* L.) during postharvest using ozone in aqueous phase: Microbiological, structure, and mechanical issues. *Food and Bioprocess Technology*, *12*(12), 2136–2147. <https://doi.org/10.1007/s11947-019-02358-z>.
- Jin, P., Zhu, H., Wang, J., Chen, J., Wang, X., & Zheng, Y. (2013). Effect of methyl jasmonate on energy metabolism in peach fruit during chilling stress. *Journal of the Science of Food and Agriculture*, *93*(8), 1827–1832. <https://doi.org/10.1002/jsfa.5973>.
- Jin, P., Zhu, H., Wang, L., Shan, T., & Zheng, Y. (2014). Oxalic acid alleviates chilling injury in peach fruit by regulating energy metabolism and fatty acid contents. *Food Chemistry*, *161*, 87–93. <https://doi.org/10.1016/j.foodchem.2014.03.103>.
- Kruger, N. J. (1994). The Bradford method for protein quantitation. *Methods in Molecular Biology*, *32*, 9–15.
- Li, S., Jijang, H., Wang, Y., Lyu, L., Prusky, D., Ji, Y., Zheng, X., & Bi, Y. (2020). Effect of benzothiazazole treatment on improving the mitochondrial energy metabolism involved in induced resistance of apple fruit during postharvest storage. *Food Chemistry*, *302*, 125288. <https://doi.org/10.1016/j.foodchem.2019.125288>.
- Lin, Y., Lin, Y., Lin, H., Ritenour, M., Shi, J., Zhang, S., Chen, Y., & Wang, H. (2017). Hydrogen peroxide-induced pericarp browning of harvested longan fruit in association with energy metabolism. *Food Chemistry*, *225*, 31–36. <https://doi.org/10.1016/j.foodchem.2016.12.088>.
- Liu, H., Jiang, Y., Luo, Y., & Jijang, W. (2006). A simple and rapid determination of ATP, ADP and AMP concentrations in pericarp tissue of litchi fruit by high performance liquid chromatography. *Food Technology and Biotechnology*, *44*(4), 531–534.
- Moller, I. M., Jensen, P. E., & Hansson, A. (2007). Oxidative modifications to cellular components in plants. *Annual Review of Plant Biology*, *58*(1), 459–481. <https://doi.org/10.1146/annurev.arplant.58.032806.103946>.
- Piechowiak, T., & Balawejder, M. (2019). Impact of ozonation process on the level of selected oxidative stress markers in raspberries stored at room temperature. *Food Chemistry*, *298*, 125093. <https://doi.org/10.1016/j.foodchem.2019.125093>.
- Piechowiak, T., Antos, P., Kosowski, P., Skrobacz, K., Józefczyk, R., & Balawejder, M. (2019). Impact of ozonation process on the microbiological and antioxidant status of raspberries (*Rubus idaeus* L.) during storage at room temperature. *Agricultural and Food Science*, *28*(1), 35–44. <https://doi.org/10.23986/afsci.70291>.



- Piechowiak, T., Grzelak-Błaszczak, K., Sójka, M., & Balawejder, M. (2020a). Changes in phenolic compounds profile and glutathione status in raspberry fruit during storage in ozone-enriched atmosphere. *Postharvest Biology and Technology*, *168*, 111277. <https://doi.org/10.1016/j.postharvbio.2020.111277>.
- Piechowiak, T., Skóra, B., & Balawejder, M. (2020b). Ozone treatment induces changes in antioxidative defense system in blueberry fruit during storage. *Food and Bioprocess Technology*, *13*(7), 1240–1245. <https://doi.org/10.1007/s11947-020-02450-9>.
- Pinto, L., Palma, A., Cefola, M., Pace, B., D'Aquino, S., Carboni, C., & Baruzzi, F. (2020). Effect of modified atmosphere packaging (MAP) and gaseous ozone pre-packaging treatment on the physico-chemical, microbiological and sensory quality of small berry fruit. *Food Packaging and Shelf Life*, *26*, 100573. <https://doi.org/10.1016/j.foodchem.2020.100573>.
- Shu, C., Zhang, W., Zhao, H., Cao, J., & Jijang, W. (2020). Chlorogenic acid treatment alleviates the adverse physiological responses of vibration injury in apple fruit through the regulation of energy metabolism. *Postharvest Biology and Technology*, *159*, 110997. <https://doi.org/10.1016/j.postharvbio.2019.110997>.
- Su, X., Jiang, Y., Duan, X., Liu, H., Li, H., Lin, W., & Zheng, Y. (2005). Effect of pure oxygen on the rate of skin browning and energy status in longan fruit. *Food Technology and Biotechnology*, *43*(4), 359–365.
- Yaseen, T., Ricelli, A., Turan, B., Albanese, P., & D'Onghia, A. M. (2015). Ozone for postharvest treatment of apple fruits. *Phytopathologia Mediterranea*, *54*(1), 94–103. [https://doi.org/10.14601/Phytopathol\\_Mediterr-14478](https://doi.org/10.14601/Phytopathol_Mediterr-14478).
- Zhou, Q., Zhang, C., Cheng, S., Wei, B., Liu, X., & Ji, S. (2014). Changes in energy metabolism accompanying pitting in blueberries stored at low temperature. *Food Chemistry*, *164*, 493–501. <https://doi.org/10.1016/j.foodchem.2014.05.063>.

**Publisher's Note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.