Effect of ozone treatment on the microstructure, chemical composition and sensory quality of apple fruits

Karina Juhnevica-Radenkova[1], Vitalijs Radenkovs[1], Karlis Kundzins[2] and Dalija Seglina[1]

Abstract

The aim of this study was to assess the effect of O_3 treatment on the quality of different cultivars of apples (*Malus domestica* Borkh.). Apples were stored for six months at different concentrations of ozone. During the research, minor differences between ozone-treated and control fruits were found in terms of cell integrity and epicuticular wax structure. Ozone application for apple treatment could accelerate the natural ageing of the waxes found on the surface of apples, thereby reducing the thickness of the waxes. The rate of degradation for the epicuticular wax was found to be cultivar dependent. After six months of storage, the ozonation process prevented the decay of 'Iedzenu', 'Auksis' and 'Belorusskoje Malinovoje' apple cultivars, but it accelerated damage in the 'Gita' apple cultivar. A positive impact of ozone during long-term storage was found regarding flesh firmness of 'Iedzenu' apple cultivar samples subjected to O_3 exposure at concentrations of 0.8 ppm and 3.0 ppm. In other cultivars of apples, significant differences between ozonation and cold storage (control) were not found. In general, ozone treatment has a potential to be applied in order to maintain the sensory quality and biologically active compound level in apples during six-month storage; however, the degree of effectiveness depends both on the cultivar and on the concentration of ozone.

Keywords

Apple treatment, microstructure, postharvest storage, sensory evaluation, quality

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INTRODUCTION

Apple (*Malus domestica* Borkh.) is a popular temperate fruit, consumed both fresh and processed and ranked third in global fruit production at 80.8 million tonnes in 2013 (FAOSTAT, 2013). Global production is centred on the high-value fresh market, which requires maintaining fruit quality during long-term storage and shipping (Greene et al., 2014; McCluskey et al., 2007).

Ozone (O_3) is widely used as an anti-microbial agent to inactivate bacteria, fungi, viruses and protozoa, allowing water in the food industry to be disinfected and wastewater to be reused, as well as controlling the alkalinity and pH of shrimp pond water (Kim et al., 1999). Ozone has been approved for use in food by the United States Food and Drug Administration (2018) and is thought to reduce decay in some fruits and vegetables, although results have been inconsistent (Forney, 2003). Both advantages (Harding, 1968; Jin et al., 1989; Liew and Prange, 1994; Palou et al., 2002; Palou et al., 2003; Pinilla et al., 1996; Sarig et al., 1996; Skog and Chu, 2001) and disadvantages (Pérez et al., 1999) of ozone in air

[1]Institute of Horticulture, Latvia University of Life Sciences and Technologies, Dobele, Latvia
[2]Institute of Solid State Physics, University of Latvia, Riga, Latvia

Corresponding author:
Vitalijs Radenkovs, Institute of Horticulture, Latvia University of Life Sciences and Technologies, Graudu Street 1, Dobele LV-3701, Latvia.
Email: vitalijs.radenkovs@lvai.lv

use in fruit and vegetable storage rooms have been reported. It has been reported by Forney (2003) that the degree of injury depends on O_3 exposure time, concentration and apple cultivar. For instance, Smilanick (2003) noted that no fruits were wounded by daily exposure during five months at 1.95 ppm. However, the aroma of all cultivars tested except Golden Delicious was aggravated by 3.25 ppm of ozone. Furthermore, the author concluded that no impairment was caused by 1.95 ppm on any variety tested. In terms of structural changes, it is obvious that some cultivars of apples promptly becoming sticky and covered by varnish may be observed. In terms of physiological properties, no differences were found between treated and untreated fruit (Smilanick, 2003). Changes in the fruit surface gloss suggested probable modifications to the light reflectance characteristics which could be caused by either a loss of cuticular and/or ultra-structural changes in the crystalline structure of the surface wax. Any modification in the fruit surface could also contribute to a reduction in the adherence of the pathogen, thereby reducing colonisation (Charles et al., 2008).

Due to its strong oxidizing activity, ozone may cause physiological damages to fruits and vegetables (Horvath et al., 1985). Bananas treated with ozone developed black spots after eight days of exposure to 25 to 30 ppm of gaseous ozone. Carrots exposed to ozone gas during storage had a lighter, less intense colour than untreated carrots (Liew and Prange, 1994). A study on kiwi fruit showed that sugar reduction was lower in cold storage with ozone treatment than in cold storage between 15 and 29 weeks of storage, and that cold storage better conserved organic acids (citric, quinic and malic acids) than in ozone-treated fruits at 29 weeks (Barboni et al., 2010). A study on tomatoes showed that short-term gaseous ozone treatment (10 μL/L; 10 min) increased the total phenolics content immediately after treatment and after six days of storage at 20 °C (Rodoni et al., 2009). Onions, citrus fruit, russet potatoes, cantaloupes, waxed apples and kiwi fruit were unharmed when examined one week after treatment, while stone fruit, mushrooms, bananas, leafy vegetables of many kinds, snow peas, mangos, broccoli, Brussels sprouts and un-waxed apples and pears were severely harmed (Smilanick, 2003). Skog and Chu (2001) showed that ozone could effectively prevent ethylene accumulation in apples and pears in storage rooms at a concentration of 0.4 ppm. Mushrooms, apples, pears, broccoli and cucumbers tolerated this low concentration without harm. On the whole, the effect of ozone on fruit quality is inconsistent in different fruit species, and the ozone concentration, application method and period seem to be very important in optimising the treatment effect.

The aim of this study was to assess the effect of O_3 treatment on the quality of different cultivar of apples (*Malus domestica* Borkh.).

MATERIAL AND METHODS

The research was carried out between 2015 and 2016 at the Experimental Processing Department of the Latvia State Institute of Fruit-Growing (currently, Institute of Horticulture, Latvia University of Life Sciences and Technologies) in Dobele and at the Institute of Solid State Physics, University of Latvia.

The following apple cultivars were chosen for the experiment – autumn cultivars: 'Auksis' and 'Gita' and winter cultivars: 'Iedzenu', 'Belorusskoje Malinovoje'. All apple trees were grafted on the rootstock B9 and grown under the same conditions in the orchard run in an integrated system. Shortly after harvesting, apples were air-cooled for 24 h in a cooling chamber at 4 °C ± 0.5 °C. Forty fruits (approximately 6 kg) were sampled per cultivar/per treatment/storage technology. Samples were then placed in polypropylene boxes with perforated walls. The cooled down apples were divided into three groups for post-harvest storage: (1) cold storage – control storage under traditional conditions at air temperature +2 ± 1 °C and relative air humidity of 85%; (2) cold storage + ozone treatment at a concentration of 0.8 ppm; (3) cold storage + ozone treatment at a concentration of 3.0 ppm. Analyses were carried out after long-term apple storage (six months) and after an additional five days of shelf life. In the research, apple treatment with an ozone concentration of 0.8 ppm (150 mg/h, FM-300 ozone generator, Baifeng Ozone Technical Co., Ltd, Guangzhou, China) and 3 ppm (1000 mg/h, L-1000 ozone generator, Baifeng Ozone Technical Co., Ltd, Guangzhou, China) was conducted in a 1 m^3 cold storage room set to maintain a room concentration of ozone. This concentration of O_3 was selected based on the previous small scale in vitro experiments as well as according to the manufacturer's recommendations. Control apple fruits were stored under the same environmental conditions in conventional atmosphere (cold storage/control).

Ten fruits were used individually for the analysis of flesh firmness (N), soluble solids content (°Brix), and titratable acidity (%). Fresh weight loss was determined by the scaling method provided by Billiard (1999). Flesh firmness was measured on two opposite sides of each apple without skin using a digital penetrometer (model TR 53205, Italy) which was equipped with 11 mm diameter probe; peak destructive force was expressed in Newtons (N). Titratable acidity was determined using standard method (LVS EN 12147:2001) and quantified by titration of 1 ml of juice (automatic titration DL 21, Mettler Toledo, Swiss) with 0.1 M

NaOH to a pH of 8.1. Expended amount of NaOH was expressed in percentage of malic acid. The soluble solid content was determined using standard method (LVS EN 12143:2001). Ten fruits were selected and ground with the hand blender 'Bamix®' (Switzerland, model SwissLine, Liechtensteinn) into a puree. The content of soluble solids (in °Brix) was determined using a digital electronic refractometer (type Pal-1, Tokyo, Japan).

Apple deterioration was determined using a method provided by Juhnevica et al. (2013). Apple skin colour was measured by using a ColorTec-PCM Plus 30 mm Benchtop colorimeter (Clinton, New York). The values for L^* (+ = lighter, − = darker), a^* (+ = redder, − = greener), and b^* (+ = yellower, − = bluer) were recorded to evaluate the colour changes of apples after six months' storage (Biller et al., 2007).

SEM analysis was performed by using a Tescan Mira/LMU scanning electron microscope operating in low vacuum mode ($PH_2O = 0.1$–1.0 torr) using a large field detector (LFD) and in environmental mode (ESEM, $PH_2O = 1.0$–4.8 torr) using a gaseous secondary electron detector. Specimens approximately 1 mm thick were cut from apple samples. For the low vacuum analysis, they were mounted onto SEM stubs by means of double sided adhesive carbon discs and observed at 10–20 kV acceleration voltage. For the ESEM analysis, specimens were placed on the cooling stage, setting the temperature to 1 °C and observing at 20–30 kV acceleration voltage.

The total phenolic content was determined spectrophotometrically using the Folin-Ciocalteu method (Singleton et al., 1999). Determination of the vitamin C content was performed using standard method (LVS EN 14130:2003). Tannin content was determined spectrophotometrically (Paaver et al., 2010). Antioxidant activity of extracts was evaluated spectrophotometrically (Brand-Williams et al., 1995).

Fifteen well-trained panellists (5 men and 10 women), aged between 25 and 50, participated in the current study. The sensory attributes of apples were evaluated using: *Hedonic scale* evaluation by the standard method 'ISO 4121:2003 – Sensory analysis – Guidelines for the use of quantitative response scales'. All samples were coded with random three-digit numbers. The panellists were provided with five slices of apples for every experimental sample and asked to score different sensory attributes. To avoid unwanted browning, apples were cut just before being served and placed on each serving tray in a randomized order. To evaluate the overall acceptability of the apple (external quality), slices were served together with a whole uncut apple sample. To evaluate sensory attributes such as appearance, aroma, taste, maturity stage, acidity, sweetness and juiciness for all apple samples, assessment was carried out using five-point *Hedonic scale* with the following ratings: 1 – 'dislike very much', 2 – 'dislike', 3 – 'neither like or dislike', 4 – 'like', 5 – 'like very much'.

Data analysis was carried out using the General Linear Model functions in the IBM® SPSS® Statistics programme 20.0 (SPSS Inc., Chicago, IL). The obtained data were analysed using descriptive statistics. Significant differences determined using *UNIANOVA*, by 'Least Significant Difference' (LSD) criteria. The significance of differences was determined at $p < 0.05$. Mean and standard deviation values were calculated for all parameters. In order to compare sensory data obtained from Line scale evaluation, as well as to classify the samples in terms of chemical composition, the results were processed by PanelCheck V1.4.2, programmed by Oliver Tomic and Henning Risvik software using principal component analysis (PCA) (Næs et al., 2010). PCA provides a representation of the dataset in a small number of dimensions called principal components, which explain the majority of the variance of the dataset. In the current research, PCA represents the similarities and differences among the samples, as well as their relationship with the evaluated sensory attributes. For instance, if the average score of a particular attribute is equal to another score, points are located on the plot close to each other and enclosed in an ellipse. Furthermore, if a particular attribute strongly correlates with the particular sample, its location is near the sample and enclosed in an ellipse (Piqueras-Fiszman et al., 2015).

RESULTS AND DISCUSSION

Little is known about the effect of O_3 treatment on apples during post-harvest long-term storage (Yaseen et al., 2015); therefore, it is important to evaluate the quality of apples that have been exposed to different concentrations of O_3. Epicuticular waxes are of interest because they are involved in preventing excessive water loss, protecting the living tissue of the plant from external attack including UV light and influencing the uptake of chemicals such as pesticides (Kochar, 2015). As can be seen (Figures 1 to 4), fresh apple shows non-uniform epicuticular covering the cells with clearly expressed epicuticular crystals. In turn, after apple treatment with O_3, there was a noticeable decrease in the deposition and morphology of epicuticular wax crystals on the apple surface (Figures 1, 3 and 4). After apple treatment with O_3 at concentrations of 0.8 ppm and 3.0 ppm, the surface of 'Iedzenu', 'Belorusskoje Malinovoje' and 'Gita' apples became smoother and more homogenous with no pronounced epicuticular peaks of wax crystals. Due to the lack of scientific literature towards structural changes of apples after O_3 exposure, a direct comparison is not possible. To the best of our knowledge and on the basis of some

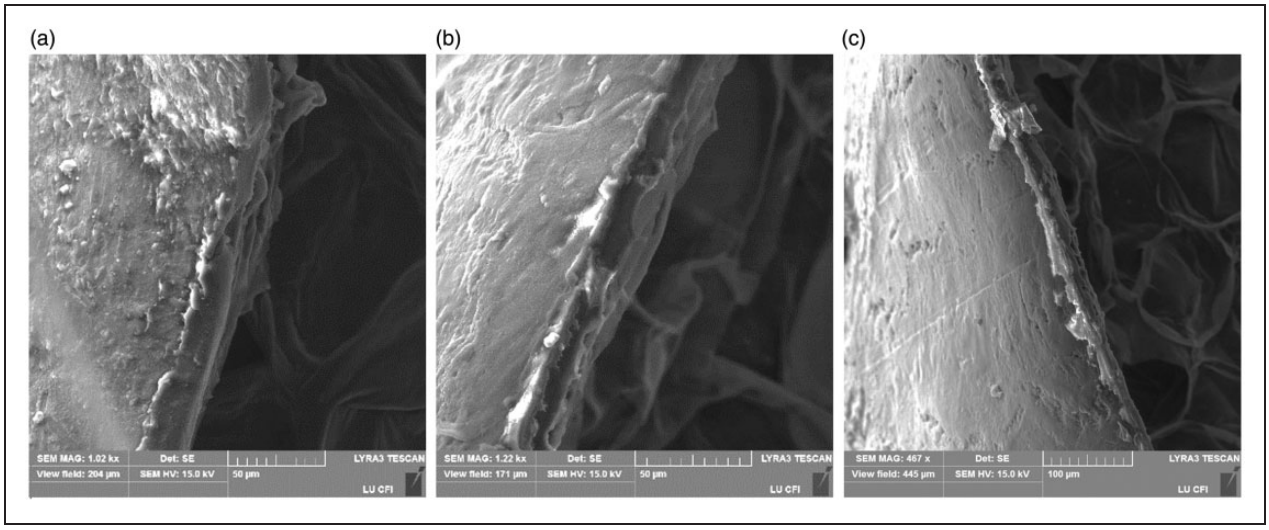

Figure 1. Structure of the epidermal layer with epicuticular crystals of cultivar 'Iedzenu' apples, (a) cold storage (control), (b) ozone concentration 0.8 ppm, (c) ozone concentration 3.0 ppm.

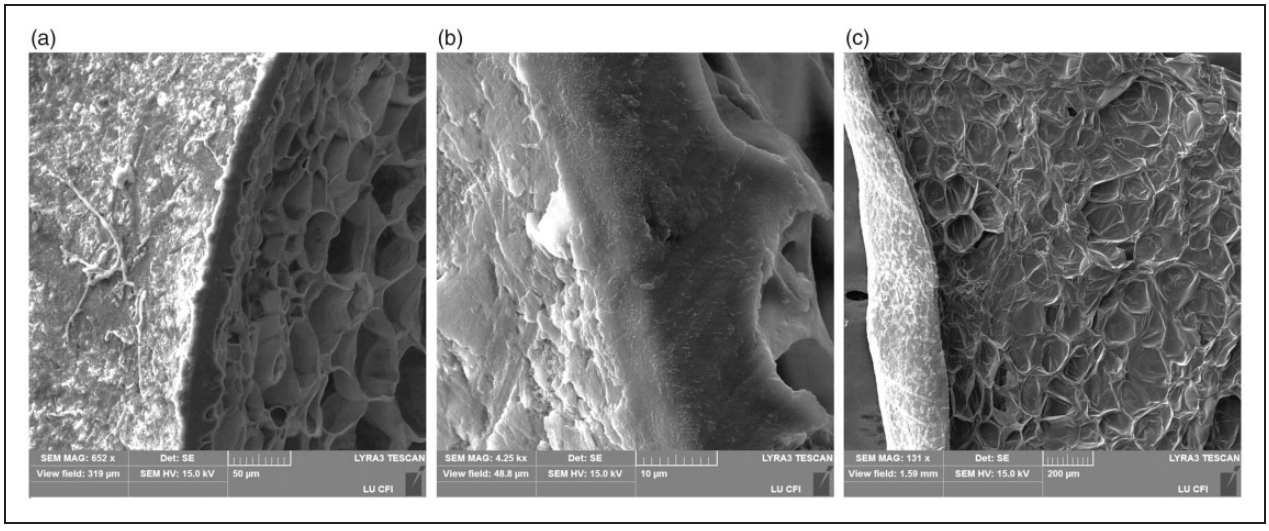

Figure 2. Structure of the epidermal layer with epicuticular crystals of cultivar 'Auksis' apples, (a) cold storage (control), (b) ozone concentration 0.8 ppm, (c) ozone concentration 3.0 ppm.

papers Percy et al. (2009) and Kochar (2015) related to exposure to different concentrations of O_3, one can conclude that the composition of plant epicuticular layers can be affected and changed by chemicals, particularly by secondary pollutant – ozone. Ozonizing of fruits during storage did not show any positive effect on the surface of the apple. Conversely, ozone application for apple treatment could accelerate the natural ageing of the waxes found on the surface of apples, thereby reducing the thickness of the waxes and facilitating a greater rate of transpiration.

Apple structure is a critical quality feature for the consumer, as it is strictly correlated to the firmness of the fruit. The structure of fruits is determined by physical characteristics that arise from structural organization of cells and tissues. Cell integrity strongly impacts structural quality (Laurienzo et al., 2013). The control apple samples (Figures 5(a), 6(a), 7(a) and 8(a)) had a regular structure, while cells of cultivar 'Belorusskoje Malinovoje' and 'Auksis' apples those exposed to ozone treatment of concentrations of 0.8 ppm and 3.0 ppm collapsed with non-uniform homogeneity (Figure 7(b) and (c)). Severe shrinkage is due to the thickness of the skin typical for these cultivars. It was observed that such cultivars have very thin skin and therefore higher permeability of gaseous ozone

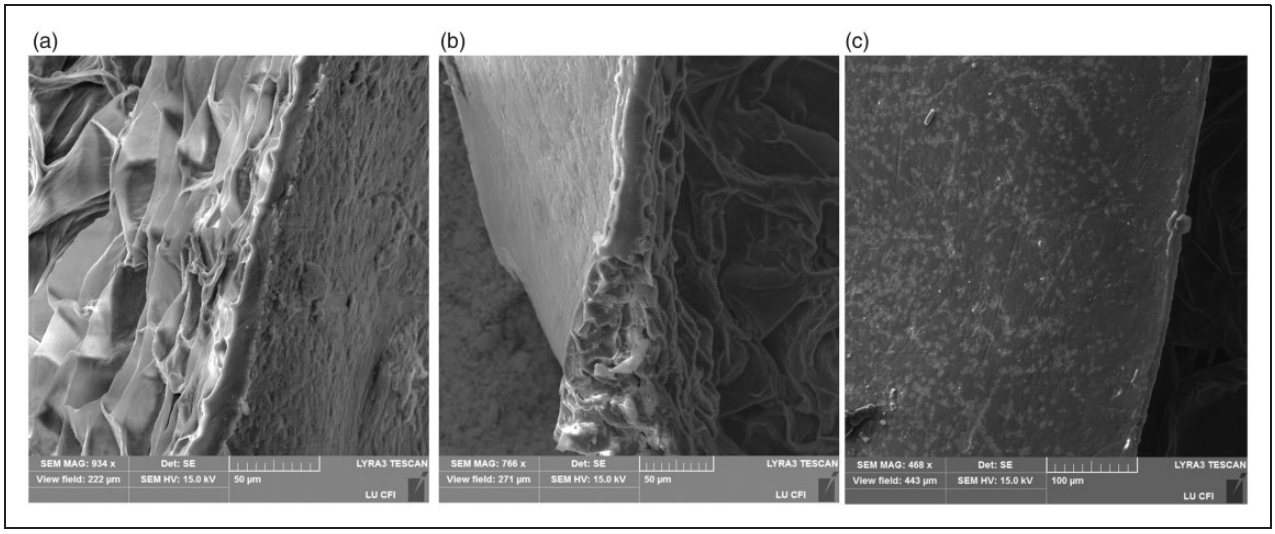

Figure 3. Structure of the epidermal layer with epicuticular crystals of cultivar 'Belorusskoje Malinovoje' apples, (a) cold storage (control), (b) ozone concentration 0.8 ppm, (c) ozone concentration 3.0 ppm.

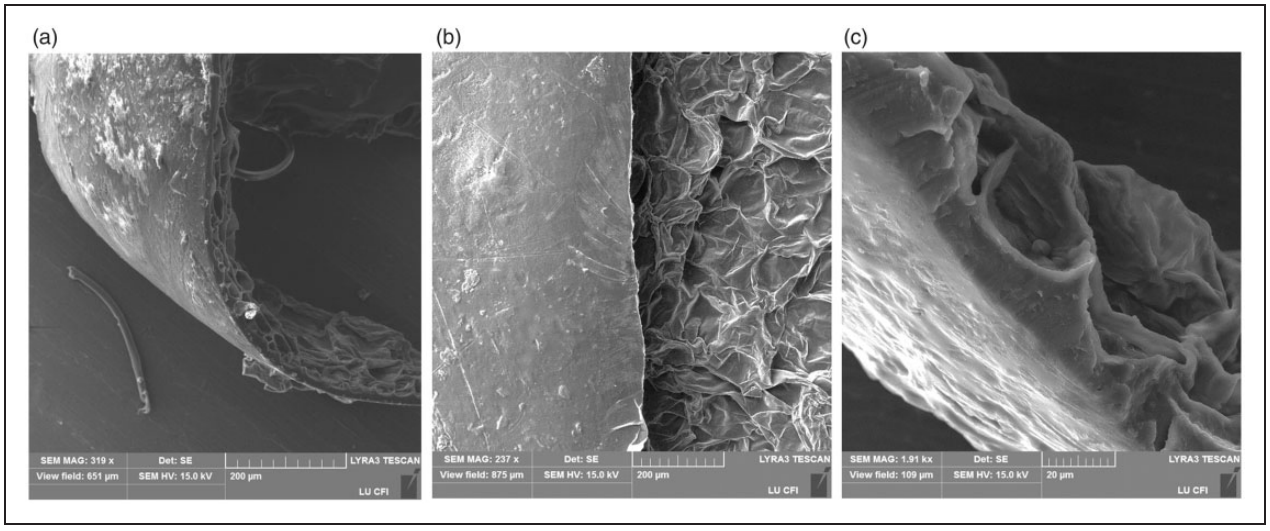

Figure 4. Structure of the epidermal layer with epicuticular crystals of cultivar 'Gita' apples, (a) cold storage (control), (b) ozone concentration 0.8 ppm, (c) ozone concentration 3.0 ppm.

compared to other fruit. Authors from Latvia (Radenkovs and Juhnevica-Radenkova, 2018) found significantly greater weight loss and firmness decline in a cultivar of 'Belorusskoje Malinovoje' apples than in other fruit. Moreover, membrane cracks and fibre development (Figure 6(b) and (c)) can be observed in the cultivar 'Auksis' apples, indicating that ozone treatment causes cells membrane damage. However, the degree of damage is cultivar dependent.

In general, it should be noted that minor differences concerning cell integrity were found between ozone-treated and control fruits. The vast majority of the changes were found towards epicuticular wax structure; in presence of ozone epicuticular, the surface became stickier, thinner and varnish-like.

A number of researchers have recommended the use of ozonation to reduce fruit decay and extend the storage period (Harding 1968; Jin et al., 1989; Liew and Prange, 1994; Palou et al., 2002; Palou et al., 2003; Pinilla et al., 1996; Sarig et al., 1996; Skog and Chu, 2001) of fruits. Some papers have shown that ozone has little or no effect on fruit decay (Pérez et al., 1999), or fruits decayed even more in the presence of ozone than in the normal atmosphere.

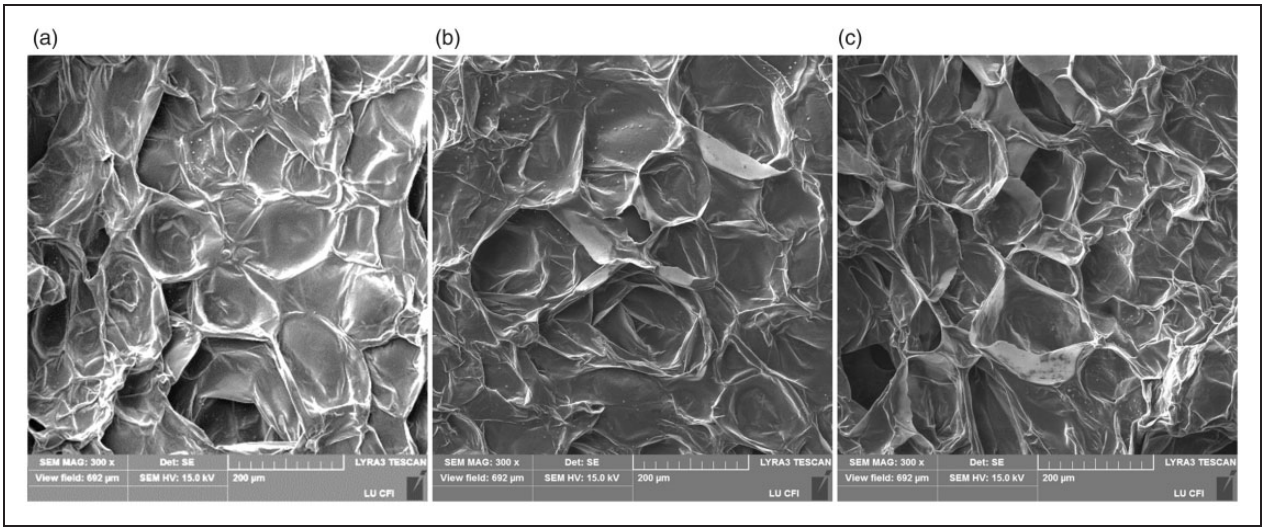

Figure 5. Cell structure of cultivar 'Iedzenu' apples, (a) cold storage (control), (b) ozone concentration 0.8 ppm, (c) ozone concentration 3.0 ppm.

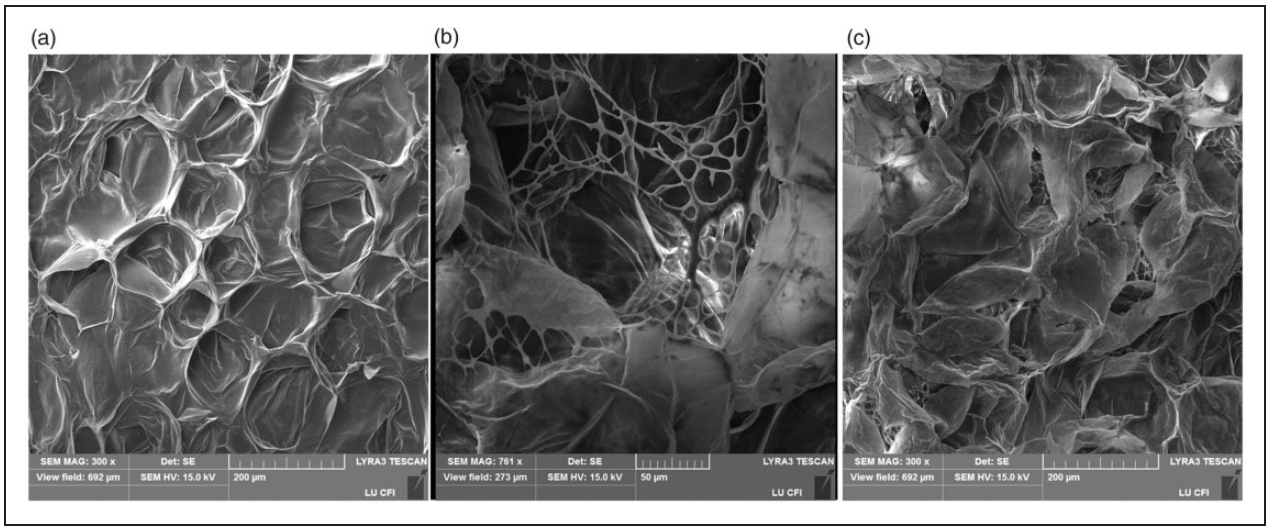

Figure 6. Cell structure of cultivar '*Auksis*' apples, (a) cold storage (control), (b) ozone concentration 0.8 ppm, (c) ozone concentration 3.0 ppm.

Data depicted in Table 1 show that after six months of apple storage, severity of spoilage fluctuated in a range from 3.57% to 26.67% (control samples), from 0.00% to 30.61% (ozone treated at 0.8 ppm) and from 0.00% to 27.08% (ozone treated at 3.0 ppm). The amount of damaged apples after shelf life was from 0.00% to 2.23% (control samples), from 1.09% to 2.04% (ozone treated at 0.8 ppm) and from 1.51% to 5.83% (ozone treated at 3.0 ppm). The occurrence of spoilage after long-term storage was associated with physiological disorders (data not shown), particularly decay caused by superficial and soft scald. Deterioration in the quality of apple during shelf life was related with pronounced softening of fruits. In the paper presented by Skog and Chu (2001), it was shown that ozone could effectively prevent ethylene accumulation, thereby reducing the softening in apple and pear in storage rooms at a concentration of 0.4 ppm. Our results partially coincide with the abovementioned statement. In general, it should be noted that the application of ozone at concentrations of 0.8 ppm and 3.0 ppm could be used for the preservation of apple fruits during postharvest storage. The ozonation process prevented decay of 'Iedzenu', 'Auksis' and 'Belorusskoje Malinovoje' apple cultivars, but it accelerated damage in the 'Gita' cultivar.

Postharvest softening of apple is a serious problem for many growers, including Latvia. Recently,

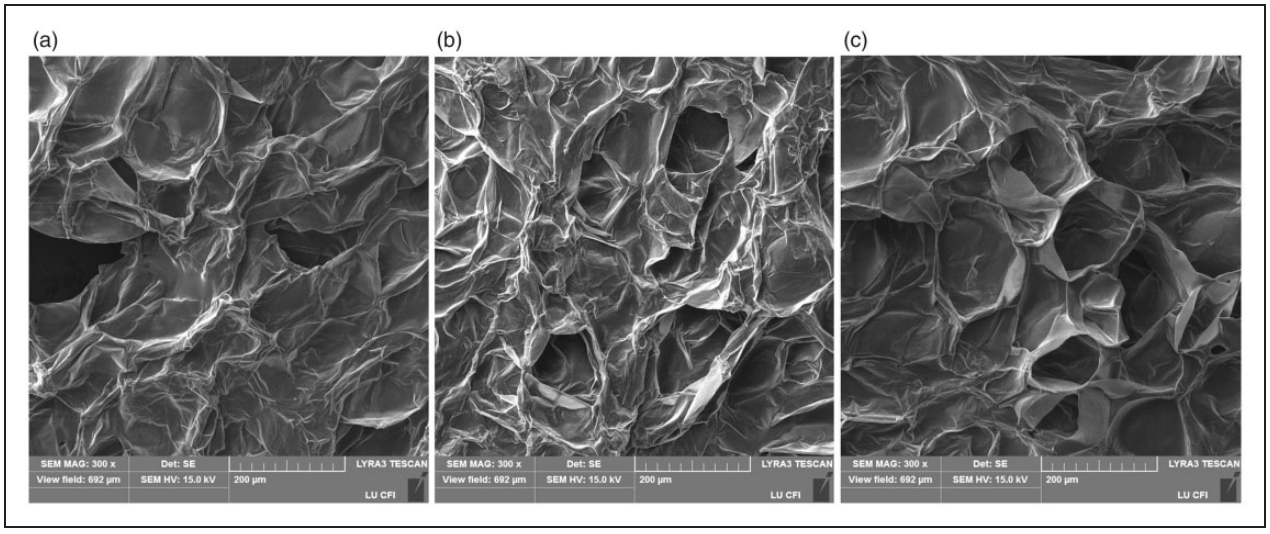

Figure 7. Cell structure of cultivar 'Belorusskoje Malinovoje' apples, (a) cold storage (control), (b) ozone concentration 0.8 ppm, (c) ozone concentration 3.0 ppm.

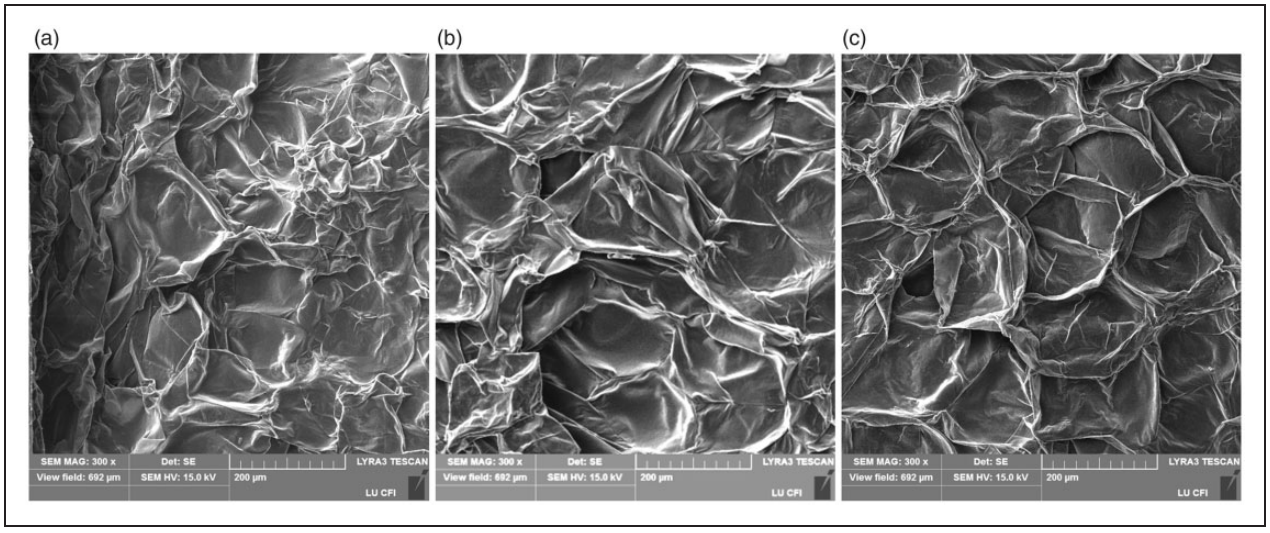

Figure 8. Cell structure of cultivar '*Gita*' apples, (a) cold storage (control), (b) ozone concentration 0.8 ppm, (c) ozone concentration 3.0 ppm.

considerable research has been carried out aimed to identify the biological causes of softening, so that this process can be managed or regulated more effectively. Softening is generally considered an undesirable ripening process in apple fruit, as firmer apples tend to be juicier, crisper, crunchier and less mealy than softer fruit (Conforti and Totty, 2007). Results showed that after six months of apple storage (Table 2), weight loss was significantly higher in 'Gita' apples (29.41%) treated with ozone at a concentration of 3.0 ppm, while for other cultivars differences between treatments were less pronounced. During the shelf life, the same trend is observed, where highest weight loss noted in ozone treated (0.8 ppm and 3.0 ppm) 'Gita' apples (0.08% and 0.10%, respectively) and 3.0 ppm treated 'Iedzenu' apples (0.24%). Forney et al. (2003) and Palou et al. (2002) found an increase in peaches and broccoli weight loss, respectively, and assigned this effect to cuticle degradation. Moreover, a strong correlation has been found between fruit weight loss and thickness of a cuticular layer (Veravrbeke et al., 2003). Taking into consideration weight loss of ozone-treated samples and structure of apple samples presented previously, the line of interrelation was not found. Our observation does not coincide with above-mentioned researchers.

Table 1. Number of damaged apples stored under different conditions, %.

Cultivar	Ozone concentration	Storage time	
		After six months of storage	After shelf life of five days
'Iedzenu'	Control	21.62a	1.80b
	0.8 ppm	14.29b	1.98b
	3.0 ppm	13.16c	5.83a
'Auksis'	Control	5.97a	1.81a
	0.8 ppm	0.00c	1.09a
	3.0 ppm	3.13b	2.88a
'Belorusskoje Malinovoje'	Control	3.57b	2.23a
	0.8 ppm	4.92a	2.04a
	3.0 ppm	0.00c	1.51a
'Gita'	Control	26.67b	0.00b
	0.8 ppm	30.61a	1.97a
	3.0 ppm	27.08b	2.52a

Note: Values for the same cultivar followed by different small letters are significantly different by the LSD at 0.05 level (differences between storage conditions).

Table 2. Fresh weight loss of apples stored under different conditions, %.

Cultivar	Ozone concentration	Storage time	
		After six months of storage	After shelf life of five days
'Iedzenu'	Control	10.00a	0.06b
	0.8 ppm	10.25a	0.08b
	3.0 ppm	6.50b	0.24a
'Auksis'	Control	1.67c	0.10a
	0.8 ppm	15.19a	0.06b
	3.0 ppm	6.73b	0.18a
'Belorusskoje Malinovoje'	Control	4.90a	0.12a
	0.8 ppm	5.00a	0.12a
	3.0 ppm	4.51b	0.10a
'Gita'	Control	6.12c	0.00b
	0.8 ppm	7.45b	0.08a
	3.0 ppm	29.41a	0.10a

Note: Values for the same cultivar followed by different small letters are significantly different by the LSD at 0.05 level (differences between storage conditions).

Apple flesh firmness is dependent on the degree of ripeness, place of growing, weather conditions and cultivar. Flesh firmness decreases during fruit ripening; therefore, for all cultivars, the highest firmness has been observed before storage, and storage technology significantly affects the firmness of the apples (Juhņeviča-Radenkova and Radenkovs, 2016).

Data depicted in Table 3 indicate that at the beginning of long-term storage, flesh firmness fluctuated in a range from 34.58 N to 63.46 N.

During long-term storage, considerably lower loss of firmness was noted in the case of cold storage without ozone treatment, as well as when ozone-treated at a concentration of 0.8 ppm was applied. Significantly higher flesh firmness was noted in 'Iedzenu' apples (43.23 N and 42.60 N) subjected to O_3 exposure at concentrations of 0.8 ppm and 3.0 ppm, respectively. With regard to other cultivars, no significant differences were found between treated and untreated fruit. Taking into account the results obtained after shelf life, it is evident that significantly better preservation of flesh firmness was achieved when 'Iedzenu' (38.54 N and 40.61 N) apples were treated with ozone at the concentrations of 0.8 and 3.0 ppm, respectively. Due to a lack of scientific research concerning O_3 influence on apple quality, it is not possible to compare the results obtained within this research. In some papers, O_3 has made a significant (Liu et al., 2016) and little (Miller et al., 2013) impact on maintaining the firmness of fresh-cut apples.

The main acids in apples are malic acid, citric acid and tartaric acid, and their levels depend on the cultivar and the degree of ripeness. During ripening, as well as storage, the level of acids in apples decreases due to the activity of endogenous enzymes. Data depicted in Table 4 indicate that at the beginning of storage, a higher titratable acidity was observed, corresponding to values from 0.41% to 0.73%, while after long-term storage, it considerably declined. The analysis of variance showed no significant difference ($p > 0.05$) for titratable acidity from the interaction of treatment or no treatment with O_3, except the cultivar 'Gita' apples (concentration of 0.8 ppm).

After shelf life, a significantly higher ($p < 0.05$) titratable acidity was found in untreated apples, which were kept under normal conditions, while no impact was observed on a cultivar of 'Gita' apples. Our findings coincide with the results of Alencar et al. (2014), who pointed out that there was no statistically significant effect of ($p < 0.05$) of O_3 at a concentration of 100 ppm and flow rate of 4.6 L min^{-1} for 60 min on titratable acidity in pears (*Pyrus communis* cv. 'Williams'). Contrarily, a positive impact (Heleno et al., 2015) on table grape (*Vitis vinifera* L.) fruits subjected to treatment with 2 mg L^{-1} of gaseous O_3 within seven weeks of storage has been achieved.

The analysis of variance showed a significant difference ($p < 0.05$) after long-term apple storage for total soluble solids (TSS) from the interaction of treatment

Table 3. Flesh firmness of apples stored under different conditions, n.

Cultivar	Ozone concentration	Storage time		
		Before storage	After six months of storage	After shelf life of five days
'Iedzenu'	Control	63.46 ± 5.54	39.60b ± 5.73	28.91b ± 6.38
	0.8 ppm		43.23a ± 9.51	38.54a ± 5. 45
	3.0 ppm		42.60a ± 6.87	40.61a ± 7.83
'Auksis'	Control	38.64 ± 4.19	38.14a ± 7.68	28.65a ± 3.47
	0.8 ppm		31.92b ± 7.98	29.95a ± 6.31
	3.0 ppm		36.51a ± 5.52	31.02a ± 6.05
'Belorusskoje Malinovoje'	Control	49.55 ± 3.57	32.64a ± 2.93	31.51a ± 3.21
	0.8 ppm		31.36a ± 2.25	31.30a ± 3.40
	3.0 ppm		32.10a ± 3.29	26.25b ± 6.33
'Gita'	Control	34.58 ± 4.19	32.02a ± 4.47	22.72a ± 4.13
	0.8 ppm		29.79a ± 4.70	26.46a ± 3.34
	3.0 ppm		33.13a ± 5.20	23.16a ± 6.27

Note: Values for the same cultivar followed by different small letters are significantly different by the LSD at 0.05 level (differences between storage conditions).

Table 4. Titratable acidity of apples stored under different conditions, %.

Cultivar	Ozone concentration	Storage time		
		Before storage	After six months of storage	After shelf life of five days
'Iedzenu'	Control	0.41	0.38a	0.51a
	0.8 ppm		0.38a	0.38b
	3.0 ppm		0.38a	0.38b
'Auksis'	Control	0.45	0.38a	0.38a
	0.8 ppm		0.38a	0.38a
	3.0 ppm		0.38a	0.38a
'Belorusskoje Malinovoje'	Control	0.73	0.57a	0.64a
	0.8 ppm		0.51a	0.51b
	3.0 ppm		0.51a	0.51b
'Gita'	Control	0.67	0.51a	0.45a
	0.8 ppm		0.45b	0.38a
	3.0 ppm		0.57a	0.45a

Note: Values for the same cultivar followed by different small letters are significantly different by the LSD at 0.05 level (differences between storage conditions).

or no treatment with ozone. Mean values with standard deviation are shown in Table 5. Ozone-treated (concentration of 3.0 ppm) apples as well as untreated apples reached the highest TSS concentration after long-term storage. As can be seen, the higher TSS value is observed in the cultivar 'Iedzenu' samples that were ozone treated with a concentration of 3.0 ppm, cultivar 'Auksis' samples treated with a concentration of 0.8 ppm and cultivar 'Gita' samples: both treated (concentration of 3.0 ppm) and untreated apples. Taking into consideration the results presented in literature, it becomes apparent that in the majority of published papers, there is no evidence regarding significant influence of ozonation on TSS content (Miller et al., 2013).

However, Alegria et al. (2009) noted a significant decrease of TSS content in ozonated carrots. The authors came to the conclusion that the decrease of

Table 5. Total soluble solids content of apples stored under different conditions, °Brix.

Cultivar	Ozone concentration	Storage time		
		Before storage	After six months of storage	After shelf life of five days
'Iedzenu'	Control	11.75 ± 0.15	12.41b ± 0.25	11.43a ± 0.05
	0.8 ppm		11.65c ± 0.20	11.04a ± 0.11
	3.0 ppm		13.42a ± 0.13	11.09a ± 0.13
'Auksis'	Control	12.33 ± 0.19	11.16b ± 0.39	11.63a ± 0.15
	0.8 ppm		12.16a ± 0.57	11.63a ± 0.07
	3.0 ppm		11.61ab ± 0.05	11.78a ± 0.10
'Belorusskoje Malinovoje'	Control	11.03 ± 0.20	10.51a ± 0.27	9.72b ± 0.38
	0.8 ppm		9.95b ± 0.18	11.06a ± 0.09
	3.0 ppm		10.54a ± 0.50	9.19b ± 0.03
'Gita'	Control	12.16 ± 0.25	11.66a ± 0.29	10.97a ± 0.05
	0.8 ppm		10.64b ± 0.41	10.07a ± 0.17
	3.0 ppm		11.24a ± 0.14	10.91a ± 0.14

Note: Values for the same cultivar followed by different small letters are significantly different by the LSD at 0.05 level (differences between storage conditions).

TSS was caused by the leaching process. The analysis of variance showed that after apple shelf life, only in one case a significant difference ($p < 0.05$) was observed for 'Belorusskoje Malinovoje', where higher TSS recorded in treated with 0.8 ppm fruits. Our results indicate that there were no significant differences for TSS between treated and untreated apple fruits. Similar observations have been introduced by various researchers, who pointed out that O_3 treatment has no effect on preservation of TSS in table grape (Tzortzakis et al., 2007), melon (Selma et al., 2007) and tomatoes (Venta et al., 2010). The content of TSS decreased overall for all samples after five days of shelf life, indicating a continuation of the ripening process.

Different chemical compounds are liable for the colour changes, depending on the type of fruits. For instance, different phenolics, such as flavonols, phloridzin and hydroxycinnamic acids, can contribute to colour characteristics in apple (Sanoner et al., 1999). The Hunter colour values of apples after long-term storage under normal atmosphere conditions only or in combination with treatment of ozone are shown in Table 6. L^*, a^* and b^* values of treated samples were significantly different ($p > 0.05$) from those of the control samples. After ozonation, the apple samples became lighter ('Gita' – 0.8 ppm), redder ('Auksis' – 3.0 ppm) and yellower ('Gita' – 0.8 ppm and 'Iedzenu' – 3.0 ppm) in colour, i.e. increased L^*, a^* and b^* with values. However, the change in colour depended on the cultivar of apples. Within the literature, contradictory observations were found, which stated that the colour of apple juice (Patil et al., 2010; Torres et al., 2011) and grape (Tiwari et al., 2009) could be deteriorated due to ozone processing.

Table 6. Colour of apples stored under different conditions, L^*, a^* and b^*.

Cultivar	Ozone concentration	After six months of storage		
		L^*	a^*	b^*
Control	50.45a	7.23b	29.68c	
'Iedzenu'	0.8 ppm	52.65a	4.47c	35.67b
	3.0 ppm	52.3a	12.4a	44.27a
'Auksis'	Control	62.12a	−1.77b	39.29a
	0.8 ppm	61.9a	−1.12b	40.09a
	3.0 ppm	40.47b	20.71a	26.6b
'Belorusskoje Malinovoje'	Control	49.85a	2.63b	25.79c
	0.8 ppm	49.96a	4.93a	31.4b
	3.0 ppm	47.66a	4.11a	34.36a
'Gita'	Control	60.95b	−2.67ab	37.41b
	0.8 ppm	67.51a	−3.84a	45.96a
	3.0 ppm	62.74b	−1.09b	33.25c

Note: Values for the same cultivar followed by different small letters are significantly different by the LSD at 0.05 level (differences between storage conditions).

In contrast, Sung et al. (2014) noted that ozone treatment had no effect on the colour value of apple juice. To the best of our knowledge, this is the first report that examines the effect of ozone treatment of apple quality.

PCA was performed on the sensory data (Tables 7 and 8) of the four analysed apple cultivars that were

Table 7. Sensory evaluation results of samples after six months of storage.

Cultivar	Ozone concentration	After six months of storage						
		App	Aro	Tas	Pre	Aci	Swe	Jui
	Control	3.5bc ± 0.4	2.7ab ± 1.2	3.7a ± 0.4	4.8a ± 0.4	2.9abc ± 0.9	3.6ab ± 0.5	3.0ab ± 0.6
'Iedzenu'	0.8 ppm	3.0d ± 0.7	2.5 ± bc ± 1.0	3.5ab ± 0.4	4.6ab ± 0.9	3.1ab ± 0.7	3.7ab ± 0.4	2.5b ± 0.5
	3.0 ppm	2.7e ± 0.8	2.0 ± d ± 0.7	3.4ab ± c0.7	4.5ab ± 0.9	2.7bcd ± 1.0	3.6ab ± 0.7	2.9b ± 0.3
'Auksis'	Control	4.1a ± 0.2	2.6ab ± 1.1	3.3abc ± 0.8	4.4ab ± 1.3	2.3d ± 1.0	3.1cd ± 0.8	2.6b ± 0.7
	0.8 ppm	3.7ab ± 0.5	2.3c ± 1.0	2.9cd ± 1.0	4.4ab ± 1.3	2.5cd ± 0.9	3.1cd ± 1.0	2.9b ± 0.2
	3.0 ppm	3.5bc ± 0.4	2.3c ± 0.4	2.7d ± 0.5	4.4ab ± 1.3	2.6bcd ± 0.4	3.4bc ± 0.8	2.9b ± 0.2
'Belorusskoje Malinovoje'	Control	3.1d ± 0.4	2.5bc ± 0.1	3.2bc ± 0.9	4.4ab ± 1.3	2.9abc ± 1.0	2.6e ± 1.0	3.4ab ± 0.5
	0.8 ppm	2.5ef ± 0.8	2.4c ± 1.0	2.1e ± 0.6	4.2b ± 0.9	2.3d ± 0.7	2.4e ± 0.7	1.7c ± 0.8
	3.0 ppm	2.3f ± 0.5	2.3c ± 0.4	2.6d ± 1.0	4.4ab ± 1.3	3.0abc ± 0.8	2.8de ± 0.5	2.5b ± 0.7
'Gita'	Control	3.2cd ± 1.2	3.0a ± 1.2	3.5ab ± 0.8	4.4ab ± 1.3	3.3a ± 0.9	2.9cde ± 0.8	3.0ab ± 1.4
	0.8 ppm	3.1d ± 0.5	2.5bc ± 1.1	3.0bcd ± 1.1	4.5ab ± 1.8	3.1ab ± 1.3	2.5e ± 1.0	3.0ab ± 0.7
	3.0 ppm	3.0d ± 0.4	2.3c ± 1.0	2.9cd ± 0.5	4.4ab ± 1.3	2.8abc ± 1.0	2.6e ± 0.8	2.9b ± 0.9

Note: Sensory attributes: App: appearance, Aro: aroma, Tas: taste, Pre: maturity stage, Aci: acidity, Swe: sweetness, Jui: juiciness. Values for the same cultivar followed by different small letters are significantly different by the LSD at 0.05 level (differences between storage conditions).

Table 8. Sensory evaluation results of samples after shelf-life storage.

Cultivar	Ozone concentration	After additional five days of shelf-life						
		App	Aro	Tas	Pre	Aci	Swe	Jui
	Control	3.4ab ± 0.5	2.7ab ± 0.6	3.4a ± 0.5	3.7ab ± 1.2	2.6cd ± 1.1	3.2ab ± 0.5	3.0bc ± 0.3
'Iedzenu'	0.8 ppm	2.1ef ± 0.7	2.6adc ± 0.5	3.2a ± 0.5	3.6ab ± 1.2	2.8bc ± 1.1	3.4a ± 0.5	3.8a ± 0.4
	3.0 ppm	1.9ef ± 0.5	2.5abcd ± 0.5	3.3a ± 0.8	3.8a ± 1.2	2.6cd ± 1.0	3.4a ± 0.9	3.7a ± 0.5
'Auksis'	Control	3.0b ± 0.3	2.6abc ± 1.0	3.1a ± 0.8	3.2cd ± 1.6	2.6cd ± 1.3	3.2a ± 0.8	2.6c ± 0.8
	0.8 ppm	2.8cd ± 1.0	2.3cd ± 0.4	3.0a ± 0.8	3.3b ± 1.4	2.6cd ± 0.8	2.8bc ± 0.5	3.0b ± 0.3
	3.0 ppm	3.7a ± 0.4	2.7ab ± 0.8	3.1a ± 0.5	3.7ab ± 1.2	3.2ab ± 0.8	3.1a ± 0.5	3.4b ± 0.5
'Belorusskoje Malinovoje'	Control	3.1bc ± 0.4	2.8ab ± 0.8	3.0a ± 0.5	3.3b ± 1.4	3.1a ± 0.5	2.4cd ± 0.9	2.8c ± 1.1
	0.8 ppm	2.8cd ± 1.2	2.7ab ± 0.5	3.0a ± 0.6	3.5a ± 1.2	3.1a ± 0.6	3.0a ± 0.6	3.4a ± 0.5
	3.0 ppm	3.3a ± 0.8	3.0a ± 0.7	3.1a ± 0.5	3.7ab ± 1.2	3.6a ± 0.7	3.1a ± 0.9	3.5a ± 0.5
'Gita'	Control	2.4de ± 0.9	2.4b ± 1.1	2.2b ± 0.8	2.3e ± 1.5	2.3d ± 1.3	1.7e ± 0.5	1.8d ± 0.9
	0.8 ppm	1.6f ± 0.7	2.0d ± 1.3	2.0b ± 1.1	2.8d ± 1.8	2.2de ± 1.3	2.0de ± 1.3	2.0d ± 1.3
	3.0 ppm	2.1ef ± 0.7	2.2d ± 1.2	1.8b ± 0.8	2.3e ± 1.5	1.8e ± 1.0	2.2d ± 1.0	1.8d ± 1.0

Note: Sensory attributes: App: appearance, Aro: aroma, Tas: taste, Pre: maturity stage, Aci: acidity, Swe: sweetness, Jui: juiciness. Values for the same cultivar followed by different small letters are significantly different by the LSD at 0.05 level (differences between storage conditions).

kept under different long-term conditions (Figure 9(a) – after six months cold storage; (b) – after six months cold storage + additionally 5 days of shelf life). PC1 and PC2 together explain 78.9 and 91.3% of the samples' variance, respectively. As can be seen in Figure 9(a), clear separation (the upper and the lower left side of cultivar 'Iedzenu' apples (IE_C and IE_0.8 ppm) is observed, and those samples were kept separate of other apple samples. These apple samples were characterized as fruits with the most pronounced aroma, acidity, sweetness, taste and better maturity stage. Furthermore, apple samples: BM_C; BM_0.8 ppm; BM_3.0 ppm; AU_C; AU_0.8 ppm have been highlighted as samples with pronounced juiciness and appearance. Taking into account the results after long-term apple storage + five additional days of shelf life, one can conclude that apples that were ozone treated (with concentrations 0.8 and 3.0 ppm), particularly cultivars 'Belorusskoje Malinovoje' and 'Auksis' (BM_3.0 ppm; AU_3.0 ppm), were characterised as the most acidic, fragrant and having the best appearance, whereas cultivar of 'Iedzenu' apples treated with ozone at both concentrations of 0.8 and 3.0 ppm had the most pronounced sweetness, taste, juiciness

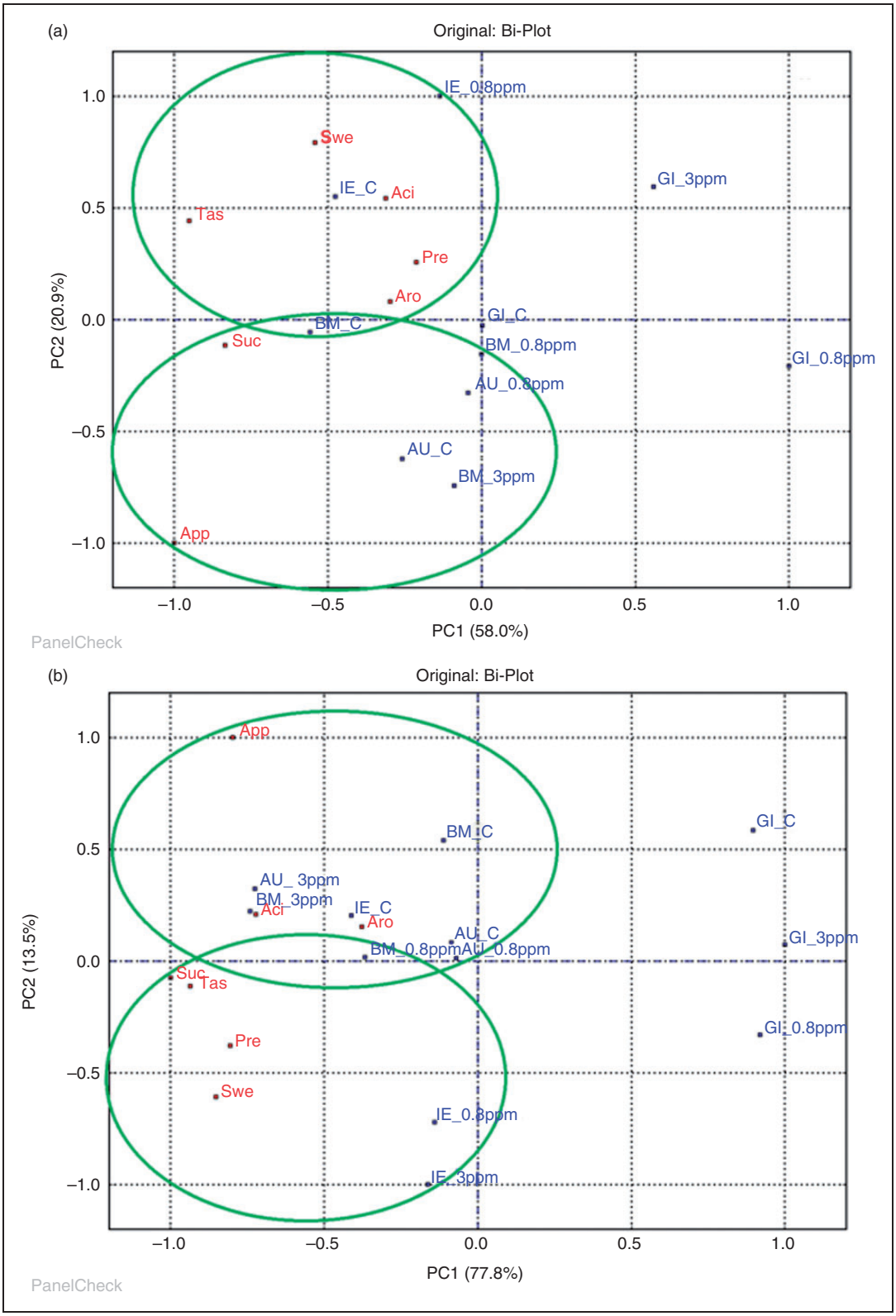

Figure 9. Biplot presenting the scores and loadings of the first two principal components of apple sensory data (a – after six months' storage, b – after five days of shelf life).
Note: Letters represented in the figures indicate the types of storage: C: cold storage; 0.8 ppm: ozone concentration 0.8 ppm; 3 ppm: ozone concentration 3 ppm. Cultivars: Au: 'Auksis'; GI: 'Gita'; IE: 'Iedzenu'; BM: 'Belorusskoje Malinovoje'. Attributes: App: appearance; Aro: aroma; Tas: taste; Pre: maturity stage; Aci: acidity; Swe: sweetness; Suc: juiciness.

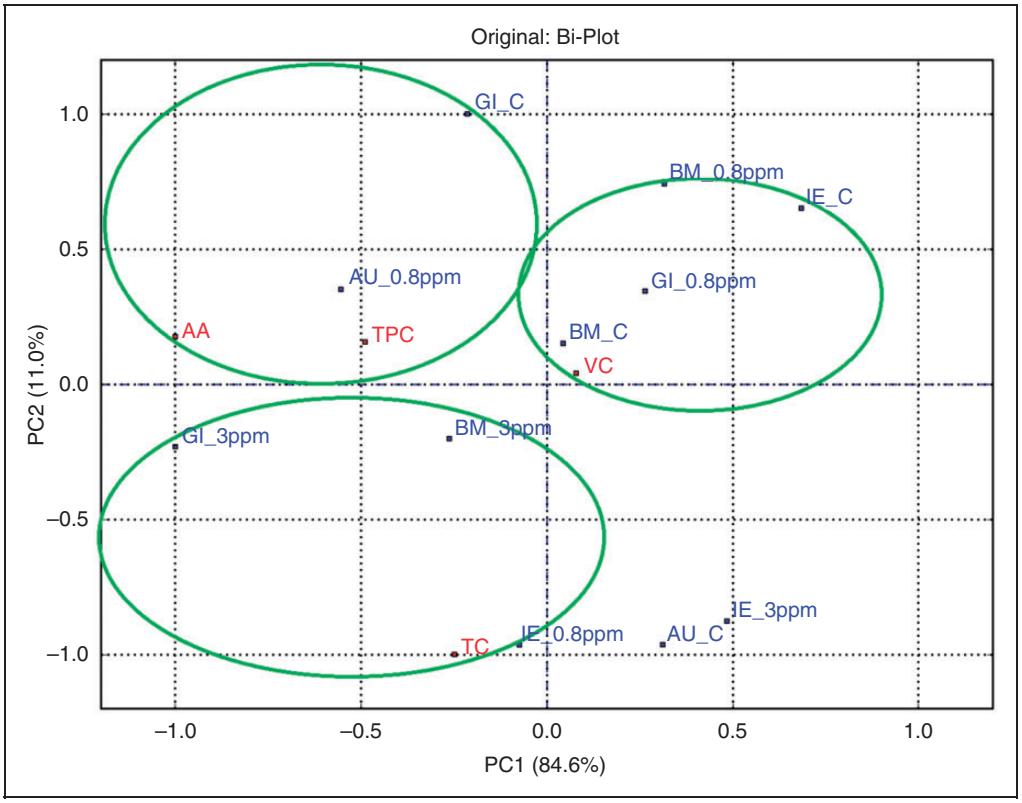

Figure 10. Biplot presenting scores and loadings of the first two principal components of apple chemical composition. Notes: Letters represented in the figures indicate the types of storage: C: cold storage; 0.8 ppm: ozone concentration 0.8 ppm; 3 ppm: ozone concentration 3 ppm. Cultivars: Au –'Auksis'; GI: 'Gita'; IE –'Iedzenu'; BM –'Belorusskoje Malinovoje'. Attributes: VC: Vitamin C; TPC: total phenolic content; TC: tannin content; AA: antioxidant activity.

and better maturity stage. In conclusion, it should be mentioned that apple cold storage with a combination of ozone treatments can be used as a novel technology that is capable of preserving sensory quality of apples during long-term storage and during shelf life as well.

Antioxidants comprise a wide range of constituents that have the ability to scavenge free radicals, preventing oxidative food damage. These substances act as antimicrobial and anticarcinogenic agents, and they possess health-protective functions. The antioxidants capacity in fruits mainly depends on the presence of polyphenols, vitamin C, anthocyanins and carotenoids (Miller et al., 2013). Therefore, it is desirable to determine the content of antioxidants in apples and their activity against free radicals and to quantitatively define the effect of ozone on the biochemical profile of apples.

PCA was performed on the data of the four analysed apple cultivars that were kept for under different long-term conditions (Figure 10 and Table 9). PC1 and PC2 together explain 95.6% of the samples' variance. In Figure 10, clear separation of three groups is evident (the upper and the lower left side and the upper right side). It was observed that for the 'Gita' (GI_C) and 'Auksis' (AU_0.8 ppm) samples, the antioxidant capacity (determined by using DPPH method) had a strong positive correlation with total phenolics. Cultivar 'Auksis' (AU_0.8 ppm) apples among other samples contained the highest amount of phenolics. In turn, total tannins were found to be dominant in the apple cultivars 'Gita' (GI_3 ppm), 'Iedzenu' (IE_0.8 ppm) and 'Belorusskoje Malinovoje' (BM_3.0 ppm), while vitamin C was dominant in the 'Belorusskoje Malinovoje' (BM_C), 'Gita' (GI_0.8 ppm), and 'Iedzenu' (IE_C) samples. This phenomenon could be explained by the ability of ozone induce or even enhance the production of the phenolics in plants (Forney, 2003). It is also explainable by the plant's natural tolerance to environmental abiotic stress conditions caused by many factors (heat, frost, cold, ozone, salinity, etc.). Torres et al. (2011) found that ozonation as a preservation technique for processing of apple juice is very useful; nutritional value of the produce can be altered, with the key factor being exposure time.

Table 9. Total vitamin C, phenolic, tannin content and antioxidant activity of four apple cultivars stored under different conditions.

Cultivar	Ozone concentration	After six months of storage			
		VC, mg 100 g^{-1}	TPC, mg 100 g^{-1}	TC, mg 100 g^{-1}	AA, mmol Trolox 100 g^{-1}
'Iedzenu'	Control	16.86a ± 0.17	66.33b ± 5.56	25.16c ± 5.19	109.40c ± 4.69
	0.8 ppm	12.94c ± 0.42	91.97a ± 5.58	58.15a ± 3.65	138.43a ± 5.02
	3.0 ppm	14.60b ± 0.42	64.98c ± 1.81	48.96b ± 3.32	117.18b ± 3.26
'Auksis'	Control	7.43b ± 0.42	72.14c ± 4.69	52.23a ± 3.34	123.71c ± 3.15
	0.8 ppm	8.01a ± 0.33	107.80a ± 0.95	44.63b ± 4.07	171.93b ± 4.91
	3.0 ppm	8.10a ± 0.58	100.57a ± 1.94	49.04a ± 3.54	198.25a ± 5.30
'Belorusskoje Malinovoje'	Control	16.36a ± 0.33	90.01a ± 6.14	40.98b ± 4.57	136.15b ± 5.89
	0.8 ppm	13.10b ± 0.58	85.88c ± 0.97	29.09c ± 4.81	122.90c ± 6.36
	3.0 ppm	13.09b ± 0.17	87.06b ± 0.84	53.64a ± 4.23	155.56a ± 4.58
'Gita'	Control	9.01b ± 0.19	89.97b ± 6.22	31.43c ± 2.88	155.10b ± 3.49
	0.8 ppm	10.60a ± 0.42	72.97c ± 4.50	34.24b ± 3.08	131.04c ± 5.82
	3.0 ppm	7.51c ± 0.50	94.37b ± 2.17	58.75a ± 1.75	191.23a ± 1.45

Note: Values for the same cultivar followed by different small letters are significantly different by the LSD at 0.05 level (differences between storage conditions).
Attributes: VC: vitamin C; TPC: total phenolic content; TC: tannin content; AA: antioxidant activity.

CONCLUSIONS

The present work investigated the effect of ozone treatment on the postharvest quality of four cultivars of apples. The results have shown that the ozone treatment of fruits during long-term storage has a minor effect on cellular membranes, cell integrity and changes in epicuticular waxes structure. Ozone application could accelerate the natural ageing of the waxes found on the surface of apples, thereby reducing the thickness of the waxes. The rate of degradation of waxes appears to be cultivar dependent. After six months of storage, the ozonation process prevented decay of 'Iedzenu', 'Auksis' and 'Belorusskoje Malinovoje' apple cultivars, but it accelerated damage in cultivar 'Gita' apple samples. Moreover, a positive impact of ozone during long-term storage was also found for flesh firmness of 'Iedzenu' apples subjected to O_3 exposure at concentrations of 0.8. ppm and 3.0 ppm. In turn, no significant differences were found for other cultivars of apples between ozonation and cold storage (control). The data obtained show that after six months of storage, 'Iedzenu' cultivar samples, stored both under normal atmosphere conditions and treated with ozone at a concentration of 0.8 ppm (IE_C and IE_0.8 ppm), were characterized as fruits with the most pronounced aroma, acidity, sweetness, taste and better maturity stage. Furthermore, apple samples: BM_C; BM_0.8 ppm; BM_3.0 ppm; AU_C; AU_0.8 ppm have been highlighted as samples with pronounced juiciness and appearance. In general, the results indicate that conventional cold storage with a combination of ozone treatments could be used as a novel technology that is capable of preserving both chemical and sensory quality of apples during long-term storage. However, the degree of effectiveness depends both on the cultivar and on the concentration of ozone.

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