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# Influence of pre-harvest calcium spray on fruit quality of apple cultivars during cold storage

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**Abstract:** Effect of pre-harvest calcium chloride ($CaCl_2$) treatment on fruit quality attributes of three apple cultivars ('Golden Delicious Reinders®', 'Granny Smith' and 'Red Chief') at harvest and after two and four months of cold storage during two consecutive years was studied. Trees were sprayed four times starting from the beginning of June at 20-day intervals. After harvest, fruits were stored in a regular atmosphere (RA) for further analysis. Measurements included fruit weight, weight loss, firmness, total soluble solids (TSS), titrable acidity (TA), total sugars (TS), TS/TA, total phenolic content (TPC) and antioxidant activity (AA). $CaCl_2$ had a positive impact on fruit firmness of all cultivars in both years. Higher values of TSS and TS of 'Golden Delicious Reinders®' were determined in $CaCl_2$ treatment in both years. TSS, TS and TS/TA were increased by prolonging storage in 'Red Chief' in $CaCl_2$ treatment and control, in both years. TSS and TS proved similarly stable in 'Granny Smith' during storage in $CaCl_2$ treatment and control in the second year of examination, but a decrease of TA and TS/TA has been detected in both years of storage. The AA of cultivars 'Golden Delicious Reinders®' and 'Red Chief' was stronger affected by the storage compared to $CaCl_2$ treatment. Through a comprehensive analysis of apple cultivars it can be concluded that pre-harvest application of $CaCl_2$ in cultivars 'Golden Delicious Reinders®' and 'Red Chief' can be used to improve fruit quality during storage.

**Keywords:** *Malus domestica*; calcium-chloride; physicochemical traits of fruit; fruit storage

Apple (*Malus domestica* Borkh.) is the most important fruit species in terms of world production. According to official FAO statistics (2022), average annual apple production in the world was more than 86 million tonnes in 2020 while production in Republic of Serbia was about 490 000 tonnes, and the areas under apple orchards were larger than 26 000 ha. The area of new apple orchards on average increases every year by 200 to 400 ha (Statistical yearbook 2018). The current trends involve setting up highly intensive orchards in dense planting system, with anti-hail nets and irrigation systems, accompanied by introduction of quality standards and modern assortment in response to the changing market demands (Radivojević

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et al. 2019). Such plantations ought to incorporate fruit cultivar assortments based on market demands (e.g. 'Golden Delicious', 'Red Delicious' and 'Granny Smith') (Lukač-Bulatović et al. 2019).

Apple fruits are usually harvested during August and September and part of the harvested fruit can be sold to the markets for fresh consumption, although the prices are generally low during the harvesting season (Ali et al. 2004). The price of an apple and the profit itself depend on many factors. One of the ways to achieve a better market price is to use storage for 4–6 months in order to provide the market with fruit out of season as long as possible (Farooq et al. 2012). The apple fruit quality is achieved in the orchard, and during storage it cannot be improved but can be preserved (Pašalić 2006). The genetic background, environmental conditions, cultural and developmental pre-harvest factors (Skic et al. 2016), as well as ripening stage (Korićanac et al. 2019) are factors that affect fruit quality at harvest. During storage, intensive metabolic processes take place in apple fruits, which result in a loss of quality (Fallahi et al. 2010), therefore storage conditions play important role in preservation of fruit quality (Soliva-Fortuny et al. 2002; Blažek et al. 2003; Saletnik et al. 2022). Increasing of apple production in the Republic of Serbia is not completely followed by construction of cold storage, especially ULO or DA cold storages, which presently cover less than one half of yearly apple fruit production (Radivojević et al. 2019). Because of that, most of the harvested fruits are commonly stored for long periods in a regular atmosphere (RA).

Many previous studies have focused on developing new agricultural and biotechnological practices that may improve apple quality and maintain its freshness after harvest (Tijero et al. 2021). Calcium has been extensively reviewed as both an essential element and its potential role in maintaining postharvest quality of fruit especially bearing in mind its function in stabilizing cellular membranes and delaying senescence in different horticultural crops (Shirzadeh et al. 2011). Optimal supply of apple fruits with this element is associated with better fruit firmness, higher content of soluble solids and total acids, better fruit coloration (Kadir 2005) and reduced occurrence of physiological diseases during storage (Fallahi et al. 2010) without detrimental effect on consumer acceptance (Lester, Gursak 1999). $CaCl_2$ and calcium nitrate ($Ca(NO_3)_2$) are common calcium fertilizers used for spraying of apple (Ghorbani et al. 2017). Experimental results showed different effects of applying calcium fertilizers (Lanauskas, Kvikliene 2006) that were conditioned by application time (Schleg, Schönherr 2002; Neilsen et al. 2005; Lötze et al. 2008), cultivar (Hahn et al. 2022) and growing conditions (Kalcsits 2017). Also, the temperature and relative humidity during storage affect fruit firmness, weight loss, content of total acids and soluble solids and have great importance for preservation of apple quality (Saletnik et al. 2022).

The subject of this research was to examine the influence of $CaCl_2$ treatment on 'Golden Delicious Reinders®', 'Red Chief' and 'Granny Smith' apple cultivars before harvest on fruit quality at harvest and after two and four months of storage in RA. The obtained results would be a guideline for the preharvest application of $CaCl_2$ and the optimal storage duration of fruits in a RA for mentioned cultivars.

## MATERIAL AND METODS

**Plant material.** During two consecutive growing seasons (2018 and 2019), orchard trial with three apple (*Malus domestica* Borkh.) cultivars ('Golden Delicious Reinders®', 'Red Chief' and 'Granny Smith') grafted on M9 rootstock was established at experimental plantation of the Fruit Research Institute, Čačak (Serbia) at the village Donja Trepča (43°53'36"N latitude; 20°25'157"E longitude; 233 m a.s.l.), Western Serbia. Trees were planted at a spacing of 4 m × 1.25 m (2 000 trees/ha), except cultivar 'Red Chief' that was planted at distance 4 m × 1.00 m (2 500 trees/ha) and trained to the Spindle Bush system. Soil maintenance was performed according to the principles of integrated production, which meant grassing space among rows, while the space in rows was maintained without weeds. Treatments against diseases and pests were performed in accordance with standard recommendations for commercial apple orchards.

The experiment was set up as a randomized block design with 80 trees in $CaCl_2$treatment and 80 trees in control (20 trees repeated four times) for each cultivar. Treated trees were sprayed with $CaCl_2$ (YaraVita Stopit), while control represented trees without calcium chloride treatment. The $CaCl_2$ was applied in the concentration of 169 g/L w/w and amount of applied solution was 7.5 L/ha. The treatments for each cultivar started when a diameter was up

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to 40 mm–T stage fruit (BBCH 74) and was repeated four times at every 20 days until one month before fruits were ripe for picking.

**Fruit quality analysis.** Apples were harvested at commercial maturity stage which was determined according to the iodine-starch test. Harvested fruits of uniform size, without visible damages and infections, randomly selected from the same pool of trees were transported to the laboratory of Fruit Research Institute. 40 fruits of each examined cultivars in four repetitions (160 fruits in total) per treatment were sampled and divided into three groups of 50 fruits (remaining 10 fruits were kept as reserves in case any of the fruits were damaged during storage). The first group consisted of fruits harvested at the full maturity stage, the second group were fruits stored for two months and the third group consisted of fruits that were stored for four months. Immediately after harvesting, the physical properties of all sampled fruits were measured and the fruits from the first group were used for chemical analyses. The remaining fruits were stored in RA for two and four months, after which fruit physical measurements were done and samples further used for chemical analysis.

**Weight and weight loss.** Fruit weight is determined by measuring on a technical scale Adventurer Pro AV812M (Ohaus Corporation, Switzerland), with accuracy of ± 0.01 g. Weight loss was calculated using formula (Ranjbar et al. 2018):

$$\textit{Fruit weight loss}\ (\%) = \frac{\textit{Initial weight} - \textit{final weight}}{\textit{Initial weight}} \times 100 \quad (1)$$

**Firmness.** Fruit firmness is determined with a hand penetrometer (FHT-803, EFFEGI, Italy) fitted with 11.1 mm diameter round tip. The skin of the fruit was removed at the place where the firmness was measured. Values were expressed as kg/cm$^2$.

**Total soluble solids**. The juice extracted by squeezing the homogenized fruit pulp through muslin cloth was used to measure total soluble solids (TSS). It was expressed as °Brix, were measured with a digital refractometer (Pocket PAL-1, Atago, Japan).

**Sugar and acid content.** The content of total sugars (TS) was determined volumetrically, using the Luff-Schoorl method (Egan et al. 1981) and expressed in %. Titratable acidity (TA) was measured by titration with 0.1 N sodium hydroxide to increase the pH of the samples to 8.1 using an automatic burette (Brand® Titrette®) and results were expressed as % malic acid. The sugar/organic acid ratio were calculated by the following equation:

$$\textit{Sugar / organic acid ratio} = \frac{\textit{Total sugars content}}{\textit{Total acid content}} \quad (2)$$

**Total phenolic content.** Total phenolic content (TPC) was determined using a modified Folin-Ciocalteau method (Singleton et al. 1999; Liu et al. 2012). A 0.2 mL aliquot of the 40-fold water diluted apple extract was added to a 15 mL tube and 0.2 mL of 1 : 10 Folin-Ciocalteau reagent: water solution was added to the mixture. The tube was allowed to stand at room temperature for 1 min. Then, 2 mL of 7.5% $Na_2CO_3$ were added to the mixture. After 2 hours at room temperature, absorbance was measured at 765 nm by UV/Vis spectrophotometer (Jenway 6300, Cole-Parmer, UK). The results were expressed as mg of gallic acid equivalents per 100 g fresh weight of the sample (mg GAE/100 g FW). Calibration was performed by analyzing the standard gallic acid (Sigma – Aldrich CO., USA) three times at four different concentrations.

**Antioxidant activity.** Antioxidant activity (AA) was determined using ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) assay (Pulido et al. 2003). The ABTS•+ radical cation, was prepared by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulphate and allowing the mixture to remain in the dark for 16 h at room temperature. The ABTS•+ solution was diluted with methanol until it reached an absorbance of 0.70 ± 0.02 at 658 nm (Pulido et al. 2003). An aliquot of methanol solutions containing different standard concentrations (at 4 different concentrations in three replicates) and samples was added to 3.9 mL of ABTS•+ in methanol. The decreasing of absorbance was measured at 658 nm by UV/Vis spectrophotometer (Jenway 6300, Cole-Parmer, UK). The results were expressed in µmol Trolox equivalents per 100 g of fresh weight (µmol TE 100 g FW).

**Statistical analysis.** Experimental data were processed with two-way analyses of variance (ANOVA). Source of variation were application of $CaCl_2$ (treatment and control) and storage lenght (0, 2 and 4 months). The data were analyzed using Statgraphic Centurion 18 program (Manugistics, Inc., Rockville, MD, USA). The analyses were performed in four replications and mean values were compared by Duncan's Multiple Range at the 5% level ($P$ < 0.05) of probability. Results were expressed as the mean ± standard error.

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**RESULTS**

The weight of apple fruit is a cultivar-specific characteristic, and represents important indicators of fruit quality that contribute to the yield. Fruit weight at harvest of cultivar 'Golden Delicious Reinders®' (Table 1) in treatment with $CaCl_2$ (144.63 g and 151.32 g) was higher in comparison with control (142.50 g and 148.72 g) at harvest time in both studied years, but differences were not statistically significant. The fruit weight decreased during storage period without significant differences. Weight loss and fruit firmness of mentioned cultivar were conditioned by treatment and storage period. The regularity of $CaCl_2$ effect on weight loss was not observed, i.e. the results obtained in studied years were opposite. On the other hand, the firmness was higher in fruits picked from sprayed trees (8.56 and 7.69 kg/cm$^2$) compared to control (7.27 and 6.94 kg/cm$^2$) and was decreased during storage in both years.

Foliar application of $CaCl_2$ in the cultivar 'Red Chief' (Table 2) had a significant effect only on fruit firmness in 2019. In addition, in this cultivar, a significant influence of the storage period on all tested parameters was found, except for fruit weight in the first year of testing.

As expected, fruit weight and firmness decreased, and weight loss increased during storage. Treatments showed a different trend of weight loss during storage in the first year, so the ANOVA revealed that this parameter was affected by the interaction of treatment and storage period (Figure 1). Weight loss was less pronounced during harvest and after two months of storage in the $CaCl_2$ application, but later (after four months of storage) weight loss was lower in the control.

The 'Granny Smith' (Table 3) was characterised by higher fruit weight (195.87 g and 172.09 g) and weight loss (4.83 and 4.34%) in control, but differences in weight loss were not significant. The fruit weight significantly decreased after four months in cold storage for approximately 10 g in both years and consequently weight loss increased. It is important to note that the fruit weight in both years, as well as the weight loss and firmness in the first examined year did not differ significantly after two and four months of fruit storage.

The foliar application of $CaCl_2$ had a positive effect on fruit firmness. Also, it was determined that the firmness of the fruit significantly decreased during storage in both years.

Content of TSS and TS in fruits of 'Golden Delicious Reinders®' was significantly higher under preharvest spraying with $CaCl_2$, while other studied parameters of chemical composition of fruits have not differed in comparison with control (Table 4). Values of TSS and content of primary metabolites (sugars and acids) of mentioned cultivar, in 2018, decreased during storage. On the other hand, the ratio of sugars and acids was high after two and four months of storage in both years of testing. Contrary to the content of essential nutrients, the content of TPC and AA of the fruit showed a completely opposite trend during years of the examination. Namely, TP and AA increased during the storage period in the first year of the testing, in contrast to the second year when this content decreased with storage. Also, the ANOVA revealed that treatment by storage length interaction

Table 1. Effect of preharvest $CaCl_2$ application and storage length on physical traits of apple cultivar 'Golden Delicious Reinders®'

| | | Fruit weight (g) | | Weight loss (%) | | Fruit firmness (kg/cm$^2$) | |
|---|---|---|---|---|---|---|---|
| | | 2019 | 2018 | 2019 | 2018 | 2018 | 2019 |
| Application of $CaCl_2$ (A) | $CaCl_2$ | 144.6 ± 1.3$^a$ | 151.3 ± 2.6$^a$ | 4.21 ± 0.36$^a$ | 4.39 ± 0.2$^b$ | 8.56 ± 0.32$^a$ | 7.69 ± 0.35$^a$ |
| | Control | 142.5 ± 0.4$^a$ | 148.7 ± 2.3$^a$ | 3.27 ± 0.43$^b$ | 5.67 ± 0.40$^a$ | 7.27 ± 0.31$^b$ | 6.94 ± 0.33$^b$ |
| Storage length (B) | 0 months | 147.2 ± 2.4$^a$ | 155.2 ± 3.2$^a$ | / | / | 9.00 ± 0.34$^a$ | 8.83 ± 0.17$^a$ |
| | 2 months | 143.2 ± 1.9$^a$ | 148.2 ± 2.7$^a$ | 3.96 ± 0.46$^a$ | 4.57 ± 0.25$^a$ | 7.83 ± 0.32$^b$ | 6.94 ± 0.14$^b$ |
| | 4 months | 140.3 ± 2.6$^a$ | 146.8 ± 2.9$^a$ | 4.95 ± 0.63$^a$ | 5.50 ± 0.39$^a$ | 6.90 ± 0.34$^c$ | 6.17 ± 0.18$^c$ |
| A | | ns | ns | * | * | * | * |
| B | | ns | ns | * | * | * | * |
| A × B | | ns | ns | ns | ns | ns | ns |

$^{a-c}$Represent the means of four replicates, each represented by 40 fruits; ± standard error; different letters within the column indicate significant differences ($P$ ≤0.05) according to the Duncan test

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Table 2. Effect of preharvest $CaCl_2$ application and storage length on physical traits of apple cultivar 'Red Chief'

| | | Fruit weight (g) | | Weight loss (%) | | Fruit firmness (kg/cm$^2$) | |
|---|---|---|---|---|---|---|---|
| | | 2019 | 2018 | 2019 | 2018 | 2018 | 2019 |
| Application of $CaCl_2$ (A) | $CaCl_2$ | 193.4 ± 3.7$^a$ | 192.1 ± 3.8$^a$ | 3.72 ± 0.72$^a$ | 6.18 ± 0.54$^a$ | 8.97 ± 0.38$^a$ | 7.43 ± 0.48$^a$ |
| | Control | 187.3 ± 1.9$^a$ | 185.7 ± 3.1$^a$ | 3.63 ± 0.15$^a$ | 6.45 ± 0.79$^a$ | 8.41 ± 0.28$^a$ | 6.77 ± 0.43$^b$ |
| Storage length (B) | 0 | 195.1 ± 2.6$^a$ | 197.3 ± 3.7$^a$ | / | / | 9.88 ± 0.28$^a$ | 9.03 ± 0.18$^a$ |
| | 2 | 189.9 ± 0.5$^a$ | 188.1 ± 4.4$^b$ | 2.71 ± 0.56$^a$ | 4.66 ± 0.19$^a$ | 8.41 ± 0.34$^b$ | 6.79 ± 0.23$^b$ |
| | 4 | 186.0 ± 1.4$^a$ | 181.4 ± 3.5$^b$ | 4.64 ± 0.39$^a$ | 7.97 ± 0.31$^a$ | 7.78 ± 0.22$^b$ | 5.49 ± 0.10$^c$ |
| A | | ns | ns | ns | ns | ns | * |
| B | | ns | * | * | * | * | * |
| A × B | | ns | ns | * | ns | ns | ns |

$^{a-c}$Represent the means of four replicates, each represented by 40 fruits; ± standard error; different letters within the column indicate significant differences ($P \leq 0.05$) according to the Duncan test

induced TSS in both years, TS and TPC in 2018, as well as TA and TS/TA in 2019.

According to the results shown in Table 5, the positive effect of $CaCl_2$ preharvest application on TA and AA of fruits of apple cultivar 'Red Chief' in 2018 only has been found. Contrary to the cultivar 'Golden Delicious Reinders®', the increasing trend of TSS, TS and TS/TA during storage in this cultivar was observed, while in respect of TA opposite results in different years were obtained. The content of total acids in samples on the harvest time was the highest in 2018 in both, application $CaCl_2$ and control, while during storage this content has been significantly reduced. Interestingly, TA increased after four months of storage in $CaCl_2$ application and after two months of storage in control in 2019. Regarding the content of total phenols, no significant difference was found between freshly picked fruits and fruits stored for two and four months, while the AA of the fruit was the highest after two months of storage in both years. Treatment and duration of cold storage interaction significantly impacted TA and TS/TA in both year, as well as TPC and AA in the first and TS in second year of investigation. In general, TSS, TS and TS/TA increased with storage duration and reached the highest values after four months of fruit storage. Further, the highest values of TPC and AA were recorded after two months of cold storage.

Among the studied parameters of the chemical composition of the 'Granny Smith' apples, higher values in the $CaCl_2$ preharvest application compared to the control were found for TPC in the first year of testing (Table 6). During cold storage of 'Granny Smith' fruits, an increasing content of TSS after two months of cold storage in comparison with harvest was observed, while after four months of cold storage there was a decrease in the TSS. Also, an increasing trend of TS/TA during storage was observed, while

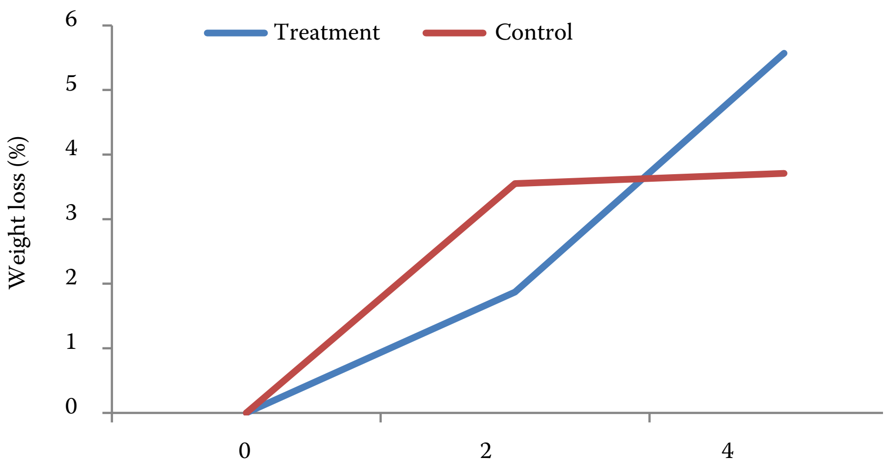


Figure 1. Weight loss of the apple cultivar 'Red Chief' depending on interaction storage length and $CaCl_2$ application in 2018

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Table 3. Effect of preharvest $CaCl_2$ treatment and storage length on physical traits of apple cultivar 'Granny Smith'

| | | Fruit weight (g) | | Weight loss (%) | | Fruit firmness (kg/cm$^2$) | |
|---|---|---|---|---|---|---|---|
| | | 2019 | 2018 | 2019 | 2018 | 2019 | 2018 |
| Application of $CaCl_2$ (A) | $CaCl_2$ | 183.3 ± 2.6$^b$ | 162.6 ± 2.3$^b$ | 4.07 ± 0.72$^a$ | 4.21 ± 0.83$^a$ | 9.27 ± 0.37$^a$ | 8.52 ± 0.39$^a$ |
| | Control | 195.8 ± 0.4$^a$ | 172.1 ± 0.6$^a$ | 4.83 ± 0.34$^a$ | 4.34 ± 0.46$^a$ | 8.21 ± 0.29$^b$ | 8.08 ± 0.48$^b$ |
| Storage length(B) | 0 | 195.7 ± 3.2$^a$ | 172.3 ± 3.4$^a$ | / | / | 10.18 ± 0.29$^a$ | 9.83 ± 0.12$^a$ |
| | 2 | 187.6 ± 2.7$^b$ | 166.6 ± 2.5$^b$ | 3.96 ± 0.46$^a$ | 3.34 ± 0.40$^b$ | 8.25 ± 0.27$^b$ | 8.64 ± 0.14$^b$ |
| | 4 | 185.6 ± 3.7$^b$ | 163.1 ± 3.8$^b$ | 4.95 ± 0.63$^a$ | 5.20 ± 0.71$^a$ | 7.93 ± 0.18$^b$ | 6.43 ± 0.20$^c$ |
| A | | * | * | ns | ns | * | * |
| B | | * | * | * | * | * | * |
| A × B | | ns | ns | ns | ns | ns | ns |

$^{a-c}$Represent the means of four replicates, each represented by 40 fruits; ± standard error; different letters within the column indicate significant differences ($P$ ≤0.05) according to the Duncan test

values of TA and TPC decreased in comparison with the harvest time. In respect of TS opposite results in different years were obtained. The interaction between the main factors of variability had no effect on antioxidant activity during the entire study period, as well as on TA in the first year and TPC in the second year of the study.

## DISCUSSION

Acceptance of apple fruits by consumers is based on visual (appearance, size, color, uniformity and freshness) and on internal (taste, aroma, texture, nutritional value and healthiness) fruit quality parameters (Mikulič-Petkovšek et al. 2009). Regardless of the treatment, the obtained values of the fruit weight of apple cultivars investigated in present study are in agreement with the previously published results of Blažek and Hlušičková (2007). Comparably, they measured similar fruit weight in cultivars 'Golden Delicious Reinders®' (165.1 g) and 'Granny Smith' (153.0 g) in commercial orchards situated in the major growing regions of the Czech Republic. Due to metabolic processes that continue after harvesting, the apple fruit lose water during storage leading to weight loss. Results related to changes of values of fruit weight and weight loss during storage obtained in our study are in accordance with Kader (2002) and Kwon et al. (2021) who reported that longer duration of storage resulted in increasing the percentage of fruit weight loss. Comparable fruit weight of apple cultivar 'Golden Delicious' was also described by Waghmode et al. (2021) who concluded that fruit weight slightly decreased with the progression of storage (from 0 to 90 days). The weight loss of 5% can be considered as an acceptable limit for apples (Ozdemir et al. 2005). Higher values of weight loss than mentioned were observed in 2019 in all cultivars after four months of storage. The positive influence of preharvest application of $CaCl_2$ on the fruit weight at harvest and during storage was not observed in the present study.

The flesh firmness of apples after storage is closely correlated with their firmness at the harvest time (Konopacka, Płocharski 2002). The significant positive impact of foliar application of $CaCl_2$ on fruit firmness of investigated cultivars in both experimental years in present study was observed. This can be explained by the fact that more than 60% of calcium is found in the cell wall, where it is bound to pectic acids, hemicellulose and proteins (Štampar et al. 2009) and plays a role in strengthening bonds and reducing cell wall porosity (White, Broadley 2003) and increasing fruit firmness (Gilliham et al. 2011). Also, in our research fruit firmness decreased continuously during cold storage as previously reported by Kwon et al. (2021) and Saletnik et al. (2022). Reasons for this are water losses and thinning of cell wall which are accompanied by a large amount of degradation of the cell wall materials and the pectin (Zhang et al. 2022). A critical level of the firmness which is considered acceptable for apple fruit quality amounts to 50 N (Blažek et al. 2003) and it was reached after four months of cold storage in all investigated cultivars in our study.

The content of soluble solids is good indicator of the internal fruit quality of apple fruit (Zhang et al. 2021). According to Jemrić et al. (2005), optimal values of soluble solids in apple fruit, which represent the limit for better acceptability by con-

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Table 4. Effect of preharvest $CaCl_2$ application and storage length on chemical composition of apple cultivar 'Golden Delicious Reinders®'

| | | TSS (°Brix) | | TS (%) | | TA (%) | |
|---|---|---|---|---|---|---|---|
| | | 2018 | 2019 | 2018 | 2019 | 2018 | 2018 |
| **Application of $CaCl_2$ (A)** | | | | | | | |
| $CaCl_2$ | | 12.81 ± 0.21[a] | 15.96 ± 0.13[a] | 11.72 ± 0.24[a] | 13.04 ± 0.14[a] | 0.25 ± 0.02[a] | 0.30 ± 0.01[a] |
| Control | | 11.91 ± 0.18[b] | 15.62 ± 0.24[b] | 10.58 ± 0.21[b] | 12.53 ± 0.15[b] | 0.24 ± 0.03[a] | 0.31 ± 0.02[a] |
| **Storage length (months) (B)** | | | | | | | |
| 0 | | 12.61 ± 0.38[a] | 16.20 ± 0.09[a] | 11.67 ± 0.4[a] | 13.17 ± 0.17[a] | 0.35 ± 0.01[a] | 0.33 ± 0.01[a] |
| 2 | | 12.69 ± 0.14[a] | 16.13 ± 0.17[a] | 11.36 ± 0.08[a] | 12.36 ± 0.18[b] | 0.21 ± 00.1 [b] | 0.33 ± 0.01[a] |
| 4 | | 11.78 ± 0.15[b] | 15.05 ± 0.19[b] | 10.42 ± 0.27[b] | 12.83 ± 0.12[a] | 0.19 ± 0.01[b] | 0.26 ± 0.01[b] |
| **Application of $CaCl_2$ × Storage length (A × B)** | | | | | | | |
| $CaCl_2$ | 0 | 13.59 ± 0.12[a] | 16.25 ± 0.09[a] | 12.64 ± 0.03[a] | 13.52 ± 0.04[a] | 0.35 ± 0.01[a] | 0.30 ± 0.01[c] |
| | 2 | 12.81 ± 0.18[b] | 16.11 ± 0.25[a] | 11.52 ± 0.04[b] | 12.64 ± 0.03[a] | 0.22 ± 0.01[a] | 0.33 ± 0.01[b] |
| | 4 | 12.04 ± 0.16[cd] | 15.53 ± 0.09[b] | 11.02 ± 0.04[c] | 12.95 ± 0.28[a] | 0.20 ± 0.01[a] | 0.28 ± .0.01[c] |
| Control | 0 | 11.63 ± 0.19[d] | 16.15 ± 0.16[a] | 10.70 ± 0.14[d] | 12.83 ± 0.14[a] | 0.35 ± 0.01[a] | 0.36 ± 0.01[a] |
| | 2 | 12.58 ± 0.23[bc] | 16.16 ± 0.25[a] | 11.21 ± 0.07[c] | 12.07 ± 0.29[a] | 0.19 ± 0.02[a] | 0.33 ± 0.01[b] |
| | 4 | 11.52 ± 0.21[d] | 14.57 ± 0.07[c] | 9.82 ± 0.07[e] | 12.70 ± 0.07[a] | 0.19 ± 0.01[a] | 0.24 ± 0.01[d] |
| **ANOVA** | | | | | | | |
| A | | * | * | * | * | ns | ns |
| B | | * | * | * | * | * | * |
| A × B | | * | * | * | ns | ns | * |

| | | TS/TA | | TPC (mg GAE 100 g/FW) | | AA (µmol TE 100 g/FW) | |
|---|---|---|---|---|---|---|---|
| | | 2018 | 2019 | 2018 | 2019 | 2018 | 2019 |
| **Application of $CaCl_2$ (A)** | | | | | | | |
| $CaCl_2$ | | 48.47 ± 3.02[a] | 43.75 ± 1.39[a] | 109.78 ± 8.40[a] | 64.79 ± 9.44[a] | 0.64 ± 0.07[a] | 0.55 ± 0.17[a] |
| Control | | 48.35 ± 5.04[a] | 42.01 ± 2.90[a] | 123.77 ± 8.90[a] | 59.23 ± 15.52[a] | 0.63 ± 0.05[a] | 0.48 ± 0.13[a] |
| **Storage length (months) (B)** | | | | | | | |
| 0 | | 33.83 ± 1.26[b] | 40.75 ± 2.32[b] | 104.73 ± 12.81[a] | 94.87 ± 12.05[a] | 0.42 ± 0.02[c] | 1.10 ± 0.08[a] |
| 2 | | 57.24 ± 3.94[a] | 37.85 ± 1.22[b] | 118.38 ± 9.28[a] | 58.22 ± 5.48[b] | 0.66 ± 0.02[b] | 0.18 ± 0.03[b] |
| 4 | | 54.16 ± 1.07[a] | 50.04 ± 1.50[a] | 127.23 ± 9.36[a] | 32.94 ± 5.64 [b] | 0.82 ± 0.03[a] | 0.26 ± 0.03[b] |
| **Application of $CaCl_2$ × Storage length (A × B)** | | | | | | | |
| $CaCl_2$ | 0 | 36.64 ± 0.21[a] | 45.82 ± 0.58[b] | 83.50 ± 7.02[c] | 97.65 ± 7.88[a] | 0.39 ± 0.03[a] | 2.29 ± 0.08[a] |
| | 2 | 53.58 ± 0.89[a] | 38.33 ± 0.78[c] | 138.09 ± 4.97[a] | 55.18 ± 8.76[a] | 0.66 ± 0.02[a] | 1.43 ± 0.05[a] |
| | 4 | 55.18 ± 1.78[a] | 47.08 ± 0.28[b] | 107.76 ± 5.06[bc] | 41.53 ± 8.62[a] | 0.85 ± 0.04[a] | 0.92 ± 0.23[a] |
| Control | 0 | 31.01 ± 0.16[a] | 35.67 ± 0.97[c] | 125.96 ± 17.89[ab] | 92.09 ± 25.62[a] | 0.45 ± 0.01[a] | 1.95 ± 0.12[a] |
| | 2 | 60.89 ± 7.96[a] | 37.37 ± 2.57[c] | 98.66 ± 4.14[bc] | 61.25 ± 8.03[a] | 0.65 ± 0.04[a] | 1.59 ± 1.44[a] |
| | 4 | 53.15 ± 1.22[a] | 52.99 ± 1.56[a] | 146.69 ± 5.83[a] | 24.34 ± 3.31[a] | 0.79 ± 0.06[a] | 1.33 ± 3.40[a] |
| **ANOVA** | | | | | | | |
| A | | ns | ns | ns | ns | ns | ns |
| B | | * | * | ns | * | * | * |
| A × B | | ns | * | * | ns | ns | ns |

TSS – total soluble solids; TA – titrable acidity; TS – total sugars; TS/TA – ratio between total sugars and total acids; TPC – total phenolic content; AA – antioxidant activity; ANOVA – analyses of variance

[a–d]Represent the means of four replicates, each represented by 40 fruits, ± standard error; different letters within the column indicate significant differences ($P \leq 0.05$) according to the Duncan test

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Table 5. Effect of preharvest $CaCl_2$ application and storage length on chemical composition of apple cultivar 'Red Chief'

| | | TSS (°Brix) | | TS (%) | | TA (%) | |
|---|---|---|---|---|---|---|---|
| | | 2018 | 2019 | 2018 | 2019 | 2018 | 2019 |
| **Application of $CaCl_2$ (A)** | | | | | | | |
| $CaCl_2$ | | 10.96 ± 0.39$^{b}$ | 15.96 ± 0.65$^{b}$ | 9.94 ± 0.47$^{b}$ | 12.39 ± 0.35$^{b}$ | 0.27 ± 0.02$^{a}$ | 0.28 ± 0.01$^{a}$ |
| Control | | 12.05 ± 0.39$^{a}$ | 16.36 ± 0.60$^{a}$ | 10.34 ± 0.45$^{a}$ | 13.39 ± 0.71$^{a}$ | 0.26 ± 0.01$^{b}$ | 0.27 ± 0.01$^{a}$ |
| **Storage length (months) (B)** | | | | | | | |
| 0 | | 9.81 ± 0.24$^{c}$ | 13.28 ± 0.17$^{c}$ | 8.58 ± 0.11$^{c}$ | 10.92 ± 0.17$^{c}$ | 0.31 ± 0.01$^{a}$ | 0.25 ± 0.01$^{b}$ |
| 2 | | 11.81 ± 0.20$^{b}$ | 17.90 ± 0.05$^{a}$ | 10.11 ± 0.14$^{b}$ | 13.33 ± 0.25$^{b}$ | 0.26 ± 0.01$^{b}$ | 0.28 ± 0.01$^{a}$ |
| 4 | | 12.89 ± 0.22$^{a}$ | 17.29 ± 0.15$^{b}$ | 11.74 ± 0.11$^{a}$ | 14.42 ± 0.53$^{a}$ | 0.23 ± 0.01$^{c}$ | 0.28 ± 0.01$^{a}$ |
| **Application of $CaCl_2$ × Storage length (A × B)** | | | | | | | |
| | 0 | 9.28 ± 0.24$^{a}$ | 12.96 ± 0.14$^{a}$ | 8.34 ± 0.03$^{a}$ | 11.08 ± 0.22$^{d}$ | 0.34 ± 0.01$^{a}$ | 0.26 ± 0.01$^{bc}$ |
| $CaCl_2$ | 2 | 11.29 ± 0.02$^{a}$ | 17.78 ± 0.04$^{a}$ | 9.88 ± 0.04$^{a}$ | 12.83 ± 0.22$^{c}$ | 0.25 ± 0.01$^{c}$ | 0.27 ± 0.01$^{b}$ |
| | 4 | 12.31 ± 0.07$^{a}$ | 14.12 ± 0.28$^{a}$ | 11.58 ± 0.00$^{a}$ | 13.27 ± 0.18$^{bc}$ | 0.24 ± 0.01$^{c}$ | 0.30 ± 0.18$^{a}$ |
| | 0 | 10.34 ± 0.12$^{a}$ | 13.60 ± 0.21$^{a}$ | 8.82 ± 0.03$^{a}$ | 10.76 ± 0.25$^{d}$ | 0.28 ± 0.01$^{b}$ | 0.25 ± 0.01$^{c}$ |
| Control | 2 | 12.33 ± 0.12$^{a}$ | 18.01 ± 0.05$^{a}$ | 10.33 ± 0.22$^{a}$ | 13.83 ± 0.07$^{b}$ | 0.27 ± 0.01$^{b}$ | 0.30 ± 0.01$^{a}$ |
| | 4 | 13.47 ± 0.08$^{a}$ | 17.46 ± 0.06$^{a}$ | 11.89 ± 0.18$^{a}$ | 15.58 ± 0.07$^{a}$ | 0.23 ± 0.01$^{c}$ | 0.26 ± 0.01$^{bc}$ |
| **ANOVA** | | | | | | | |
| A | | * | * | * | * | * | ns |
| B | | * | * | * | * | * | * |
| A × B | | ns | ns | ns | * | * | * |

| | | TS/TA | | TPC (mg GAE 100 g/FW) | | AA (μmol TE 100 g/FW) | |
|---|---|---|---|---|---|---|---|
| | | 2018 | 2019 | 2018 | 2019 | 2018 | 2019 |
| **Application of $CaCl_2$ (A)** | | | | | | | |
| $CaCl_2$ | | 38.22 ± 3.60$^{b}$ | 45.08 ± 1.07$^{b}$ | 174.33 ± 11.73$^{a}$ | 56.36 ± 9.89$^{a}$ | 0.82 ± 0.06$^{a}$ | 0.32 ± 0.05$^{a}$ |
| Control | | 40.91 ± 3.19$^{a}$ | 50.78 ± 2.97$^{a}$ | 173.65 ± 10.19$^{a}$ | 37.15 ± 5.11$^{a}$ | 0.84 ± 0.03$^{a}$ | 0.20 ± 0.04$^{b}$ |
| **Storage length (months) (B)** | | | | | | | |
| 0 | | 28.22 ± 1.52$^{c}$ | 43.32 ± 1.10$^{b}$ | 171.46 ± 9.62$^{a}$ | 56.96 ± 56.96$^{a}$ | 0.71 ± 0.04$^{b}$ | 0.15 ± 0.02$^{b}$ |
| 2 | | 39.39 ± 0.93$^{b}$ | 47.23 ± 0.89$^{b}$ | 176.26 ± 8.01$^{a}$ | 47.60 ± 7.11$^{a}$ | 0.96 ± 0.06$^{a}$ | 0.34 ± 0.08$^{a}$ |
| 4 | | 51.09 ± 1.07$^{a}$ | 53.25 ± 4.09$^{a}$ | 174.24 ± 12.54$^{a}$ | 35.72 ± 13.28$^{a}$ | 0.82 ± 0.03$^{b}$ | 0.29 ± 0.02$^{a}$ |
| **Application of $CaCl_2$ × Storage length (A × B)** | | | | | | | |
| | 0 | 24.89 ± 0.11$^{e}$ | 42.68 ± 1.78$^{b}$ | 157.81 ± 16.55$^{bc}$ | 61.25 ± 14.01$^{a}$ | 0.64 ± 0.03$^{c}$ | 0.16 ± 0.05$^{a}$ |
| $CaCl_2$ | 2 | 40.47 ± 1.58$^{c}$ | 47.58 ± 1.82$^{b}$ | 214.94 ± 10.81$^{a}$ | 65.80 ± 6.13$^{a}$ | 1.04 ± 0.05$^{a}$ | 0.48 ± 0.02$^{a}$ |
| | 4 | 49.29 ± 0.64$^{b}$ | 44.99 ± 1.06$^{b}$ | 150.23 ± 3.08$^{c}$ | 42.04 ± 27.93$^{a}$ | 0.78 ± 0.03$^{bc}$ | 0.32 ± 0.02$^{a}$ |
| | 0 | 31.53 ± 0.77$^{d}$ | 43.95 ± 1.55$^{b}$ | 185.11 ± 1.82$^{ab}$ | 52.66 ± 6.15$^{a}$ | 0.78 ± 0.05$^{bc}$ | 0.14 ± 0.02$^{a}$ |
| Control | 2 | 38.31 ± 0.84$^{c}$ | 46.89 ± 0.70$^{b}$ | 137.59 ± 5.06$^{c}$ | 29.40 ± 5.74$^{a}$ | 0.88 ± 0.08$^{b}$ | 0.20 ± 0.11$^{a}$ |
| | 4 | 52.88 ± 1.47$^{a}$ | 61.51 ± 3.78$^{a}$ | 198.26 ± 14.26$^{a}$ | 29.40 ± 7.88$^{a}$ | 0.86 ± 0.05$^{b}$ | 0.25 ± 0.03$^{a}$ |
| **ANOVA** | | | | | | | |
| A | | * | * | ns | ns | ns | * |
| B | | * | * | ns | ns | * | * |
| A × B | | * | * | * | ns | * | ns |

TSS – total soluble solids; TA – titrable acidity; TS – total sugars; TS/TA – ratio between total sugars and total acids; TPC – total phenolic content; AA – antioxidant activity; ANOVA – analyses of variance

$^{a-c}$Represent the means of four replicates, each represented by 40 fruits, ± standard error; different letters within the column indicate significant differences ($P$ ≤0.05) according to the Duncan test

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Table 6. Effect of preharvest $CaCl_2$ application and storage length on chemical composition of apple cultivar 'Granny Smith'

| | | TSS (°Brix) | | TS (%) | | TA (%) | |
|---|---|---|---|---|---|---|---|
| | | 2018 | 2019 | 2018 | 2019 | 2018 | 2019 |
| **Application of $CaCl_2$ (A)** | | | | | | | |
| $CaCl_2$ | | 11.55 ± 0.14$^a$ | 13.34 ± 0.17$^b$ | 10.45 ± 0.17$^a$ | 10.11 ± 0.35$^b$ | 0.55 ± 0.02$^a$ | 0.54 ± 0.02$^b$ |
| Control | | 11.87 ± 0.16$^a$ | 14.01 ± 0.21$^a$ | 10.33 ± 0.18$^a$ | 10.72 ± 0.23$^a$ | 0.52 ± 0.03$^a$ | 0.57 ± 0.03$^a$ |
| Storage length (months) (B) | | | | | | | |
| 0 | | 11.50 ± 0.11$^a$ | 13.51 ± 0.35$^b$ | 9.92 ± 0.19$^c$ | 9.93 ± 0.29$^b$ | 0.62 ± 0.02$^a$ | 0.62 ± 0.03$^a$ |
| 2 | | 11.97 ± 0.16$^a$ | 14.12 ± 0.18$^a$ | 10.89 ± 0.11$^a$ | 9.80 ± 0.20$^b$ | 0.52 ± 0.02$^b$ | 0.57 ± 0.01$^b$ |
| 4 | | 11.66 ± 0.25$^a$ | 13.39 ± 0.15$^b$ | 10.36 ± 0.10$^b$ | 11.51 ± 0.05$^a$ | 0.46 ± 0.01$^c$ | 0.48 ± 0.01$^c$ |
| **Application of $CaCl_2$ × Storage length (A × B)** | | | | | | | |
| $CaCl_2$ | 0 | 11.64 ± 0.19$^{ab}$ | 12.62 ± 0.08$^c$ | 10.20 ± 0.29$^b$ | 9.35 ± 0.06$^c$ | 0.62 ± 0.03$^a$ | 0.57 ± 0.01$^b$ |
| | 2 | 11.88 ± 0.30$^{ab}$ | 13.71 ± 0.14$^b$ | 11.01 ± 0.18$^a$ | 9.47 ± 0.13$^c$ | 0.55 ± 0.04$^a$ | 0.58 ± 0.00$^b$ |
| | 4 | 11.13 ± 0.03$^c$ | 13.70 ± 0.13$^b$ | 10.14 ± 0.03$^b$ | 11.51 ± 0.11$^a$ | 0.49 ± 0.01$^a$ | 0.47 ± 0.01$^c$ |
| Control | 0 | 11.36 ± 0.09$^{bc}$ | 14.41 ± 0.17$^a$ | 9.65 ± 0.11$^c$ | 10.52 ± 0.25$^b$ | 0.63 ± 0.01$^a$ | 0.67 ± 0.03$^a$ |
| | 2 | 12.05 ± 0.15$^a$ | 14.54 ± 0.12$^a$ | 10.77 ± 0.11$^a$ | 10.14 ± 0.25$^b$ | 0.49 ± 0.01$^a$ | 0.56 ± 0.01$^b$ |
| | 4 | 12.20 ± 0.33$^a$ | 13.08 ± 0.14$^c$ | 10.58 ± 0.07$^{ab}$ | 11.52 ± 0.04$^a$ | 0.44 ± 0.01 $^a$ | 0.49 ± 0.01$^c$ |
| **ANOVA** | | | | | | | |
| A | | ns | * | ns | * | ns | * |
| B | | ns | * | * | * | * | * |
| A × B | | * | * | * | * | ns | * |

| | | TS/TA | | TPC (mg GAE 100 g/FW) | | AA (μmol TE 100 g/FW) | |
|---|---|---|---|---|---|---|---|
| | | 2018 | 2019 | 2018 | 2019 | 2018 | 2019 |
| **Application of $CaCl_2$ (A)** | | | | | | | |
| $CaCl_2$ | | 19.22 ± 0.86$^a$ | 19.21 ± 1.39$^a$ | 213.59 ± 21.99$^a$ | 88.89 ± 6.22$^b$ | 0.83 ± 0.04$^a$ | 0.54 ± 0.06$^a$ |
| Control | | 20.58 ± 0.35$^a$ | 19.29 ± 0.12$^a$ | 176.52 ± 15.43$^b$ | 195.39 ± 12.09$^a$ | 0.78 ± 0.03$^a$ | 0.65 ± 0.26$^a$ |
| **Storage length (months) (B)** | | | | | | | |
| 0 | | 15.97 ± 0.33$^b$ | 16.20 ± 0.23$^c$ | 246.03 ± 18.85$^a$ | 163.12 ± 25.82$^a$ | 0.85 ± 0.03$^a$ | 0.46 ± 0.03$^a$ |
| 2 | | 22.23 ± 0.92$^a$ | 17.30 ± 0.16$^b$ | 192.95 ± 22.38$^b$ | 147.70 ± 29.81$^a$ | 0.84 ± 0.05$^a$ | 0.39 ± 0.34$^a$ |
| 4 | | 22.51 ± 0.83$^a$ | 18.82 ± 0.08$^a$ | 146.18 ± 9.919$^c$ | 115.60 ± 18.85$^b$ | 0.72 ± .0.04$^a$ | 0.95 ± 0.11$^a$ |
| **Application of $CaCl_2$ × Storage length (A × B)** | | | | | | | |
| $CaCl_2$ | 0 | 16.50 ± 0.46$^c$ | 16.54 ± 0.19$^c$ | 270.04 ± 14.16$^a$ | 110.29 ± 7.45$^a$ | 0.91 ± 0.04$^a$ | 0.53 ± 0.01$^a$ |
| | 2 | 20.45 ± 1.75$^b$ | 16.33 ± 0.23$^c$ | 241.23 ± 6.57$^a$ | 86.47 ± 6.33$^a$ | 0.86 ± 0.06$^a$ | 0.39 ± 0.07$^a$ |
| | 4 | 20.69 ± 0.17$^b$ | 24.76 ± 0.40$^a$ | 129.50 ± 7.17$^b$ | 74.90 ± 3.82$^a$ | 0.71 ± 0.08$^a$ | 0.71 ± 0.09$^a$ |
| Control | 0 | 15.43 ± 0.24$^c$ | 15.52 ± 0.38$^c$ | 222.02 ± 31.61$^a$ | 215.95 ± 22.10$^a$ | 0.79 ± 0.12$^a$ | 0.38 ± 0.09$^a$ |
| | 2 | 22.01 ± 0.74$^{ab}$ | 18.27 ± 0.25$^b$ | 144.67 ± 11.38$^b$ | 213.93 ± 3.95$^a$ | 0.82 ± 0.08$^a$ | 0.39 ± 0.76$^a$ |
| | 4 | 24.31 ± 0.33$^a$ | 23.75 ± 0.35$^a$ | 162.87 ± 9.63$^b$ | 156.30 ± 10.26$^a$ | 0.72 ± 0.03$^a$ | 1.19 ± 0.03$^a$ |
| ANOVA | | | | | | | |
| A | | ns | ns | * | * | ns | ns |
| B | | * | * | * | * | ns | ns |
| A × B | | * | * | * | ns | ns | ns |

TSS – total soluble solids; TA – titrable acidity; TS – total sugars; TS/TA – ratio between total sugars and total acids; TPC – total phenolic content; AA – antioxidant activity; ANOVA – analyses of variance

$^{a–c}$represent the means of four replicates, each represented by 40 fruits, ± standard error; different letters within the column indicate significant differences ($P$ ≤ 0.05) according to the Duncan test

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sumers, are ≥ 11.0%, while values > 13.5%, guarantee safe placement of apple fruit. The apples contain different level of soluble solids at harvest time depending on cultivar, growing season and growing technology (Shewa et al. 2022). The differences between investigated cultivars, as well as differences between individual growing seasons of the same cultivar in present study were found. The same tendency of the content of total sugars was also evident, which can be explained by the fact that the sugars (sucrose, glucose and fructose) and sugar alcohols (e.g. sorbitol and manitol) constitute the majority of total soluble solids (Magwaza, Opara 2015). Our results showed a positive influence of foliar application of $CaCl_2$ on the TSS and TS at harvest only in the 'Golden Delicious Reinders®'. In respect to impact of $CaCl_2$ on TSS levels, different findings have been published (Casero et al. 2009; Wojcik et al. 2010; Farag, Nagy 2012; Ghorbani et al. 2021). Ghorbani et al. (2021) reported that $CaCl_2$ negatively affects the SSC TSS in 'Granny Smith' apples and explained that reason for this can be that calcium decreases respiration and metabolic activity and delay the process of fruit ripening, which is consistent with the results of our research. Also, a decreasing trend of the TSS and TS during cold storage in the 'Golden Delicious Reinders®' was observed. The similar trend for the same cultivar was refereed by Díaz et al. (2019). Ahmad et al. (2021) described that the initial decrease of TSS can be attributed to the conversion of starch to the sugars. An increasing trend of TSS and TS during storage was observed in 'Red Chief' apples, while TSS and TS in 'Granny Smith' fruits firstly increased and then decreased. Similar results were published by Cheng et al. (2018) and were explained by weight loss and conversion of starch to monosaccharides (Ahmad et al. 2021). Also, the cited authors reported a trend of decreasing content of TA during storage, which is in accordance with our results. The increasing trend of the ratio between TS and TA during storage was determined, while the positive influence of foliar $CaCl_2$ application on this parameter was not confirmed in the present study. It is known that the content of different health-promoting compounds of apples depends on the cultivar (Mitić et al. 2013), growing technology (Fotirić-Akšić et al. 2022) and cold storage technique (Korićanac et al. 2019). The results of our studies showed variations in terms of the TPC and the AA of the fruit, both among cultivars and among years. Despite the fact that the content of total phenols decreased during storage in both experimental years in fruits of all examined cultivars regardless of treatment, 'Red Chief' and 'Granny Smith' remained with high total phenols content during most of storage periods in 2018 contrary to 2019, when these values were lower. In accordance with significantly higher TPC, antioxidant capacity was higher in 2018 compared to 2019. Different values of the mentioned parameters were determined for the same cultivar in certain years of study, which is in agreement with Matthes and Schmitz-Eiberger (2008) who reported that the antioxidant activity of apple fruit is affected by environmental conditions. On the other hand, van der Sluis et al. (2001) found that long-term storage, both at refrigerator temperature and under controlled atmosphere conditions do not influence flavonoid concentration or antioxidant activity in apple cultivars ('Jonagold', 'Golden Delicious', 'Cox's Orange', and 'Elstar') in three different harvest years unlike our study where AA of all cultivars was significantly influenced by the storage period with significantly higher values after two and four months of storage.

## CONCLUSION

Apple fruits are generally stored for certain periods at low temperatures to ensure market supply (with fruits) for as long as possible. Changes in the physical characteristics of the fruit as well as the loss of primary (sugars and acids) and secondary (phenols) metabolites in our study was expected in all samples after harvest, but particularly in those stored for two and four months in RA. It was concluded that foliar application of $CaCl_2$ improved firmness of the apple fruit compared to control in both years. Also, the mentioned treatment had a positive effect on the fruit weight in cultivars 'Golden Delicious Reinders®' and 'Red Chief' as well as on prevention of weight loss during storage in cultivar 'Golden Delicious Reinders®' in 2019, which had generally the lowest weight loss among tested cultivars in both years. Less weight loss in foliar application of $CaCl_2$ was also confirmed in the cultivar 'Red Chief' after two months of cold storage.

Results showed that soluble solids and total sugars content increased in fruit of 'Golden Delicious

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Reinders®' treated with $CaCl_2$ as compared to control fruit in both experimental years. Also, the increasing trend of TSS, TS and TS/TA in apple cultivar 'Red Chief' in $CaCl_2$ application and control with storage progress, in both experimental years was recorded.

Phenolic content increased in cultivar 'Granny Smith' during storage, but this increase was significantly affected by the interaction of variability factors ($CaCl_2$ application × storage length). Moreover, the highest value of total phenols was recorded in the $CaCl_2$ application of fresh and two-month-stored fruits of the cultivar 'Granny Smith' in the first year of the study. The antioxidant capacity of the cultivars 'Golden Delicious Reinders®' and 'Red Chief' was significantly affected by the storage length and increasing values by prolonging storage time were recorded.

In conclusion, pre-harvest $CaCl_2$ application in cultivars 'Golden Delicious Reinders®' and 'Red Chief' had a significant effect on apple fruit quality preservation and storability, while the extended cold storage period enhanced the nutritional composition of these cultivars.

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