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[1]Fruit Research Institute Cacak, Department for Technology of Fruit Growing, Cacak, Serbia
[2]University of Belgrade, Faculty of Agriculture, Belgrade-Zemun, Serbia

Application of 1-methylcyclopropene in fruit of five apple cultivars grown in Serbia

M. Milinkovic[1*], B. Lalevic[2], V. Raicević[2], S.M. Paunovic[1]

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Summary

Fruits of five apple cultivars were treated using 1-methylcyclopropene (1-MCP or SmartFresh™) after cropping and were stored at normal atmosphere 2 ± 0.5 °C, 90 ± 5% relative humidity (RH) and 20.9 kPa O_2 + <0.5 kPa CO_2. Fruit firmness was assessed at three periods: 7 d after storing, 120 d after storing and 30 d after the second assessment and storing at room temperature. Contents of K in all of the cultivars and in all years of study varied within the average values between 1390.5 and 2028.0 mg kg^{-1}, while the Ca content varied between 21.7 and 59.5 mg kg^{-1}. The K:Ca ratio was the lowest in cultivar 'Granny Smith' (24.0) and the highest in 'Redchief' (99.1). Application of 1-MCP made the strongest impact on fruit firmness of the cultivars 'Granny Smith' and 'Idared' in all measuring periods. Cultivars 'Redchief', 'Čadel' and 'Morrens Ionagored' responded well to the application of 1-MCP in the storage conditions, whereas the effect of its application influenced conservability of the fruits stored at room temperature except in fruits of the cultivar 'Morens Jonagored'. Application of 1-MCP made an important effect on the preservation of fruit firmness, all in accordance with the degree of ripeness of the fruits subjected to the treatment and the contents of K, Ca and K:Ca ratio. This study indicates that the use of 1-MCP treatment in post harvest handling of apples is promising for maintaining the freshness and quality of fruits.

Key words: apple, degree of ripeness, firmness of fruits, storage quality, 1-methylcyclopropene.

Introduction

As one of the world's most commonly cultivated crops, apple is undoubtedly a top global fruit (SHEFFIELD et al., 2016). Apple is nowadays the most commonly grown and the most significant cultivar in the world's temperate areas (ZOHARY and HOPF, 2000; CORNILLE et al., 2012). As the most important pome fruit species, it covers the surface of 23,737 ha in Serbia coming right after plum in the respective area (KESEROVIC and MAGAZIN, 2014). Different assortments and varied fruits storing conditions are an increasingly important topic for the fruit growers, especially from the aspect of preserving the quality of the fruits intended for sale in the market several months after the harvest, when they reach a higher price. For fresh fruit consumption, harvest maturity is determined differently depending on species, cultivar, storage conditions, remoteness of consumers, etc. Choosing apple cultivars – with long-term preservation characteristics and resistance to transportation – is as important as any other work or process carried out in the orchard before harvesting or during handling and storing of fruits after harvest (MOLINA et al., 2006; PINEIRO and LUZ, 2007). Along the fruit supply chain from farm to fork, the intrinsic quality of fruits is becoming increasingly important, with external colour, fruit size and fruit firmness being the dominant factors in consumer acceptance of apple. The storability of apples depends, to a great extent, on the harvest date (SZALAY et al., 2013). Starch hydrolysis begins at the end of the fruit development process, around 2 to 3 weeks before the start of ethylene production LAU (1985). A close correlation has been found between the rate of starch degradation and the ethylene production (TOMALA and PIESTRZENIEWICZ, 1998). Harvest date in different maturity periods is determined using iodine-starch test index (PASHAZADEH et al., 2017). Quality of apple changes during storage and thus, substantially affects consumer acceptability (VIEIRA et al., 2009). At first instance, it is judged by appearance comprising colour, gloss, size and secondly by texture, total soluble solids (TSS) content and/or titratable acidity. Dehydration reduces the weight and quality of fruits affecting both organoleptic characteristics and appearance of fruits as it comes to skin traction. The loss of fruit weight occurs as a result of respiration and combustion of organic materials such as sugars (HAJNAJARI et al., 2010). Fruits harvested later than at optimum harvesting stage contain heightened values of TSS (KVIKLIENE and VALIUŠKAITE, 2009). TSS value points to the amount of converted starch into sugars, which may serve as ripe index (TROMP et al., 2005). Optimum nutrition of fruits provides balance in the composition of mineral substances in fruits. Calcium is believed to be the most important element to determine the conservability of apple fruits as it affects the reduction of physiological disorders: fruit firmness and water content, skin traction, ethylene production and other physiological disorders (CONWAY et al., 2002). Content of calcium (Ca) and its relation with potassium is also important for the conservability of apple (LANAUSKAS and KVIKLIENE, 2006).

Storage of apples is mainly carried out in the conditions of cooling and additionally under controlled atmosphere (CA) to delay ripening and provide longer shelf-life (BEKELE et al., 2015). Such system of storing apples requires cooling in a controlled atmosphere with partial pressure and an increased concentration of carbon dioxide. Application of cooling systems with controlled atmosphere (CA) and the treatment of apple fruits with 1-methylcyclopropene (1-MCP) contributes to preserving the fruit quality after harvest. Preservation of fruit firmness after storage is one of the most important quality parameters. VALERO and SERRANO (2010) have stated that fruit softening is closely related to ethylene. The application of 1-MCP inhibits the maturation of fruits by blocking ethylene receptors (GWANPUA et al., 2017). It has been found that the inhibition of ethylene production, with 1-MCP causes changes in the quality parameters such as colour, strength, and weight loss (POYESH et al., 2017). Incorrect storage conditions can lead to storage disorders and loss of quality, which can make entire batches unsuitable for consumption (THOMPSON, 1998).

Considering that in our country the predominant method for storing apple are normal atmosphere conditions with cooling, the aim of this study is to determine the impact made by application of 1-MCP on fruit firmness from different assortment in the stated storing conditions.

Materials and methods

Plant material

Apple fruits were collected from the plantations established in 2007 plantations, grafted on M-9 rootstock at the experimental plantation

* Corresponding author

of the Fruit Research Institute in Cacak in the village Donja Trepča (Serbia), (N 43° 53''63.4'', E 20° 26' 07.6'', 232 m), under conditions of temperate continental climate, during 2015 and 2016. The study included the following cultivars: 'Red Chief', 'Morrens Jonagored', 'Čadel', 'Idared' and 'Granny Smith'. The fruits of the assessed cultivars were harvested at two dates: the first was in September 16th and the second was in October 22nd, in both of the trial years. While the fruits harvested during the first date were subjected to iodine-starch test (SZALAY, 2013) and measurement of total soluble solids, the fruits collected in the second harvest period were used for chemical analyses, measuring of the ethylene levels, determination of fruit firmness and treatment with 1-methylcyclopropene (1-MCP).

Climatic factors

Perennial average air temperature for the period 1997-2016 in the experimental field was 12.5 °C. In the first year of study in 2015, the annual average temperature was 12.8 °C, with the average highest mean temperatures in July, 23.6 °C and 22.4 °C in August. In 2016, the average annual air temperature was 11.3 °C, with the average high temperature of air in June (20.7 °C), compared to the same month of 2015 (19.3 °C), while in other months the average temperature was lower compared to 2015. Fig. 1 shows average monthly air temperatures for several years, average score and period of investigation.
The average amount of rainfall (1997-2016) was 672.77 mm m^{-2}, the highest values of precipitations in the months of June or July (76.96-79.03 mm m^{-2}). The total rainfall in 2015 amounted to 432.3 mm m^{-2}, with the highest rainfall in June, 86.0 mm m^{-2}. In the second year of study (2016), > 100 mm m^{-2}, it was noted in March, May, August and October, which contributed to the amount of rainfall to be 789.6 mm m^{-2} (Fig. 2).

1-methylcyclopropene (1-MCP) treatment

Apple fruits of all the examined cultivars of even shape (of 70-85 mm diameter depending on the cultivar) and with no visible mechanical damage were selected for the purposes of the experiment, to include 30 fruits in three replications for each cultivar, whereas an equal number of samples were set aside in the cold storage, to serve as the control variant. After selecting the samples for the analysis, the fruits were placed inside the cold storage with normal atmosphere (NA) for 2 days, at the temperature of 10 °C. Fruits selected for treatment were arranged into crates, which were then placed into plastic bags of 1 m^3 volume. Treating the fruits with the agent acting as an ethylene blocker was performed by dissolving three tablets of 1-MCP (pink SmartFresh^{TM} research tablets) with a single activator tablet (blue activator tablets) in 20±0.5 ml of citric acid (20 °C). After diluting 1-MCP (SmartFresh^{TM} protab), the plastic bag was sealed airtight for the next 24 hours, until the gas concentration of 937 ppb was achieved. A small battery-drive air fan was attached to the jar, for securing a better distribution of 1-MCP gas inside the plastic bag. After this, the bag was removed and the fruits were kept in a cold storage for the next 120 days.

Fruit storage and sampling

All of the replications of the fruits selected for treatment, as well as the ones that were not subjected to the 1-MCP treatment, were placed in wooden crates and kept in vertical tiers. The fruits were kept in cold atmosphere: (2 ± 0.5) °C, (90 ± 5) % relative humidity (20.9kPa 0_2 + <0.5kPa CO_2), over a period of 120 days. In order to determine fruit firmness, the sampling of both treated and non-treated apple fruits was conducted at three periods: 7 days and 120 days following the application of 1-MCP on fruits kept in the cold storage and 30 days following the 2nd measurement. After taking the samples from the cold storage, the fruits were left in conditions of normal atmosphere: 21 ± 1 °C and 60 ± 5% RH, for 2 hours. Fruits for the 3rd measurement were kept in the same conditions of at room temperature, over a period of 30 days. The fruit measuring was performed using the penetro-meter Fruit Pressure Tester FT 327 (Winopal Forschungsbedarf GmbH, Germany) with a 11-mm probe. Two measurements were made

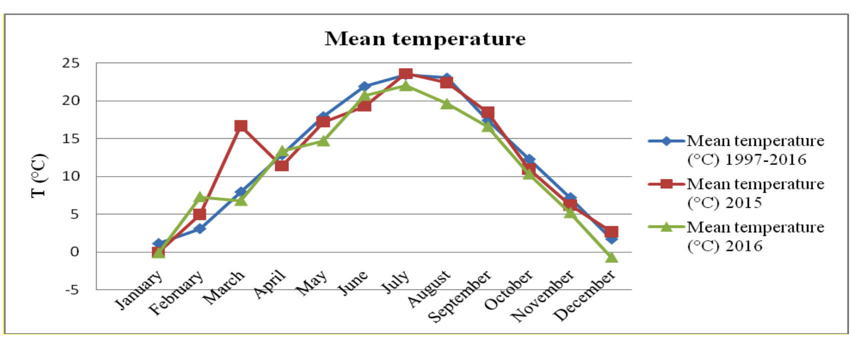

Fig. 1: Average monthly air temperature for the period 1997-2016, 2015 and 2016 (°C)

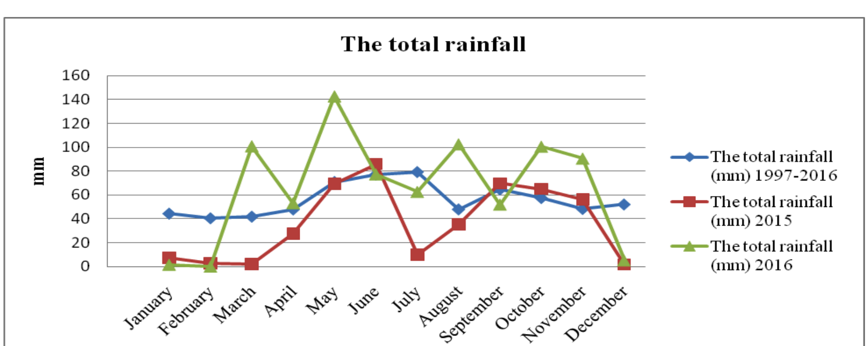

Fig. 2: Average rainfall for the period 1997-2016, 2015 and in 2016 (mm m^{-2})

in six fruits per each replication on both sides expressed in Newton (N/cm^2) = fruit firmness value (kg/cm^2) × 9.81.

Assessments

The starch index (SI) was evaluated using the starch iodine test using the solution of 1% iodine flakes and 4% potassium iodide. Five fruits each were picked from five trees of each cultivar (25 fruits/ per cultivar). The fruits were cut into equatorial halves and left for 10 seconds to soak in the solution after which dried for at least 5-10 min with surface up in the air or face down. Determining, reading the starch-iodine test and calculating the index was performed according to the (Code Amidon, Ctifl, 2002). Total soluble solids (TSS) were determined using a hand refractometer (0 to 32%), Carl Zeiss 3828, Germany. Chemical analysis of the fruits included: the analysis of ethylene content $\mu L\ L^{-1}$ and determination of calcium (Ca), potassium (K) content and the K:Ca ratio. Sampling of fruits for the ethylene content analysis was performed before the SmartFresh^{tm} treatment and 24 h after treating the fruits, which included six apple fruits per cultivar. Both treated and non-treated (control) fruits were kept for 7 days at room temperature before individual samples were laid for 6 hours in the airtight vessel of 3.7 L volume, in order to measure the ethylene production using the ICA 56 device. The content of macro-elements was determined before the experiment setup, by sampling 2 kg of average samples in three replications, for each cultivar. The macro-elements content and the K:Ca ratio were determined using the following methods: K (by atomic absorption spectrophotometry-emission) and Ca (by atomic absorption spectrophotometry-absorbance), whereas K:Ca ratio was determined using calculation. In addition to measuring apple fruit firmness, the occurrence of scald and fruit rot was also monitored, after each sampling for fruit firmness and storing at room temperature.

Statistical analysis

The data was subjected to analysis of variance (*ANOVA*) using statistical package MSTAT-C (Michigan State University, USA). The least significance difference (LSD) was used to compare treatment means and treatments declared different at $p = 0.05$ level of significance.

Results and discussion

Fruit ripeness degree

Determination of the ripeness degree in apple fruits using the iodine starch method is based on the hydrolysis of starch into sugar, which takes place with the gradual ripening of the fruits. The ripening process starts from the core (inner parts) of the fruits and progresses towards the perimeter. The process may vary in length, depending on the cultivar-specific characteristics. There are different optimum maturity levels for the same cultivars, depending on the intended use and storage life desired. The maturity level of fruits in the first and second measurement time period was substantially greater in 2016 than in 2015, which was determined by the comparative analysis (AXB) of measurement date and the study year on five tested apple cultivars. Ripeness degree was a major factor in the impact of 1-MCP application, as a cultivar-specific characteristic in combination with climatic conditions (Tab. 1). If the fruits were harvested when overmature, problems with fruit drop would appear and apples could no longer be used for long-term storage as this could lead to a soft mealy texture, off flavours and greasy skin (Little and Holmes, 2000). Although on-tree softening was slow, different harvest dates resulted in different initial firmness values affecting fruit quality throughout the whole postharvest life.

The contents of total soluble solids Tab. 2 showed significant differences between two different measurements in all of the tested cultivars, whereas the impact of the year of trial was of a statistical importance only in the cultivar ‘Idared’. The established TSS values were slightly higher, compared to the research (Jha et al., 2012). According to the results shown in Soska and Tomala (2006), in addition to the impact made by the cultivar-specific characteristics, the TSS variations were also under the influence of the climate, which was established by our research in the cultivar ‘Idared’ (Fig. 1, 2).

In their investigations, Skic et al. (2016) have indicated that the choice of optimum fruit maturity is determined by several parameters: Total Soluble Solid Content (TSSC), Starch Index (SI) Firmness (F) and others. This methodology has an indestructible character in comparison with chemical analysis and allows farmers to determine their harvest time and storage using alternative methods. It has been found that there is a correlation between the examined parameters of fruit maturity. Juhņeviča-Radenkova and Radenkovs (2016) have found that harvest time impacts physical and chemical properties of apples stored after harvest. Furthermore, it has been found that after 6 months of storage, apples are sweeter and more aromatic preserving the juiciness and acidity.

The plant hormone, ethylene, produced during metabolism of fruits is mainly responsible for intensifying ripening and consequently, reducing the shelf life of fruits (Watkins, 2006). Based on the results shown in Tab. 3, there is a statistically significant difference in the ethylene content between the apple fruits treated with 1-MCP and those not treated, in all of the examined cultivars. Statistically, differences in the ethylene content in different years of trial were

Tab. 1: Values the starch index (SI) on a different assortment of apples in periods.

Factor	Measurements	‘Red Chief’	‘Morrens Jonagored’	‘Čadel’	‘Idared’	‘Granny Smith’
A	1st measurement	2.10±0.19b	4.95±0.49b	5.55±0.37b	2.48±0.26b	2.48±0.22b
	2nd measurement	6.55±0.35a	6.75±0.32a	7.05±0.25a	5.00±0.19a	5.15±0.43a
B	2015	3.30±0.44b	4.20±0.35b	5.05±0.29b	3.10±0.35b	2.50±0.22b
	2016	5.35±0.61a	7.50±0.14a	7.56±0.11a	4.38±0.32a	5.13±0.44a
A × B	1st measurement 2015	1.50±0.22d	2.90±0.23d	4.00±0.21d	1.70±0.21d	1.70±0.21c
	1st measurement 2016	2.70±0.15c	7.00±0.15b	7.10±0.10b	3.25±0.31c	3.25±0.13b
	2nd measurement 2015	5.10±0.23b	5.50±0.31c	6.10±0.23c	4.50±0.22b	3.30±0.15b
	2nd measurement 2016	8.00±0.00a	8.00±0.00a	8.00±0.00a	5.50±0.22a	7.00±0.00a
	ANOVA					
	A	*	*	*	*	*
	B	*	*	*	*	*
	A × B	*	*	*	*	*

Note: Means with different letters are significantly different (Fisher’s LSD, $P = 0.05$); * – statistically significant.

Tab. 2: Content of total soluble solids (TSS %) at different measuring periods on a different assortment of apples in two measurement periods.

Factor	Measurements	'Red Chief'	'Morrens Jonagored'	'Čadel'	'Idared'	'Granny Smith'
A	1st measurement	12.59±0.17b	12.81±0.26b	12.86±0.14b	10.99±0.12b	12.18±0.36b
	2nd measurement	15.21±0.15a	13.96±0.20a	14.10±0.21a	12.94±0.26a	14.53±0.12a
B	2015	14.10±0.29a	13.18±0.34a	13.66±0.27a	12.30±0.37a	13.30±0.35a
	2016	13.70±0.38a	13.59±0.15a	13.30±0.16a	11.63±0.19b	13.41±0.41a
A × B	1st measurement 2015	12.99±0.21b	11.86±0.21c	12.68±0.20c	10.94±0.19c	11.97±0.28b
	1st measurement 2016	12.18±0.22c	13.76±0.23b	13.04±0.18bc	11.03±0.15c	12.38±0.68b
	2nd measurement 2015	15.21±0.21a	14.50±0.25a	14.63±0.22a	13.66±0.36a	14.62±0.19a
	2nd measurement 2016	15.21±0.21a	13.42±0.19b	13.56±0.26b	12.22±0.21b	14.43±0.16a
	ANOVA					
	A	*	*	*	*	*
	B	ns	ns	ns	*	ns
	A × B	*	*	*	*	ns

Note: Means with different letters are significantly different (Fisher's LSD, $P = 0.05$); ns – not significant, * – statistically significant.

Tab. 3: Content ($\mu L\ L^{-1}$) of ethylene in apple fruits prior to and following 1-methylcyclopropene (1-MCP) application.

Factor	Treatments	'Red Chief'	'Morrens Jonagored'	'Čadel'	'Idared'	'Granny Smith'
A	Control	46.45±0.10a	58.95±2.77a	14.20±0.98a	43.00±3.31a	33.60±3.41a
	1-MCP	12.15±4.58b	12.25±4.89b	2.30±0.38b	16.95±2.44b	8.90±3.66b
B	2015	24.80±10.08b	30.80±13.35b	8.40±2.95a	31.20±8.15a	13.85±5.84b
	2016	33.80±5.47a	40.40±7.89a	8.10±2.56a	28.75±4.22a	28.65±5.48a
A × B	Control 2015	47.30±1.45a	60.20±5.16a	14.80±1.48a	49.20±2.57a	26.80±1.66b
	Control 2016	45.60±1.46a	57.70±3.20a	13.60±1.51a	36.80±3.12b	40.40±3.02a
	1-MCP 2015	2.30±0.21c	1.40±0.26c	2.00±0.67b	13.20±1.14c	0.90±0.15d
	1-MCP 2016	22.00±2.83b	23.10±1.27b	2.60±0.44b	20.70±3.79c	16.90±1.76c
	ANOVA					
	A	*	*	*	*	*
	B	*	*	ns	ns	*
	A × B	*	*	ns	*	*

Note: Means with different letters are significantly different (Fisher's LSD, $P = 0.05$); ns – not significant, * – statistically significant.

significantly different in cultivars 'Red Chief', 'Morrens Jonagored' and 'Granny Smith'. Different correlations between the optimal time of harvest and the ethylene production rate can be found depending on the cultivar (Watkins, 1989). A lot of effort has been invested in profiling the changes in ethylene biosynthesis and quality in apple dependent on: harvesting (Lin and Walsh, 2008), harvest maturity (Johnston, 2002), application of chilling conditions and re-warming (Lara and Vendrell, 2003; Vilaplana et al., 2007), or shelf-life conditions (Xuan and Streif, 2005; Dal, 2007). By treating apple fruits with 1-MCP, ethylene biosynthesis is almost completely suppressed during the storage period, except when fruits are harvested beyond optimal deadline, i.e. at late harvest, because such fruits get the ability to form ethylene in two weeks (Bulens at al., 2012). In their study, Valero and Serrano (2010) found a decrease in the production of ethylene in a large number of cultivars. The results obtained correspond to our research results.

Content of K, Ca and K:Ca ratio

Chemical composition of fruits is determined by a number of both ecological and genetic factors. Fruits with low Ca and high K content, and consequently, a high K:Ca ratio, are susceptible to bitter pit occurrence. Moreover, development of this disorder is also influenced by storage conditions and the activity of the enzymes involved in fruit respiration (Wińska-Krysiak and Łata, 2010).

Potassium (K) is often considered to be detrimental to fruit quality through interference with the uptake and utilization of Ca. However, over-emphasis on this issue can sometimes lead to K deficiency, particularly in high-density production systems on coarse-textured soils, or wherever soils are naturally low in K, due to the parent material or presence of K-fixing clay minerals (Neilsen and Neilsen, 2009). K content was manifested depending on the cultivar, whereas the differences in the content relative to the year of study were significant in all of the tested cultivars except cultivar 'Granny Smith'. On average, the lowest K content (1390.5 mg kg^{-1}) was observed in the 'Granny Smith', whereas the highest K content (2028.0 mg kg^{-1}) was observed in 'Morrens Jonagored' (Tab. 4).

Calcium (Ca) is a mineral nutrient, which has been most highly implicated in the quality of fruits, particularly with respect to disorders, which affect storage. Unlike the content of K, presence of Ca in the trial years revealed differences in cultivars 'Red Chief', 'Čadel' and 'Idared'. The highest average value of Ca was recorded in the cultivar 'Granny Smith', whereas the lowest average Ca value was observed in 'Red Chief', with all the other cultivars recording similar values of this parameter, in the range 39.4 to 43.4 mg kg^{-1}. In their manuscript, Lu et al. (2015) have found that application of larger quantities of K after flowering stage leads to decreased quantities of Ca in fruits with negative influence on the fruit quality.

Tab. 4: Content (mg kg^{-1}) of potassium (K) in fruits of different apple cultivars.

Factor	Measurements	'Red Chief'	'Morrens Jonagored'	'Čadel'	'Idared'	'Granny Smith'
A	2015	1783.0±19.08a	2082.0±7.55a	1860.0±14.29a	1659.0±18.88a	1450.0±52.54a
	2016	1442.3±109.44b	1974.0±25.38b	1741.7±33.46b	1523.3±20.28b	1331.0±49.34a
	average	1612.5	2028.0	1800.9	1591.2	1390.5
	ANOVA	*	*	*	*	ns

Note: Means with different letters are significantly different (Fisher's LSD, $P = 0.05$); ns – not significant, * – statistically significant.

The K:Ca ratio did not reveal significant differences in the tested cultivars in different years of the study. The average values of the K:Ca ratio were in the range from 24.0 in 'Granny Smith' to 99.1 in 'Red Chief'(Tab. 6). Wińska-Krysiak and Łata (2010) found that the content of Ca in fruits differed depending on the cultivar, whereas the content of K was identical in both sampling periods. The same authors established that the mean of the K:Ca ratio was 33.2, which was a lower value than the one observed in our study (Tab. 6).

Comparing the research results with other authors, in their work, Demuth and Sundrud (2012) have reported that if the calcium content in apple is greater than 30 mg kg^{-1} then it is unlikely for physiological disorders to be developed, and if the content is lower than 19 mg kg^{-1}, the greater the chance of the incidence of bitter pit and other physiological disorders. For values between 19 to 30 index mg kg^{-1}, the content of Mg and K is important as well as their relationship with Ca. The aforementioned authors have also found the most common concentrations of cations in the fruits of apple, for calcium 10 to 50 mg kg^{-1} and potassium 500 to 1500 mg kg^{-1}. In their study, they have found that in different apple cultivars the content of calcium ranges 28-110 mg kg^{-1}, whereas potassium content is 690 to 1200 mg kg^{-1}. The most common values of Ca content are 10-50 mg kg^{-1}. In our studies, the average content of K is 1390.5 to 2028.0 mg kg^{-1}, Ca 21.7 to 59.5 mg kg^{-1}, and K: Ca 24.0 to 99.1. Most of the tested cultivars have increased values of K and average values of Ca in accordance with aforementioned authors. The difference exists only in the cultivar 'Granny Smith'. In the investigations by Piestrzenievicz and Tomala (2001), it has been confirmed that when ratio K:Ca in ripe 'Jonagold' fruits does not exceed 28. Values of the content of certain ions in apple fruits depend on the cultivar and genetic resources.

Firmness of apple fruits

Firmness of fruits of different apple cultivars was significantly different at all different measurement periods (Tab. 7). The highest value of the initial fruit firmness was recorded in 'Granny Smith', whereas the cultivars 'Red Chief', 'Čadel' and 'Idared'showed no statistically significant difference of this parameter. During the different measuring periods, including the final measurement, the largest firmness of fruits was established in the cultivar 'Granny Smith', compared to the initial measurement. In South Tyrol (Italy), a region that grows approximately 1/10 of European apples, these methods are therefore more commonly used on cultivars susceptible to superficial scald approx. 30% of the whole production – respectively distributed among the cultivars 'Red Delicious', 'Granny Smith', 'Rome Beauty', 'Fuji', 'Winesape' and 'Cripps Pink', than on those resistant to this post-harvest disorder (Zanella and Stürz, 2013), where the advantage could be the improved firmness. While ripening on-tree, 'Jonagold' apples softened slowly (0.5 N/day), but during shelf-life after extended storage the rate of firmness loss was much higher (1.07 N/day).

Application of 1-MCP resulted in increased fruit firmness, compared to non-treated fruits. The fruit firmness showed significant differences in the different years of the trial, with the exception of the non-treated fruits from the 1st measurement period, and the 3rd measurement period of all the examined cultivars. Applying the 1-MCP treatment, fruit firmness losses during storage can be reduced (DeEll et al., 2007; Hoehn et al., 2008). The extent to which these losses may be reduced varies in relation to several factors, such as cultivar, ripening stage, storage conditions, temperatures at application, timing of application, and duration of application (DeEll et al., 2002; Blankenship and Dole, 2003). Different harvest dates resulted in

Tab. 5: Content (mg kg^{-1}) of calcium (Ca) in apple fruits.

Factor	Measurements	'Red Chief'	'Morrens Jonagored'	'Čadel'	'Idared'	'Granny Smith'
A	2015	28.0±8.74a	49.0±4.36a	48.0±3.46a	47.0±2.31a	57.0±6.24a
	2016	15.3±5.04b	37.7±2.40a	30.7±2.96b	34.3±2.96b	62.0±5.51a
	average	21.7	43.4	39.4	40.7	59.5
	ANOVA	*	ns	*	*	ns

Note: Means with different letters are significantly different (Fisher's LSD, $P = 0.05$); ns – not significant, * – statistically significant.

Tab. 6: Potassium and calcium (K:Ca) ratio in apple fruits.

Factor	Measurements	'Red Chief'	'Morrens Jonagored'	'Čadel'	'Idared'	'Granny Smith'
A	2015	87.3±38.47a	43.2±3.76a	39.2±2.96a	35.5±2.06a	26.0±2.85a
	2016	110.9±26.19a	52.9±3.67a	58.2±7.09a	45.1±4.04a	22.0±2.86a
	average	99.1	48.1	48.7	40.3	24.0
	ANOVA	ns	ns	ns	ns	ns

Note: Means with different letters are significantly different (Fisher's LSD, $P = 0.05$); ns – not significant, * – statistically significant.

Tab. 7: Firmness (N/cm^{-2}) of apple fruits at different measurement periods: 7 and 120 days following the application of 1-MCP on fruits kept in the cold storage and 30 days following the 2nd measurement in terms of room temperature.

			Firmness					
Factor	Parameter	Control Ø	1st measurement – 7 days		2nd measurement – 120 days		3rd measurement – 120 + 30 days	
			control	1-MCP	control	1-MCP	control	1-MCP
A	'Red Chief'	71.12±0.47b	53.37±0.16d	71.52±0.35b	45.22±0.22d	59.74±0.38c	35.02±0.16d	51.80±0.35cd
	'Morrens Jonagored'	64.35±0.23c	51.99±0.17d	61.80±0.18c	45.71±0.08d	56.11±0.19c	47.09±0.16c	47.58±0.09d
	'Čadel'	70.14±0.39b	58.57±0.14c	62.69±0.23c	51.50±0.14c	56.21±0.11c	49.83±0.12c	52.39±0.11c
	'Idared'	67.10±0.15bc	64.45±0.09b	72.89±0.14b	56.60±0.16b	65.83±0.17b	55.03±0.28b	65.33±0.27b
	'Granny Smith'	92.41±0.25a	92.02±0.17a	93.59±0.23a	84.37±0.27a	92.12±0.21a	76.22±0.26a	88.39±0.22a
B	2015	82.70±0.23a	64.55±0.26a	77.98±0.23a	52.19±0.19b	71.71±0.23a	46.50±0.18b	62.20±0.25a
	2016	63.37±0.18b	63.57±0.20a	67.00±0.19b	60.72±0.27a	60.33±0.23b	58.76±0.25a	59.94±0.27a
A × B	'Red Chief' 2015	88.58±0.42b	57.09±0.26e	82.60±0.43b	36.69±0.15h	74.75±0.23c	30.71±0.19g	63.08±0.38d
	'Red Chief' 2016	60.53±0.23d	53.66±0.11d	53.76±0.26e	49.74±0.13fd	44.73±0.22g	39.34±0.18d	40.61±0.27g
	'Morrens Jonagored' 2015	69.36±0.38c	45.81±0.11f	62.20±0.34d	45.03±0.10fg	61.90±0.25e	41.79±0.18d	46.21±0.11fg
	'Morrens Jonagored' 2016	59.25±0.15de	58.27±0.15e	59.06±0.13d	52.48±0.10d	50.33±0.13f	44.54±0.12d	49.05±0.12ef
	'Čadel' 2015	85.45±0.22b	60.23±0.25de	72.10±0.14c	54.35±0.20d	59.45±0.14e	50.13±0.19c	55.13±0.16e
	'Čadel' 2016	56.99±0.14e	54.94±0.21e	53.37±0.10e	48.66±0.14ef	53.07±0.07f	49.54±0.17c	49.74±0.11ef
	'Idared' 2015	72.40±0.09c	63.37±0.09cd	68.47±0.28c	50.62±0.12de	62.88±0.15e	43.46±0.09d	55.13±0.19e
	'Idared' 2016	73.48±0.28c	65.53±0.14c	65.83±0.13cd	66.61±0.10c	68.87±0.27d	62.49±0.12b	75.54±0.17c
	'Granny Smith' 2015	101.93±0.18a	96.53±0.20a	100.94±0.28a	74.36±0.18b	99.47±0.22a	66.32±0.14b	91.63±0.27a
	'Granny Smith' 2016	94.47±0.19a	87.60±0.18b	86.33±0.15b	86.23±0.021a	84.76±0.12b	82.99±0.15a	85.05±0.32b
	ANOVA							
	A	*	*	*	*	*	*	*
	B	*	ns	*	*	*	*	ns
	A × B	*	*	*	*	*	*	*

Note: Means with different letters are significantly different (Fisher's LSD, $P = 0.05$); ns – not significant, * – statistically significant.

different initial firmness values affecting fruit quality throughout the whole postharvest life (BULENS et al., 2012).

Monitoring the impact, made by 1-MCP on fruit firmness in the 2nd measurement (120 days), it is possible to observe a significant difference between the treated and non-treated fruits. In cultivars 'Red Chief' and 'Morrens Jonagored', the treated fruits had considerably higher firmness compared to non-treated fruits, in a situation when the initial firmness was higher, and vice versa. In both the cultivars 'Čadel' and 'Idared', it is possible to observe the effect made by the ethylene blockers in both trial years, whereas this effect was the most prominent in the cultivar 'Red Chief', and, in 'Granny Smith', a substantial fruit firmness in all measuring periods has been preserved. MAGAZIN et al., (2010) have found a positive effect of 1-MCP on the quality of cultivar 'Granny Smith' fruits was established. In the cultivars 'Morrens Jonagored' and 'Čadel', no significant difference was established in the 3rd measurement regarding the fruit firmness in relation to the year of study, despite a significant difference in the initial firmness of fruits. This finding combined with the difference between treated and non-treated fruits confirmed that the application of 1-MCP had no impact on preservation of fruit firmness in fruits of this cultivar kept at room temperature for a prolonged period of time (Tab. 7).

The effect of treatment with 1-MCP on the ethylene production rate was much more profound than the effect of the different harvest dates. In 1-MCP treated apples respiration also increased but to a lower level compared to the untreated apples (BULENS et al., 2012).

PASHAZADEH et al., (2017) have found that by application of 1-MCP the loss of weight and firmness of fruit flesh is reduced compared to control. It is notable also that the survey results differ between the cultivars studied. According to the available results (JEZIOREK et al., 2010; SARDABI et al., 2014), MCP-1 treatment promotes the preservation of fruit firmness of the cultivar 'Golden Delicious' whereas the same effect is noticed in other treated apple cultivars. Similar results have been found by ZANELLA and ROSSI (2015) in the cultivar 'Red Delicious' by storing fruits in different storing conditions compared to normal cold storage, where application of 1-MPC contributes to preservation of fruit firmness. Positive effects of the 1-MCP application are shown in studies (AKBUDAK et al., 2009; POYESH et al., 2017).

The act of harvesting did not affect fruit softening on the short term except in the case of late harvested apples, which showed an increased softening during the shelf life directly after harvest. These results are in accordance with studies described in LIN and WALSH (2008). In accordance with our research results, other authors have found similar results indicating that fruit firmness after storage and application of 1-MCP depends on the cultivar.

Conclusions

The impact made by application of 1-MCP on the quality and firmness of fruits depends on the degree of fruit ripeness and cultivar-specific characteristics, which may be under the influence of agro-ecological conditions in the year of the trial.

Each of the examined cultivars has its own optimum harvesting period, enabling optimum storage and application of ethylene blocker, and these periods tend not to coincide in different years of study, which was additionally confirmed by this experiment in Red Chief, Morrens Jonagored and Čadel cultivars. For this reason, it is necessary to examine maturity in several periods to select the appropriate one for storing with cultivars of the same ripening period. In cultivars, characterized by later technological maturity, there was a smaller difference in the initial hardness, but the impact made by 1-MCP produced different effects in different periods when the hardness of fruits was measured, occurring as a result of the interaction of other parameters of ripeness and contents of macro-elements, as shown in the results of our research.

The strongest impact of 1-MCP application was observed in the Granny Smith cultivar where the fruits retained firmness comparable to the one existing during the harvest, after storing the fruits for 30 days at room temperature. A strong effect of the 1-MCP application was observed in the Idared cultivar and 'Red Chief', whereas the fruit firmness of other cultivars ('Morrens Jonagored' i 'Čadel') preserved in the cold storage cannot be sustained at room temperature over a prolonged period.

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Address of the corresponding author:
E-mail: miramilinkovic@yahoo.com