UNIVERSITY OF LJUBLJANA
BIOTECHNICAL FACULTY
INTERNATIONAL MASTER OF FRUIT SCIENCE

Tamara PLEIĆ

THE CHANGES IN THE FRUIT CHEMICAL COMPOSITION OF FOUR APPLE CULTIVARS (*Malus domestica* Borkh.) FROM HARVEST TILL SPRING

M. SC. THESIS
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MAGISTRSKO DELO
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Master Degree Thesis

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AB Market for apple (*Malus domestica* Borkh.) is a demanding one, so we try to keep our products as good at the end of the sales season as they were at the begining. Therefore, the aim of this study was to measure fruit firmness, content of soluble solids, total acid content, ascorbic acid, hydroxycinnamic acid, total sugar content, total phenolic content, flavanols, dihydrochalcones, flavonoles and anthocyanins in three different terms (27. 9.2012, 10. 1. and 13. 3. 2013) in fruits of four different cultivars (‘Granny Smith’, ‘Golden Delicious’, ‘Idared’ and ‘Braeburn’) held in two types of storage: ULO and standard one. We compared the data between the measurement terms and we compared the two types of storage on a measurement term 10. 1. 2013. In comparison of the measurement terms we found that, generally, there are statistically great differences between them. Moreover, we found that there are greater differences between the first measuring term (27. 9. 2012) and the second one (10. 1. 2013), than between the second and the third one (13. 3. 2013). As for the comparison of the two types of storage on a measuring term 10. 1. 2013. we concluded that one of the characteristics of apple, its firmness of fruit, was generally higher in ULO storage than in standard one. The other feaures had higher results in standard cold-storage than in ULO. We can conclude that this a result of a greater loss of water from fruits held in standard cold-storage than in ULO, so the concentration of all its components was greater in fruits held in standard cold-storage.

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AI Trg za jabolka (*Malus domestica* Borkh.) je zelo zahteven in plodovi morajo biti ves čas prodajne sezone podobne kakovosti. Zato je bil cilj naše raziskave, da izmerimo, kako se med tremi termini skladiščenja (27. 9. 2012, 10. 1. in 13. 3. 2013), pri štirih različnih sortah (‘Granny Smith’, ‘Zlati delišes’, ‘Idared’ in ‘Braeburn’) in dveh načinih skladiščenja (ULO in navadna atmosfera) spreminjajo trdota plodov, vsebnost topne suhe snovi, vsebnost sladkorjev in organskih kislin, vsebnost askorbinske kisline, skupni fenoli, vsebnost hidroksicimentnih kislin, flavanolov, dihidrohalkonov, flavonov in antocianinov. Rezultate pri obeh nčinih skladiščenja smo primerjali 10. 1. 2013. Prav tako smo pri vsakem načinu skladiščenja spremljali spreminjanje parametrov z njegovim trajanjem. Ugotovili smo, da obstajajo statistično značilne razlike med obema načinoma skladiščenja in med termini. Prav tako smo ugotovili, da obstajajo večje razlike med med prvim (27. 9. 2012) in drugim vzorčenjem (10. 1. 2013), kot pa med drugim in tretjim (13. 3. 2013). Ob primerjavi obeh načinov skladiščenja (termin 10. 1. 2013) lahko zaključimo, da je bila trdota plodov večja pri plodovih skladiščenih v ULO atmosferi v primerjavi z navadno atmosfero. Ostali izmerjeni parametri skladiščenega sadja so bili praviloma nekoliko večji v navadni atmosferi. Zaključimo lahko, da je to verjetno posledica hitrejših metabolnih procesov in večje izgube vode v navadni atmosferi, kjer se kontrolira samo nizka temperaturea kot pa v ULO atmosferi, kjer se dodatno kontrolira tudi sestava plinov.

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Ann. A2: Average temperature (mm) in each month for last 30 years, meteorological station in Sisak (data given by DHMZ, Metorological and hydrological service of Croatia, December 2014)

GLOSSARY

Abbreviation	Meaning
ULO	Ultra-low oxygen
'Golden Del.'	'Golden Delicious'
stand.	standard cold-storage
WAPA	World Apple and Pear Association
HPLC	High Performance Liquid Chromotography
TPC	Total phenolic content
TSC	Total sugars content
LSD	multiple comparison test
SSC	soluble solids content
hom. group	Homogenous group
JAN	January
FEB	February
MAR	March
APR	April
JUN	June
JUL	July
AUG	August
SEP	September
OCT	October
NOV	November
DEC	December
DHMZ	Državni hidrometeorološki zavod (Meteorological and hydrological service of Croatia)

1 INTRODUCTION

1.1 REASON OF RESEARCH

On an apple market in Europe and in the World, there is almost no room for mistakes in fruit quality. Products should be flawless. As there are certain requirements of production, there are some in storage as well. Apples have to be picked in the optimal ripening time and only the ones of the best quality are stored for longer period. Storage creates a large part of the final price, so it must be as cheap as possible and efficient. It should protect your product from aging and all the other changes in its composition that go with it. There is lack of data available what is happening with the chemical composition of fruits during prolonged time of storage from harvest till spring when the demand and price of apples are high.

1.2 WORKING HYPOTHESIS

Our hypothesis was that good quality fruits held in a proper storage (Ultra Low Oxygen chamber) mantain their quality better than those held in a standard cold-storage, without a controlled atmosphere. Proper storage conditions required also higher costs which should be balanced with more fruits of better quality.

1.3 PURPOSE OF THE RESEARCH

The purpose of the research was to prove what are really the benefits of the ULO chamber compared with standard storage treatment. We followed the apple quality during the storage period based on measurements of different quality parameters.

2 LITERATURE REVIEW

2.1 APPLE (*Malus domestica* Borkh.)

Apple is a fruit species that has a full right to have the title of the queen of fruits. Fruits ripen from the early summer till the late autumn. That fact is not surprising because we are familiar with more than 10 000 apple cultivars (Adamič, 1963).

Apple is considered as a member of Rosaceae family. Today's form of apple is a descendent of *Malus sieversii,* but it is considered that *Malus orientalis* and *Malus sylvestris* have also had an impact on its evolution (Štampar et al., 2009).

It is grown throughout the World, but China is leading country with 39 682 618 t, followed by The United States of America, Turkey and Poland. Croatia is on the 48th place with 128 211 t (FAOSTAT, 2013).

2.2 ECOLOGICAL REQUIREMENTS OF APPLE

Apple is a continental fruit species and its environmental requirements are consistent with that. For a successful apple growing following environmental conditions are needed: average annual temperature between 8 and 12 °C (Mišić, 1978), moderate to relatively high air humidity (60-75 %), snow during cold winter temperatures etc., soil quality – deep, sandy-loam structure, slightly sour – pH 5,5, with no more than 5 % of $CaCO_3$, and relief position - altitude between 120 and 600 m, slope angle around 4 % etc. (Krpina, 2004).

2.3 DEVELOPMENT OF APPLE FRUIT

In fruit development there are three major developmental stages: growth, maturation and senescence. The developmental stages can overlap and are in thight connection with tree physiology (Zadravec et al., 2014).

Cell division and subsequent cell enlargement are involved in the first physiological stage, growth. It leads to a generally irreversible physical increase of the developing plant or fruit (Zadravec et al., 2014).

Maturation is the time between the stages of growth and senescence. During maturation phase, many physical and chemical changes occur. The fruit is in most cases still uneatable but has reached the ability to ripen. The final eating quality of climacteric fruit is affected not only by postharvest handling, but also with the stage of maturity at the harvest date (Giovannoni et al., 2004).

Ripening is the process by which the physiologically mature but inedible fruit attains its characteristic appearance and quality. The term is restricted to changes within the fruit and is considered to begin during the final stages of maturation until the first stages of senescence. Loss of chlorophyll, synthesis of carotenoids and anthocyanins, loss of acidity, increase in sweetness (starch-sugar conversion, sugar-starch conversion, conversion of starch and sugar to CO_2 and water through respiration, breakdown of pectins and other polysacharides, increase in lignin content), tissue softening and formation of flavor volatiles are changes that occur during ripening (Giovanonni et al., 2004).

Growth and development are mainly completed while fruit is attached to the tree, while senescence may proceed on or off the plant.

Senescence is the final physiological stage and it is characterized by degradative processes which lead to deterioration and subsequently death of the plant tissue. With effective postharvest storage regimes and proper handling senescence can be delayed and slowed down. Storability of fruit might be prolonged with delayed senescence (Thompson, 2010).

Maturation of stored horticultural products can be accelerated by the influence of some biological factors (respiration, ethylene production, compositional changes, transpiration, physiological breadown, physical damage and pathological breakdown) and environmental factors (temperature, relative humidy, atmospheric composition, ethylene, light and other) (Thompson, 2010).

Fruits can be classified into two groups according to their pattern of respiration and ethylene production rates: climacteric and non-climacteric. During ripening, climacteric fruit, such as apple and pear indicate a distinct inrease in respiration rates and ethylene production rates. They generally reach fully ripe stage after the respiratory peak. Most climacteric fruit can be harvested mature and can ripen on or off the plant. Ethylene is necessary for the completion of ripening. Faster and more uniform ripening can be achieved by exogenously applying ethylene. Non-climacteric fruits, such as berries, cherries or grapes are not able to ripen after harvest and produce very small quantities of ethylene (Thompson, 2010).

Fruit species are divided into five different classes, according to their ethylene production rate: very low, low, moderate, high and very high. Apple has an ethylene production rate between 10 and 100, what puts her in “high” class (Thompson, 2010).

2.4 PRIMARY AND SECONDARY METABOLITES

Growers strive to improve the quality of fruits which are produced, in terms of eating potential and its ability to store well after harvest. Assessment of quality is made via a set of recognised parameters, which, except fruit firmness and soluble bsolids content, include content of many secondary metabolites (Travers, 2013).

The compounds in living organisms are divided into two main groups: primary and secondary metabolites. Plants synthesize a large range of organic compounds that are traditionally classified as primary and secondary metabolites although the precise boundaries between the two groups can be unclear (Veberič, 2010).

Primary metabolites are those produced by the plant and involved in primary metabolic processes, such as respiration and photosynthesis (Seigler, 2012). They are synthesized by the cell because they are indispensable for their growth (Renneberg and Demain, 2008).

Primary metabolites include molecules such as sugars, amino acids, proteins, polysaccharides etc., of which many are considered as "building blocks" and energy sources (Seigler, 2012). They have essential roles associated with photosynthesis, respiration, and growth and development (these include simple sugars, lipids, nucleotides, amino acids and organic acids, etc.) (Veberič, 2010). Most influence on taste of apples have sugars and organic acids (Veberič et al., 2010).

Secondary metabolites have an important ecological and protective functions against different forms of stress and don't have a direct function in growth and maintenance of the cells. Even though secondary compounds are a normal part of the metabolism of an organism, they are often produced in specialized cells or tissue, and can be more complex than primary ones. Plants produce secondary metabolites as a response to adverse environmental conditions or in specific developmental stages. Their biosynthesis starts from some primary metabolite or from intermediates of the primary metabolism and many of them accumulate in surprisingly high concentrations in some species. Thousands of these chemicals have been identified in several major classes. These chemichals can sometimes be used as taxonomic characters in classifying plants as each plant family, genus, and species produces a characteristic mix of them. They have a key role in protecting plants from herbivores and microbial infection, as attractants for pollinators and seed-dispersing animals etc. Based on their biosynthetic origins, plant secondary metabolites can be divided into three main groups: phenolics, terpenoids and nitrogen-containing compounds (Veberič, 2010).

2.4.1 Phenols

The phenolics are found all over the plant kingdom and about 10,000 phenolic structures have been reported. The content of phenolic compounds in plants varies with the species, the cultivar, the considered organ, the physiological stage and the pedoclimatic conditions (Scalbert and Williamson, 2000).

The phenol compounds form one of the main classes of secondary metabolites with a large range of chemical structure and contribute to the organoleptic and nutritional quality of fruits and vegetables. Phenols have great antioxidant activities and their possible beneficial implications to human health is evident. They also have proven biological activity in the prevention of cancer and cardiovascular diseases (Pearson et al., 1999).

Phenols are present in high concentrations in the epidermis of leaves and the skin of fruits and contribute to all aspects of plant responses towards biotic and abiotic stimuli. They are not only indicators of plant stress responses to variation of light or mineral treatment, but are also key mediators of plant resistance towards pests. Lignin, suberin and similar phenolic-based polymers contribute substantially to the stability and robustness of gymnosperms and angiosperms. That greatly helps in prevention of plants mechanical or environmental damage, like drought or wounding. Phenolics are characterized by having at least one aromatic ring with one or more hydroxyl groups attached. They extent from simple, low molecular-weight, single aromatic ringed compounds to large and complex tannins and derived polyphenols. They can be classified based on the number and arrangement of their carbon atoms and are usually found to be conjugated to sugars and organic acids. Phenolics can be classified into two groups: flavonoids and the non-flavonoids (Veberič, 2010).

2.4.1.1 Phenolic acids and related compounds

2.4.1.1.1 Hydroxycinnamic acids

Hydroxycinnamic acids have a C6 - C3 skeleton (Figure 1). They are often present in plants. Most common are: cinnamic acid, *p*-coumaric acid, caffeic acid etc. Cinnamic acids are commonly found in plants as esters of quinic acid, shikimic acid and tartaric acid (Veberič, 2010).

Hydroxycinnamic acids are found free in plants to only a small extent. Usually they are bound to a sugar either as glycosides or as esters (Hess, 2012). Most important found in apple fruit are chlorogenic, coumaric and caffeic (Spanos and Wrolstad, 1992).

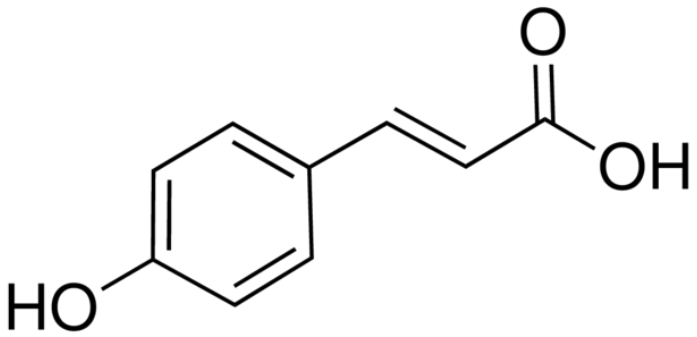

Figure 1. Skeletal formula of hydroxycinnamic acid (Chemfaces, 2010)
Slika 1: Skeletna formula hidroksicimetne kisline (Chemfaces, 2010)

2.4.1.1.2 Chalcones

Chalcones and dihydrochalocones have a linear chain connecting the two aromatic rings. The C3- chain of chalcones contains a double bond (Figure 2), whereas the C3-chain of dihydrochalcones is saturated. An example of a dihydrochalcone is phloridzin which is a compound found primarily in apple leaves, and which has been reported to have an important role in plant defense systems against different diseases (e.g. apple scab). (Veberič, 2010)

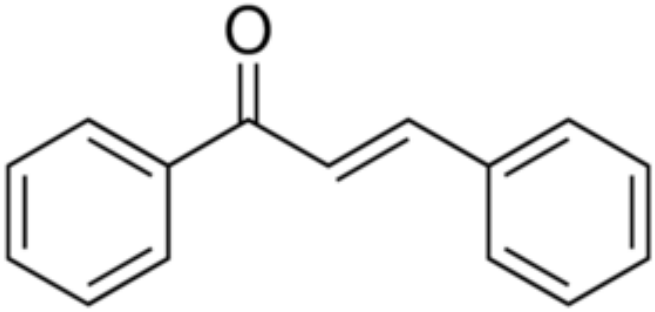

Figure 2. Skeletal formula of chalcone (Sigma Aldrich, 2010)
Slika 2: Skeletna formula halkona (Sigma Aldrich, 2010)

2.4.1.2 Flavonoids

A major class of polyphenols found habitually in fruits and vegetables are flavonoids. Apples are one of the most important dietary sources of this kind of phytochemicals in human diet (Boyer and Liu, 2004).

In apples, flavonoids are important components and located mostly in the skin. Phenolic substances also play a crucial role in determination of the quality characteristics of colour and taste of fresh apples and its processed products. There is even indication that

flavonoids and phenolic acids may influence fruit firmness. However, the literature concerning changes in phenolics during storage is much more contradictory (Awad and de Jager, 2000).

2.4.1.2.1 Flavonols

Flavonols are the most widely spread subclass of flavonoids, being abundant in almost all fruit species. The distribution and structural variations of flavonols are extensive and have been well documented. The levels of flavonols found in commonly consumed fruits can vary due to seasonal changes, varietal differences, used technology as well as storage and processing (Veberič, 2010).

Most abundant flavonols in apple are quercetin-3-O-rutinoside, quertcetine-3-O-galactoside and quercetin-3-O-glucoside (Zupan et al., 2014).

2.4.1.2.2 Flavanols

Flavanols are a complex subclass of flavonoids ranging from the simple monomers, to the oligomeric and polymeric proanthocyanidins, which are also known as condensed tannins (Veberič, 2010).

They may vary from one cultivar to other, but they are always more abundant in fruit skin (Macheix and Fleuriet, 1990). Most abundant flavanols in apple are epicatechin (Figure 3), catechin and procyanidin (Zupan et al., 2014).

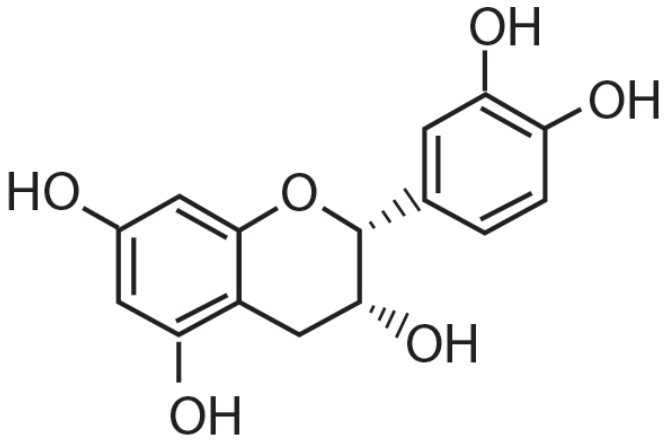

Figure 3. Skeletal structure of the epicatechin (Mars Cocoa Science, 2010)
Slika 3: Skeletna formula epikatehina (Mars Cocoa Science, 2010)

2.4.1.2.3 Anthocyanidins and anthocyanins

The physiological roles of anthocyanidins and their conjugated derivatives, anthocyanins, in vegetative tissues have puzzled scientists for over a century. Anthocyanins can be found in the vacuoles of almost every cell type in the epidermal, ground and vascular tissues of all vegetative organs. They occur in roots, hypocotyls, coleoptiles, rhizomes, stolons, bulbs, leaves etc. (Gould et al., 2008).

They are involved in the protection of plants against excessive light by shading leaf mesophyll cells and also have an important role to play in attracting pollinating insects. The most common anthocyanidins are pelargonidin, cyanidin, petunidin and malvidin (Veberič, 2010).

Cyanidin 3-galactoside was detected as the major anthocyanin in apple skin, accounting for 80 % of total anthocyanins. Cyanidin 3-glucoside, cyanidin 3-arabinoside, cyanidin 3-rutinoside and cyanidin 3-xyloside were detected as the remaining anthocyanindins (Liu et al., 2013).

2.5 FRUIT FIRMNESS

Fruit firmness is considered one of the most important criteria concerning eating quality of apples. Facing demands in this respect represents a big challenge for the industry. Many factors such as genetics, growing factors, mineral nutrition, harvest maturity and storage regimes affect firmness (Saei et al., 2011).

Storage has a great impact on maintaining firmness and has been the subject of many studies. Storage regimes have been optimised encompassing minimal losses due to physiological disorders and maximum retention of firmness during long-term storage. Progress has been vital in the past few decades paralleling the introduction of ultra low oxygen storage. Many studies have also demonstrated improvement of fruit firmness retention in a wide range of apple cultivars while stored in low and ultra-low oxygen storage (Hoehn et al., 2003).

Fruit quality assessments usually involve measuring flesh firmness using a penetrometer. Usually a probe, with either a flat or convex tip, is driven into the flesh, and the maximum force is recorded. Magness and Taylor have developed early hand-held penetrometers and from these devices, a range of devices have been developed. The wide use of firmness measurements has led to the graduall development of new devices for measuring firmness (Harker et al., 1996).

2.6 SOLUBLE SOLIDS CONTENT

As apples mature, starch is converted into sugars thereby increasing soluble solids content (SSC) and making fruit taste sweeter. Sugars are the major soluble solids in apples. The extractable juice in apples contains soluble compounds including fructose, glucose, sucrose, sorbitol, organic acids and inorganic salts (Kingston, 1992). The ratio of sugars varies depending on the fruit and the cultivar (Wu et al., 2007) and influences taste. Fructose is sweeter than sucrose, which is sweeter than glucose (Kader, 2002).

Soluble solids can be measured in many different scales, but °Brix are the most commonly used. One of the simplest and also most common used way to measure soluble solids is usage of refractometer (Ventura et al., 1998).

2.7 ACIDITY

Acidity is an important component of fruit flavour and in combination with SSC, contributes to overall organoleptic quality. Total organic acid content declines in fruit as they mature, ripen and store, with apples having a reasonably high acid content compared to pears. However fruit acidity should be considered in conjunction with other quality parameters, especially firmness and soluble solids content as consumer studies show a strong relationship between those three parameters (Harker et al., 2008). The principle acid in apples and pears is malic acid (Colarič et al., 2007).

3 MATERIAL AND METHODS

3.1 ORCHARD

The apples were picked from the orchard in autumn 2012. The orchard is situated in Katoličko Selišće, in the municipality of Velika Ludina in central Croatia, on an altitude of 132-138 m above sea level and coordinates 45°37'32" N and 16°36'56" E. It was planted from 2008 to 2011. There are 13 apple cultivars growing on 18 hectars of a modern apple orhard. The trees are distributed in 410 rows, mostly 150 meters long. The rows are planted in direction North-South with a distance of 3.2 m between the rows, and 60-100 cm between the trees, depending of the cultivar. The whole plantation is covered by anti-hail nets and has irrigation. The orcard is owned by company Fructus Ltd. from Velika Ludina.

3.2 WEATHER CONDITIONS

The municipaility Velika Ludina is situated in central Croatia, and so it has a continental climate. During the vegetational year 2011/2012 we had a dry autumn (2011), an average winter, dry and very hot spring, very dry and extremely hot summer and a rainy and very hot autumn (2012). Overall, the year of 2012 was dry and extremely hot.

As the average year percipitation on meteorological station Sisak (in last 30 years, data given by DHMZ, Metorological and hydrological service of Croatia) was 907.6 mm, and in 2012 there were only 810.9 mm, we can conclude that the year of 2012 was dry (Figure 4 and Figure 6). The data received from Meteorological and hydrological service of Croatia for percipitation per month in last 30 years, measured in weather station Sisak is shown in Annex A1.

Regarding the temperature (data given by DHMZ, Metorological and hydrological service of Croatia) the data says that the average year temperature in last 30 years was 11.5 °C, and in 2012 we had 12.4 °C. We can conclude that the year of 2012 was extremely hot (Figure 5 and Figure 7). The data received from Meteorological and hydrological service of Croatia for average temperature per month in last 30 years, measured in weather station Sisak is shown in Annex A2.

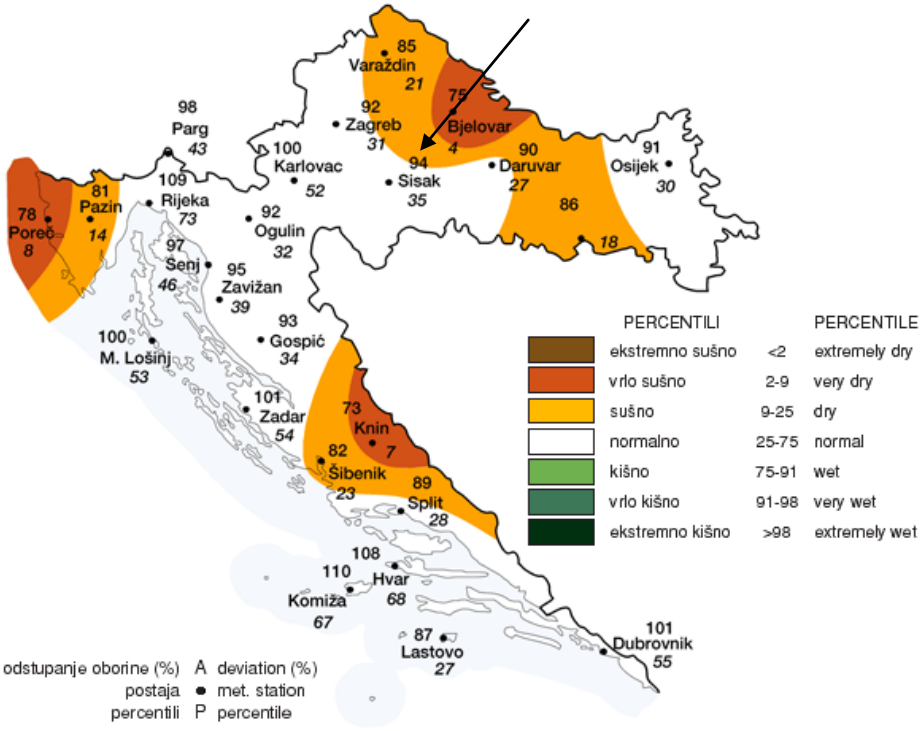

Figure 4. Deviation of the amount of precipitation in year 2012 with an arrow pointing to an approximate location of the orchard (DHMZ, 2014)

Slika 4: Odstopanje količine padavin v letu 2012 z označeno lokacijo nasada (DHMZ, 2014)

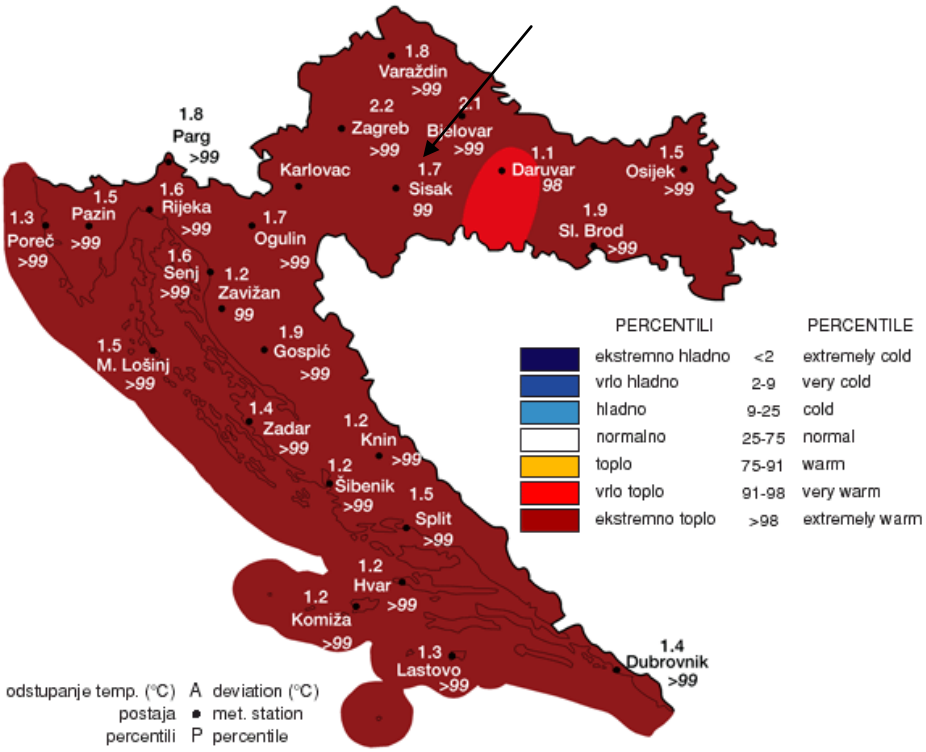

Figure 5. Deviation of the mean air temperature in year 2012 with an arrow pointing to an approximate location of the orchard (DHMZ, 2014)

Slika 5: Odstopanje povprečne temperature zraka v letu 2012 z označeno lokacijo nasada (DHMZ, 2014)

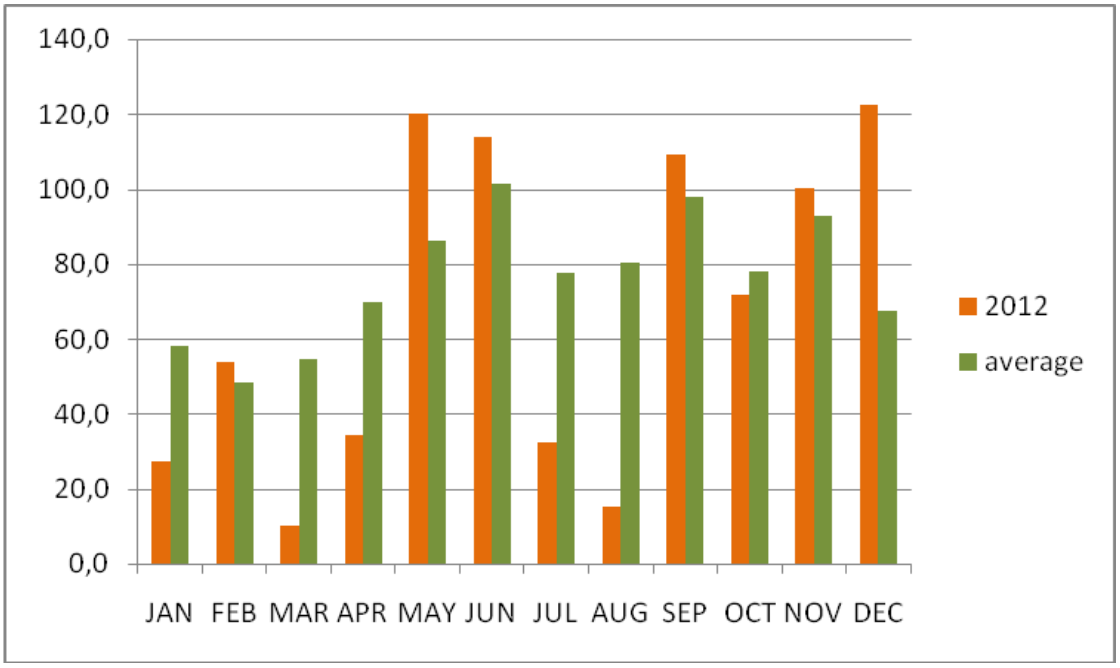

Figure 6. Relations between long-term (1983-2012) average month percipitation and percipitation in 2012 (in mm), meteorological station Sisak

Slika 6: Povprečna mesečna količina padavin v dolgoletnem obdobju (1983-2012) in v letu 2012 (v mm), meteorološka postaja Sisak

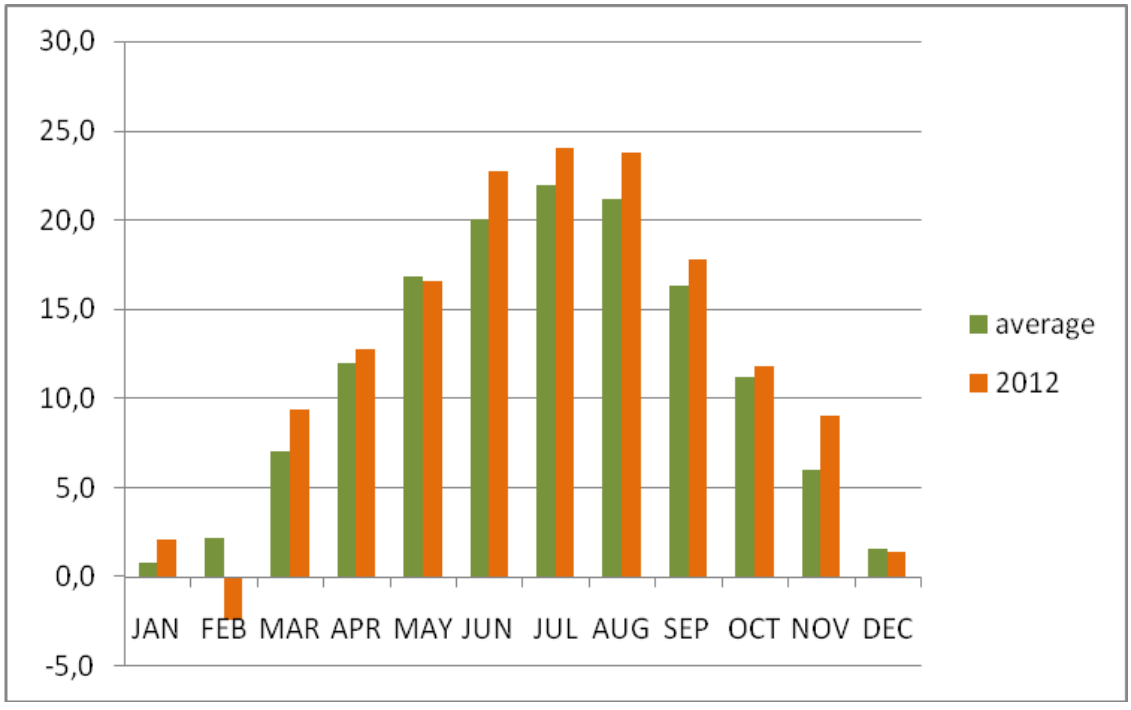

Figure 7. Relations between long-term (1983-2012) average month temperature and temperature in 2012 (in °C), meteorological station Sisak

Slika 7: Povprečna mesečna temperatura zraka v dolgoletnem obdobju (1983-2012) in v letu 2012 (v °C), meteorološka postaja Sisak

3.3 PLANT MATERIAL

3.3.1 Cultivar ‘Braeburn’

'Braeburn' (Figure 8) was introduced in 1950's in New Zealand, with Lady Hamilton as one of the parents. Its flesh is yellow and very aromatic. The skin is yellow with partial or complete coverage of orange or red stripes. It is quite susceptible to diseases and pests. It ripens in late October. It's yield slowly and gradually grows each year, so nowadays in Europe we pick arround 300 thousand tonnes (World apple …, 2015).

Figure 8. The fruit of the ‘Braeburn’ cultivar (private photo, 17. 10. 2014)
Slika 8: Plod sorte ‘Braeburn’ (privatna fotografija, 17. 10. 2014)

3.3.2 Cultivar ‘Golden Delicious’

'Golden Delicious' (Figure 9) was discovered in 1890's in West Virginia, USA. It's flesh is white to pale yellow and sweet, while the skin is yellowish to light green. It has some susceptibility to diseases, but at the moment it is the most grown apple cultivar in the World. Forecast for 2015 made by WAPA says that only EU will pick more than 2,5 million tonnes of this cultivar. That is 21,75 % of the total European apple yield. It has many famous offsprings, such as 'Gala', 'Jonagold', 'Cripps Pink' and 'Elstar' (World apple …, 2015).

Figure 9. The fruit of the 'Golden Delicious' cultivar (private photo, 17. 10. 2014)
Slika 9: Plod sorte 'Zlati delišes' (privatna fotografija, 17. 10. 2014)

3.3.3 Cultivar 'Granny Smith'

'Granny Smith' (Figure 10) was introduced in 1860's in Australia. Its flesh is white and sour and sharp and its skin is green. It has a low-chill requirement. It has some suscepatability to pests ad diseases. In the last 10 years its yield in EU is even- arround 300-400 thousand tonnes (World apple …, 2015).

Figure 10. The fruit of the 'Granny Smith' cultivar (private photo, 17. 10. 2014)
Slika 10: Plod sorte 'Granny Smith' (privatna fotografija, 17. 10. 2014)

3.3.4 Cultivar ‘Idared’

‘Idared’ (Figure 11) is a a cultivar commonly grown in Croatia, and there it represents more than half of produced apples. The cultivar itself was developed in Idaho, USA, in 1930's by crossing cultivars 'Jonathan' and 'Wagener'. It is highly productive and very popular. Its flesh is white to pale yellow, and skin is green with partial or complete red coverage. It ripens in October. It is very resistant to frost. With over 1,1 million tonnes, it's the third most grown cultivar in Europe, right after ‘Golden Delicious’ and 'Gala' (World apple …, 2015).

Figure 11. The fruit of the ‘Idared’ cultivar (private photo, 17. 10. 2014)
Slika 11: Plod sorte ‘Idared’ (privatna fotografija, 17. 10. 2014)

3.4 METHODS

Analysis of fruit firmness and content of phenols, organic acids, vitamin C, sugars and soluble solids in apple were carried out in the Chair for fruit growing, viticulture and vegetable growing, Biotechnical Faculty, University of Ljubljana. All four different cultivars were picked at optimal ripening time and the fruits randomly divided into two types of storage (ULO and standard cold storage).

Temperature conditions in standard cold-storage were not completely stable, the temperature varied between 5 °C and 8 °C and the ratios of gases in atmosphere in the chamber was the same as the one in normal atmosphere, while in ULO storage the temperature and gases ratios were stable during the whole time of storage. The temperature was between 1°C and 2°C, level of oxygen was held between 0,5 % and 1.5 % and the level of CO_2 between 1 % and 2 %, all depending of the cultivar.

Apples were sampled in five repetitions (each repetition consisted of 10 apples) three times during storage (27th September, 10th January and 13th March). At the last sampling the fruits in the standard cold storage treatment were no longer available due to previous internal breakdown.

Soluble solids and fruit firmness were measured instantly on each date of measurement. The skin and the pulp were separately extracted and analysed for the content of anthocyanins, TPC, hydroxycinnamic acids, flavanols, dihydrochalcones and flavonols. Ascorbic acid content, total organic acids and total sugar content were measured in samples where both pulp and peel were used.

3.4.1 Extraction of sugars and organic acids

For the extraction of sugars and organic acids 10 grams of sample (skin and pulp) was extracted with 50 ml of bidistilled water. The samples were extracted at room temperature for 30 minutes. Then the samples were centrifuged for 7 minutes on 10000 rpm and 4 °C (Eppendorf Centrifuge 5810, Hamburg, Germany). The supernatant was then filtered through a 0,2 μm filter. Further analyses were made by a HPLC system Thermo Finnigan Surveyor. Method was previously described by Zupan (2012).

3.4.2 Extraction of vitamin C

Extraction of vitamin C was made from 5 g of edible part of the fruit (peel and pulp were taken). Samples were cut into small pieces and extracted with 10 ml of 2 % metaphosphoric acid. The samples were then extracted on room temperature for 30 minutes. Then the samples were centrifuged for 7 minutes on 10000 rpm and 4 °C (Eppendorf Centrifuge 5810, Hamburg, Germany). The supernatant was then filtered through a 0,2 μm filter. Further analyses were made by a HPLC system Thermo Finnigan Surveyor. Extraction and analysis was done according to the method previously described by Veberič et al. (2014).

3.4.3 Extraction of phenolics

Extraction of phenolics was made separately from pulp and skin. Plant material was cut into small pieces. For the measurement of phenols in skin, 5 grams of sample were extracted with 18 ml of methanol containing 3 % of formic acid; and for the measurements in pulp, 10 grams of sample were extracted with 10 ml of methanol containing 3 % of formic acid. Samples were then put on ice and homogenized with Ultra-Turrax T-25 (Ika-Labortechnik, Germany). Then the samples were centrifuged for 7 minutes on 10000 rpm and 4 °C (Eppendorf Centrifuge 5810, Hamburg, Germany). The supernatant was then

filtered through a 0,2 μm filter. Further analyses were made by a HPLC system Thermo Finnigan Surveyor. Method was previously described by Zupan (2012).

3.4.4 HPLC analysis

HPLC is an abbreviation for High Performance Liquid Chromatography. Chromatographic separation can be attained for compounds that migrate at different speeds through a so called "chromatographic bed". The phenomenon of different migration velocities is based on different retentions of the migrating compounds caused by the elementary process of different distributions of the separands between two phases: a mobile phase and a stationary phase (Corradini et al., 2011).

3.4.4.1 HPLC analysis of sugars and organic acids

HPLC analysis of sugars and organic acids was done according to Zupan (2012). For the analysis of sugars we used 20 μl of prepared sample. For the separation of samples we used Razex RCM-monosaccharide column (300 mm x 7.8 mm) and an IR detector. Mobile phase was bidistilled water, and the flow rate was 0.6 ml/min. The sample was analysed for 30 minutes on a temperature of 65 °C.

For the analysis of organic acids we used 20 μl of prepared sample. We used column Razex ROA-organic acid (300 mm x 7.8 mm), the UV detector was set to 210 nm, and the column was heated up to 65 °C. The mobile phase was 4 mM sulfuric acid in bidistilled water with the flow rate of 0.6 ml/min. The analysis took 30 minutes.

Concentration of individual acids (ascorbic acid) was determined by the method of external standard and recalculated regarding the calibration curve, which was made on the basis of standard solutions.

For the analysis of ascorbic acid we used 20 μl of prepared sample. We used column Razex ROA-organic acid (300 mm x 7.8 mm), the UV detector was set to 245 nm, and the column was heated up to 20 °C. The mobile phase was 4 mM sulfuric acid in bidistilled water with the flow rate of 0.6 ml/min. The analysis took 30 minutes.

3.4.4.2 HPLC analysis of phenolics

HPLC analysis of phenolics was done according to Mikulič-Petkovšek et al. (2014). Phenolic supstances were analysed with a HPLC system Thermo Finnigan Surveyor (San Jose, USA). The volume of injected sample was 20 μl, flow rate 1 ml/min. The detection took place at 280 and 350 nm wave length. The column that we used was a Phenomenex

Gemini C18 (150 mm x 4.5 mm, 3 μm) at the temperature of 25 °C. Mobile phases were aqueuous 0,1 % formic acid in bidistilled water and 0,1 % formic acid in acetonitrile.

Identification of individual supstances started with a comparison of retention time and spectrum. All phenolic supstances were also confirmed by usage of mass spectrometer (Thermo Scientific, LCQ Deca XP MAX) with an electrospray ionisation. Concentrations of phenolic supstances were calculated regarding the peaks of chromatography for samples and adequate standards. Concentrations were expressed with mg per kg of fruit.

3.4.4.3 Analysis of TPC

Content of TPC was estimated with usage of Colin-Ciocalteu reagent. In 100 μl of sample we added 6 ml of bidistilled water and 500 μl Colin-Ciocalteu 20 % sodium carbonate and 1,9 ml bidistilled water. Samples were then mixed and were held for 30 minutes on 40 °C. Afterwords we measured absorbance of samples with a spectrophotometer (Perkin-Elmer, UV/visible Lambda Bio 20) at wave length of 765 nm. The mixture of reagents and bidestilled water was used as a blind sample. Amount of total phenols was expressed in mg of gallic acid per kg of fresh fruit. Absorption was measured in three repetitions. The analysis of total phenolic content was done according to Novljan (2013).

3.4.5 Measurement of fruit firmness

When measuring fruit firmness, we had to make sure that all the samples were about the same temperature, since warm fruits are usually softer than cold ones. On each fruit we made 2 puncture tests, one on each side (on opposite sides), midway between the stem-end and calyx-end. Before using the manual penetrometer, we removed a disc of skin (about 2 cm in diameter) with a ceramic knife. We used an appropriate tip (plunger) for apple (11 mm). Before penetrating the plunger into the fruit up to a marked line on the plunger, we had to make sure that the penetrometer was restarted. Fruit firmness was expressed in kg/cm^2.

3.4.6 Measurement of soluble solids content

During the measurements of soluble solids in fruits, we used a digital refractometer that automatically showed (in ºBrix) the amount of soluble solids in fruit. We made sure that the juice from two opposite sides of fruit was taken for analysis.

3.5 STATISTICAL ANALYSIS

Data obtained from chemical analysis was statistically analyzed with a statistics program Statgrapfic Plus for Windows 4.0, program Microsoft Excell 2007 and statistics program

R. Statistically significant differences were noted by analysis of variances (ANOVA). The examination of differences between the measurements was done by LSD test (multiple comparison test), $p \leq 0.05$. Statistically significant differences were marked with different letters.

4 RESULTS

4.1 FRUIT FIRMNESS

Figure 12 shows average fruit firmness in 4 different cultivars, in 2 types of storage and in 3 different terms. In ULO storage apples of most cultivars (except 'Granny Smith') show a decline in average fruit firmness during the storage period. In standard cold-storage results show the decline of fruit firmness in all cultivars.

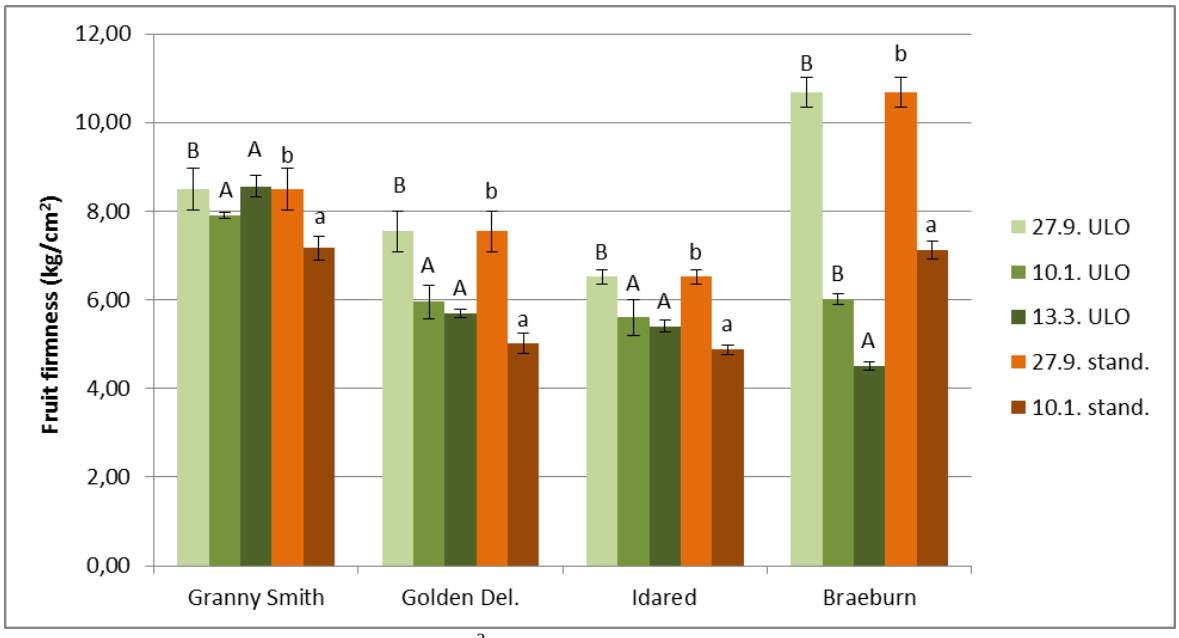

Figure 12. Average fruit firmness (kg/cm^2) in 4 different cultivars of apple ('Granny Smith', 'Golden Delicious', 'Idared' and 'Braeburn') in 2 types of storage (ULO and standard) and in 3 different terms (27. 9. 2012, 10. 1. 2013 and 13. 3. 2013). A different letter above the columns indicates a statisticaly significant difference ($p \leq 0.05$) in fruit firmness of a certain cultivar within one type of storage. Capital letter marks a result in ULO storage, and a small one marks a result in standard cold-storage. Types of storage are also marked in different colors.

Slika 12: Povprečna trdota mesa (kg/cm^2) pri štirih sortah jabolk ('Granny Smith', 'Zlati delišes', 'Idared' in 'Braeburn') in 2 načinih skladiščenja (ULO in navadna atmosfera) ter treh terminih vzorčenja (27. 9. 2012, 10. 1. 2013 in 13. 3. 2013). Različne črke nad stolpci označujejo statistično značilne razlike ($p \leq 0,05$) v trdoti mesa za vsako sorto znotraj enega načina skladiščenja. Velike črke označujejo rezultate pri skladiščenju v ULO atmosferi in majhne črke pri skladiščenju v navadni atmosferi. Načini skladiščenja so označeni tudi z različnima barvama.

Table 1 shows average fruit firmness in 4 different cultivars, in 2 types of storage on measuring term 10. 1. 2013. Apples of all cultivars except 'Braeburn' showed higher values in ULO than in standard cold-storage.

Table 1. Average fruit firmness (kg/cm^2) in 4 different cultivars of apple (‘Granny Smith’, ‘Golden Delicious’, ‘Idared’ and ‘Braeburn’) in 2 types of storage (ULO and standard) on measuring term 10. 1. 2013. Standard error is shown next to average result, and a different letter in the right column indicates a statisticaly significant difference ($p \leq 0.05$) in fruit firmness of a different types of storage within each cultivar.
Preglednica 1: Povprečna trdota mesa (kg/cm^2) pri štirih sortah jabolk (‘Granny Smith’, ‘Zlati delišes’, ‘Idared’ in ‘Braeburn’) in 2 načinih skladiščenja (ULO in navadna atmosfera) pri terminu vzorčenja 10. 1. 2013. Standardna napaka je zapisana zraven povprečne vrednosti, različne črke v desnem stolpcu označujejo statistično značilne razlike ($p \leq 0,05$) v trdoti mesa med načini skladiščenja znotraj posamezne sorte.

FRUIT FIRMNESS	10.1. 2013			
	ULO		STANDARD STORAGE	
	average +/- standard error	hom. group	average +/- standard error	hom. group
‘Granny Smith’	7.90 +/- 0.07	b	7.17 +/- 0.27	a
‘Golden Del.’	5.95 +/- 0.38	b	5.01 +/- 0.23	a
‘Idared’	5.60 +/- 0.40	b	4.88 +/- 0.11	a
‘Braeburn’	6.02 +/- 0.12	a	7.12 +/- 0.21	b

4.2 SOLUBLE SOLIDS CONTENT

Figure 13 shows the average content of soluble solids in 4 different cultivars, in 2 types of storage and in 3 different terms. In ULO storage apples of the most cultivars (except ‘Idared’) show an average increase of the content of soluble solids when the first and the last date are compared. In standard cold-storage results are different. Apples of ‘Golden Delicious’ and ‘Breaburn’ showed no statistical difference in soluble solids values, whereby apples of ‘Granny Smith’ showed an increase of these values but applesof ‘Idared’ a decline of the values.

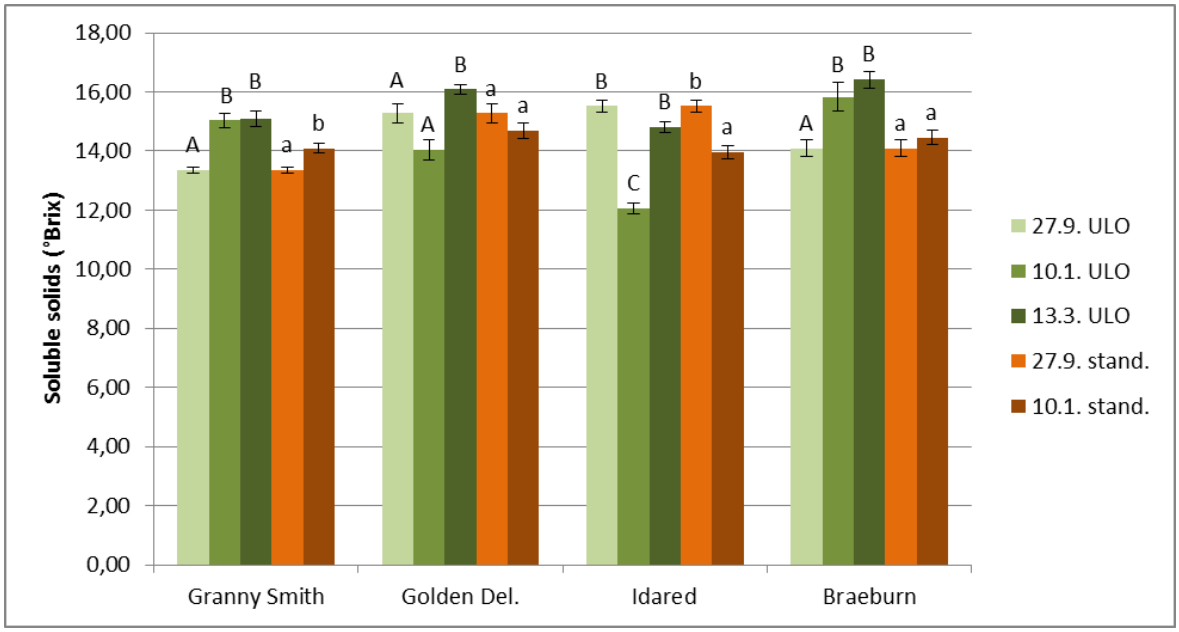

Figure 13. Average content of soluble solids in 4 different cultivars of apple (‘Granny Smith’, ‘Golden Delicious’, ‘Idared’ and ‘Braeburn’) in 2 types of storage (ULO and standard) and in 3 different terms (27. 9. 2012, 10. 1. 2013 and 13. 3. 2013). A different letter above the columns indicates a statisticaly significant difference ($p \leq 0.05$) in the content of soluble solids of a certain cultivar within one type of storage. Capital letter marks a result in ULO storage, and a small one marks a result in standard cold-storage. Types of storage are also marked in different colors.
Slika 13: Povprečna vsebnost suhe snovi pri štirih sortah jabolk (‘Granny Smith’, ‘Zlati delišes’, ‘Idared’ in ‘Braeburn’) in 2 načinih skladiščenja (ULO in navadna atmosfera) ter treh terminih vzorčenja (27. 9. 2012, 10. 1. 2013 in 13. 3. 2013). Različne črke nad stolpci označujejo statistično značilne razlike ($p \leq 0,05$) v vsebnosti suhe snovi za vsako sorto znotraj enega načina skladiščenja. Velike črke označujejo rezultate pri skladiščenju v ULO atmosferi in majhne črke pri skladiščenju v navadni atmosferi. Načini skladiščenja so označeni tudi z različnima barvama.

Table 2 shows an average content of soluble solids in 4 different cultivars, in 2 types of storage on measuring term 10. 1. 2013. Apples of ‘Granny Smith’ and ‘Idared’ showed higher values stored in ULO, while apples of ‘Golden Delicious’ and ‘Idared’ showed higher values in standard cold-storage.

Table 2. Average content of soluble solids (°Brix) in 4 different cultivars of apple (‘Granny Smith’, ‘Golden Delicious’, ‘Idared’ and ‘Braeburn’) in 2 types of storage (ULO and standard) on measuring term 10. 1. 2013. Standard error is shown next to average result, and a different letter in the right column indicates a statisticaly significant difference ($p \leq 0.05$) in the content of soluble solids of a different types of storage within each cultivar.
Preglednica 2: Povprečna vsebnost suhe snovi (°Brix) pri štirih sortah jabolk (‘Granny Smith’, ‘Zlati delišes’, ‘Idared’ in ‘Braeburn’) in 2 načinih skladiščenja (ULO in navadna atmosfera) pri terminu vzorčenja 10. 1. 2013. Standardna napaka je zapisana zraven povprečne vrednosti, različne črke v desnem stolpcu označujejo statistično značilne razlike ($p \leq 0,05$) v vsebnosti suhe snovi med načini skladiščenja znotraj posamezne sorte.

SOLUBLE SOLIDS	10. 1. 2013			
	ULO		STANDARD STORAGE	
	average +/- standard error	hom. group	average +/- standard error	hom. group
‘Granny Smith’	15.04 +/- 0.23	b	14.10 +/- 0.17	a
‘Golden Del.’	14.05 +/- 0.34	a	14.69 +/- 0.27	b
‘Idared’	12.06 +/- 0.17	a	13.96 +/- 0.22	b
‘Braeburn’	15.84 +/- 0.48	b	14.46 +/- 0.25	a

4.3 TOTAL SUGAR CONTENT

Figure 14 shows an average total sugar content in 4 different cultivars, in 2 types of storage and in 3 different terms. In ULO storage all cultivars mostly showed a decline of total sugar content. In standard cold-storage apples of all cultivars exept ‘Granny Smith’ showed statistically significant decline of total sugar content.

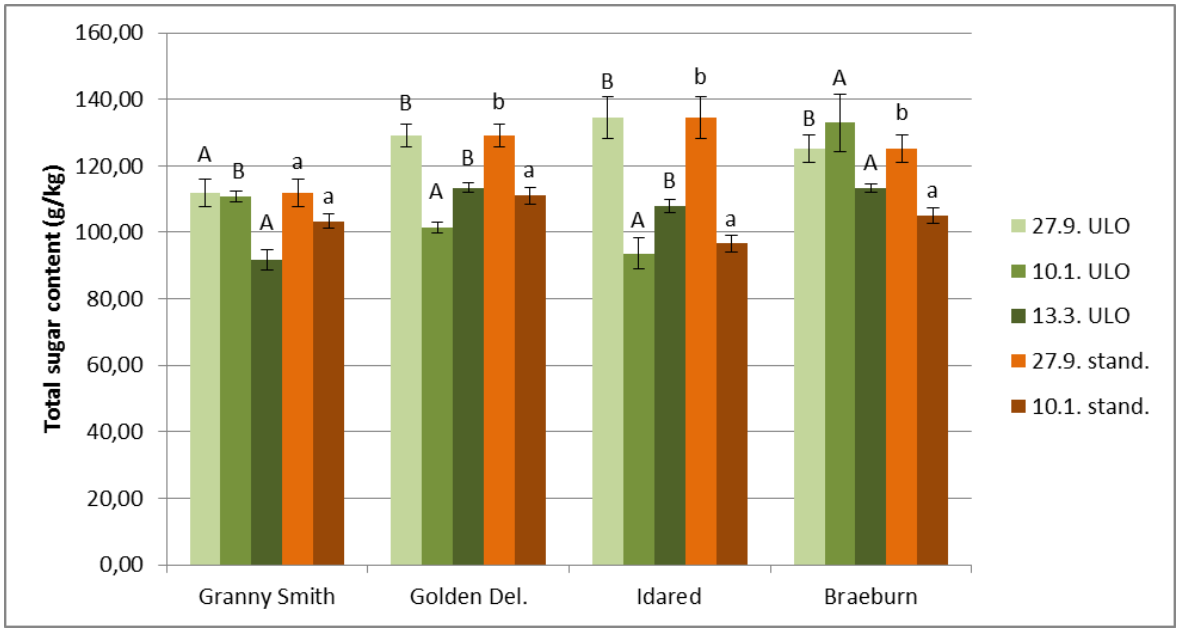

Figure 14. Average total sugar content (mg/kg) in 4 different cultivars of apple (‘Granny Smith’, ‘Golden Delicious’, ‘Idared’ and ‘Braeburn’) in 2 types of storage (ULO and standard) and in 3 different terms (27. 9. 2012, 10. 1. 2013 and 13. 3. 2013). A different letter above the columns indicates a statisticaly significant difference ($p \leq 0.05$) in total sugar content of a certain cultivar within one type of storage. Capital letter marks a result in ULO storage, and a small one marks a result in standard cold-storage. Types of storage are also marked in different colors.

Slika 14: Povprečna vsebnost skupnih sladkorjev (mg/kg) pri štirih sortah jabolk (‘Granny Smith’, ‘Zlati delišes’, ‘Idared’ in ‘Braeburn’) in 2 načinih skladiščenja (ULO in navadna atmosfera) ter treh terminih vzorčenja (27. 9. 2012, 10. 1. 2013 in 13. 3. 2013). Različne črke nad stolpci označujejo statistično značilne razlike ($p \leq 0,05$) v vsebnosti skupnih sladkorjev za vsako sorto znotraj enega načina skladiščenja. Velike črke označujejo rezultate pri skladiščenju v ULO atmosferi in majhne črke pri skladiščenju v navadni atmosferi. Načini skladiščenja so označeni tudi z različnima barvama.

Table 3 showsan average total sugar content in 4 different cultivars, in 2 types of storage on measuring term 10. 1. 2013. Apples of ‘Idared’ showed no statistical significance, whereby apples of ‘Golden Delicious’ showed higher values in standard cold-storage, compared with apples of ‘Granny Smith’ and ‘Braeburn’ with higher values in ULO storage.

Table 3. Average total sugar content (mg/kg) in 4 different cultivars of apple (‘Granny Smith’, ‘Golden Delicious’, ‘Idared’ and ‘Braeburn’) in 2 types of storage (ULO and standard) on measuring term 10. 1. 2013. Standard error is shown next to average result, and a different letter in the right column indicates a statisticaly significant difference ($p \leq 0.05$) in total sugar content of different types of storage within each cultivar.
Preglednica 3: Povprečna vsebnost skupnih sladkorjev (mg/kg) pri štirih sortah jabolk (‘Granny Smith’, ‘Zlati delišes’, ‘Idared’ in ‘Braeburn’) in 2 načinih skladiščenja (ULO in navadna atmosfera) pri terminu vzorčenja 10. 1. 2013. Standardna napaka je zapisana zraven povprečne vrednosti, različne črke v desnem stolpcu označujejo statistično značilne razlike ($p \leq 0,05$) v vsebnosti skupnih sladkorjev med načini skladiščenja znotraj posamezne sorte.

TOTAL SUGAR CONTENT	10. 1. 2013			
	ULO		STANDARD STORAGE	
	average +/- standard error	hom. group	average +/- standard error	hom. group
‘Granny Smith’	110.76 +/- 1.77	b	103.38 +/- 2.29	a
‘Golden Del.’	101.31 +/- 1.67	a	111.05 +/- 2.52	b
‘Idared’	93.55 +/- 4.65	a	96.58 +/- 2.52	a
‘Braeburn’	133.00 +/- 8.58	b	104.97 +/- 2.23	a

4.4 TOTAL ORGANIC ACIDS CONTENT

Figure 15 shows average total acids content in 4 different cultivars, in 2 types of storage and in 3 different terms. Apples of all cultivars in ULO storage mostly showed a decline in total acids content compared with standard cold-storage.

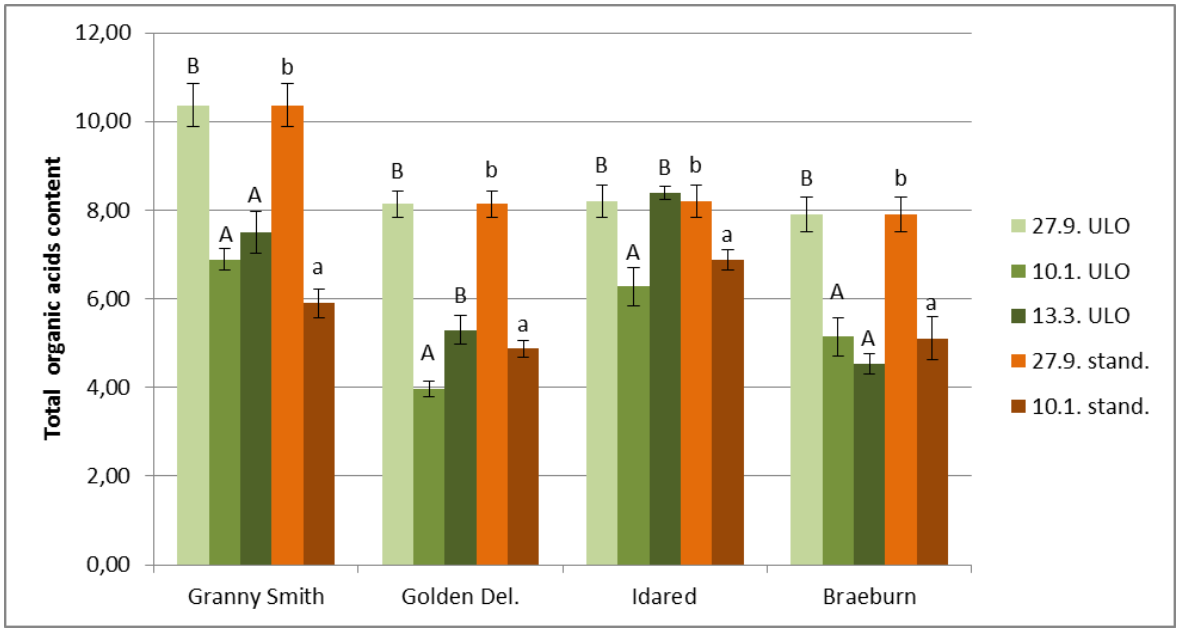

Figure 15. Average total acids content (mg/kg) in 4 different cultivars of apple (‘Granny Smith’, ‘Golden Delicious’, ‘Idared’ and ‘Braeburn’) in 2 types of storage (ULO and standard) and in 3 different terms (27. 9. 2012, 10. 1. 2013 and 13. 3. 2013). A different letter above the columns indicates a statisticaly significant difference ($p \leq 0.05$) in total acids content of a certain cultivar within one type of storage. Capital letter marks a result in ULO storage, and a small one marks a result in standard cold-storage. Types of storage are also marked in different colors.

Slika 15: Povprečna vsebnost skupnih kislin (mg/kg) pri štirih sortah jabolk (‘Granny Smith’, ‘Zlati delišes’, ‘Idared’ in ‘Braeburn’) in 2 načinih skladiščenja (ULO in navadna atmosfera) ter treh terminih vzorčenja (27. 9. 2012, 10. 1. 2013 in 13. 3. 2013). Različne črke nad stolpci označujejo statistično značilne razlike ($p \leq 0,05$) v vsebnosti skupnih kislin za vsako sorto znotraj enega načina skladiščenja. Velike črke označujejo rezultate pri skladiščenju v ULO atmosferi in majhne črke pri skladiščenju v navadni atmosferi. Načini skladiščenja so označeni tudi z različnima barvama.

Table 4 shows average total acids content in 4 different cultivars, in 2 types of storage on measuring term 10. 1. 2013. Apples of ‘Idared’ and ‘Braeburn’ showed no statistical significance. Apples of ‘Golden Delicious’ showed higher values in standard cold-storage, while apples of ‘Granny Smith’ showed higher values in ULO storage.

Table 4. Average total acids content (mg/kg) in 4 different cultivars of apple (‘Granny Smith’, ‘Golden Delicious’, ‘Idared’ and ‘Braeburn’) in 2 types of storage (ULO and standard) on measuring term 10. 1. 2013. Standard error is shown next to average result, and a different letter in the right column indicates a statisticaly significant difference ($p \leq 0.05$) in total acids content of different types of storage within each cultivar.
Preglednica 4: Povprečna vsebnost skupnih kislin (mg/kg) pri štirih sortah jabolk (‘Granny Smith’, ‘Zlati delišes’, ‘Idared’ in ‘Braeburn’) in 2 načinih skladiščenja (ULO in navadna atmosfera) pri terminu vzorčenja 10. 1. 2013. Standardna napaka je zapisana zraven povprečne vrednosti, različne črke v desnem stolpcu označujejo statistično značilne razlike ($p \leq 0,05$) v vsebnosti skupnih kislin med načini skladiščenja znotraj posamezne sorte.

TOTAL ACIDS CONTENT	10. 1. 2013			
	ULO		STANDARD STORAGE	
	average +/- standard error	hom. group	average +/- standard error	hom. group
‘Granny Smith’	6.89 +/- 0.24	b	5.90 +/- 0.33	a
‘Golden Del.’	3.97 +/- 0.18	a	4.87 +/- 0.19	b
‘Idared’	6.28 +/- 0.43	a	6.88 +/- 0.24	a
‘Braeburn’	5.14 +/- 0.44	a	5.11 +/- 0.49	a

4.5 ASCORBIC ACID CONTENT

Figure 16 shows average ascorbic acid content in 4 different cultivars, in 2 types of storage and in 3 different terms. In ULO storage apples of ‘Granny Smith’ and ‘Golden Delicious’ showed a decline of ascorbic acid content during storage time, while apples of ‘Idared’ and ‘Braeburn’ even showed an increase of this parameter. In standard cold-storage apples of ‘Granny Smith’ and ‘Golden Delicious’ showed a decline of the ascorbic acid content, while differences regarding apples of ‘Idared’ and ‘Braeburn’ were not statistical significance.

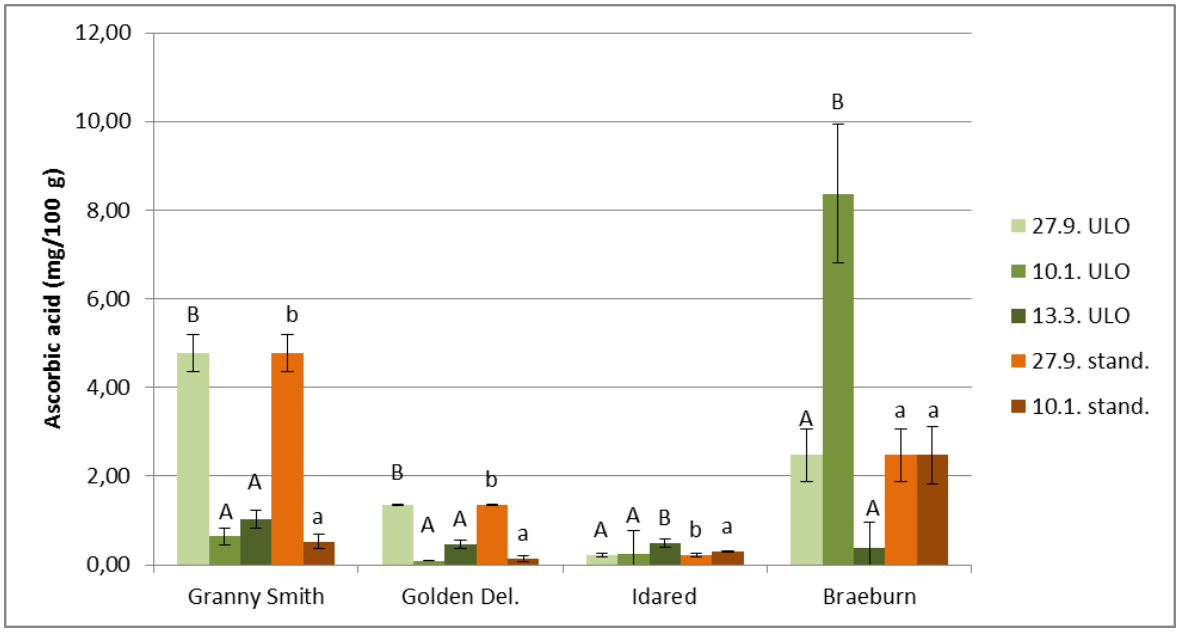

Figure 16. Average content of ascorbic acid (mg/100 g) in 4 different cultivars of apple ('Granny Smith', 'Golden Delicious', 'Idared' and 'Braeburn') in 2 types of storage (ULO and standard) and in 3 different terms (27. 9. 2012, 10. 1. 2013 and 13. 3. 2013). A different letter above the columns indicates a statisticaly significant difference ($p \leq 0.05$) in content of ascorbic acid of a certain cultivar within one type of storage. Capital letter marks a result in ULO storage, and a small one marks a result in standard cold-storage. Types of storage are also marked in different colors.

Slika 16: Povprečna vsebnost askorbinske kisline (mg/100 g) pri štirih sortah jabolk ('Granny Smith', 'Zlati delišes', 'Idared' in 'Braeburn') in 2 načinih skladiščenja (ULO in navadna atmosfera) ter treh terminih vzorčenja (27. 9. 2012, 10. 1. 2013 in 13. 3. 2013). Različne črke nad stolpci označujejo statistično značilne razlike ($p \leq 0,05$) v vsebnosti askorbinske kisline za vsako sorto znotraj enega načina skladiščenja. Velike črke označujejo rezultate pri skladiščenju v ULO atmosferi in majhne črke pri skladiščenju v navadni atmosferi. Načini skladiščenja so označeni tudi z različnima barvama.

Table 5 shows average ascorbic acid content in 4 different cultivars, in 2 types of storage on measuring term 10. 1. 2013. All cultivars except 'Braeburn' showed no statistical significance. 'Braeburn' shows higher values in ULO storage.

Table 5. Average content of ascorbic acid (mg/100 g) in 4 different cultivars of apple (‘Granny Smith’, ‘Golden Delicious’, ‘Idared’ and ‘Braeburn’) in 2 types of storage (ULO and standard) on measuring term 10. 1. 2013. Standard error is shown next to average result, and a different letter in the right column indicates a statisticaly significant difference ($p \leq 0.05$) in content of ascorbic acid of different types of storage within each cultivar.
Preglednica 5: Povprečna vsebnost askorbinske kisline (mg/100 g) pri štirih sortah jabolk (‘Granny Smith’, ‘Zlati delišes’, ‘Idared’ in ‘Braeburn’) in 2 načinih skladiščenja (ULO in navadna atmosfera) pri terminu vzorčenja 10. 1. 2013. Standardna napaka je zapisana zraven povprečne vrednosti, različne črke v desnem stolpcu označujejo statistično značilne razlike ($p \leq 0,05$) v vsebnosti askorbinske kisline med načini skladiščenja znotraj posamezne sorte.

ASCORBIC ACID	10. 1. 2013			
	ULO		STANDARD STORAGE	
	average +/- standard error	hom. group	average +/- standard error	hom. group
‘Granny Smith’	0.64 +/- 0.19	a	0.52 +/- 0.16	a
‘Golden Del.’	0.09 +/- 0.01	a	0.14 +/- 0.06	a
‘Idared’	0.24 +/- 0.53	a	0.30 +/- 0.01	a
‘Braeburn’	8.37 +/- 1.57	b	2.47 +/- 0.65	a

4.6 TOTAL PHENOLIC CONTENT

4.6.1 Total phenolic content in fruit skin

Figure 17 shows an average total phenolic content in fruit skin in 4 different cultivars, in 2 types of storage and in 3 different terms. In ULO storage apples of all cultivars showed a decline in total phenolic content in fruit skin, but in ‘Golden Delicious’ and ‘Granny Smith’ that decline was not statistically significant. In standard cold-storage apples of all cultivars showed a decline in that parameter except in ‘Golden Delicious’, where the difference between the two measurements was not statistical significant.

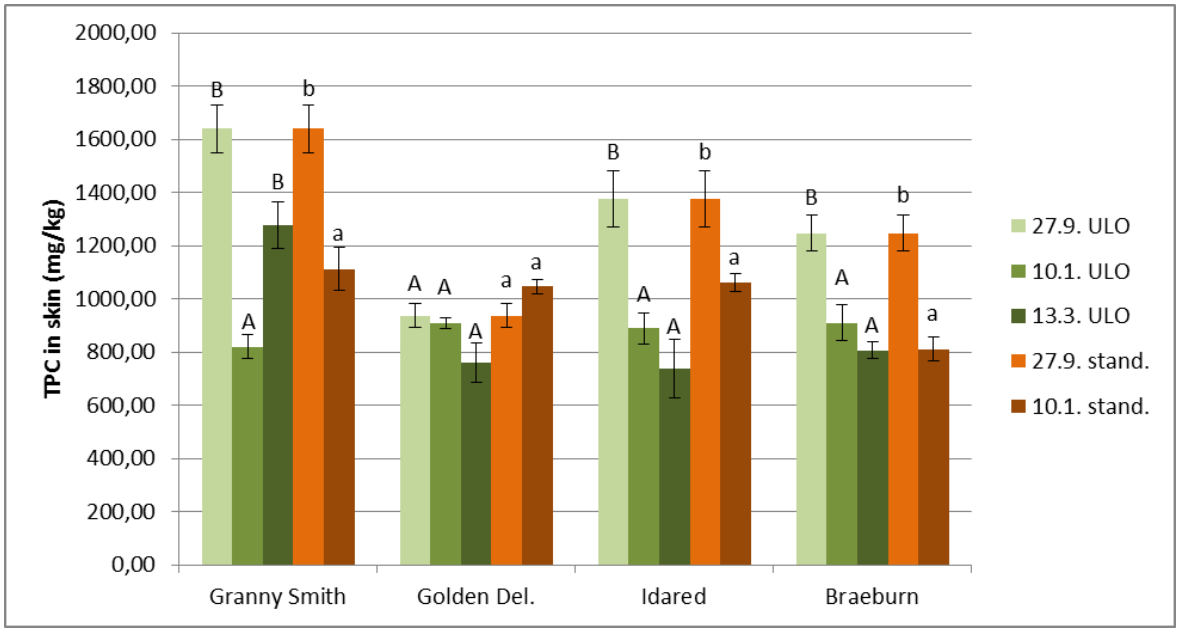

Figure 17. Average total phenolic content in skin (mg/kg) in 4 different cultivars of apple (‘Granny Smith’, ‘Golden Delicious’, ‘Idared’ and ‘Braeburn’) in 2 types of storage (ULO and standard) and in 3 different terms (27. 9. 2012, 10. 1. 2013 and 13. 3. 2013). A different letter above the columns indicates a statisticaly significant difference ($p \leq 0.05$) in total phenolic content in skin of a certain cultivar within one type of storage. Capital letter marks a result in ULO storage, and a small one marks a result in standard cold-storage. Types of storage are also marked in different colors.
Slika 17: Povprečna vsebnost skupnih fenolov v kožici (mg/kg) pri štirih sortah jabolk (‘Granny Smith’, ‘Zlati delišes’, ‘Idared’ in ‘Braeburn’) in 2 načinih skladiščenja (ULO in navadna atmosfera) ter treh terminih vzorčenja (27. 9. 2012, 10. 1. 2013 in 13. 3. 2013). Različne črke nad stolpci označujejo statistično značilne razlike ($p \leq 0{,}05$) v vsebnosti skupnih fenolov v kožici za vsako sorto znotraj enega načina skladiščenja. Velike črke označujejo rezultate pri skladiščenju v ULO atmosferi in majhne črke pri skladiščenju v navadni atmosferi. Načini skladiščenja so označeni tudi z različnima barvama.

Table 6 shows an average total phenolic content in fruit skin in 4 different cultivars, in 2 types of storage on measuring term 10. 1. 2013. Aples of ‘Braeburn’ shows no statistical significance, whereby apples of other cultivars show higher values in standard cold-storage.

Table 6. Average total phenolic content in skin (mg/kg) in 4 different cultivars of apple (‘Granny Smith’, ‘Golden Delicious’, ‘Idared’ and ‘Braeburn’) in 2 types of storage (ULO and standard) on measuring term 10. 1. 2013. Standard error is shown next to average result, and a different letter in the right column indicates a statisticaly significant difference ($p \leq 0.05$) total phenolic content in skin of different types of storage within each cultivar.
Preglednica 6: Povprečna vsebnost skupnih fenolov v kožici (mg/kg) pri štirih sortah jabolk (‘Granny Smith’, ‘Zlati delišes’, ‘Idared’ in ‘Braeburn’) in 2 načinih skladiščenja (ULO in navadna atmosfera) pri terminu vzorčenja 10. 1. 2013. Standardna napaka je zapisana zraven povprečne vrednosti, različne črke v desnem stolpcu označujejo statistično značilne razlike ($p \leq 0,05$) v vsebnosti skupnih fenolov v kožici med načini skladiščenja znotraj posamezne sorte.

TOTAL PHENOLIC CONTENT (SKIN)	10. 1. 2013			
	ULO		STANDARD STORAGE	
	average +/- standard error	hom. group	average +/- standard error	hom. group
‘Granny Smith’	819.90 +/- 44.13	a	1111.75 +/- 81.74	b
‘Golden Del.’	908.44 +/- 20.06	a	1045.99 +/- 25.57	b
‘Idared’	889.23 +/- 57.48	a	1063.01 +/- 33.58	b
‘Braeburn’	909.54 +/- 66.37	a	809.42 +/- 44.89	a

4.6.2 Total phenolic content in fruit pulp

Figure 18 shows average total phenolic content in pulp in 4 different cultivars, in 2 types of storage and in 3 different terms. In ULO storage fruits of all cultivars mostly show growth in total phenolic content in pulp, as they do in standard cold-storage, except for apples of ‘Idared’, where differences in average total phenolic content in pulp were not statistically significant. In the case of ‘Braeburn’ apples TPC in the standard cold-storage is decreasing.

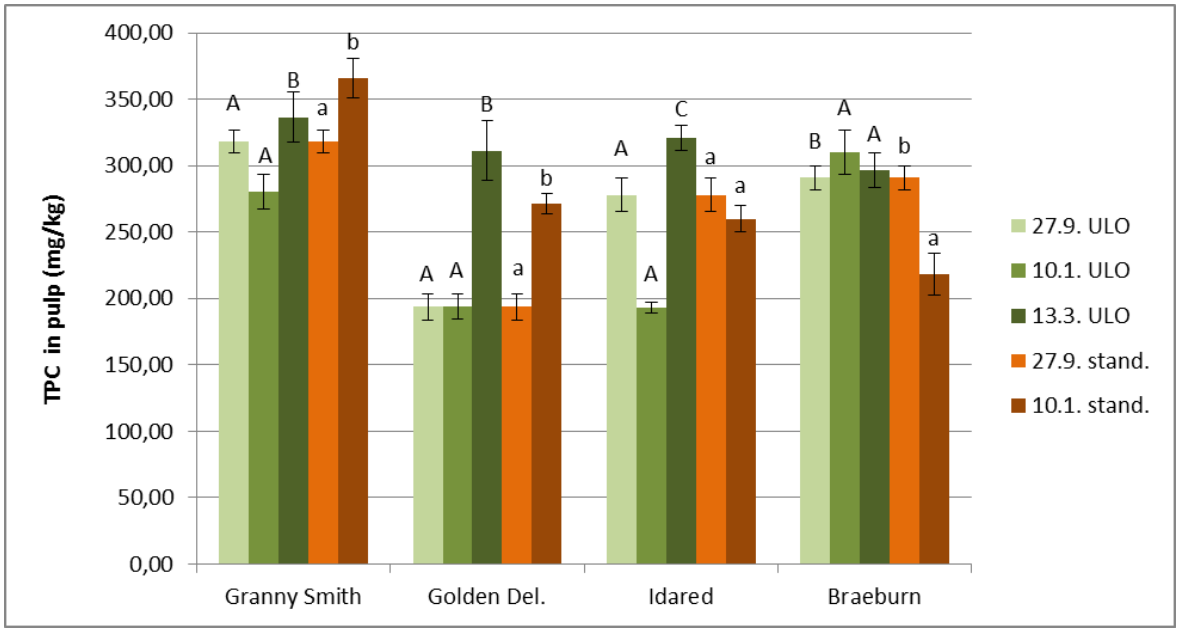

Figure 18. Average total phenolic content in pulp (mg/kg) in 4 different cultivars of apple ('Granny Smith', 'Golden Delicious', 'Idared' and 'Braeburn') in 2 types of storage (ULO and standard) and in 3 different terms (27. 9. 2012, 10. 1. 2013 and 13. 3. 2013). A different letter above the columns indicates a statisticaly significant difference ($p \leq 0.05$) in total phenolic content in pulp of a certain cultivar within one type of storage. Capital letter marks a result in ULO storage, and a small one marks a result in standard cold-storage. Types of storage are also marked in different colors.
Slika 18: Povprečna vsebnost skupnih fenolov v mesu (mg/kg) pri štirih sortah jabolk ('Granny Smith', 'Zlati delišes', 'Idared' in 'Braeburn') in 2 načinih skladiščenja (ULO in navadna atmosfera) ter treh terminih vzorčenja (27. 9. 2012, 10. 1. 2013 in 13. 3. 2013). Različne črke nad stolpci označujejo statistično značilne razlike ($p \leq 0,05$) v vsebnosti skupnih fenolov v mesu za vsako sorto znotraj enega načina skladiščenja. Velike črke označujejo rezultate pri skladiščenju v ULO atmosferi in majhne črke pri skladiščenju v navadni atmosferi. Načini skladiščenja so označeni tudi z različnima barvama.

Table 7 shows average total phenolic content in pulp in 4 different cultivars, in 2 types of storage on measuring term 10. 1. 2013. All cultivars show higher values in standard cold-storage than in ULO storage, except in 'Braeburn'.

Table 7. Average total phenolic content in pulp (mg/kg) in 4 different cultivars of apple (‘Granny Smith’, ‘Golden Delicious’, ‘Idared’ and ‘Braeburn’) in 2 types of storage (ULO and standard) on measuring term 10. 1. 2013. Standard error is shown next to average result, and a different letter in the right column indicates a statisticaly significant difference ($p \leq 0.05$) total phenolic content in pulp of different types of storage within each cultivar.
Preglednica 7: Povprečna vsebnost skupnih fenolov v mesu (mg/kg) pri štirih sortah jabolk (‘Granny Smith’, ‘Zlati delišes’, ‘Idared’ in ‘Braeburn’) in 2 načinih skladiščenja (ULO in navadna atmosfera) pri terminu vzorčenja 10. 1. 2013. Standardna napaka je zapisana zraven povprečne vrednosti, različne črke v desnem stolpcu označujejo statistično značilne razlike ($p \leq 0,05$) v vsebnosti skupnih fenolov v mesu med načini skladiščenja znotraj posamezne sorte.

TOTAL PHENOLIC CONTENT (PULP)	10. 1. 2013			
	ULO		STANDARD STORAGE	
	average +/- standard error	hom. group	average +/- standard error	hom. group
‘Granny Smith’	280.47 +/- 13.17	a	365.88 +/- 14.62	b
‘Golden Del.’	193.89 +/- 9.54	a	271.29 +/- 7.75	b
‘Idared’	192.96 +/- 4.27	a	259.98 +/- 10.08	b
‘Braeburn’	309.82 +/- 16.60	b	218.58 +/- 15.69	a

4.7 HYDROXYCINNAMIC ACID CONTENT

4.7.1 Hydroxycinnamic acid content in fruit skin

Figure 19 shows an average of hydroxycinnamic acid content in fruit skin in 4 different cultivars, in 2 types of storage and in 3 different terms. In ULO storage apples of ‘Granny Smith’, ‘Golden Delicious’ and ‘Braeburn’ showed an increase of hydroxycinnamic acid content, but the results in ‘Golden Delicious’ apples were not statistically significant. Cultivar ‘Idared’ showed a decline of values of this parameter. As for the results in standard cold-storage, ‘Braeburn’ apples showed a decline of the values, but values in ‘Granny Smith’ and ‘Idared’ apples showed no statistical significance between the terms or even an increase in case of ‘Golden Delicious’.

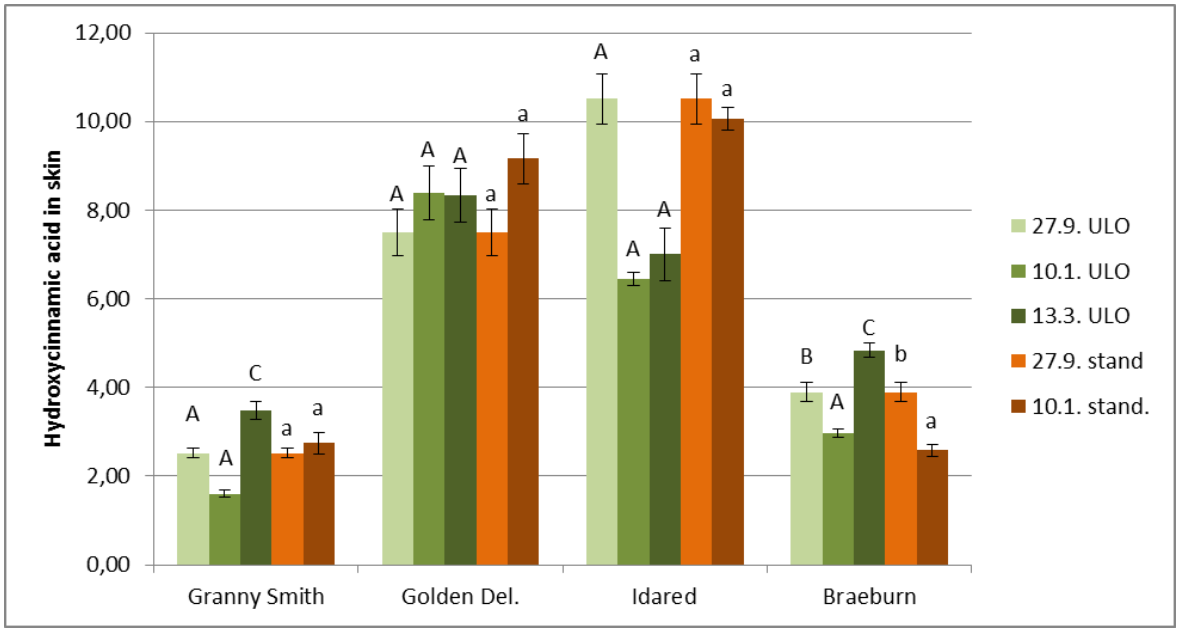

Figure 19. Average hydroxycinnamic acid content in skin (mg/kg) in 4 different cultivars of apple ('Granny Smith', 'Golden Delicious', 'Idared' and 'Braeburn') in 2 types of storage (ULO and standard) and in 3 different terms (27. 9. 2012, 10. 1. 2013 and 13. 3. 2013). A different letter above the columns indicates a statisticaly significant difference ($p \leq 0.05$) in hydroxycinnamic acid content in skin of a certain cultivar within one type of storage. Capital letter marks a result in ULO storage, and a small one marks a result in standard cold-storage. Types of storage are also marked in different colors.
Slika 19: Povprečna vsebnost hidroksicimetne kisline v kožici (mg/kg) pri štirih sortah jabolk ('Granny Smith', 'Zlati delišes', 'Idared' in 'Braeburn') in 2 načinih skladiščenja (ULO in navadna atmosfera) ter treh terminih vzorčenja (27. 9. 2012, 10. 1. 2013 in 13. 3. 2013). Različne črke nad stolpci označujejo statistično značilne razlike ($p \leq 0,05$) v vsebnosti hidroksicimetne kisline v kožici za vsako sorto znotraj enega načina skladiščenja. Velike črke označujejo rezultate pri skladiščenju v ULO atmosferi in majhne črke pri skladiščenju v navadni atmosferi. Načini skladiščenja so označeni tudi z različnima barvama.

Table 8 shows an average of hydroxycinnamic acid content in skin in 4 different cultivars, in 2 types of storage on measuring term 10. 1. 2013. Apples of 'Granny Smith' and 'Idared' showed higher values of hydroxycinnamic acid in standard cold-storage, while apples of 'Golden Delicious' showed no statistical significance. Apples of 'Braeburn' showed higher values of hydroxycinnamic acid in ULO storage.

Table 8. Average hydroxycinnamic acid content in skin (mg/kg) in 4 different cultivars of apple (‘Granny Smith’, ‘Golden Delicious’, ‘Idared’ and ‘Braeburn’) in 2 types of storage (ULO and standard) on measuring term 10. 1. 2013. Standard error is shown next to average result, and a different letter in the right column indicates a statisticaly significant difference ($p \leq 0.05$) hydroxycinnamic acid content in skin of different types of storage within each cultivar.
Preglednica 8: Povprečna vsebnost hidroksicimetne kisline v kožici (mg/kg) pri štirih sortah jabolk (‘Granny Smith’, ‘Zlati delišes’, ‘Idared’ in ‘Braeburn’) in 2 načinih skladiščenja (ULO in navadna atmosfera) pri terminu vzorčenja 10. 1. 2013. Standardna napaka je zapisana zraven povprečne vrednosti, različne črke v desnem stolpcu označujejo statistično značilne razlike ($p \leq 0,05$) v vsebnosti hidroksicimetne kisline v kožici med načini skladiščenja znotraj posamezne sorte.

HYDROXY-CINNAMIC ACID (SKIN)	10. 1. 2013			
	ULO		STANDARD STORAGE	
	average +/- standard error	hom. group	average +/- standard error	hom. group
‘Granny Smith’	1.60 +/- 0.08	a	2.74 +/- 0.25	b
‘Golden Del.’	8.39 +/- 0.61	a	9.16 +/- 0.56	a
‘Idared’	6.45 +/- 0.14	a	10.07 +/- 0.26	b
‘Braeburn’	2.97 +/- 0.09	b	2.58 +/- 0.14	a

4.7.2 Hydroxycinnamic acid content in fruit pulp

Figure 20 shows an average of hydroxycinnamic acid content in pulp in 4 different cultivars, in 2 types of storage and in 3 different terms. In ULO storage apples of all cultivars mostly showed an increase of hydroxycinnamic acid content. As for the results in standard cold-storage, apples of ‘Granny Smith’ and ‘Golden Delicious’ showed an increase in values, ‘Idared’ and ‘Braeburn’ showed no statistical significance between measurments.

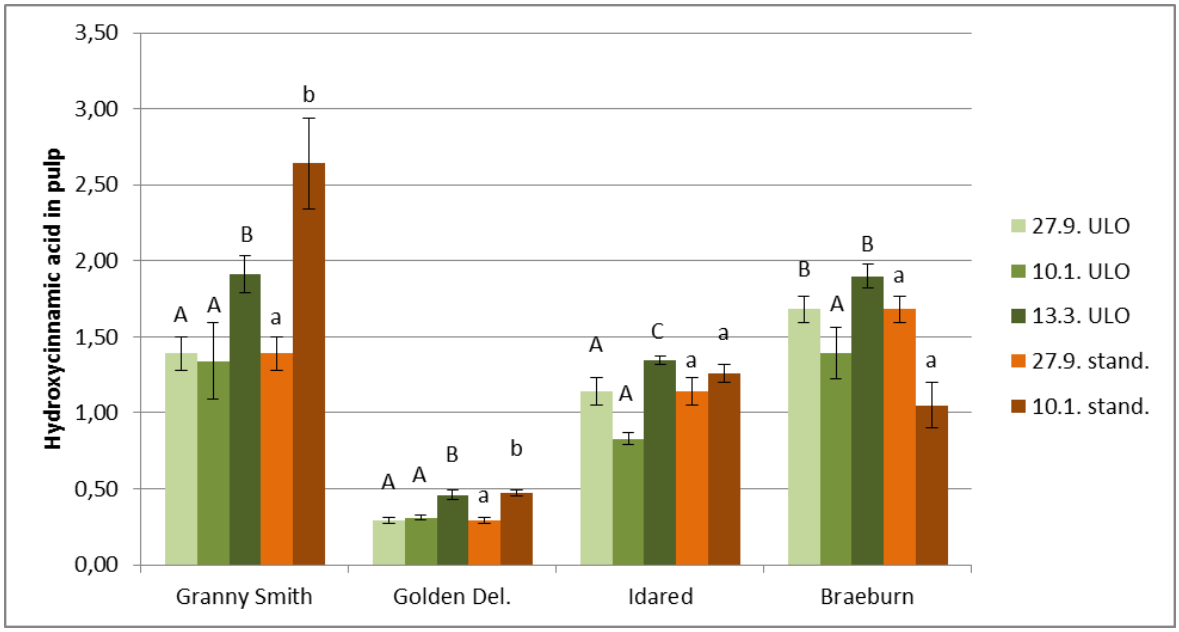

Figure 20. Average hydroxycinnamic acid content in pulp (mg/kg) in 4 different cultivars of apple ('Granny Smith', 'Golden Delicious', 'Idared' and 'Braeburn') in 2 types of storage (ULO and standard) and in 3 different terms (27. 9. 2012, 10. 1. 2013 and 13. 3. 2013). A different letter above the columns indicates a statisticaly significant difference ($p \leq 0.05$) in hydroxycinnamic acid content in pulp of a certain cultivar within one type of storage. Capital letter marks a result in ULO storage, and a small one marks a result in standard cold-storage. Types of storage are also marked in different colors.

Slika 20: Povprečna vsebnost hidroksicimetne kisline v mesu (mg/kg) pri štirih sortah jabolk ('Granny Smith', 'Zlati delišes', 'Idared' in 'Braeburn') in 2 načinih skladiščenja (ULO in navadna atmosfera) ter treh terminih vzorčenja (27. 9. 2012, 10. 1. 2013 in 13. 3. 2013). Različne črke nad stolpci označujejo statistično značilne razlike ($p \leq 0,05$) v vsebnosti hidroksicimetne kisline v mesu za vsako sorto znotraj enega načina skladiščenja. Velike črke označujejo rezultate pri skladiščenju v ULO atmosferi in majhne črke pri skladiščenju v navadni atmosferi. Načini skladiščenja so označeni tudi z različnima barvama.

Table 9 shows an average of hydroxycinnamic acid content in fruit pulp in 4 different cultivars, in 2 types of storage on measuring term 10. 1. 2013. Only 'Braeburn' showed higher values in ULO, and all the other ones show higher values in standard cold-storage treatment.

Table 9. Average hydroxycinnamic acid content in pulp (mg/kg) in 4 different cultivars of apple (‘Granny Smith’, ‘Golden Delicious’, ‘Idared’ and ‘Braeburn’) in 2 types of storage (ULO and standard) on measuring term 10. 1. 2013. Standard error is shown next to average result, and a different letter in the right column indicates a statisticaly significant difference ($p \leq 0.05$) hydroxycinnamic acid content in pulp of different types of storage within each cultivar.

Preglednica 9: Povprečna vsebnost hidroksicimetne kisline v mesu (mg/kg) pri štirih sortah jabolk (‘Granny Smith’, ‘Zlati delišes’, ‘Idared’ in ‘Braeburn’) in 2 načinih skladiščenja (ULO in navadna atmosfera) pri terminu vzorčenja 10. 1. 2013. Standardna napaka je zapisana zraven povprečne vrednosti, različne črke v desnem stolpcu označujejo statistično značilne razlike ($p \leq 0,05$) v vsebnosti hidroksicimetne kisline v mesu med načini skladiščenja znotraj posamezne sorte.

HYDROXY-CINNAMIC ACID (PULP)	10. 1. 2013			
	ULO		STANDARD STORAGE	
	average +/- standard error	hom. group	average +/- standard error	hom. group
‘Granny Smith’	1.34 +/- 0.25	a	2.64 +/- 0.30	b
‘Golden Del.’	0.31 +/- 0.01	a	0.47 +/- 0.02	b
‘Idared’	0.83 +/- 0.04	a	1.26 +/- 0.06	b
‘Braeburn’	1.39 +/- 0.17	b	1.05 +/- 0.15	a

4.8 FLAVANOLS CONTENT

4.8.1 Flavanols content in fruit skin

Figure 21 shows average flavanols content in fruit skin in 4 different cultivars, in 2 types of storage and in 3 different terms. In ULO storage apples of ‘Granny Smith’ and ‘Golden Delicious’ showed an increase of flavanol contents, and ‘Idared’ and ‘Braeburn’ showed a decline of the values. The decline in apples of ‘Braeburn’ was not statistically significant. As the results in standard cold-storage, apples of ‘Granny Smith’ and ‘Braeburn’ showed no statistical significant differences in flavanol contents, apples of ‘Golden Delicious’ showed an increase and ‘Idared’ apples a decline of values.

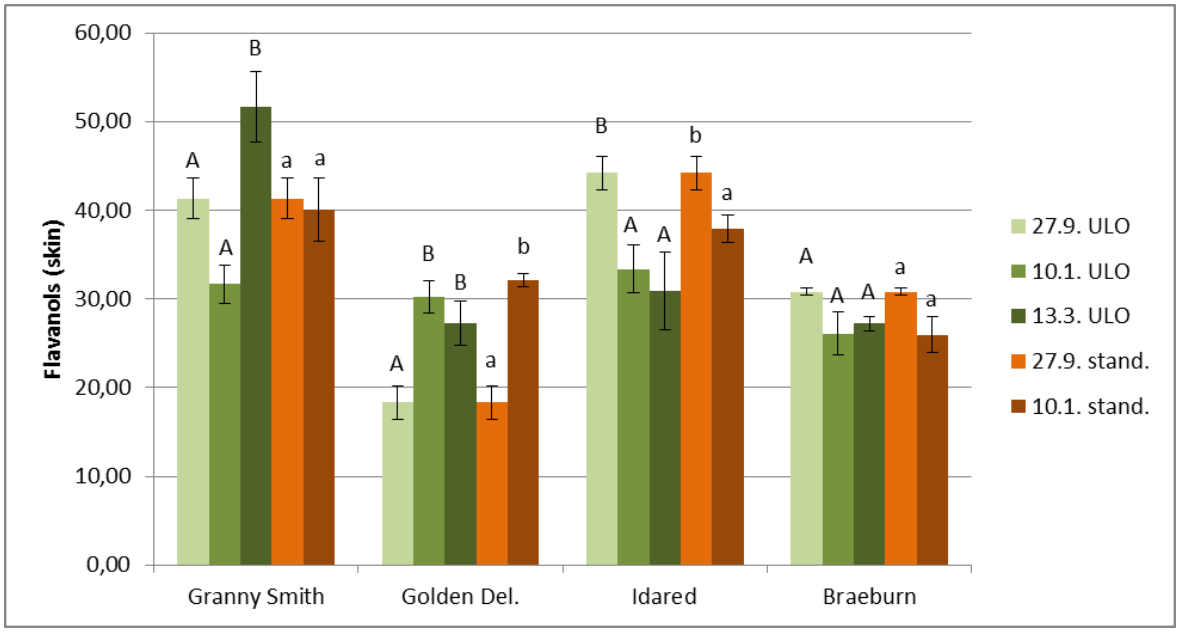

Figure 21. Average flavanols content in skin (mg/kg) in 4 different cultivars of apple (‘Granny Smith’, ‘Golden Delicious’, ‘Idared’ and ‘Braeburn’) in 2 types of storage (ULO and standard) and in 3 different terms (27. 9. 2012, 10. 1. 2013 and 13. 3. 2013). A different letter above the columns indicates a statisticaly significant difference ($p \leq 0.05$) in flavanols content in skin of a certain cultivar within one type of storage. Capital letter marks a result in ULO storage, and a small one marks a result in standard cold-storage. Types of storage are also marked in different colors.
Slika 21: Povprečna vsebnost flavanolov v kožici (mg/kg) pri štirih sortah jabolk (‘Granny Smith’, ‘Zlati delišes’, ‘Idared’ in ‘Braeburn’) in 2 načinih skladiščenja (ULO in navadna atmosfera) ter treh terminih vzorčenja (27. 9. 2012, 10. 1. 2013 in 13. 3. 2013). Različne črke nad stolpci označujejo statistično značilne razlike ($p \leq 0,05$) v vsebnosti flavanolov v kožici za vsako sorto znotraj enega načina skladiščenja. Velike črke označujejo rezultate pri skladiščenju v ULO atmosferi in majhne črke pri skladiščenju v navadni atmosferi. Načini skladiščenja so označeni tudi z različnima barvama.

Table 10 shows average flavanols content in skin in 4 different cultivars, in 2 types of storage on measuring term 10. 1. 2013. ‘Granny Smith’ and ‘Idared’ showed higher values in standard cold-storage, while ‘Golden Delicious’ and ‘Braeburn’ showed no statistical significance differences.

Table 10. Average flavanols content in skin (mg/kg) in 4 different cultivars of apple (‘Granny Smith’, ‘Golden Delicious’, ‘Idared’ and ‘Braeburn’) in 2 types of storage (ULO and standard) on measuring term 10. 1. 2013. Standard error is shown next to average result, and a different letter in the right column indicates a statisticaly significant difference (p≤0.05) flavanols content in skin of different types of storage within each cultivar.
Preglednica 10: Povprečna vsebnost flavanolov v kožici (mg/kg) pri štirih sortah jabolk (‘Granny Smith’, ‘Zlati delišes’, ‘Idared’ in ‘Braeburn’) in 2 načinih skladiščenja (ULO in navadna atmosfera) pri terminu vzorčenja 10. 1. 2013. Standardna napaka je zapisana zraven povprečne vrednosti, različne črke v desnem stolpcu označujejo statistično značilne razlike (p≤0,05) v vsebnosti flavanolov v kožici med načini skladiščenja znotraj posamezne sorte.

FLAVANOLS (SKIN)	10. 1. 2013			
	ULO		STANDARD STORAGE	
	average +/- standard error	hom. group	average +/- standard error	hom. group
‘Granny Smith’	31.66 +/- 2.11	a	40.13 +/- 3.57	b
‘Golden Del.’	30.23 +/- 1.77	a	32.07 +/- 0.76	a
‘Idared’	33.34 +/- 2.69	a	37.86 +/- 1.55	b
‘Braeburn’	26.07 +/- 2.43	a	25.96 +/- 2.03	a

4.8.2 Flavanols content in fruit pulp

Figure 22 shows average flavanols content in fruit pulp in 4 different cultivars, in 2 types of storage and in 3 different terms. In ULO storage apples of all cultivars showed an increase of values, but in cultivar ‘Braeburn’ difference was not statistically significant. As for the results in standard cold-storage, apples of ‘Braeburn’ showed a decline of values, and all the other cultivars showed an increase, but in ‘Idared’ the difference was not statistically significant.

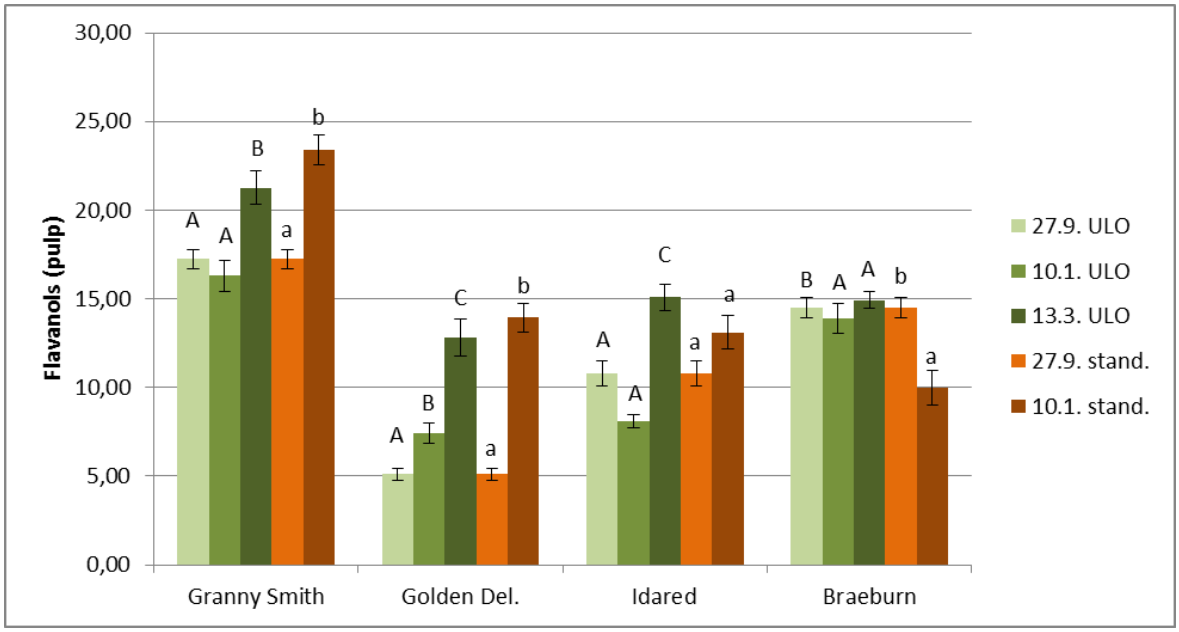

Figure 22. Average flavanols content in pulp (mg/kg) in 4 different cultivars of apple ('Granny Smith', 'Golden Delicious', 'Idared' and 'Braeburn') in 2 types of storage (ULO and standard) and in 3 different terms (2727. 9. 2012, 10. 1. 2013 and 13. 3. 2013). A different letter above the columns indicates a statisticaly significant difference ($p \leq 0.05$) in flavanols content in pulp of a certain cultivar within one type of storage. Capital letter marks a result in ULO storage, and a small one marks a result in standard cold-storage. Types of storage are also marked in different colors.
Slika 22: Povprečna vsebnost flavanolov v mesu (mg/kg) pri štirih sortah jabolk ('Granny Smith', 'Zlati delišes', 'Idared' in 'Braeburn') in 2 načinih skladiščenja (ULO in navadna atmosfera) ter treh terminih vzorčenja (27. 9. 2012, 10. 1. 2013 in 13. 3. 2013). Različne črke nad stolpci označujejo statistično značilne razlike ($p \leq 0,05$) v vsebnosti flavanolov v mesu za vsako sorto znotraj enega načina skladiščenja. Velike črke označujejo rezultate pri skladiščenju v ULO atmosferi in majhne črke pri skladiščenju v navadni atmosferi. Načini skladiščenja so označeni tudi z različnima barvama.

Table 11 shows average flavanols content in fruit pulp in 4 different cultivars, in 2 types of storage on measuring term 10. 1. 2013. Apples of 'Braeburn' showed a higher value of the measuring parameter in ULO storage, apples of all other cultivars showed higher values in standard cold-storage.

Table 11. Average flavanols content in pulp (mg/kg) in 4 different cultivars of apple (‘Granny Smith’, ‘Golden Delicious’, ‘Idared’ and ‘Braeburn’) in 2 types of storage (ULO and standard) on measuring term 10. 1. 2013. Standard error is shown next to average result, and a different letter in the right column indicates a statisticaly significant difference (p≤0.05) flavanols content in pulp of different types of storage within each cultivar.
Preglednica 11: Povprečna vsebnost flavanolov v mesu (mg/kg) pri štirih sortah jabolk (‘Granny Smith’, ‘Zlati delišes’, ‘Idared’ in ‘Braeburn’) in 2 načinih skladiščenja (ULO in navadna atmosfera) pri terminu vzorčenja 10. 1. 2013. Standardna napaka je zapisana zraven povprečne vrednosti, različne črke v desnem stolpcu označujejo statistično značilne razlike (p≤0,05) v vsebnosti flavanolov v mesu med načini skladiščenja znotraj posamezne sorte.

FLAVANOLS (PULP)	10. 1. 2013			
	ULO		STANDARD STORAGE	
	average +/- standard error	hom. group	average +/- standard error	hom. group
‘Granny Smith’	16.29 +/- 0.86	a	23.38 +/- 0.84	b
‘Golden Del.’	7.43 +/- 0.56	a	13.94 +/- 0.79	b
‘Idared’	8.09 +/- 0.35	a	13.10 +/- 0.95	b
‘Braeburn’	13.88 +/- 0.86	b	9.98 +/- 0.96	a

4.9 DIHYDROCHALCONE CONTENT

4.9.1 Dihydrochalcone content in fruit skin

Figure 23 shows average dihydrochalcones content in fruit skin in 4 different cultivars, in 2 types of storage and in 3 different terms. In ULO storage apples of all cultivars mostly showed a decline of the measuring parameter. A decline of values was present in standard cold-storage, but in cultivars ‘Golden Delicious’ and ‘Idared’ that decline was not statistically significant.

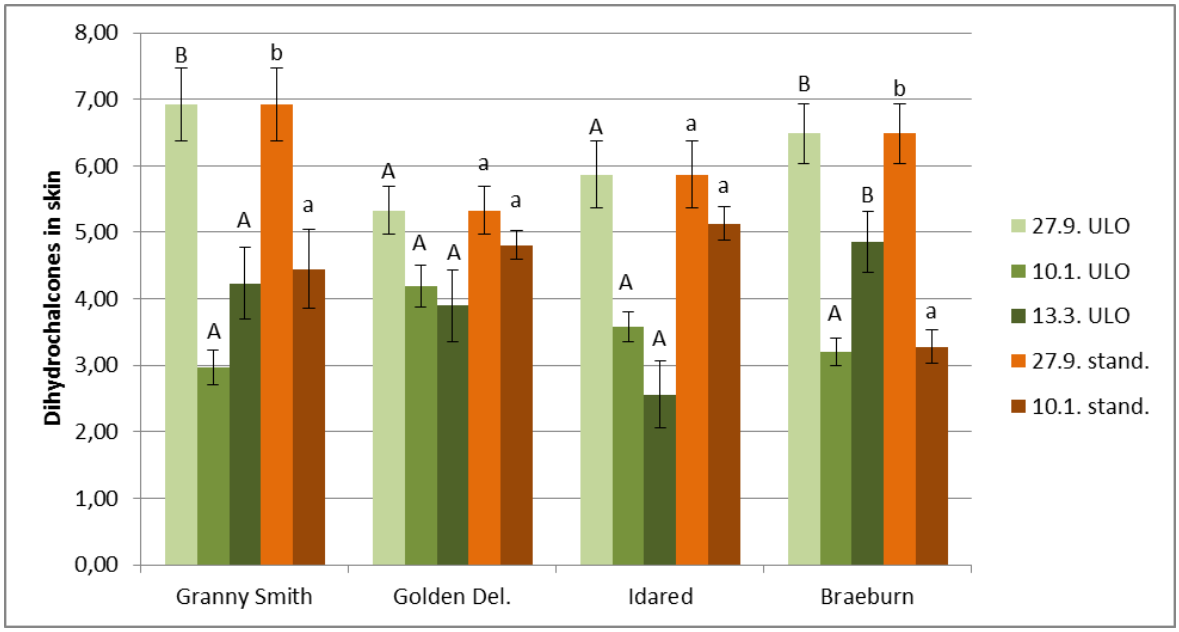

Figure 23. Average dihydrochalcone content in skin (mg/kg) in 4 different cultivars of apple ('Granny Smith', 'Golden Delicious', 'Idared' and 'Braeburn') in 2 types of storage (ULO and standard) and in 3 different terms (27. 9. 2012, 10. 1. 2013 and 13. 3. 2013). A different letter above the columns indicates a statisticaly significant difference ($p \leq 0.05$) in dihydrochalcone content in skin of a certain cultivar within one type of storage. Capital letter marks a result in ULO storage, and a small one marks a result in standard cold-storage. Types of storage are also marked in different colors.
Slika 23: Povprečna vsebnost dihidrohalkonov v kožici (mg/kg) pri štirih sortah jabolk ('Granny Smith', 'Zlati delišes', 'Idared' in 'Braeburn') in 2 načinih skladiščenja (ULO in navadna atmosfera) ter treh terminih vzorčenja (27. 9. 2012, 10. 1. 2013 in 13. 3. 2013). Različne črke nad stolpci označujejo statistično značilne razlike ($p \leq 0,05$) v vsebnosti dihidrohalkonov v kožici za vsako sorto znotraj enega načina skladiščenja. Velike črke označujejo rezultate pri skladiščenju v ULO atmosferi in majhne črke pri skladiščenju v navadni atmosferi. Načini skladiščenja so označeni tudi z različnima barvama.

Table 12 shows average dihydrochalcone content in fruit skin in 4 different cultivars, in 2 types of storage on measuring term 10. 1. 2013. Apples of 'Braeburn' showed no statistical significant differences between the two types of storage, but apples of all the other cultivars showed higher values in standard than in ULO storage.

Table 12. Average dihydrochalcone content in skin (mg/kg) in 4 different cultivars of apple (‘Granny Smith’, ‘Golden Delicious’, ‘Idared’ and ‘Braeburn’) in 2 types of storage (ULO and standard) on measuring term 10. 1. 2013. Standard error is shown next to average result, and a different letter in the right column indicates a statisticaly significant difference ($p \leq 0.05$) dihydrochalcone content in skin of different types of storage within each cultivar.
Preglednica 12: Povprečna vsebnost dihidrohalkonov v kožici (mg/kg) pri štirih sortah jabolk (‘Granny Smith’, ‘Zlati delišes’, ‘Idared’ in ‘Braeburn’) in 2 načinih skladiščenja (ULO in navadna atmosfera) pri terminu vzorčenja 10. 1. 2013. Standardna napaka je zapisana zraven povprečne vrednosti, različne črke v desnem stolpcu označujejo statistično značilne razlike ($p \leq 0,05$) v vsebnosti dihidrohalkonov v kožici med načini skladiščenja znotraj posamezne sorte.

DIHYDRO-CHALCONE (SKIN)	10. 1. 2013			
	ULO		STANDARD STORAGE	
	average +/- standard error	hom. group	average +/- standard error	hom. group
‘Granny Smith’	2.97 +/- 0.26	a	4.45 +/- 0.59	b
‘Golden Del.’	4.19 +/- 0.32	a	4.81 +/- 0.21	b
‘Idared’	3.58 +/- 0.23	a	5.13 +/- 0.25	b
‘Braeburn’	3.20 +/- 0.2	a	3.28 +/- 0.25	a

4.9.2 Dihydrochalcone content in fruit pulp

Figure 24 shows an average of dihydrochalcone content in fruit pulp in 4 different cultivars, in 2 types of storage and in 3 different terms. In ULO storage apples of all cultivars showed an increase of values. Results are mostly the same in standard cold-storage, except for apples of ‘Braeburn’, which showed no statistical significance between the measuring terms.

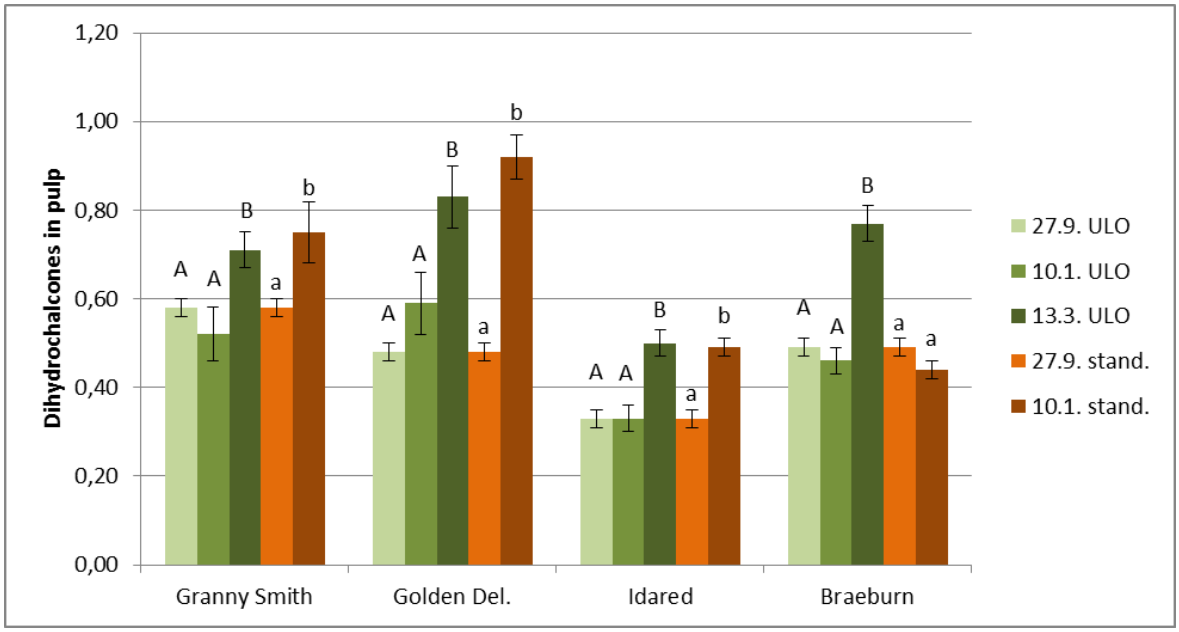

Figure 24. Average dihydrochalcone content in pulp (mg/kg) in 4 different cultivars of apple ('Granny Smith', 'Golden Delicious', 'Idared' and 'Braeburn') in 2 types of storage (ULO and standard) and in 3 different terms (27. 9. 2012, 10. 1. 2013 and 13. 3. 2013). A different letter above the columns indicates a statisticaly significant difference ($p \leq 0.05$) in dihydrochalcone content in pulp of a certain cultivar within one type of storage. Capital letter marks a result in ULO storage, and a small one marks a result in standard cold-storage. Types of storage are also marked in different colors.
Slika 24: Povprečna vsebnost dihidrohalkonov v mesu (mg/kg) pri štirih sortah jabolk ('Granny Smith', 'Zlati delišes', 'Idared' in 'Braeburn') in 2 načinih skladiščenja (ULO in navadna atmosfera) ter treh terminih vzorčenja (27. 9. 2012, 10. 1. 2013 in 13. 3. 2013). Različne črke nad stolpci označujejo statistično značilne razlike ($p \leq 0,05$) v vsebnosti dihidrohalkonov v mesu za vsako sorto znotraj enega načina skladiščenja. Velike črke označujejo rezultate pri skladiščenju v ULO atmosferi in majhne črke pri skladiščenju v navadni atmosferi. Načini skladiščenja so označeni tudi z različnima barvama.

Table 13 shows an average of dihydrochalcone content in fruit pulp in 4 different cultivars, in 2 types of storage on measuring term 10. 1. 2013. 'Braeburn' apples showed no statistical significant differences between the two types of storage, but apples of all the other cultivars showed higher values in standard compared with ULO storage.

Table 13. Average dihydrochalcone content in pulp (mg/kg) in 4 different cultivars of apple (‘Granny Smith’, ‘Golden Delicious’, ‘Idared’ and ‘Braeburn’) in 2 types of storage (ULO and standard) on measuring term 10. 1. 2013. Standard error is shown next to average result, and a different letter in the right column indicates a statisticaly significant difference ($p \leq 0.05$) dihydrochalcone content in pulp of different types of storage within each cultivar.
Preglednica 13: Povprečna vsebnost dihidrohalkonov v mesu (mg/kg) pri štirih sortah jabolk (‘Granny Smith’, ‘Zlati delišes’, ‘Idared’ in ‘Braeburn’) in 2 načinih skladiščenja (ULO in navadna atmosfera) pri terminu vzorčenja 10. 1. 2013. Standardna napaka je zapisana zraven povprečne vrednosti, različne črke v desnem stolpcu označujejo statistično značilne razlike ($p \leq 0,05$) v vsebnosti dihidrohalkonov v mesu med načini skladiščenja znotraj posamezne sorte.

DIHYDRO-CHALCONE (PULP)	10. 1. 2013			
	ULO		STANDARD STORAGE	
	average +/- standard error	hom. group	average +/- standard error	hom. group
‘Granny Smith’	0.52 +/- 0.06	a	0.75 +/- 0.07	b
‘Golden Del.’	0.59 +/- 0.07	a	0.92 +/- 0.05	b
‘Idared’	0.33 +/- 0.03	a	0.49 +/- 0.02	b
‘Braeburn’	0.46 +/- 0.03	a	0.44 +/- 0.02	a

4.10 FLAVONOLS CONTENT

4.10.1 Flavonols content in fruit skin

Figure 25 shows average flavonol content in skin in 4 different cultivars, in 2 types of storage and in 3 different terms. In ULO storage all cultivars mostly show a decline, but in ‘Golden Delicious’ that decline is not statistically significant. Decline is present in standard cold-storage, but in cultivar ‘Idared’ that decline is not statistically significant. ‘Golden Delicious’ here shows a growth.

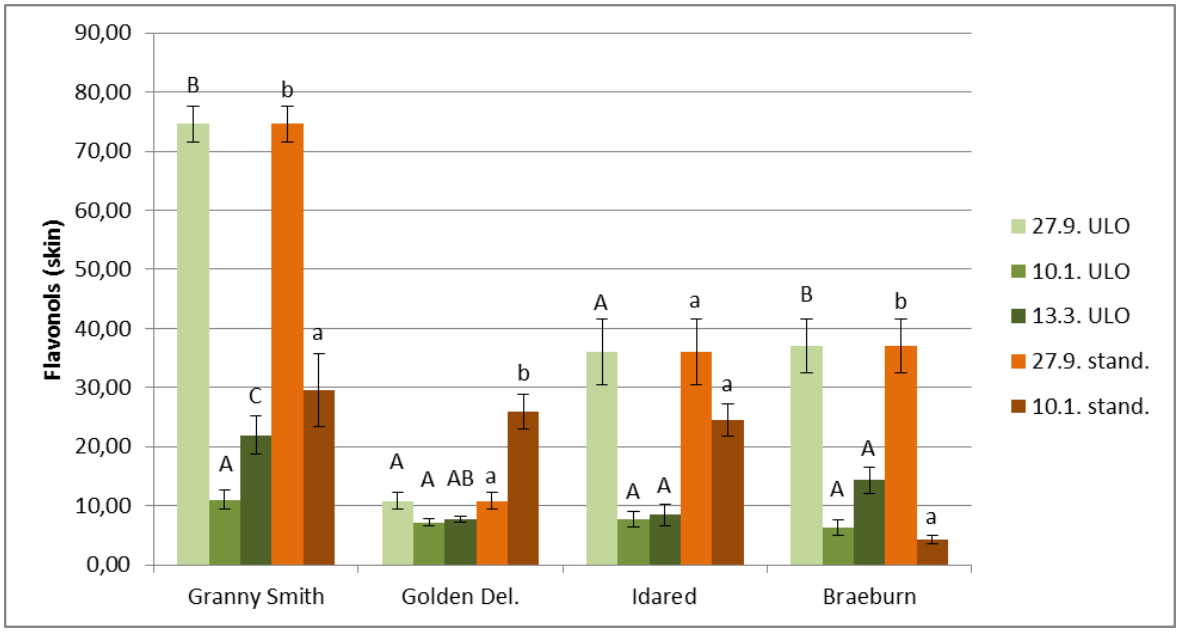

Figure 25. Average flavonol content in skin (mg/kg) in 4 different cultivars of apple (‘Granny Smith’, ‘Golden Delicious’, ‘Idared’ and ‘Braeburn’) in 2 types of storage (ULO and standard) and in 3 different terms (27. 9. 2012, 10. 1. 2013 and 13. 3. 2013). A different letter above the columns indicates a statisticaly significant difference ($p \leq 0.05$) in flavonol content in skin of a certain cultivar within one type of storage. Capital letter marks a result in ULO storage, and a small one marks a result in standard cold-storage. Types of storage are also marked in different colors.
Slika 25: Povprečna vsebnost flavonolov v kožici (mg/kg) pri štirih sortah jabolk (‘Granny Smith’, ‘Zlati delišes’, ‘Idared’ in ‘Braeburn’) in 2 načinih skladiščenja (ULO in navadna atmosfera) ter treh terminih vzorčenja (27. 9. 2012, 10. 1. 2013 in 13. 3. 2013). Različne črke nad stolpci označujejo statistično značilne razlike ($p \leq 0,05$) v vsebnosti flavonolov v kožici za vsako sorto znotraj enega načina skladiščenja. Velike črke označujejo rezultate pri skladiščenju v ULO atmosferi in majhne črke pri skladiščenju v navadni atmosferi. Načini skladiščenja so označeni tudi z različnima barvama.

Table 14 shows average flavonol content in skin in 4 different cultivars, in 2 types of storage on measuring term 10. 1. 2013. ‘Braeburn’ shows no statistical significance between the two types of storage, but all the other cultivars show higher values in standard than in ULO storage.

Table 14. Average flavonol content in skin (mg/kg) in 4 different cultivars of apple (‘Granny Smith’, ‘Golden Delicious’, ‘Idared’ and ‘Braeburn’) in 2 types of storage (ULO and standard) on measuring term 10. 1. 2013. Standard error is shown next to average result, and a different letter in the right column indicates a statisticaly significant difference ($p \leq 0.05$) flavonol content in skin of different types of storage within each cultivar.
Preglednica 14: Povprečna vsebnost flavonolov v kožici (mg/kg) pri štirih sortah jabolk (‘Granny Smith’, ‘Zlati delišes’, ‘Idared’ in ‘Braeburn’) in 2 načinih skladiščenja (ULO in navadna atmosfera) pri terminu vzorčenja 10. 1. 2013. Standardna napaka je zapisana zraven povprečne vrednosti, različne črke v desnem stolpcu označujejo statistično značilne razlike ($p \leq 0,05$) v vsebnosti flavonolov v kožici med načini skladiščenja znotraj posamezne sorte.

FLAVONOL (SKIN)	10. 1. 2013			
	ULO		STANDARD STORAGE	
	average +/- standard error	hom. group	average +/- standard error	hom. group
‘Granny Smith’	11.05 +/- 1.59	a	29.49 +/- 6.19	b
‘Golden Del.’	7.21 +/- 0.59	a	25.95 +/- 2.99	b
‘Idared’	7.73 +/- 1.40	a	24.50 +/- 2.71	b
‘Braeburn’	6.35 +/- 1.29	a	4.34 +/- 0.72	a

4.10.2 Flavonols content in fruit pulp

Figure 26 shows average flavonol content in pulp in 4 different cultivars, in 2 types of storage and in 3 different terms. In ULO storage none of the cultivars show a statistically significant difference, and in standard cold-storage only ‘Braeburn’ doesn’t show a growth.

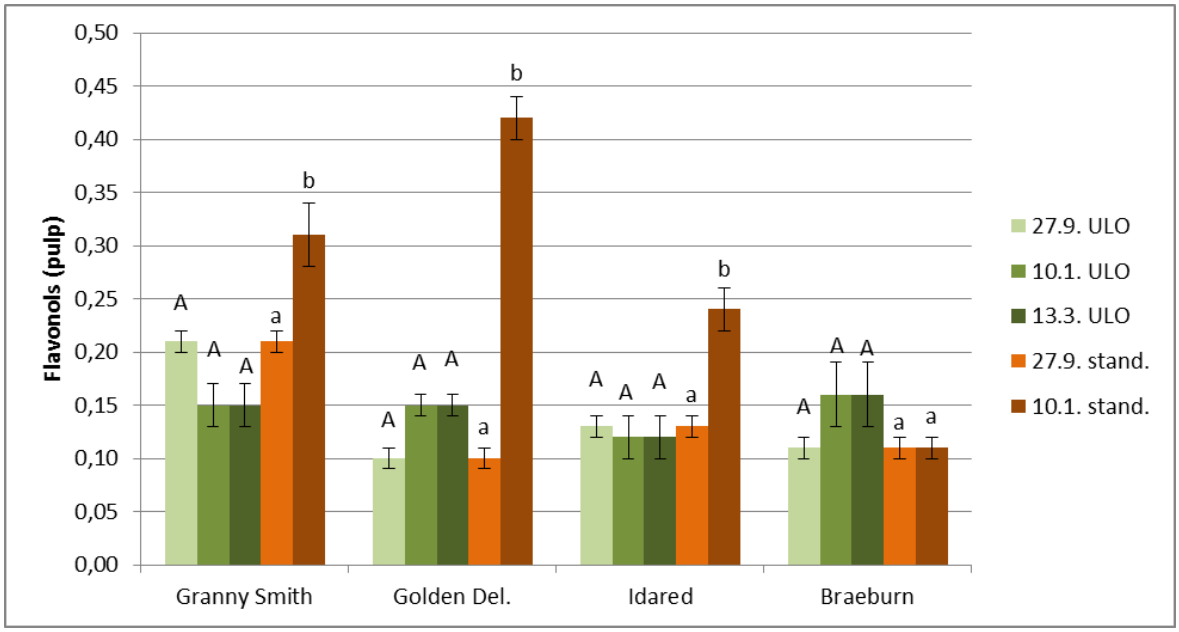

Figure 26. Average flavonol content in pulp (mg/kg) in 4 different cultivars of apple ('Granny Smith', 'Golden Delicious', 'Idared' and 'Braeburn') in 2 types of storage (ULO and standard) and in 3 different terms (27. 9. 2012, 10. 1. 2013 and 13. 3. 2013). A different letter above the columns indicates a statisticaly significant difference ($p \leq 0.05$) in flavonol content in pulp of a certain cultivar within one type of storage. Capital letter marks a result in ULO storage, and a small one marks a result in standard cold-storage. Types of storage are also marked in different colors.

Slika 26: Povprečna vsebnost flavonolov v mesu (mg/kg) pri štirih sortah jabolk ('Granny Smith', 'Zlati delišes', 'Idared' in 'Braeburn') in 2 načinih skladiščenja (ULO in navadna atmosfera) ter treh terminih vzorčenja (27. 9. 2012, 10. 1. 2013 in 13. 3. 2013). Različne črke nad stolpci označujejo statistično značilne razlike ($p \leq 0,05$) v vsebnosti flavonolov v mesu za vsako sorto znotraj enega načina skladiščenja. Velike črke označujejo rezultate pri skladiščenju v ULO atmosferi in majhne črke pri skladiščenju v navadni atmosferi. Načini skladiščenja so označeni tudi z različnima barvama.

Table 15 shows average flavonol content in pulp in 4 different cultivars, in 2 types of storage on measuring term 10. 1. 2013. 'Braeburn' shows higher values in ULO storage, and all the other cultivars show higher values in standard cold-storage.

Table 15. Average flavonols content in pulp (mg/kg) in 4 different cultivars of apple (‘Granny Smith’, ‘Golden Delicious’, ‘Idared’ and ‘Braeburn’) in 2 types of storage (ULO and standard) on measuring term 10. 1. 2013. Standard error is shown next to average result, and a different letter in the right column indicates a statisticaly significant difference ($p \leq 0.05$) flavonols content in pulp of different types of storage within each cultivar.
Preglednica 15: Povprečna vsebnost flavonolov v mesu (mg/kg) pri štirih sortah jabolk (‘Granny Smith’, ‘Zlati delišes’, ‘Idared’ in ‘Braeburn’) in 2 načinih skladiščenja (ULO in navadna atmosfera) pri terminu vzorčenja 10. 1. 2013. Standardna napaka je zapisana zraven povprečne vrednosti, različne črke v desnem stolpcu označujejo statistično značilne razlike ($p \leq 0,05$) v vsebnosti flavonolov v mesu med načini skladiščenja znotraj posamezne sorte.

FLAVONOL (PULP)	10. 1. 2013			
	ULO		STANDARD STORAGE	
	average +/- standard error	hom. group	average +/- standard error	hom. group
‘Granny Smith’	0.15 +/- 0.02	a	0.31 +/- 0.03	b
‘Golden Del.’	0.15 +/- 0.01	a	0.42 +/- 0.02	b
‘Idared’	0.12 +/- 0.02	a	0.24 +/- 0.02	b
‘Braeburn’	0.16 +/- 0.03	b	0.11 +/- 0.01	a

4.11 ANTHOCYANINS CONTENT

Figure 27 shows average anthocyanins in 2 different cultivars, in 2 types of storage and in 3 different terms. ‘Idared’ shows a decline in both types of storage, and ‘Braeburn’ mostly does in none.

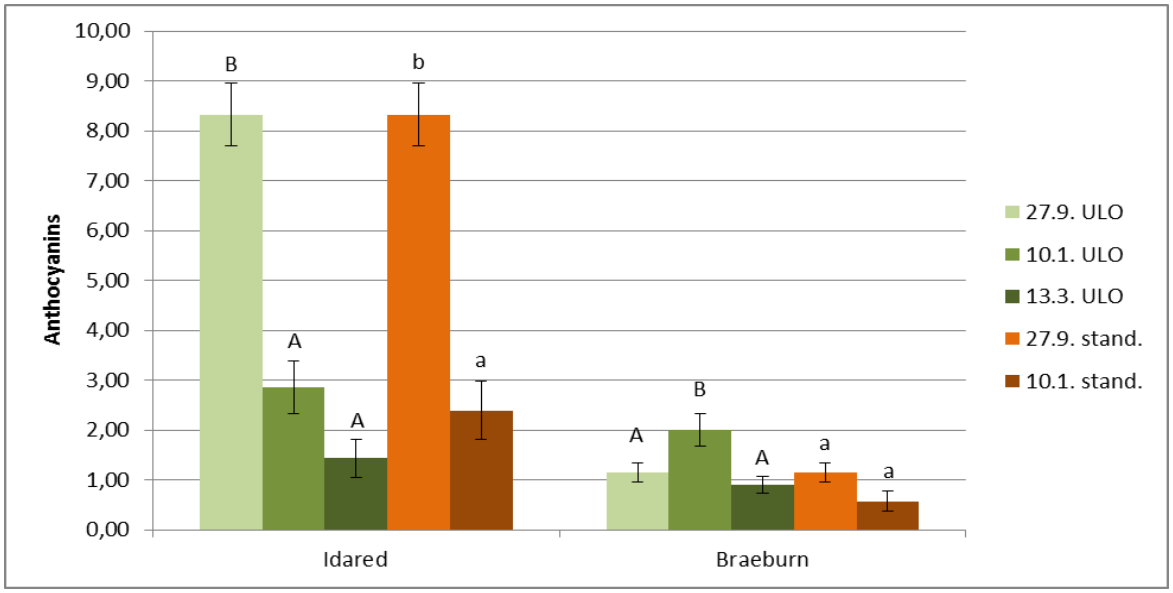

Figure 27. Average anthocyanins content (mg/kg) in 2 different cultivars of apple (‘Idared’ and ‘Braeburn’) in 2 types of storage (ULO and standard) and in 3 different terms (27. 9. 2012, 10. 1. 2013 and 13. 3. 2013). A different letter above the columns indicates a statisticaly significant difference ($p \leq 0.05$) in anthocyanins content of a certain cultivar within one type of storage. Capital letter marks a result in ULO storage, and a small one marks a result in standard cold-storage. Types of storage are also marked in different colors.
Slika 27: Povprečna vsebnost antocianinov (mg/kg) pri dveh sortah jabolk (‘Idared’ in ‘Braeburn’) in 2 načinih skladiščenja (ULO in navadna atmosfera) ter treh terminih vzorčenja (27. 9. 2012, 10. 1. 2013 in 13. 3. 2013). Različne črke nad stolpci označujejo statistično značilne razlike ($p \leq 0,05$) v vsebnosti antocianinov za vsako sorto znotraj enega načina skladiščenja. Velike črke označujejo rezultate pri skladiščenju v ULO atmosferi in majhne črke pri skladiščenju v navadni atmosferi. Načini skladiščenja so označeni tudi z različnima barvama.

Table 16 shows an average of anthocyanin contents in 2 different cultivars, in 2 types of storage on measuring term 10. 1. 2013. Apples of ‘Braeburn’ showed higher values in ULO storage, and apples of ‘Idared’ did not show any statistical significant differences between the two types of storage.

Table 16. Average anthocyanins content (mg/kg) in 2 different cultivars of apple (‘Idared’ and ‘Braeburn’) in 2 types of storage (ULO and standard) on measuring term 10. 1. 2013. Standard error is shown next to average result, and a different letter in the right column indicates a statisticaly significant difference ($p \leq 0.05$) anthocyanins content in pulp of different types of storage within each cultivar.
Preglednica 16: Povprečna vsebnost antocianinov (mg/kg) pri sveh sortah jabolk (‘Idared’ in ‘Braeburn’) in 2 načinih skladiščenja (ULO in navadna atmosfera) pri terminu vzorčenja 10. 1. 2013. Standardna napaka je zapisana zraven povprečne vrednosti, različne črke v desnem stolpcu označujejo statistično značilne razlike ($p \leq 0{,}05$) v vsebnosti antocianinov med načini skladiščenja znotraj posamezne sorte.

ANTHOCYANINS	10. 1. 2013			
	ULO		STANDARD STORAGE	
	average +/- standard error	hom. group	average +/- standard error	hom. group
‘Idared’	2.85 +/- 0.53	a	2.39 +/- 0.59	a
‘Braeburn’	2.00 +/- 0.32	b	0.57 +/- 0.20	a

5 DISSCUSION

5.1 FRUIT FIRMNESS

In fruit firmness we noticed a trend of decline in fruits of almost all cultivars and in both types of storage. In ULO storage differences between the measurement terms were not as drastic as those in standard cold-storage. As the values of fruit firmness of apples held in standard cold-storage of cultivars 'Idared' and 'Golden Delicious' drop below 5 kg/cm^2, we have to conclude that the fruits were on a border of being marketable.

'Braeburn' shows the biggest differences in three measurement terms in ULO storage, and also in two measurement terms in standard cold-storage.

Only 'Granny Smith' shows no statistically significant difference between the initial and final measurement, and only the middle measurement, after 14 weeks of storage, is statistically lower than the initial and final measurement. That seems to be a normal occurrence in cultivar 'Granny Smith', according to Watkins et al. (1991).

Also, lower values were observed (in difference between ULO and standard cold-storage) in standard cold-storage after 14 weeks of storage (measurement term 10. 1. 2013) in all cultivars except 'Braeburn'.

Generally, our assumption was confirmed – lower values were achieved in time in both types of storage. The same results were confirmed by many authors, one of which is Kovač et al. (2010), where after 30 weeks of storage, flesh firmness of 'Cripps Pink' declined from 8.53 kg/cm^2 to 6.78 kg/cm^2.

5.2 SOLUBLE SOLIDS CONTENT

In soluble solids content we noticed a trend of increase in all cultivars, except in 'Idared'. Apples of 'Idared' showed the lowest value on a measurement made after 15 weeks of storage, and a statistically insignificant difference between the initial and final measuring term. As for the measurements of apples held in standard cold-storage, results are mainly the same, except, again, in 'Idared', where we notice a decline. Also growth is not statistically significant in cultivar 'Braeburn' in standard cold-storage.

Regarding the comparison of the two types of storage on a measuring date 10. 1. 2013 (15 weeks after harvest), results show that in cultivars 'Granny Smith' and 'Braeburn' soluble solids content was higher in ULO storage, and in cultivars 'Golden Delicious' and 'Braeburn', it was higher in standard cold-storage.

Generally, our assumption was confirmed – higher values of soluble solids with time were expected. What was not expected was a lower value in ‘Idared’ after 15 weeks of storage, and a higher one after 24 weeks. Different results were achieved by Kovač et al. (2010), where they also had 3 terms of measurements (immediately after harvest, after 14 weeks and after 30 weeks). The lowest value was achieved on the final measurement term, and a gradual decline with time was noticed.

5.3 TOTAL SUGAR CONTENT

Measurement results of total sugar content were mostly uniform. In fruits of all cultivars in both types of storage we noticed a decline, statistically significant in all examples except in ‘Granny Smith’ in standard cold-storage.

Results were not so straightforward when comparing two types of storage on measuring term 10. 1. 2013. ‘Granny Smith’ and ‘Braeburn’ showed higher values in ULO, and ‘Golden Delicious’ and ‘Idared’ in standard cold-storage. The difference was not statistically significant in cultivar ‘Idared’. Other authors, such as Ackermann at al. (1992) reported a drop at the beginning of storage and growth until 15th week of storage, where it reaches its peek, and stagnation afterwards, until the end of experiment at week 25. The sudden drop of sugars at the beginning of storage period can be explained by the fact that the apples were harvested just before the climacteric phase. This phase is characterized by a period of increased respiration during which the sugars (and acids) are rapidly used as substrates in the metabolic processes. Once the cell growth period is terminated, the sugar content does not vary much anymore.

5.4 TOTAL ORGANIC ACIDS CONTENT

In total acids content, there is a visible decline trend in all cultivars and in both types of storage. We noticed a slight increase in final measurement with cultivars ‘Golden Delicious’ and ‘Idared’.

As for the differences between the two types of storage on measuring term 10. 1. 2013, statistically there were no difference in cultivars ‘Idared’ and ‘Braeburn’, while there were higher values in ULO storage regarding ‘Granny Smith’, and lower regarding ‘Golden Delicious’. Our results were mostly in accordance with the results of other authors, such as Napolitano et al. (2004) or Goliáš et al. (2008), where values with the passing time of storage also decreased.

5.5 ASCORBIC ACID CONTENT

In ascorbic acid content, the trend in 'Granny Smith' and 'Golden Delicious' was, as we expected - decline in time, both in ULO and standard cold-storage. In cultivar 'Idared', in standard cold-storage there was no statistically significant difference between the data, and in ULO storage, higher values were measured on the final measurement term. As for the cultivar 'Braeburn', there is a decline (not a statistically significant one) from the initial to the final measurement, but there were a very high values measured on the second measuring term, after 14 weeks of storage. As in 'Idared', there is no difference between the terms in standard cold-storage.

As for the differences between the two types of storage on measuring term 10. 1. 2013, statistically there were none, except for the cultivar 'Braeburn', where much higher values were achieved in ULO than in standard cold-storage.

Our assumptions were generally confirmed, in time, there was a decline in ascorbic acid content. Similar results were achieved by other authors, such as Kovač et al. (2010), where with time spent in storage, gradual decline of ascorbic acid was recorded on a cultivar 'Cripps Pink'. Loss of vitamin C during storage is influenced by storage conditions such as temperature, storage duration and athmosphere. Kevers at al. (2011) in analysis of 'Jonagold' cultivar found that ascorbic acid content is, after 3 months in storage much higher in ULO storage, while after 6 months they equalize, and the apples held in standard cold-storage don't get to be analyzed on final term of measurements as they collapse.

5.6 TOTAL PHENOLIC CONTENT

Total phenolic content was measured in skin and in pulp separately. In TPC measured in skin, there is a noticeable decline trend in almost all cultivars and in both types of storage. Cultivar 'Granny Smith' shows the lowest value on the second measurement term, after 14 weeks of storage, and no statistically significant difference between the initial and final measurement. 'Golden Delicious' shows no statistical difference between any of the measuring terms, either in ULO or in standard cold-storage.

The differences between the two types of storage on measuring term 10. 1. 2013 are statistically significant (higher values in standard cold-storage) in all cultivars, except in 'Braeburn', which shows higher values in ULO storage.

In TPC measured in pulp, results are not that straightforward. Cultivar 'Granny Smith' shows growth in both types of storage, as does 'Golden Delicious'. 'Idared' shows a relative growth in ULO, and no change in standard cold-storage, while 'Braeburn' shows a growth in ULO and a decline in standard cold-storage.

Comparison of the two types of storage on measuring term 10. 1. 2013. is more simple - standard cold-storage shows higher values than ULO in all cultivars.

Results obtained by other authors can somewhat confirm our own. Kovač et al. (2010) found that there is a major drop right after picking, so there is almost no difference between the values on a measuring term after 14 weeks of storage and the one after 30 weeks. On the other hand, Napolitano et al. (2004) recorded the lowest value right after picking, the highest value after 3 months of storage and the middle one after 4 months of storage. Leja et al. (2003) reported an increase after 120 days of storage both in standard and in ULO storage. Increase was more noticeable in ULO than in standard cold-storage. The studies available on the phenols composition during storage are mainly focused on the peels.

The increase of total phenols in apple skin during cold storage could be due to the ethylene action. This hormone stimulates activity of key enzimes in biosynthesis of phenolic compounds (Leja et al., 2003).

5.7 HYDROXYCINNAMIC ACIDS

Hydroxycinnamic acid content was measured separately in skin and in pulp. Results in skin were not so uniform nor we could notice a trend. In ULO values increased with the time spent in storage in cultivars ‘Granny Smith’, ‘Golden Delicious’ (not statistically significant) and ‘Braeburn’, while they dropped in ‘Idared’. In standard cold-storage ‘Granny Smith’ and ‘Idared’ show statistically significant difference, ‘Golden Delicious’ shows a growth, and ‘Braeburn’ a decline.

The difference between the two types of storage (10. 1. 2013), shows that there was no statistically significant difference in cultivar ‘Golden Delicious’, while ‘Granny Smith’ and ‘Idared’ showed a higher value in standard cold-storage and ‘Braeburn’ in ULO storage.

Regarding the results in pulp, results show a growth with time spent in storage, with some minor fluctuations in ‘Braeburn’. As for the standard cold-storage, ‘Granny Smith’ and ‘Golden Delicious’ show growth, and ‘Idared’ and ‘Braeburn’ show no statistical difference.

In comparison of the two types of storage (10. 1. 2013), all cultivars, except ‘Braeburn’, show a higher value in standard cold-storage.

Other authors, such as Golding at al. (2001), where they stored three apple cultivars also in two types of storage, also didn’t have so straightforward results, but they conclude that there was almost (if we disregard some minor peeks) no change in hydroxycinnamic acid

content in nine months of storage. They connect the high concentrations of cinnamic acid derivates in peel with the high concentration of chlorogenic acid. The general trends in the concentrations of chlorogenic acid were similar to those of hydroxycinnamic acid.

5.8 FLAVANOLS

Flavanols content was measured in skin and separately in pulp. Regarding the results obtained in skin in ULO storage, the flavanol content is higher with time in apples of 'Granny Smith' and 'Golden Delicious' and decreasing in 'Idared' apples. 'Braeburn' shows no statistically significant difference.

As for the results in standard cold-storage, 'Braeburn' and 'Granny Smith' show no statistical difference, 'Golden Delicious' shows a growth and 'Idared' shows a decline. The difference between the two types of storage, observed on the measurement term 10. 1. 2013. shows that there was no statistically significant difference in cultivars 'Golden Delicious' and 'Braeburn', while 'Granny Smith' and 'Idared' show higher values in standard cold-storage.

Results obtained in pulp in ULO storage show a growing trend, but in cultivar 'Braeburn' that growth is not statistically significant. Results are similar in standard cold-storage, but growth is not statistically significant in cultivar 'Idared' and there is a decline present in cultivar 'Braeburn'.

Comparing of the two types of storage on measuring term 10. 1. 2013, we noticed higher values in standard cold-storage in all cultivars except in 'Braeburn'.

Carbone at al. (2011) measured flavanols immediately after harvest and after three months of storage both in skin and pulp. Results were somewhat different. In both, 'Braeburn' and 'Golden Delicious', they measured a decline in flavanols content over time. The same results were obtained in both skin and pulp. Awad and de Jager (2000) reported almost no difference between the two types of storage and a decline in flavanols with the time of storage. Results were the same for the measurements of flavanols in skin.

5.9 DIHYDROCHALCONES

Results obtained in measurements of fruit skin stored both in ULO and standard cold-storage show a declining trend in all cultivars, but 'Idared' and 'Golden Delicious' don't show a statistically significant difference between the terms in standard cold-storage. Comparing of the two types of storage on measuring term 10.1.2013, we noticed higher values in standard cold-storage in all cultivars except in 'Braeburn', which shows no statistically significant difference.

Results obtained in measurements of fruit pulp stored both in ULO and standard cold-storage show a growth trend in all cultivars, but ‘Braeburn’ doesn’t show a statistically significant difference between the terms in standard cold-storage.

Comparing of the two types of storage on measuring term 10. 1. 2013, we noticed higher values in standard cold-storage in all cultivars except in ‘Braeburn’, which shows no statistically significant difference.

Other authors, such as Golding at al. (2001) where they stored three apple cultivars also in two types of storage, reviewed that the only chalcone identified in the peel of apples was phloridzin, and it was present at in relatively low concentrations. Levels of phloridzin in ‘Granny Smith’ were practicaly identical throughout the measurement period of nine months.

5.10 FLAVONOLS

Flavonols content was measured in skin and separately in pulp. Regarding the results obtained in skin in ULO storage, we can notice a decline in all cultivars, except for ‘Golden Delicious’, where the decline is not statistically significant. In standard cold-storage, decline is present in all cultivars except in ‘Golden Delicious’, where we can see a growth.

In comparison of the two types of storage, we noticed higher values in standard cold-storage in all cultivars except for ‘Braeburn’.

Measurements of flavonols in pulp were somewhat different. Results in ULO storage show no statistical difference in either of the cultivars, but in standard cold-storage we noticed a growth in all cultivars except in ‘Braeburn’.

As for the comparison of the two types of storage, we noticed higher values in standard cold-storage in all cultivars except in ‘Braeburn’.

Patterns of changes in flavonols content during storage are variable. Piretti et al. (1994) found that they generally decreased in ‘Granny Smith’ towards the end of storage, both in air and controlled atmosphere. In contrast, Kolesnik et al. (1977) found that the concentration of flavonols increased during storage. On the other hand Ju et al. (1996) reported a slow decrease in 4-5 months of storage. This slower decrease in flavonoids might be credited to the limitation of simple phenols to initiate the oxidation reaction.

5.11 ANTHOCYANINS

In anthocyanins content in ULO storage, in cultivar ‘Idared’ we noticed a decline, while in ‘Braeburn’ the measurement made on 10. 1. 2013 showed the highest value, and the initial and final data measurement were lower and there is almost no statistically significant difference between them. In both cultivars there is a decline in standard cold-storage, but in ‘Braeburn’ it’s not statistically significant.

As for the comparison of the two types of storage, ‘Idared’ shows no statistical difference and ‘Braeburn’ shows higher value in ULO storage.

Other authors are displaying somewhat contrary results. Kolesnik et al. (1977) found that concentration of anthocyanins increased during storage, while Ju at al. (1996) reported no changes. Leja et al. (2003) reported that content of anthocyanins decreased in fruits stored in standard cold-storage, while in controlled athmosphere their level did not change.

6 CONCLUSIONS

In this experiment we studied the amount of soluble solids, fruit firmness, ascorbic acid, TSC, total acids content, TPC, hydroxycinnamic acid, flavanols, dihydrochalcones, flavonols and anthocyanins in four apple cultivars, in two types of storage and in three different measurement terms: 27th September 2012, 10th January 2013 and 13th March 2013. Fruits held in standard cold-storage did not stay fresh until the measurement term in March.

In comparison of the measurement terms we found that, generally, there are statistically great differences between them. Moreover, we found that there are greater differences between the first measuring term (27. 9. 2012) and the second one (10. 1. 2013), than between the second and the third one (13. 3. 2013)

Taking the data collected with this experiments into consideration, we can conclude that, with proper storing and handling, apple can be sold (with almost equal quality and characteristics) troughout the sales period – from one harvest to the next one.

As for the comparison of the two types of storage on a measuring term 10. 1. 2013. (15 weeks in storage) we concluded that one of the characteristics of apple, its firmness of fruit, was generally higher in ULO storage than in standard one.

The other measurement parameters were higher in standard cold-storage than in ULO. We assume that this can be partly explained by a greater loss of water from fruits held in standard cold-storage than in ULO, so the concentration of all its components was greater in fruits held in standard cold-storage.

While comparing the measurement terms in one type of storage as well as between both types of storage, the changes can be probably explained by an increased respiration and ethylene action.

Values of measurements of soluble solids content, TPC in pulp, hydroxycinammic acids, flavanols, dihydrochalcones in pulp and flavonols in pulp (in standard cold-storage) were higher at the end of storage, and those of fruit firmness, ascorbic acid, total sugar content, total acids content, TPC in skin, dihydrochalcones in skin and flavonoles in skin were lower. Concentrations of flavonols in pulp and anthocyanins did not change during the time spent in storage.

Regardless of the component that we measure or observe, or the fact that the trend is declining or growing, or that all the other cultivars had higher results in ULO, cultivar ‘Braeburn’ almost always opposed. The biggest surprise was that, when comparing the two

types of storage, it seamed as if standard cold-storage suited it better than ULO. While in standard cold-storage it didn't endure until the measurement in March, in ULO storage there was massive damage and expences caused by senescent breakdown of 'Braeburn' and 'Jonagold' (Figure 28).

Figure 28. Senescent breakdown of cultivars 'Braeburn' and 'Jonagold' (private photo, 23.01.2013)
Slika 28: Notranji zlom plodov sort 'Braeburn' in 'Jonagold' (privatna fotografija, 23. 01. 2013)

For future research it would be interesting to prolong the experiment until late summer (before picking new-seasons fruits) and also to observe the influence of fertilization on fruit characteristics and storage performance.

7 SUMMARY (POVZETEK)

7.1 SUMMARY

In an apple orcard situated in Katoličko Selišće in central Croatia, in year 2012 we picked our plant material - fruits of four different apple cultivars: 'Granny Smith', 'Golden Delicious', 'Idared' and 'Braeburn'. First measurements were made immediately after picking, on 27th September 2012. Part of the fruits was stored in standard cold-storage, and the rest in ULO chamber. The second measurement was made on 10th January 2013, after 15 weeks in storage, and the third one on 13th March 2013, after 24 weeks in storage. Frutis held in standard cold-storage didn't make it to measurements in March.

With every cultivar, on each date and on each storage type we had 5 repetitions, each contained of two apples. Analysis of soluble solids, fruit firmness, total acid content, ascorbic acid, hydroxycinnamic acid, total sugar content, total phenolic content, flavanols, dihydrochalcones, flavonoles and anthocyanins was made in laboratory of Chair for fruit growing, viticulture and vegetable growing, Biotechnical Faculty, University of Ljubljana.

In all fruits fruit firmness was measured with a penetrometer on opposite cheeks, soluble solids with a digital refractometer, and parts of fruits were than frozen for following analysis. By usage of HPLC, after chemical and physical preparation of samples, the amount of total acid content, ascorbic acid, hydroxycinnamic acid, total sugar content, total phenolic content, flavanols, dihydrochalcones, flavonoles and anthocyanins were determined. For the determination of TPC we used Folin-Ciocaleteu reagent.

We concluded that the amount af almost all components notably changes between the first and the second measurement terms. The changes were minor between the second and third measuring terms.

When comparing the two types of storage on a measuring term 10. 1. 2013, we can assume that apples held in standard cold-storage have lost greater amounts of water and so have had higher concentrations of all the components. That can also occur due to higher metabolic activity.

The only exception to the rule in both comparison of measurement terms and types of storage was cultivar 'Braeburn', which later, in ULO storage, showed significant signs of senescent breakdown.

On the basis of our results we have concluded that both of our hypothesis can be confirmed. Time spent in storage and the type of storage have influence on fruit firmness, content of soluble solids and secondary metabolites.

7.2 POVZETEK

UVOD

Na zahtevnem evropskem in svetovnem trgu jabolk skoraj ni prostora za napake v kakovosti in sestavi ploda. Pridelek mora biti brezhiben. Tako kot so določene zahteve pri pridelavi, so določene tudi pri shranjevanju in skladiščenju. Jabolka je treba obrati, ko so optimalno zrela. Samo tista najboljše kakovosti se hranijo dalj časa. Njihovo skladiščenje oblikuje precejšen del končne cene, zato mora biti čim ugodnejše in čim učinkovitejše. Skladiščenje mora zaščititi pridelek pred staranjem in drugimi spremembami v sestavi, ki spadajo zraven.

Poleg dejstva, da sadje, ki smo ga skladiščili standardno, ni zdržalo do meritev v marcu, ni niti približno imelo tako dobrih rezultatov kot tisto, ki smo ga skladiščili v ULO atmosferi. Glede na to, da smo to predvideli, saj je bila to naša hipoteza, je bil namen raziskave dokazati, kakšne so dejanske prednosti skladiščenja v ULO atmosferi, kaj izgubimo pri sadju, če ga po obiranju ne obdelamo pravilno, in kako se jabolko spremeni proti koncu sezone – katere lastnosti in vrednosti se izgubijo pri tako dolgem skladiščenju.

PREGLED LITERATURE

Jablana (*Malus domestica* Borkh.)

Jabolko je vrsta sadja, ki ga upravičeno imenujemo kralj sadja. Sadeži zorijo od zgodnjega poletja do zime, kar ne preseneča, saj poznamo več kot 10.000 sort jabolk.

Jablane sodijo v družino rožnic Rosaceae. Je naslednica *Malus sieversii*, čeprav sta tudi *Malus orientalis* in *Malus sylvestris* vplivali na njen razvoj.

Jablane rastejo po vsem svetu. Po pridelavi je vodilna Kitajska z 39.682.618 t, sledijo pa ji Združene države Amerike, Turčija in Poljska. Hrvaška je s 128.211 t na oseminštiridesetem mestu (FAOSTAT, 2013).

Okoljske zahteve jablane

Jablana je celinska sadna vrsta in skladno s tem so tudi njene okoljske zahteve. Za zanesljivejšo oceno okoljskih razmer nekaterih območij za uspešno gojenje jabolk je potrebna obsežna raziskava podnebnih razmer – jablane potrebujejo povprečno letno temperaturo 8–12 °C, zmerno do relativno visoko zračno vlažnost (60–75 %), sneg pri mrzlih zimskih temperaturah itd. Tla morajo biti globoka, peščeno-ilovnate sestave, rahlo

kisla – pH 5,5, vendar ne z več kot 5 % $CaCO_3$, pomemben pa je tudi relief – nadmorska višina mora biti 120–600 m, naklon okoli 4 % itd.

Primarni metaboliti

Spojine v rastlinah lahko delimo v dve glavni skupini: primarni in sekundarni metaboliti. Primarni metaboliti nastanejo in sodelujejo v primarnih presnovnih procesih, kot sta dihanje in fotosinteza. Sintetizirajo se v celici, kjer so nepogrešljivi za njeno rast. Primarni metaboliti vključujejo molekule sladkorjev, aminokislin, beljakovin, polisaharidov itd., izmed katerih večino štejemo za »gradnike« in izvor energije.

Sekundarni metaboliti

Rastline sintetizirajo ogromno organskih spojin, ki se tradicionalno uvrščajo med primarne in sekundarne metabolite, čeprav je točna meja med obema skupinama v določenih primerih nekoliko nejasna. Obstaja veliko opredelitev obeh vrst metabolizma in večina izmed njih opisuje primarne metabolite kot spojine, ki imajo pomembno vlogo pri fotosintezi, dihanju, rasti in razvoju (vključujejo preproste sladkorje, maščobe, nukleotide, aminokisline in organske kisline itd.), medtem ko sekundarni metaboliti nimajo neposredne funkcije pri rasti in vzdrževanju celic, vendar pa imajo pomembne ekološke in zaščitne funkcije pred različnimi oblikami stresa. Čeprav so sekundarne spojine običajni del metabolizma organizma, se pogosto proizvajajo v posebnih celicah ali tkivu in so navadno kompleksnejše kot primarne spojine.

Rastline proizvajajo sekundarne metabolite kot odziv na škodljive vremenske razmere ali v določenih fazah razvoja. Dolgo časa so bili spregledani, zdaj pa njihova funkcija v rastlinah privablja pozornost, ker nakazuje, da imajo nekateri ključno vlogo pri zaščiti rastlin pred rastlinojedci in mikrobnimi vnetji ter kot atraktanti za opraševalce in živali, ki širijo semena itd.

Glede na njihov biosintetični izvor rastlinske sekundarne metabolite lahko razdelimo v tri glavne skupine: fenolne in terpenoidne spojine ter spojine, ki vsebujejo dušik.

MATERIAL IN METODE DELA

Sadovnjak

Jabolka so se obirala v sadovnjaku jeseni 2012. Sadovnjak je v osrednji Hrvaški, natančneje v Katoličkem Selišću v občini Velika Ludina, na nadmorski višini 132–128 m ter koordinatah 45° 37' 32" severne geografske širine in 16° 36' 56" vzhodne geografske dolžine. Sadovnjak je bil posajen med letoma 2008 in 2011. Tam raste 13 sort jabolk na 18

hektarjih modernega sadovnjaka jablan. Drevesa so razporejena v 410 vrstah, večinoma dolgih 150 m. Vrste so posajene v smeri sever–jug, medtem ko je razdalja med vrstami 320 cm in med drevesi 60–100 cm, odvisno od sorte. Celotni nasad je pokrit z mrežami proti toči in ima namakanje. Lastnik sadovnjaka je podjetje Fructus, d. o. o., iz Velike Ludine.

Metode dela

Analiza trdote mesa plodov ter vsebnosti fenolov, kislin, vitamina C, sladkorja in suhe snovi v jabolkih je bila opravljena na Katedri za sadjarstvo, vinogradništvo in vrtnarstvo, Oddelek za agronomijo Biotehniške fakultete v Ljubljani. Vse štiri sorte smo shranjevali na 2 načina (z ULO in navadno atmosfero) in opravili 3 meritve (27. Septembra 2012, 10. januarja in 13. marca 2013) v 5 ponovitvah (10 jabolk). Vsak plod smo razdelila na 3 dele: samo kožica, samo meso ter mešanica kožice in mesa skupaj.

Suha snov in trdota mesa sta se merili isti dan vzorčenja. Kožica je bila pripravljena za meritve askorbinske kisline in antocianov, medtem ko se je skupna vsebnost fenolov (TPC), hidroksicinaminske kisline, flavan-3-olov, dihidrohalkonov in flavonolov merila ločeno tako iz kožice kot mesa. Skupno vsebnost kislin in sladkorjev smo izmerili v mešanici kožice in mesa.

REZULTATI IN RAZPRAVA

Primerjava ob različnih datumih meritev

Pri meritvah trdote mesa smo čez čas dosegli manjše vrednosti pri obeh načinih skladiščenja. Iste rezultate so potrdili mnogi avtorji, eni izmed njih so Napolitano in sod. (2004). Samo sorta 'Granny Smith' ni pokazala statistično značilne razlike med prvimi in končnimi meritvami. Naše domneve so se v splošnem potrdile, čez čas se je zmanjšala tudi vsebnost askorbinske kisline. Podobne rezultate so dosegli tudi drugi avtorji, na primer Kovač in sod. (2010).

Rezultati meritev skupne vsebnosti sladkorjev so bili večinoma enotni. Pri vseh sortah in obeh načinih skladiščenja smo opazili zmanjšanje, statistično značilno je bilo pri vseh sortah razen sorte 'Granny Smith' pri skladiščenju v navadni atmosferi.

Pri meritvah skupne vsebnosti fenolov v kožici smo opazili pomembno zmanjšanje pri skoraj vseh sortah in obeh načinih skladiščenja, medtem ko rezultati meritev v mesu niso bili tako jasni. Obstaja veliko nasprotujočih si rezultatov drugih avtorjev, kot so Kovač in sod. (2010), Napolitano in sod. (2004) ter Leja in sod. (2003).

Pri hidroksicinaminski kislini so meritve v mesu čez čas pokazale rast pri skladiščenju z manjšimi nihanji pri standardnem skladiščenju v navadni atmosferi.

Pridobljeni rezultati flavan-3-olov v mesu pri skladiščenju v ULO atmosferi kažejo trend rasti, medtem ko Carbone in sod. (2011) poročajo o njegovem zmanjšanju.

Rezultati meritev mesa pri skladiščenju v ULO in navadni atmosferi kažejo trend rasti pri vseh sortah, medtem ko sorta 'Braeburn' ne kaže statistično značilnih razlik med datumi meritev pri skladiščenju v navadni atmosferi.

Primerjava različnih tipov skladiščenja

Manjše vrednosti so se pokazale pri meritvah trdote mesa po 14 tednih skladiščenju v navadni atmosferi (datum meritev 10. 1. 2013) pri vseh sortah, razen pri sorti 'Braeburn'.

Pri merjenju vsebnosti askorbinske kisline ni bilo statistično značilnih razlik, razen pri sorti 'Braeburn', kjer so bile dosežene bistveno večje vrednosti pri skladiščenju v ULO atmosferi kot navadni atmosferi.

Pri meritvah skupne vsebnosti fenolov v kožici so vse sorte razen sorte 'Braeburn' pokazale večje vrednosti pri skladiščenju v navadni atmosferi. Enako se je pokazalo pri meritvah skupne vsebnosti fenolov v mesu.

Pri vsebnosti hidroksicmetne kisline so vse sorte razen sorte 'Braeburn' imele večjo vsebnost pri skladiščenju v navadni atmosferi.

Vsebnosti za flavan-3-ole v mesu so bile večje pri skladiščenju v navadni atmosferi pri vseh sortah, razen pri sorti 'Braeburn'.

Večje vrednosti so bile izmerjene pri skladiščenju v navadni atmosferi v mesu kot v kožici. Vse sorte, razen sorte 'Braeburn', kažejo večje vsebnosti flavonolov pri skladiščenju v navadni atmosferi.

ZAKLJUČKI

Sadje, ki smo ga skladiščili v navadni atmosferi, ni ostalo sveže do meritev marca 2013. Pri primerjanju datumov meritev smo ugotovili, da so na splošno med njimi bistvene razlike. Še več, odkrili smo večje razlike med prvim datumom (27. 9.) in drugim datumom meritev (10. 1.) kot pa med drugim datumom (10. 1.) in tretjim datumom meritev (13. 3.).

Glede na zbrane podatke lahko zaključimo, da se ob pravilnem shranjevanju in ravnanju jabolka lahko prodajajo (s skoraj enako kakovostjo in značilnostmi) skozi celotno prodajno obdobje.

Glede primerjave obeh načinih skladiščenja na datum merjenja 10. 1. 2013 (15 tednov skladiščenja) lahko zaključimo, da je ena izmed značilnosti jabolk, to je trdota mesa, na splošno večja pri skladiščenju v ULO atmosferi kot pri skladiščenju v navadni atmosferi.

Druge značilnosti so pokazale večje vsebnosti pri skladiščenju v navadni atmosferi kot pri skladiščenju v ULO atmosferi. Zaključimo lahko, da je to rezultat večje izgube vode iz ploda, ko se le ta skladišči v navadni atmosferi, kot pa, če se skladišči v ULO atmosferi, zato je vsebnost vseh merjenih parametrov večja v plodovih, ki jih skladiščimo v navadni atmosferi.

Ne glede na parametre, ki smo jih merili ali opazovali, ali se le-ti zmanjšujejo ali rastejo, ali da so vse druge sorte imele večje vsebnosti v ULO atmosferi, je sorta 'Braeburn' skoraj vedno izstopala. Največje presenečenje je bilo, ko smo primerjali oba načina skladiščenja in ugotovili, da tej sorti skladiščenju v navadni atmosferi bolj ustreza kot skladiščenju v ULO atmosferi. Medtem ko pri skladiščenju v navadni atmosferi sorta ni zdržala do končnih meritev marca 2013, pa smo imeli pri skladiščenju v ULO atmosferi veliko škodo in stroške zaradi notranjega zloma plodov sorte 'Braeburn'.

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ANNEX A

Average precipitation and air temperature for last 30 years

Annex A1: Average precipitation (mm) in each month for last 30 years, meteorological station in Sisak (data given by DHMZ, Metorological and hydrological service of Croatia, December 2014)

year	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	SUMA
1983	35.0	106.8	66.0	21.4	59.2	65.4	30.6	24.0	71.8	55.0	26.3	42.6	604.1
1984	169.9	32.1	40.4	54.8	129.0	62.5	89.1	51.8	156.7	97.9	102.6	19.0	1005.8
1985	33.0	21.6	90.1	81.4	54.8	129.6	24.6	38.8	26.6	10.2	118.6	48.8	678.1
1986	73.4	69.6	63.0	82.1	54.8	190.4	64.4	68.7	38.2	109.9	25.8	28.1	868.4
1987	67.6	77.8	29.6	91.2	172.7	63.7	41.9	47.1	58.8	61.8	196.5	39.8	948.5
1988	66.8	114.4	84.4	46.2	44.1	94.0	40.8	117.5	103.2	93.1	30.8	44.5	879.8
1989		30.7	68.4	65.7	176.5	91.9	105.1	160.6	86.0	30.0	55.8	32.1	
1990	22.7	34.8	59.7	46.7	75.8	81.9	63.8	22.7	140.7	60.9	134.8	84.7	829.2
1991	41.6	31.3	52.5	55.6	198.6	42.5	148.1	105.9	41.6	147.2	124.5	18.7	1008.1
1992	13.0	47.7	80.8			152.7	51.3	10.5	25.4	178.2	137.3	69.9	
1993		4.5	38.7	61.3	35.0	127.9	37.7	92.9	105.2	131.5	172.5	129.7	
1994	42.8	43.3	35.5	75.5	32.5	153.6	105.8	150.3	79.4	78.7	17.2	50.5	865.1
1995	84.9	79.4	54.9	46.9	98.9	178.6	30.5	158.1	130.0	12.8	72.2	76.2	1023.4
1996	84.5	48.4	27.6	78.3	89.7	47.4	151.5	83.2	228.3	46.3	136.8	64.9	1086.9
1997	57.4	57.3	26.7	79.9	63.9	102.5	114.2	82.1	33.9	68.8	121.9	103.7	912.3
1998	66.6	11.1	54.1	65.5	97.7	129.3	124.4	76.5	147.3	123.9	85.9	56.4	1038.7
1999	42.3	62.7	37.6	125.8	107.0	89.0	85.9	65.6	95.4	72.8	89.3	104.3	977.7
2000	29.1	36.5	63.1	76.6	26.0	47.4	73.3	20.3	82.5	71.4	88.3	133.8	748.3
2001	90.7	12.3	95.8	83.0	61.9	128.1	43.8	21.9	249.9	9.0	131.9	45.8	974.1
2002	25.7	69.5	38.9	184.9	183.3	56.2	130.0	99.3	133.0	72.7	113.0	54.0	1160.5
2003	71.2	21.3	5.1	29.6	25.8	51.4	50.1	93.0	80.1	98.9	63.5	24.8	614.8
2004	79.0	64.2	63.8	151.9	65.0	119.6	45.1	60.6	115.0	114.9	71.2	55.6	1005.9
2005	17.5	71.7	55.9	65.0	75.9	40.0	103.6	182.3	83.5	35.6	57.7	136.4	925.1
2006	43.9	36.2	57.4	145.3	92.0	63.0	73.1	201.7	39.3	26.5	85.5	27.6	891.5
2007	81.5	57.5	85.3	6.5	92.3	42.9	26.5	54.7	140.8	141.1	96.9	73.1	899.1
2008	30.5	7.9	114.5	47.6	36.2	154.5	119.8	65.2	98.3	88.1	86.1	101.1	949.8
2009	113.2	40.4	53.6	31.3	44.2	153.2	171.4	37.3	30.2	88.4	102.3	94.8	960.3
2010	105.4	88.6	66.3	59.0	156.9	146.2	64.9	156.6	172.4	64.8	140.0	63.2	1284.3
2011	15.3	13.5	21.0	31.2	31.7	125.0	88.3	42.0	30.2	79.8	1.5	75.4	554.9
2012	27.4	53.9	10.1	34.2	120.0	113.9	32.5	15.1	109.2	71.8	100.3	122.5	810.9
average	58.3	48.2	54.7	69.8	86.3	101.5	77.7	80.2	97.8	78.1	92.9	67.4	907.6

Annex A2: Average temperature (°C) in each month for last 30 years, meteorological station in Sisak (data given by DHMZ, Metorological and hydrological service of Croatia, December 2014)

year	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	average
1983	3.1	-0.5	7.4	14.4	18.0	18.8	23.2	21.2	16.4	10.9	3.1	1.4	11.5
1984	0.8	0.6	5.5	10.5	14.9	18.3	19.4	19.1	16.3	12.3	6.3	1.0	10.4
1985	-5.0	-3.1	5.4	11.2	17.1	17.6	21.2	20.6	16.2	9.8	3.4	4.9	9.9
1986	0.8	-3.5	3.6	12.2	18.5	17.9	19.9	21.0	15.2	9.8	5.9	-0.1	10.1
1987	-3.0	1.5	1.8	11.4	14.2	19.4	22.2	19.2	19.0	11.8	4.8	1.2	10.3
1988	4.2	4.4	6.1	10.5	16.2	18.6	22.5	20.8	16.0	10.5	0.9		
1989			9.6	12.5	14.6	17.4	21.1	20.0	15.7	10.2	4.2	3.2	
1990	0.1	7.0	9.3	10.6	16.9	19.2	20.4	20.7	15.0	11.4	6.5	1.4	11.5
1991	2.0	-2.0	9.2	9.5	12.7	19.6	22.3	20.6	17.7	9.6	6.7	-1.3	10.6
1992	1.3	4.1	6.7			19.0	21.5	24.0	17.0	10.4	7.4	1.6	
1993			5.8	12.6	18.3	20.1	21.3	20.8	16.1	12.2	2.0	1.9	
1994	3.5	2.4	10.7	11.2	16.4	19.8	22.7	22.1	18.5	9.3	7.7	2.0	12.2
1995	1.2	6.2	5.6	12.0	15.5	18.3	23.0	19.5	14.8	11.9	4.9	1.6	11.2
1996	-0.8	-0.7	3.2	11.2	17.2	20.2	19.7	20.0	13.1	11.4	8.0	-1.3	10.1
1997	-1.1	4.7	6.9	8.5	17.8	21.1	21.2	20.7	16.2	9.3	6.2	3.0	11.2
1998	3.5	5.5	5.6	13.4	16.3	21.7	22.1	21.3	15.8	12.1	3.5	-3.0	11.5
1999	1.1	2.1	9.1	12.6	17.3	20.2	21.8	21.1	18.6	11.7	3.6	1.8	11.8
2000	-1.7	5.1	8.0	14.6	17.8	22.0	21.4	23.1	16.6	13.4	9.9	4.8	12.9
2001	3.9	4.9	10.6	11.1	18.2	18.8	22.5	22.6	14.6	14.3	3.3	-2.4	11.9
2002	0.6	6.4	9.1	10.6	18.3	21.7	22.1	20.9	15.1	11.6	10.3	2.1	12.4
2003	-1.1	-1.8	7.3	10.8	19.2	24.4	23.6	24.5	15.5	9.4	8.2	1.7	11.8
2004	-0.3	2.7	5.7	11.8	15.0	19.3	21.3	21.0	15.6	13.4	6.6	1.9	11.2
2005	-0.7	-1.5	5.5	11.8	16.8	20.1	21.7	19.0	16.9	11.6	5.3	1.6	10.7
2006	-1.3	1.6	5.6	12.7	16.3	20.5	23.3	19.2	17.4	13.2	9.1	4.0	11.8
2007	6.7	7.2	8.8	13.8	18.0	22.5	23.5	21.7	14.7	9.7	4.7	0.4	12.6
2008	2.2	5.2	7.8	12.4	17.5	21.4	22.0	21.4	15.0	12.6	7.5	3.6	12.4
2009	-0.1	3.2	7.6	14.7	18.6	19.7	22.5	22.4	17.9	11.4	8.4	3.4	12.5
2010	-0.6	1.8	7.4	12.4	16.5	20.5	23.4	20.9	14.9	9.1	9.1	0.7	11.3
2011	2.1	1.1	7.3	13.6	16.7	21.0	22.2	22.8	19.5	10.1	2.7	3.8	11.9
2012	2.1	-2.4	9.4	12.8	16.6	22.7	24.0	23.8	17.8	11.8	9.0	1.4	12.4
average	0.8	2.2	7.1	12.0	16.8	20.1	22.0	21.2	16.3	11.2	6.0	1.6	11.5