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A BIOCHEMICAL COMPARISON OF APPLE DURING POSTHARVEST STORAGE IN CONTROLLED ATMOSPHERE CONDITIONS

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Abstract

During the cold season, mainly climacteric fruits such as apples, quinces and pears are consumed due to their content of vitamins, minerals and antioxidant compounds. The main purpose of this study was to compare the postharvest biochemical changes during storage in controlled atmosphere conditions of four apples varieties ('Topaz', 'Redix', 'Florina' and 'Rubinola') in two different years. Apples were harvested from the experimental orchard of University of Agronomical Sciences and Veterinary Medicine (USAMV) Bucharest, and stored under the same controlled atmosphere conditions, as follows: temperature (T): 1°C, O_2: 3%, CO_2: 0%, 2% and 5%, relative humidity (RH): 95%. The antioxidant capacity, ascorbic acid, total polyphenols and total flavonoids contents were monitored during entire controlled atmosphere (CA) storage period. For all analyzed apple varieties, the ascorbic acid content decreases in both years of analysis. Differences between the biochemical compounds concentrations was observed in the apples stored in CA conditions with CO_2 compared to those stored without CO_2. The influence of CO_2 concentration on biochemical characteristics of fruits depends both the variety and the harvesting moment.

Key words*: Apple, biochemical compounds, CA, postharvest.*

INTRODUCTION

The antioxidants compounds such as ascorbic acid, known as vitamin C, phenolic and flavonoids contents, are found in fruits and vegetables, with various health benefits, according to World Health Organization (1998), which are associated with protective effects against cancer, chronic and inflammatory diseases (Karar et al., 2014; Wojdylo et al., 2014; Liato et al., 2017; Pavun et al., 2018;).

Climacteric fruits like apples, quinces and pears are consumed during the cold season, due to their rich content in: vitamins, minerals and antioxidant compounds.

Apple (*Malus x domestica* Borkh.) is the most important fruit in the temperate zone (Delian et al., 2011). It is also one of the most frequently consumed (Mureşan et al., 2012; Francini et al., 2013;), common crop grown worldwide (Yildirim et al., 2017) and stored in controlled atmosphere (CA) conditions (Bessemans et al., 2016), due to its excellent source of phenolic compounds and high antioxidant capacity (Khanizadeh et al, 2008).

Ma et al., (2019) suggest that phenolics profile have different concentration in peel and flesh influenced by the cultivar, the maturity stage of fruits and the environmental conditions where there are growing (Mitić et al., 2013). Mitić et al., (2013) showed that the ascorbic acid content explains only 0.4% of the apples antioxidant capacity.

Of the many existing storage options, controlled atmosphere (CA) storage conditions is one of the most used method of postharvest to prevent fast senescence and quality loss of the fruit (Bessemans et al, 2016), maintaining the same organoleptic characteristics during storage (Oltenacu & Oltenacu, 2013).

The main purpose of this study was to compare the biochemical changes during postharvest storage, for apples varieties: 'Topaz', 'Redix', 'Florina' and 'Rubinola'. Quality parameters that have shown interest in being tracked were: antioxidant capacity and content in: total flavonoids, polyphenols, and ascorbic acid.

MATERIALS AND METHODS

Polyphenols, flavonoids, ascorbic acid contents, and antioxidant capacity, were monitored during entire CA storage period, for the entire year of storage in both years of repetition.

Samples, in the two years, were not in the same ripening stage. In the first year the harvesting period were two weeks before the optimal harvest time due to unfavourable weather conditions, while in the second year the harvesting period coincided with the optimal harvest time, according to the literature (Ghena et al., 2004). The samples, in uniform size and colour were stored bulk in plastic boxes. Four apple varieties: 'Topaz', 'Redix', 'Florina', and 'Rubinola' were harvested from the experimental orchard of the University of Agronomical Sciences and Veterinary Medicine (USAMV) Bucharest, stored and monitored in two repeat years, under the same controlled atmosphere conditions, as follows: temperature (T): 1°C, O_2: 3%, CO_2: 0%, 2% and 5%, relative humidity (RH): 95%, in the Research Center for Studies of Food Quality and Agricultural Products, of the USAMV Bucharest.

Sample preparation

For determination of total flavonoids, total polyphenols content and the antiradical activity, 50% of aqueous ethanol was used for the extractions of the apples samples, with 1:6 ratio (5 g of sample with 30 mL 50% of aqueous ethanol). Sample preparation consisted of sonication for 30 minutes the 5 g of average sample (approximately 1/3 peel and 2/3 flesh) of the apple with 30 ml of 50% aqueous ethanol and filtered through 18-30 µm filter paper. For ascorbic acid content, the samples were extracted with a ratio of 1:10 (5 g of sample in 9% metaphosphoric acid (MPA)). For filtering the extracts for ascorbic acid determination, a 0.2 µm filter (Agilent PTFE) was used.

Total flavonoids content was determined according to Asănică et al., (2016) and Mitić et al., (2013). Briefly, 250 µl extract of the sample was mixed with: 1.250 µl of distilled H_2O, 75 µl of 5% $NaNO_2$, 75 µl of a 10% $AlCl_3$ (after 5 min), 500 µl of a 1M NaOH (after 6 min), adding distilled H_2O up to a final volume of 2.500 µl. The absorbance of the samples was read after 45 minutes at 510 nm. Flavonoids content in the samples was expressed as mg of rutin equivalent in 100 g of fresh fruit weight (mg RE/100 g).

Total polyphenols content was determined by the Folin-Ciocalteu reaction according to Stojanović et al., (2017), with some modifications. The reaction mixture was prepared by mixing: the extract (25 µl), 1.975 µl distilled H_2O, 125 µl Folin-Ciocalteu reagent and 375 µl 30% Na_2CO_3. The final volume was 2.5 ml. The absorbance of the samples was read after 2 hours at 750 nm. The polyphenols content in the samples was expressed as mg of equivalent of gallic acid in 100 g of fresh fruit weight (mg GAE/100 g FW).

Antioxidant activity of the samples was determined using spectrophotometric diphenyl-1-picrylhydrazyl (DPPH) method. An amount of 100 µl ml of hydro-alcoholic extract was mixed with 900 µl bidistilled H_2O and 2,000 µl of 0.5 mM DPPH solution, and after 30 minutes in dark, at room temperature, the absorbance of the samples was read at 515 nm, in the presence of a methanol as blank. The results of inhibition of free radical with DPPH, were expressed in % in fresh weight, the radical scavenging activity (RSA) being calculated with the formula: RSA_{DPPH} (%) = $\frac{A\ control - A\ sample}{A\ control}$ x 100, where $A_{control}$ is DPPH absorbance and A_{sample} is the sample absorbance. All three determinations described above were performed with Specord 210 Plus spectrophotometer and all the samples were analysed in triplicate.

Ascorbic acid content was quantified via chromatographic separation, using ZORBAX Eclipse XDB-C18 (4.6x50 mm, 1.8µm) column with Rapid Resolution HT and a detector UV-DAD detection wavelength 220/30 nm, with reference wavelength at 400/100 nm, from the HPLC – Agilent Technologies 1200 Series equipment.

The mobile phases used, were A=99% (ultrapure water adjusted to 2.1 pH with H_2SO_4) and B=1% (90% acetonitrile with 10% A). For filtering the extracts, a 0.2 µm filter (Agilent PTFE) was used. The injection volume (2 µL) flow rate in the column compartment at 0.5 mL/min, maintained by the column at 30 ^oC, with a post time of 4 min. All

the extractions were carried out in duplicate and were expressed in mg/100g fresh weight.
The ascorbic acid content was calculated using the formula: $\frac{a \times b \times 100}{c}$, where a= is the content of ascorbic acid (mg/ml), b= the volume of solution extraction (ml) and c= the mass of the sample (g).

RESULTS AND DISCUSSIONS

Total flavonoids content (Figure 1):
At 'Topaz', in the first year of storage, it was an increase in total flavonoids content by 12.4% in control room, by 14.22% in 2% CO_2 room, while in the room with 5% CO_2 the value was maintained during the storage period, compared to the initial value after the 12 months of storage.
In the second year, although the initial value of total flavonoids content was less than 2% lower than in the previous year, the decrease in storage was much higher, decreasing by 32.8% in the control room, with 4% in 2% CO_2 room and 27.5% in 5% CO_2 compared with the initial moment.

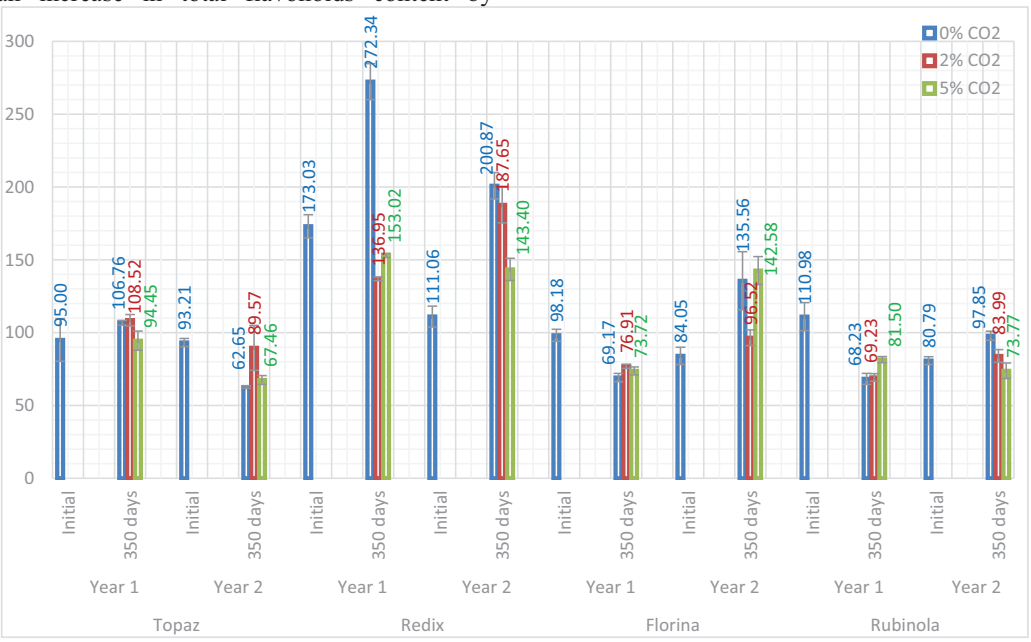

Figure 1. Variation of **total flavonoids** content (mg/100g FW) during storage period in CA for 'Topaz', 'Redix', 'Florina' and 'Rubinola' varieties for two years

In the first year of storage, 'Redix' variety recorded an increase in flavonoid content by 57.4% in control room, after 12 months of storage compared to rooms with CO_2, which recorded a decrease of 20.9% in 2% CO_2 room and 11.6% in 5% CO_2, from the initial moment, after 12 months of storage. In the second year, although the initial value was 35.9% lower than in the previous year, there was an increase in all CA rooms as follows: the increase of 80.9% and in control room, of 68.9% in 2% CO_2 room, while in the 5% CO_2 room the increase was 29.11% compared to the initial value during the storage period.

At 'Florina', the flavonoids content in the first year of storage decreased in all three CA rooms, the decrease was 30.9%, in the control room, 21.7% in 2% CO_2 room, 25% in the room with 5% CO_2 compared to the initial value during the storage period. In the 2nd year, although the initial value was 14.4% lower than in the previous year, the increase of total flavonoid content during storage was much higher, registering an increase of 61.28% in the control room, respectively 13.64% in 2% CO_2 room, and 69.64% in 5% CO_2 room, compared to the initial value, after one year of storage.

At ‘Rubinola’, in the first year of storage, the total flavonoids content decreased in all rooms with controlled atmosphere so: in the control room the decrease was 39.5%, in 2% CO_2 room decrease by 37.6%, while in the room with 5% CO_2 the decrease was 26.6%, from initial moment, after 12 months of storage. In the second year, although the initial value was 27.2% lower than in the previous year, there was an increase of 21.11% in the control room, 4% in 2% CO_2 room and a decrease of 8.7% in 5% CO_2 room, compared to the initial value, during the storage period.

Total polyphenols content (Figure 2):

The behaviour of total polyphenols content was different from that of ascorbic acid content, with an increase in both years, with slight variations depending on the varieties, so:

At ‘Topaz’, in the first year of storage, in the control room, there was an increase of the polyphenols content by 88.9% after the 12 months of storage compared to 95% in 2% CO_2 room, respectively 88% in 5% CO_2 room, from the initial moment, after 12 months of storage.

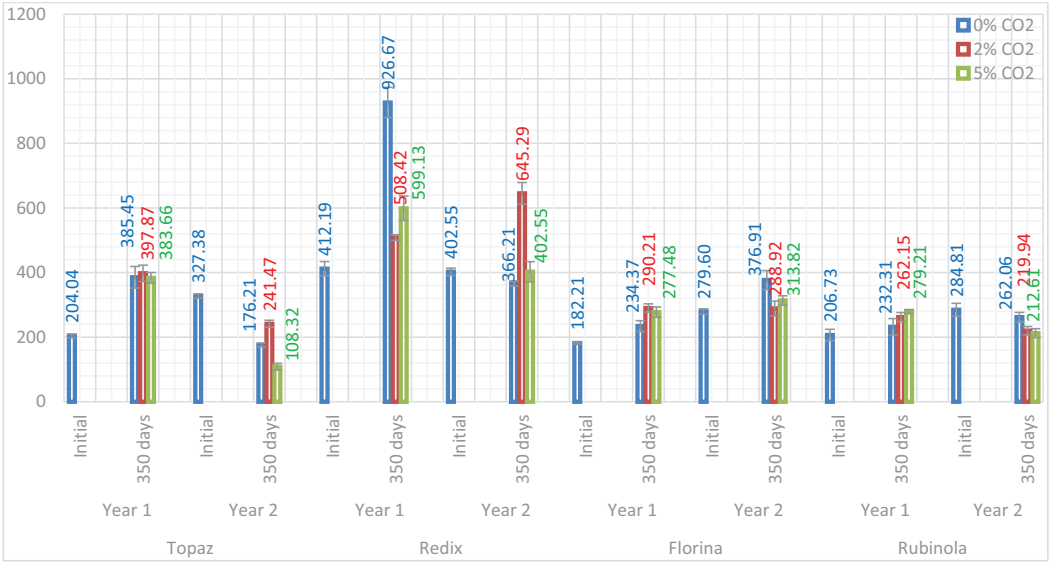

Figure 2. Variation of **total polyphenols** content (mg GAE/100 g FW) during storage period in controlled atmosphere (CA) for ‘Topaz’, ‘Redix’, ‘Florina’ and ‘Rubinola’ varieties for two years

In second year, although the initial value was 60.4% higher than in the previous year, the drop on storage was much higher, recording a decrease of 46.2% in the control room, respectively 27.25% in 2% CO_2 room and 67% in 5% CO_2 room, from the initial moment, after 12 months of storage, 50% decrease being also mentioned at ‘Topaz’ by Francini & Sebastiani (2013).

In the first year of storage, at ‘Redix’ variety, in the control room it was an increase in polyphenols content by 125% after 12 months of storage compared to 23.35% in the 2% CO_2 room, respectively 45.35% in 5% CO_2 room, compared to the initial value after the 12 months of storage. Although, the initial value, in the second year, was 2.4% lower than in the previous year, there was a 9.1% decrease in the control room, a 60.3% increase in 2% CO_2 room compared to the initial moment, while in the room with 5% CO_2 it has been maintained the initial value during storage.

For ‘Redix’ variety, in the first year, the values registered in 2% and 5% CO_2 rooms were close to value of 547.6 mg/100g FW as found by Delian et al.,(2011).

In the first year, in the control room, ‘Florina’ variety reported an increase in total polyphenol content by 28.6% after the 12 months of storage, compared to 60% in the room with 2% CO_2 respectively 52.3% in the room with 5% CO_2, from the initial value after the 12 months of storage. In second year, although the initial value was 53.5% higher than in the previous year, the growth on storage was much higher, recording an increase of 34.8% in the control

room, respectively 3.33% in 2% CO_2 room and 12.2% in 5% CO_2, compared to the initial value, after one year of storage.

In 'Rubinola', in the first year of storage, there was an increase in polyphenol content so: in the control room the increase was 12.37% compared to 26.8% in 2% CO_2 room and 35% in 5% CO_2 room, compared to the initial value, after 12 months of storage. In the second year, although the initial value was 37.77% higher than in the previous year, there was a decrease of 8.1% in the control room, 22.8% in 2% CO_2 room and 25.4% in 5% CO_2 room, compared to the initial value, after 12 months.

The results obtained, support those presented by Mureşan et al., (2014), that total phenolic varies according to storage condition, cultivar and harvest time.

Antioxidant activity (Figure 3) of the four varieties of apples has behaved similar into total

polyphenols content correlation that has been shown as well by Khanizadeh et al., (2008), with an increase in both years, with slight variations depending on the varieties, so:

At 'Topaz', in the first year of storage, in the control room, there was a bigger increase in antioxidant activity, similar with the increase of total polyphenols content, by 103.4% after the 12 months of storage compared to 112.9% in 2% CO_2 room, respectively 76.2% in 5% CO_2 room, from the initial moment, after 12 months of storage.

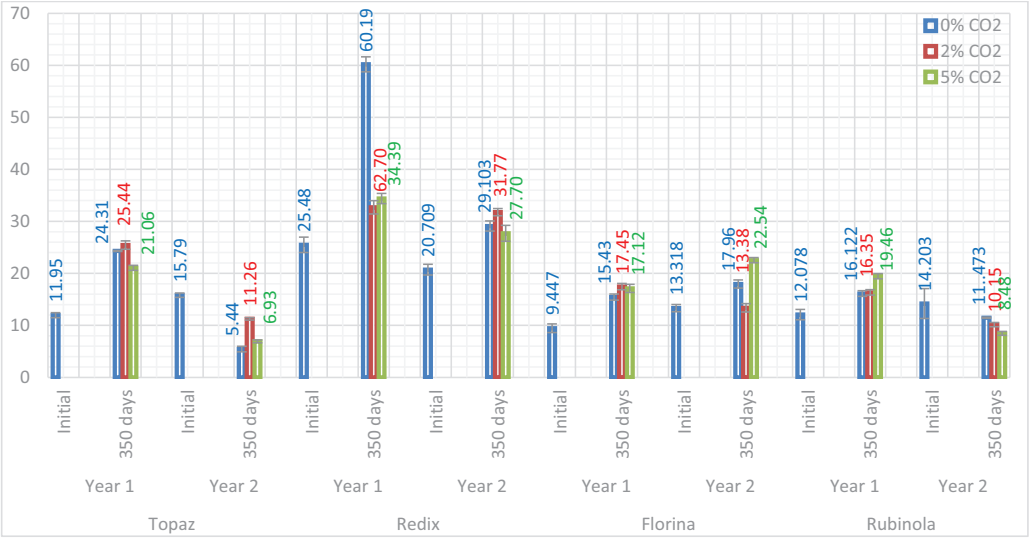

Figure 3. Variation of **antioxidant activity** (%) during storage period in CA for 'Topaz', 'Redix', 'Florina' and 'Rubinola' varieties for two years

In the second year, although the initial value was 32.2% higher than in the previous year, the drop on storage was much higher, recording a decrease of 66.6% in the control room, respectively 29.7% in 2% CO_2 room and 56.2% in 5% CO_2 room, from the initial moment, after 12 months of storage. Matthes & Schmitz-Eiberger (2009) show in their studies that antioxidant capacity decrease at 'Topaz' variety, during storage, being related to the ascorbic acid degradation, similar behavior was recorded also, in this study in the 2nd year.

In the first year of storage, at 'Redix' variety, in control room was an increase of antioxidant activity by 136.2% after 12 months of storage compared to 28.31% in the 2% CO_2 room, respectively 34.9% in 5% CO_2 room, compared to the initial value after the 12 months of storage. Although, the initial value, in the second year, was 18.74% lower than in the previous year, there were increases in all three CA rooms, so in the control room it was a 40.5% increase, a 53.4% increase in 2% CO_2 room, and 33.7% increase in 5% CO_2 room, compared to the initial moment, during storage.

'Redix' variety presents a different behaviour, during storage, comparative with the other three varieties studied, one of the genitors parents of 'Redix', being fall apple variety 'Prima'. It was observed that antioxidants compounds, such as total polyphenols, total flavonoids content and antioxidant capacity, registered much higher values in both years compared with the other three winter apples varieties: 'Topaz', 'Florina', and 'Rubinola', probably because of his fall apple genitor parent 'Prima'.

In the first year, in the control room, 'Florina' variety reported an increase in antioxidant activity by 63.4% after the 12 months of storage, compared to 84.7% in the room with 2% CO_2 respectively 81.2% in the room with 5% CO_2, from the initial value after the 12 months of storage. In the second year, although the initial value was 41% higher than in the previous year, the growth on storage was higher, recording an increase of 34.85% in the control room, 2% CO_2 room did not record any changes of the initial value, and an increase of 69.3% in 5% CO_2, compared to the initial value, after one year of storage.

In 'Rubinola', in the first year of storage, there was an increase in antioxidant activity so: in the control room the increase was 33.48% compared to 35.34% in 2% CO_2 room and 61.15% in 5% CO_2 room, compared to the initial value, after 12 months of storage. In the second year, although the initial value was 17.6% higher than in the previous year, there was a decrease of 19.3% in the control room, 28.5% in 2% CO_2 room and 40.4% in 5% CO_2 room, compared to the initial value, after 12 months of storage.

Ascorbic acid content (Figure 4):

Although ascorbic acid content decreased in both years, different behaviors were observed depending on varieties, as follow:

At 'Topaz' variety, in the first year of storage, in the room without CO_2 (the control room) there was a decrease in ascorbic acid content by 12.1%, and in the rooms with CO_2, there was a decrease in ascorbic acid content over 1/3 of the initial value after the 12 months of storage compared to the initial moment. In the second year, although the initial value was 10% lower than in the previous year, the drop on storage was much higher, with a decrease of 86% in the control room, respectively 75.5% in 2% CO_2 and 86.4% in 5% CO_2, compared to the initial moment.

In the first year of storage, for 'Redix' variety it was a decrease in ascorbic acid content by 16.9% in the control room, compared to 27.63% in the rooms with 2% CO_2, respectively 23.62% in 5% CO_2, after the 12 months of storage. In the second year, although the initial value was 4% lower than in the previous year, the decrease on storage was much higher with a decrease of 72% in the control room, respectively 81.85% in 2% CO_2 and 77.49% in 5% CO_2, compared to the initial value. At 'Florina', in the first year of storage, in the control room (0% CO_2) there was a decrease in ascorbic acid content by 45.5%, after 12 months to storage compared to 31.8% in room with 2% CO_2 respectively 20.3% in 5% CO_2 room, from the initial moment, after 12 months of storage. Although the initial value in the second year was 30.4% lower than in the previous year, the drop on storage was much higher, with a decrease of 62.6% in the control room, respectively 83.1% in 2% CO_2 room and 90.9% in 5% CO_2, compared to the initial moment, after one year.

In the first year of storage, in control room, for 'Rubinola' variety, there was a decrease in ascorbic acid content by 11.9% after 12 months of storage compared to 2.8% in the rooms with 2% CO_2, registering an increase with 13.95% in the room with 5% CO_2, from the initial value after 12 months of storage. In second year, although the initial value was 34.2% higher than in the previous year, the drop on storage was much higher, recording a 79% decrease in the control room, 80.5% in 2% CO_2 room and 73.85% in 5% CO_2 room, compared to the initial moment, after one year.

For all analysed apple varieties, the ascorbic acid content decreases in both years of analysis. It was observed the same trend of decline mentioned by Chira et al., (2014), for the first year of storage, for 'Rubinola'.

Manafu et al., (2013) showed that during storage the decrease of ascorbic acid content was on average with 75-80%, depending on the cultivar, decrease similar to the values obtained in second year of storage, at 'Topaz', 'Redix', and 'Rubinola'.

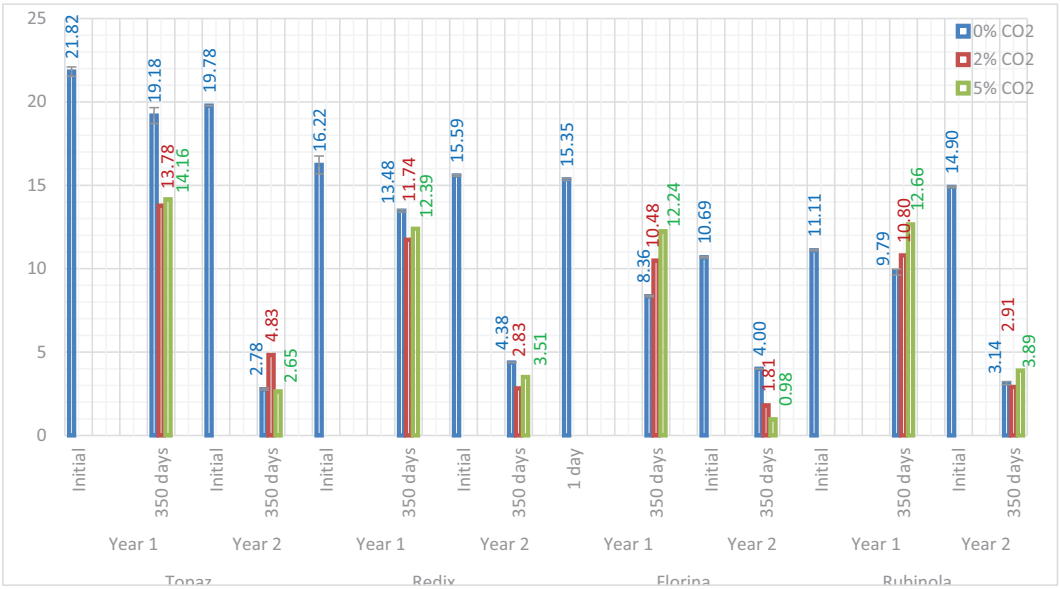

Figure 4. Variation of **ascorbic acid content** (mg/100g FW) during storage period in controlled atmosphere (CA) for 'Topaz', 'Redix', 'Florina' and 'Rubinola' varieties for two years

CONCLUSIONS

The ascorbic acid content maintained its values close of initial moment or with a slightly decrease, in the first year of storage, compared with the second year, where ascorbic acid content have dropped drastically.

The polyphenols content is correlated with the antioxidant capacity, their values tend to increase during one year storage due to the slowdown in metabolic processes in 'Topaz', 'Florina', and 'Rubinola' varieties.

The 'Redix' variety behaved differently from other varieties, which demonstrates that it should be classified as an autumn apple variety, rather than a winter one as it was classified by fruit growers, because of its good behaviour in storage, especial in CA conditions without CO_2.

Differences between the biochemical compounds concentrations was observed in the apples stored in CA conditions with CO_2 compared to those stored without CO_2.

The fruits harvested two weeks before the optimal harvesting moment maintain their bioactive compound and the antioxidant activity during storage better than others, because of the ethylene lack. Except 'Florina' variety, that have the same behaviour in every storage year. The influence of CO_2 concentration on biochemical characteristics of fruits depends both the variety and the harvesting moment.

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