


# Changes in colour parameters and anthocyanin content of aseptically filled sour cherry juice during storage

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## ABSTRACT

To overcome the problems of seasonality and geographical location in fruit production and processing, the production of aseptic semi-finished juice is an excellent solution. Even without refrigeration, aseptic pressing has a shelf life of more than a year, making it possible to produce finished products all year round. The production technology involves the addition of ascorbic acid to the pulp to fix or preserve colour. There is an increasing customer demand for ascorbic acid substitutes on the international market. In Hungary, one of the most important exports is aseptic sour cherry juice. In our work, ascorbic acid used for colour fixation was replaced by acerola concentrate. The anthocyanin content and colour coordinate values ( $L^*$ ,  $a^*$ ,  $b^*$ , H, C) of aseptically filled sour cherry juice were determined and compared with the control sample during the 12 months of storage.

## KEYWORDS

sour cherry, aseptic juice, anthocyanin, polyphenols, flavonoids

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## INTRODUCTION

In Hungary, owing to the favourable growing conditions in the Carpathian Basin, a wide range of crops are massively produced. Cherries are considered a particularly valuable fruit species and rank only second to apples. In addition to their fresh consumption, cherries are further processed into juice, puree, compote, jam, and dried fruit.

According to [Kim et al. \(2005\)](#), sour cherries are not only rich in phenols, especially anthocyanins, but also have strong anti-neurodegenerative activity and constitute a very good source of biofunctional phytochemicals in our diet. Their phenolic compounds, namely anthocyanins, protect nerve cells (PC 12) from cell-damaging oxidative stress in a dose-dependent manner. In recent years, several studies have reported that anthocyanins exhibit a wide range of biological properties, including antioxidant, antimicrobial, anti-inflammatory (COX1 and COX2), as well as anticarcinogenic and neuroprotective effects ([Kazan et al., 2016](#); [Svar-Gajic et al., 2017](#)).

Due to the above-mentioned effects of bioactive components, there have been growing demands for anthocyanin-rich fruits such as sour cherries and sour cherry products, for their effective role in the fight against the so-called diseases of civilization (e.g. obesity, cardiovascular diseases, cancers, diabetes) ([Nemes et al., 2018](#); [Pechanova et al., 2020](#)).

To diminish the variability caused by the geographical location and seasonality of sour cherry production, aseptically heat-treated, packaged, and stored juices and purees that are easy to transport and do not require refrigeration are opted for. An important step in the processing technology is to mitigate the change in the anthocyanin compounds in sour cherries. To achieve this, various colour-fixing techniques are used, e.g. the addition of ascorbic acid antioxidants during processing. A possible alternative could be the use of acerola with high ascorbic acid content. Acerola has been successfully used, among others, to increase the stability of carotenoids ([Nagamine et al., 2004](#); [Lima et al., 2011](#)).

The aim of our work was to study the effect of storage time and examine how the biologically valuable component content changes in aseptic filled sour cherry juice with added ascorbic acid (MAS), acerola juice (MACE) and control (MC) samples, respectively.

## MATERIALS AND METHODS

The sour cherry juice samples were produced from the 'Érdi Bőtermő' variety, which was planted in 2005 from a 5-ha Kiskőrös plantation with drip irrigation (Hungary, NL: 46°, 37'; EL: 19° 17'). The aseptic processing was performed by the vegetable and fruit processing plant of Juice Products Zrt. in Kiskőrös.

The fruit after the harvest was immediately processed to avoid any effect of storage. The sour cherry were first passed through on drum washer (SRAML® JP6000) and then placed in a perforated disc fruit grinder (ProXES) to be suitable for pressing. Pressing was performed with a fruit press (DELLA TOFFOLA® PEC100). After preheating to 40 °C (with FBR Elpo Mr49-6 equipment), dissolved and dispersed oxygen was removed using a deaerator (SRAML D-1000). Ascorbic acid and acerola (concentrated acerola cherry juice cloudy, 65° Brix from Eckes-Granini Ltd.) were added to the juices immediately after pressing. The heat treatment was performed with a tubular pasteurizer (Della Toffola Priamo) at 86 °C for 1 min.



The pasteurized juice was transferred to sterile 5 L polyethylene sample bags (SCHOLLE IPN<sup>®</sup>) after refrigeration within a closed system using aseptic filling system (FBR ELPO<sup>®</sup>) with ascorbic acid (MAS, 200 mg ascorbic acid L<sup>-1</sup>), with acerola (MACE, 1.42 g L<sup>-1</sup> acerola concentrate, containing the same amount of ascorbic acid as the AS sample) and the control sample (MC, without any of these). No significant change was observed in the water soluble dry matter content of samples (ranging from 12.6 to 12.8 Brix) after addition of acerola concentrate and ascorbic acid to the sour cherry juice.

Acerola juice or ascorbic acid was added to the sour cherry juice to enrich the amount of valuable component and the changes of measured parameters were monitored during the 12 months of storage at 20 °C in a dark storage. Samples were taken every 2 months (3–3 bags for each treatment and for each sampling). The anthocyanin content and colour coordinate values of aseptically filled sour cherry juice were determined and compared with the control sample.

## Methods

The Konica Minolta CR 400 type handheld digital colour measuring device (operates on the principle of reflection) served the purpose of colour measurement, which is based on the CIE Lab system. The L\* (lightness factor), the a\* (the transition from green to red) and the b\* (from blue to yellow) were measured. To evaluate the change in colour between two samples, the total colour difference parameter  $\Delta E^*$  was used according to Eq.:

$$\Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}$$

Evaluation of using  $\Delta E^*$  is presented in Table 1.

The browning index (BI) can be calculated from the L\*, a\* and b\* parameters with the following equation:  $BI = [100 (x - 0.31)]/0.172$ , where:  $x = (a^* + 1.75L^*)/(5.645L^* + a^* - 0.3012 b^*)$  (Klimczak and Gliszczyńska-Świątło, 2017). The BI value calculated from the above values is suitable for the characterization of colour changes during storage of sour cherry juice (Dalmadi et al., 2007).

The pH value of the samples was determined with a TESTO 206 handheld digital pH meter (accuracy  $\pm 0.02$  pH).

Total polyphenol content was investigated using Folin–Ciocalteu's reagent and sodium-sulphate solution according to the method of Singleton and Rossi (1965). Absorbance was monitored by Hitachi U-2900 spectrophotometer at 765 nm, and the content of soluble polyphenols was calculated from standard curve based on gallic acid (GA) concentrations. The results were expressed in mg GA 100g<sup>-1</sup>.

For the antioxidant capacity determination, the experiment was carried out by means of FRAP method proposed by Benzie and Strain (1996). FRAP assay was conducted by Hitachi

Table 1. Summary of colour difference

$\Delta E^*$	Sensible difference
0–0.5	Unnoticeable
0.5–1.5	Slightly noticeable
1.5–3.0	Noticeable
3.0–6.0	Well-visible
>6.0	Great difference



U-2900 spectrophotometer at 593 nm. FRAP value was expressed as ascorbic acid (AA) equivalents, mmol AA L<sup>-1</sup>.

The total anthocyanin content (TAC) of the methanol extract was measured using a colorimetric assay according to the protocol described by Giusti and Wrolstad (2001). The results were expressed in mg 100g<sup>-1</sup>.

The ascorbic acid content was determined using a high-performance liquid chromatography (HPLC) apparatus (Shimadzu Europa GmbH). A reversed phase C18 column (Supelco, 250 \* 4.6 mm i.d., 5 µm) was used for the analysis. To record the chromatograms, the required time, injected volume, and flow rate of 5 min, 20 µL and 1.5 mL min<sup>-1</sup> were adopted, respectively (Székely et al., 2019). Five parallel measurements were performed during the study. LC Solution Software was used for data processing and analysis. The obtained result was expressed in mg mL<sup>-1</sup>.

Phenolic acids (chlorogenic acid, gallic acid, catechin) were also measured using the high-performance liquid chromatography (HPLC) apparatus (Shimadzu Europa GmbH). The sample was prepared by mixing 0.5 ml of sour cherry juice with 9.5 ml of 20% methanol followed by a filtration through a pleated microfilter. In each case, three parallel measurements were performed. Again, a reversed phase C18 column was used for the measurements. The eluent comprised 2% acetic acid solution for pump “A” and a 30:70 mixture of 2% acetic acid and acetonitrile for pump “B”. The time required to record the chromatograms was 40 min. For each sample, the injected volume was 10 µL at a flow rate of 1.5 mL min<sup>-1</sup> (Székely et al., 2014). Chlorogenic acid, gallic acid and catechin standards were used to identify and quantify the peaks obtained and the generated results were expressed in mg mL<sup>-1</sup>.

Data were subjected to statistical analysis using SPSS Statistics v27 software for the analysis of variance (ANOVA). Pairwise comparisons between the treatments were realized using Tukey test with a 95% confidence level.

## RESULTS

### Change of pH value

Table 2 demonstrates the variation of pH values of the cherry juice samples during storage. The addition of ascorbic acid and acerola juice reduced the pH of the samples to 3.29 and 3.30, respectively, compared to the value of the control sample, which amounted to 3.33. The results are in accordance with the observations of Will et al. (2005), who found pH values ranging from 3.07 to 3.32 for different cherry cultivars.

Table 2. Changing of pH values of sour cherry juice during storage

Months	0.	2.	4.	6.	8.	10.	12.
MC	3.33±0.01 <sup>a</sup>	3.33±0.01 <sup>a</sup>	3.38±0.02 <sup>b</sup>	3.47±0.02 <sup>c</sup>	3.51±0.01 <sup>d</sup>	3.52±0.01 <sup>d</sup>	3.5±0.02 <sup>d</sup>
MAS	3.29±0.01 <sup>a</sup>	3.30±0.01 <sup>a</sup>	3.35±0.01 <sup>b</sup>	3.39±0.02 <sup>c</sup>	3.40±0.02 <sup>c</sup>	3.41±0.01 <sup>cd</sup>	3.44±0.01 <sup>d</sup>
MACE	3.30±0.01 <sup>a</sup>	3.30±0.01 <sup>a</sup>	3.34±0.01 <sup>b</sup>	3.35±0.02 <sup>b</sup>	3.38±0.02 <sup>bc</sup>	3.39±0.01 <sup>c</sup>	3.40±0.01 <sup>c</sup>

Superscript with lowercase letters indicate significance difference by time along the rows. Presented values are means ± SD. (Tukey's test,  $P < 0.05$ ).



Continuous increase in pH values was observed during the storage regardless of the applied treatments. The smallest increase was observed with the addition of acerola juice (0.1), while the addition of ascorbic acid resulted in an increase of 1.5. The largest increase of 0.2 was observed in the case of the control sample. The increase in pH during storage can be explained by the partial degradation of organic acids in the fruit. Cortés et al. (2008) reported similar results for pasteurized and PEF-treated orange juices, where they found a significant increase in pH during storage.

### Change of colour parameters

Figure 1 illustrates the colour parameters of the samples during the 12-month storage period. Up till the 6th month of the storage period, an increasing trend of  $L^*$ ,  $a^*$  and  $b^*$  values was observed for all samples, followed by a decreasing pattern.

Figure 1a shows that initially the sample treated with ascorbic acid (MAS) was the lightest while the one treated with acerola (MACE) was the darkest. There was no significant change in  $L^*$  values in the first two months, but thereafter a steadily increasing trend was observed until month 6. While the initial values measured between 18.9 and 25.5, a shift to a higher range of  $L^*$  values from 38.6 to 43.3 was obtained. During the rest of the storage period,  $L^*$  values showed a steadily decreasing trend before regaining the initial value by month 12. The ascorbic acid treated sample (MAS) becomes the darkest, while the acerola treated sample (MACE) is the brightest.

The variation of  $a^*$  values was found to be very similar to that of  $L^*$  values (Fig. 1b). At the initial phase, cherry juice treated with ascorbic acid had the reddest shade, while control and

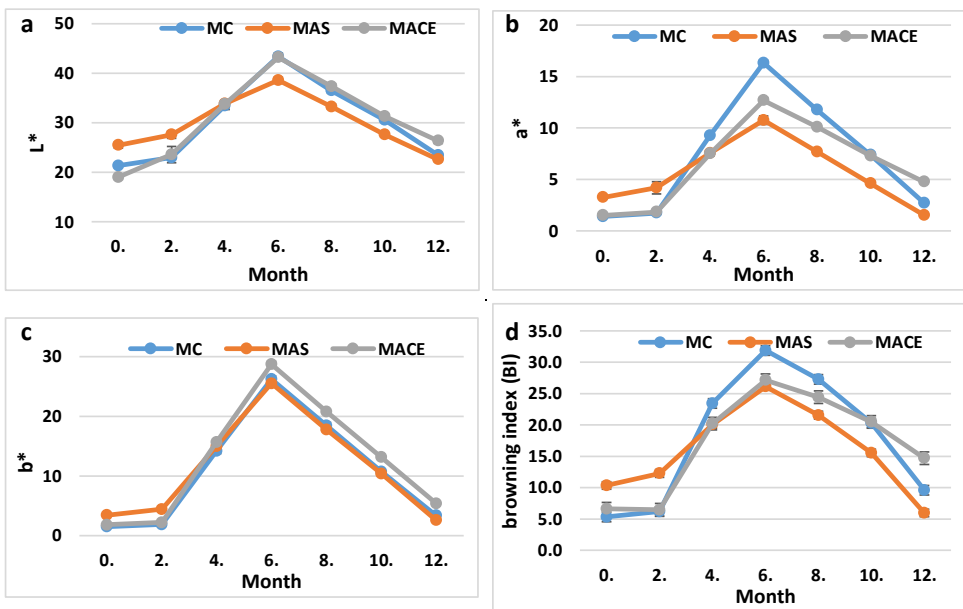


Fig. 1a-d. Changing of colour parameters ( $L^*$ ,  $a^*$ ,  $b^*$ ) and  $\Delta E^*$  of sour cherry samples during storage time



acerola-treated juice were characterized by slightly lower  $a^*$  values. No significant change was observed in the first two months. However,  $a^*$  values increased steadily until the 6th month. The largest increase was obtained in the case of the control sample, where the initial range of 1.4–3.2 reached a range of 10.7–16.3. The smallest increase, on the other hand, was observed for the ascorbic acid treated (MAS) sample. After month 6, the values showed a steady decreasing trend and returned closer to the initial values. In a similar manner to  $L^*$ , the sample with the least reddish tint at the beginning (MACE) showed the most favourable  $a^*$  value by the end of storage, i.e., it was the one with the most vibrant reddish tint.

Given the crucial role of  $a^*$  (red/green ratio) in the red colour of sour cherry juice, its variation during storage provides important information. What the results have shown is that the control sample underwent the largest change, while the ascorbic acid treated sample (MAS) showed the highest stability. Such findings accentuate the instability of the control sample, in terms of storage time, compared to the treated ones.

As outlined by (Fig. 1c), the changes in  $b^*$  values were comparable to those of  $a^*$  values. Initially, the control sample presented the lowest  $b^*$  value while the ascorbic acid treated (MAS) sample had the highest. After roughly stable results in the first two months, the  $b^*$  values also showed an increasing trend until the 6th month. The original values of 1.5–3.4 increased to range between 25.5 and 28.8 before decreasing to values that are close to the initial ones. The highest value was observed for the acerola treated (MACE) sample while the lowest was obtained in the case of the ascorbic acid (MAS) sample.

By analyzing the data of the initial samples and those stored for 12 months, it can be concluded that  $L^*$ ,  $a^*$  and  $b^*$  values increased, except for samples with added ascorbic acid (MAS).

Similar results were reported by [Wojdyło et al. \(2019\)](#), who studied the effect of storage conditions (190 days at 4 and 30 °C) on anthocyanin compounds of 25 sour cherry varieties during production under industrial conditions. The initial  $a^*$  values of the varieties ranged from 0.9 to 6.35. The  $a^*$  values of the samples stored at 4 °C remained more stable compared to the samples stored at 30 °C. Similarly, a slight upward trend in  $a^*$  values was reported and most pronounced in the 3rd month.

In their research, the authors also noticed an increase in  $L^*$  and  $b^*$ , which is a consequence of anthocyanin loss. Although a decrease in total anthocyanin content was observed during storage, the red colour remained stable until the end of storage. Such results were attributed to the co-pigmentation between anthocyanins and flavonols. [González-Manzano et al. \(2009\)](#) stipulated that the colour tends to shift towards a purple tone during storage of juices containing anthocyanins, which was observed in red wines. Similar behaviour was identified by [Wilkes et al. \(2014\)](#) and [Wojdyło et al. \(2014\)](#) for aronia and pomegranate juices, respectively. According to [González-Manzano et al. \(2009\)](#), anthocyanin polymers of flavan-3 compounds formed during the processing of juices were more resistant to degradation during storage. [Boulton \(2001\)](#) also found that different coloured polymers were presumably formed during the production and storage of anthocyanin-containing juices, or that co-pigmentation between anthocyanins and other flavonoids preserved the colour and masked adverse changes in anthocyanins during storage.

Figure 1d shows the browning index (BI), which provides a good characterization of changes during storage. Despite having the highest values at the initial stage, the ascorbic acid treated cherry juice (MAS) was the least affected during storage. Interestingly, from month 6



onwards, more favourable values were recorded compared to the other two samples. At the end of storage, at month 12, the lowest BI value was conferred to the sample treated with ascorbic acid, exhibiting the best stability. What can be observed, however, is that the trend in BI value is independent of treatment and follows the same pattern for each sample set.

$L^*$  (lightness factor) plays a role in the evolution of  $\Delta E^*$ , with values ranging from 18.9 to 43.3. The values of  $b^*$  (yellow-blue ratio) are also significant, ranging from 1.5 to 28.8, while  $a^*$  varied from 1.4 to 16.3. These results suggest that  $L^*$  and  $b^*$  played the most important role in shaping  $\Delta E^*$  values. By analyzing the data, it can be concluded that the colour change is time-dependent and not affected by the individual treatments.

When comparing the colour parameters at the initial stage and by the end of storage, the  $\Delta E^*$  values were 3.11 for the control sample, 3.46 for the ascorbic acid treated sample and 8.81 for the acerola treated sample.

The colour change is well illustrated by  $\Delta E^*$  in Fig. 2a, which shows the change in colour difference as a function of time for each of the applied treatments. Compared to the initial colour parameters, a slight but visually perceptible difference can be measured in the 2nd month for all three treatments. Such difference was most notable for the acerola-treated cherry (MACE) sample. Up to the 6th month, the difference in  $\Delta E^*$  values increased gradually for the control and acerola treated samples, while the ascorbic acid samples (MAS) showed the smallest change. A decreasing trend is observed during the rest of the storage period, but the values are still in the clearly perceptible category even nearing the end of storage (between months 10 and 12).

Figure 2b presents the pairwise comparison of  $\Delta E^*$  between treatments. In the initial stage, there is a notable difference between the control sample and the acerola treatment, whereas the ascorbic acid treatment caused an even more clearly detectable difference. There is also a great difference between the two treatments.

When comparing the ascorbic acid treatment with the control sample, it is observed that the difference between the two increases by the 2nd month but decreases to a noticeable difference by the 4th month. The difference between the two samples is largest at month 6, and thereafter the  $\Delta E^*$  value gradually decreases, with the difference between the two samples becoming

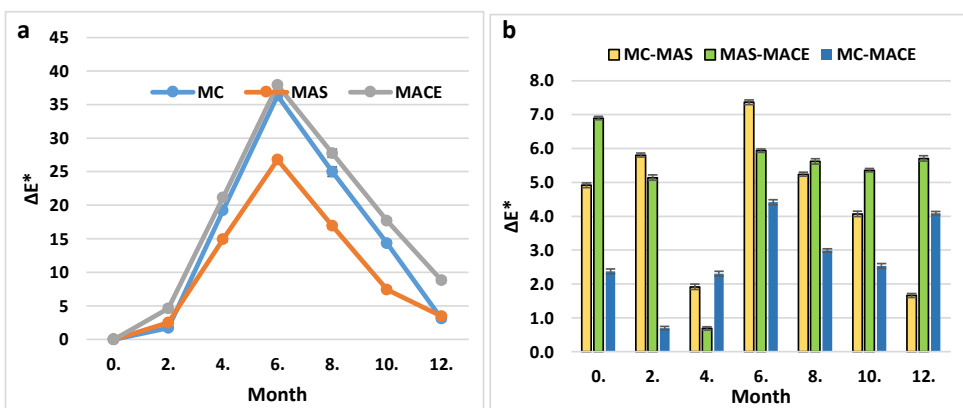


Fig. 2a-b. Changing of browning index (BI) during storage time and  $\Delta E^*$  of sour cherry samples comparing treatments



smaller. The treatment with acerola, on the other hand, induced a smaller difference compared to the control sample, becoming barely noticeable in the 2nd month of storage and then falls to clearly detectable by the 6th month. After the 6th month, the difference between the two decreases before increasing again by the end of storage. Between the two treatments (MAS-MACE) the difference is initially clearly visible, then it tends to decrease by month 4. The largest difference between the two treatments was also obtained at month 6, after which a decreasing (between MC-MAS) and changing trend was observed (between MC-MACE and MAS-MACE), with no significant change in the values that remained in the clearly detectable category.

### Change of total polyphenol content

Figure 3 shows the distribution of the total anthocyanin content in relation to the total polyphenol content. In terms of initial values, the control sample had the highest polyphenol content (138.9 mg 100 g<sup>-1</sup>), followed by the ascorbic acid treated sample (135.8 mg 100 g<sup>-1</sup>) and then the acerola treated sample (125.9 mg 100 g<sup>-1</sup>). A slight increase in total polyphenol content was observed for all three samples by the 2nd month of storage, followed by a more significant increase. The rate of increase between months 0 and 4 was 32.7% for the control sample, 29.5% for the ascorbic acid treated sample and 45.7% for the acerola treated sample.

Between months 4 and 6, while there was a significant decrease in polyphenol content for all three treatments, the values did fall below baseline values for both the control and ascorbic acid samples. Thereafter, the control sample showed a further declining trend, while the treated samples presented fluctuating values but no significant change. By the 12th month all three types of samples showed a decrease below the initial values.

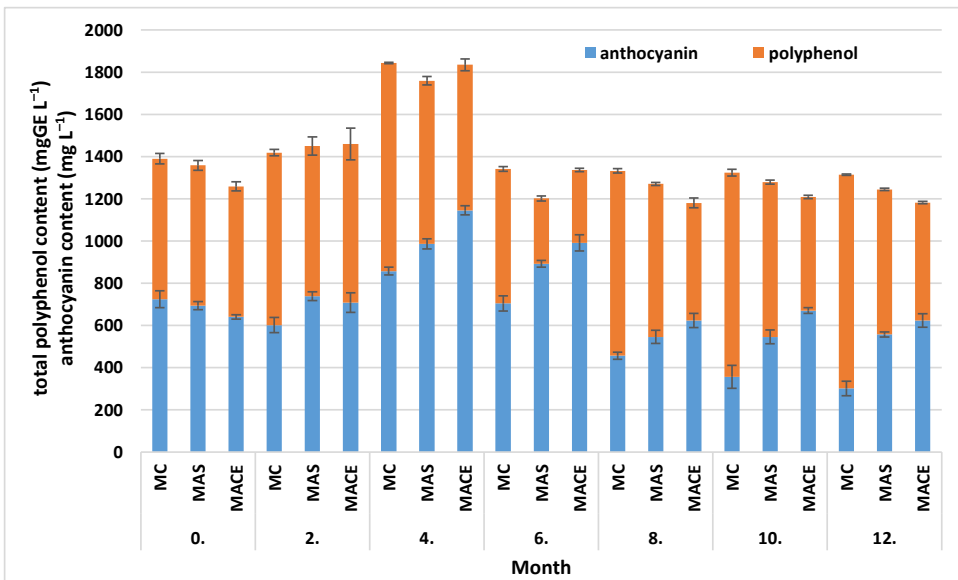


Fig. 3. Changes of total polyphenol (TPC) and total anthocyanin (TAC) content of aseptic filled sour cherry juice during storage (mg L<sup>-1</sup>)





The statistical evaluation of the total polyphenol content of the sour cherry juice samples showed that there was no significant difference between the treatments (MAS–MC,  $P = 0.714$ ; MACE–MC,  $P = 0.469$ ; MACE–MAS,  $P = 0.917$ ). Storage time had a significant effect on total polyphenol content for all treatments.

The present research shows similar results with several studies investigating the biological value and active constituents of cherry varieties. [Ficzek \(2012\)](#), for instance, determined the total polyphenol content of different sour cherry varieties over four consecutive years. The obtained results ranged from 147.7 to 527.2 mg GE 100 g<sup>-1</sup>, within which the values for the ‘Érdi bőtermő’ cultivar varied between 142.4 and 422.3 mgGE 100 g<sup>-1</sup>. Such results are similar to the ones presented herein. [Kim et al. \(2005\)](#) also examined the total phenolic compounds in cherry and sour cherry cultivars and found results ranging from 146.1 to 312.4 mgGE 100 g<sup>-1</sup>. [Damar and Ekşi \(2012\)](#), on the other hand, measured values ranging from 151 to 255 mgGE 100 g<sup>-1</sup>. For [Bonertz et al. \(2007\)](#), the total polyphenols fluctuated between 270 and 499 mgGE 100 g<sup>-1</sup> for 5 sour cherry cultivars, whereas the ‘Cigány 7’ Hungarian sour cherry cultivars contained 270 mgGE 100 g<sup>-1</sup>.

### Change of anthocyanin content

[Figure 3](#) shows the change of anthocyanin content during storage. At the beginning of storage, the anthocyanin content of the three samples was almost the same, ranging from 64.0 to 72.4 mg 100 g<sup>-1</sup>. Slight increase was observed for the treated samples by month 2, while a decrease was observed for the control sample. By month 4, a significant increase was observed for all samples, with a rate of 18.3% for the control sample, 42% for the ascorbic acid treated sample and 78.9% for the acerola treated juice. Between months 4–8, a significant decrease in anthocyanin content occurred, which continued in the case of the control sample and stabilized for the two treated samples. For the control sample, there is a continuous decrease (58.4%) while for samples with ascorbic acid and acerola an increasing trend until month 6 and then a decreasing trend is observed. The decrease for the sample with ascorbic acid is 19.7% while the decrease for the sample with acerola is 2.6%.

The statistical evaluation of the total anthocyanin content of the sour cherry juice samples showed that there was also a significant difference between the ascorbic acid-treated sour cherry juice and the control sample (MAS–MC,  $P = 0.102$ ), between the acerola-treated and control sour cherry juice samples (MACE–MC,  $P = 0.009$ ). However, no significant difference was observed in the polyphenol content of the two treatments (MACE–MAS,  $P = 0.595$ ). Storage time had a significant effect on total anthocyanin content for all treatments.

During storage time, the amount of anthocyanins ranged between 22.91 and 74.18% within the total polyphenol content. At the beginning of storage, there was no significant difference between the values as regards the treatments, with anthocyanin compounds accounting for 50.85–52.11% of the total polyphenols. From the 2<sup>nd</sup> month, treated cherry juices always had a higher rate of total anthocyanin content compared to the control sample. The highest ratio of anthocyanins was found in the 6<sup>th</sup> month of storage in the case of the ascorbic acid treated sample (MAS), 74.17%, and in the acerola treated (MACE) juice, 74.18%. During the rest of the storage period, from the 6<sup>th</sup> to the 12<sup>th</sup> month, the acerola treated (MACE) juices showed higher anthocyanin content in total polyphenol content at all time points studied. Thus, in terms of total anthocyanin content, the treatment with acerola proved to be more stable.



The study of the total anthocyanin content of sour cherry cultivars is being intensively investigated in sour cherry producing countries around the world, and numerous data is available. Kim et al. (2005) 49.1–109.2 mg 100 g<sup>-1</sup>, Blando et al. (2004) 27.8–804 mg 100 g<sup>-1</sup> and Damar and Ekşi (2012) detected total anthocyanin contents ranging from 35 to 63 mg 100 g<sup>-1</sup> in several cultivars. Homoki et al. (2014) studied the anthocyanin content of 5 Hungarian cultivars and candidate cultivars of sour cherry, with results ranging from 21 to 295 mg 100 g<sup>-1</sup>, with a particularly high value for ‘Cigánymeggy’ (C59) (206 mg 100 g<sup>-1</sup>), while total anthocyanin content was detected in the ‘Kántorjánosi’ and ‘Debreceni bőtermő’ cultivars, 21 and 63 mg 100 g<sup>-1</sup>, respectively. Bonerz et al. (2007) monitored the changes in the anthocyanin content of ‘Schattenmorelle’ cherry juice during 6 months storage at 20 °C. Based on their results they concluded that during storage the colourless phenolic compounds remained more stable, while 75% of the anthocyanins has degraded by the end of storage.

Wojdyło et al. (2019) studied anthocyanin compounds of 25 sour cherry varieties during the production of industrial cloudy juice and during 190 days at 4 and 30 °C storage. Total anthocyanin content of the samples varied from 59 to 116 mg 100<sup>-1</sup> g. Based on their results, it was found that anthocyanin content and colour characteristics were mainly influenced by variety while anthocyanin degradation during storage was more time and temperature dependent. Most of the varieties, similar to the control sample results of the present study, suffered more than 50% anthocyanin degradation during storage.

### Change of antioxidant capacity (FRAP)

The change in antioxidant capacity is shown in Fig. 4. The lowest antioxidant activity (4.61 mmolAS L<sup>-1</sup>) was determined in the control sample measured at initial months, while the highest value (6.51 mmolAS L<sup>-1</sup>) was determined also for the control sample.

At the beginning of the storage period, the lowest antioxidant capacity was found in the control sample (4.61 mmolAS L<sup>-1</sup>) while the two treated samples showed higher values, with 4.92 mmolAS L<sup>-1</sup> for the ascorbic acid (MAS)-treated sample and 5.02 mmolAS L<sup>-1</sup> for the

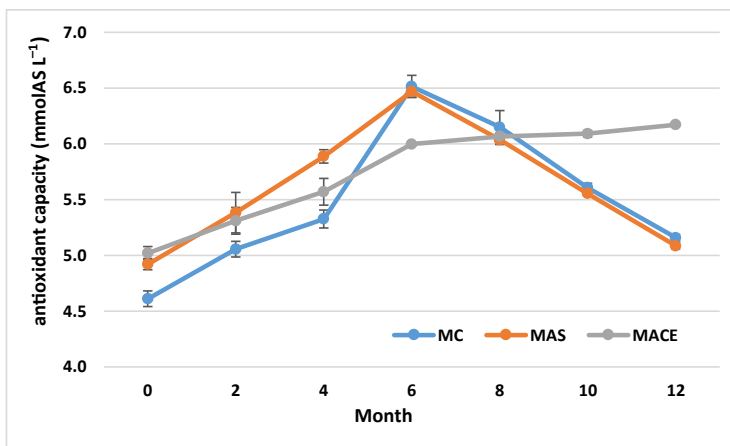


Fig. 4. Changes of antioxidant capacity (FRAP) of aseptic filled sour cherry juice during storage (mmol L<sup>-1</sup>)



acerola-treated cherry. Our results correspond with the research of Ficzek (2012), who detected values of 2.69–6.29 mmolAS L<sup>-1</sup> for 3 consecutive years in the case of the ‘Érdi bőtermő’ cultivar.

In the first 4 months of storage, all three samples showed an increasing tendency in antioxidant capacity values (control 15.6%, MAS 19.7%, MACE 10.9%). Between months 4 and 6, the increasing tendency became rapid for the control sample (22.1%), decreased for the ascorbic acid treated (MAS) sample (9.8%), while in the case of the acerola treated juice (MACE) the increasing tendency continued at a similar rate (12.9%). For the acerola-treated cherry juice, antioxidant capacity stabilized for the remainder of storage and showed a further increase of 2.7%. The antioxidant capacity of the control and ascorbic acid treated samples decreased steadily between months 6–12. However, for all three samples, the antioxidant capacity at the end of the storage period exceeded the initial values, with the most stable being the juice treated with acerola (MACE).

The statistical evaluation of the antioxidant capacity of the sour cherry juice samples showed that there was no significant difference between the treatments (MAS–MC,  $P = 0.719$ ; MACE–MC,  $P = 0.366$ ; MACE–MAS,  $P = 0.827$ ). Storage time had a significant effect on antioxidant capacity for all treatments.

Papp (2014) examined 32 sour cherry cultivars in a PhD study and the antioxidant capacity of the samples were ranged 2.5–25 mmolAS L<sup>-1</sup>. In the case of the ‘Érdi bőtermő’ variety, value was found 5 mmolAS L<sup>-1</sup>, which was in the lower 1/3 part of the varieties studied. Dragovic-Uzelac et al. (2007) determined 32.53 and 24.04 mmolAS kg<sup>-1</sup> for ‘Marasca’ and ‘Cigány-meggy’, respectively.

The antioxidant capacity values for the control (MC) and ascorbic acid treated samples (MAS) showed a strong correlation with the L\*, a\*, and b\* values, while no correlation was found for the acerola treated sample. Samples treated with acerola also showed a strong negative correlation with the phenolic components ( $r = -0.736$  for catechin;  $r = -0.928$  for chlorogenic acid;  $r = -0.920$  for gallic acid). Phenolic compounds of acerola juice are also thought to play a role in the correlation.

## Change of ascorbic acid content

Figure 5a shows the change in ascorbic acid content during storage. The initial ascorbic acid content of the control sample (MC) was 242 mg L<sup>-1</sup>. In a study by Wojdyło et al. (2014), the fruit content of 33 sour cherry varieties was investigated, the ascorbic acid content ranged from 54.5 to 221.8 mg L<sup>-1</sup> depending on the variety, with a value of 185.9 mg L<sup>-1</sup> measured for the ‘Érdi bőtermő’. As a result of the treatments (addition of 200 mg kg<sup>-1</sup> ascorbic acid), the initial values of the samples increased to 416 mg kg<sup>-1</sup> (MAS) and 376 mg kg<sup>-1</sup> (MACE). Considering that the samples were heat treated before filling, a difference in the initial values is already observed in favour of the ascorbic acid (MAS) treatment.

The decrease in ascorbic acid during storage of the control sample (MAS) is steady until month 6, after which there is an increasing and then a decreasing again. In the case of the treated samples, during storage the change is irregular, with increasing and decreasing phases, which is more pronounced for the ascorbic acid (MAS) treated sample. This type of change in ascorbic acid during storage is also observed in tomatoes treated with chitosan (Kaewklin et al., 2018). The amount of ascorbic acid added also affects the changes during storage. Özkan (2002) found that for some cherry varieties, adding 80 mg L<sup>-1</sup> ascorbic acid to the cherry juice accelerates



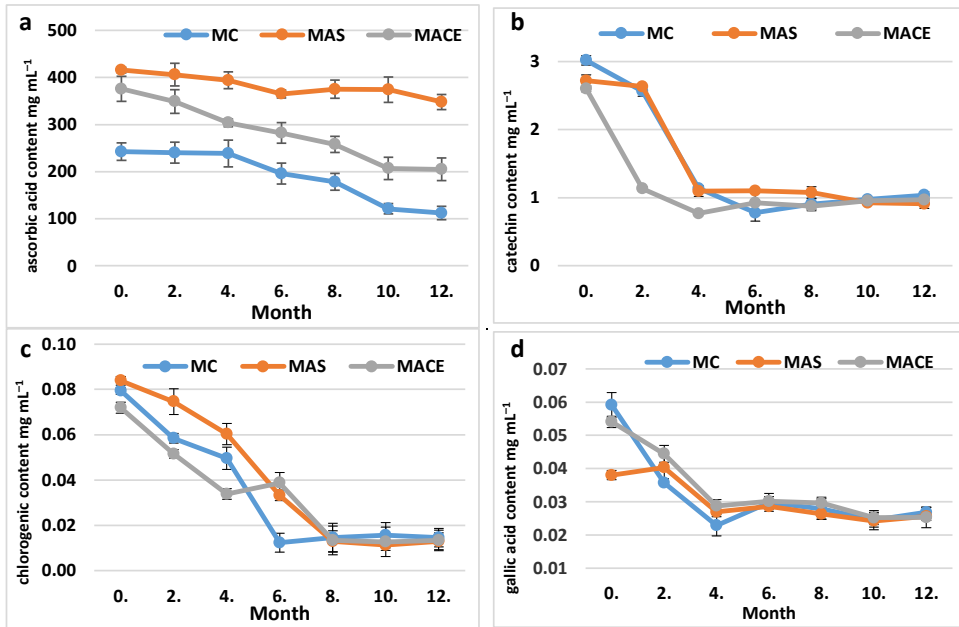


Fig. 5a-d. Changing of ascorbic acid ( $\text{mg L}^{-1}$ ) and phenolic compounds ( $\text{mg mL}^{-1}$ ) of asceptic filled sour cherry juice during storage

anthocyanin degradation during storage, whereas  $60 \text{ mg L}^{-1}$  does not have this effect. In pomegranate juice, however, both concentrations had a protective effect on anthocyanins. In the case of acai juice, De Rosso and Mercadante (2007) found that more added ascorbic acid caused more significant fading in the red-blue colours.

The statistical evaluation of the ascorbic acid content of the sour cherry juice samples showed that there was also a strong significant difference between the ascorbic acid-treated and the control sample (MAS-MC,  $P = 0.000$ ) and between the acerola-treated and control samples (MACE-MC,  $P = 0.003$ ). However, no significant difference was observed in the ascorbic acid content of the two treatments (MACE-MAS,  $P = 0.293$ ).

### Change of phenolic compounds content

Out of the three identified components, catechin was the most abundant in the samples (Fig. 5b). The highest values occurred at the beginning of storage ( $2.6534\text{--}3.0700 \text{ mg mL}^{-1}$ ). Thereafter, a decreasing trend was observed for all three samples, but not with the same intensity. For the control and ascorbic acid treated samples, a small decrease in the first two months (control 15%, MAS 3.2%) was followed by an intense decrease between months 2 and 4 (control 54.7%, MAS 57.4%). In contrast, the acerola-treated juices showed a significant decrease between months 0-2 (55.5%) and a smaller decrease between months 2-4 (31.3%).

The values stagnated during the further storage period, independent of the treatment. Overall, the decrease during 12 months of storage was 64.8% for the control sample, 65.7% for the ascorbic acid sample and 61.8% for the acerola juice sample.



The statistical evaluation of the catechin content of the sour cherry juice samples showed that there was no significant difference between the treatments (MAS–MC,  $P = 1.000$ ; MACE–MC,  $P = 0.379$ ; MACE–MAS,  $P = 0.418$ ). Storage time had a significant effect on catechin content for all treatments. The catechin content shows a negative correlation with the colour parameter  $b^*$  for the control sample (MK sample  $r = -0.725$ ).

Comparing catechin content with international publications, there is a very high variability in the results. According to the results of Bonerz et al. (2007), 1 and 14 mg L<sup>-1</sup> fall behind our catechin results. Similarly low values (11.1–29.2 mg L<sup>-1</sup>) were found by Levaj et al. (2010), despite the agreement in the amount of chlorogenic acid detected by them. The same can be observed for Alrgei et al.'s (2016) results of 1.4–5.17 mg L<sup>-1</sup>, despite the fact that there is correspondence between the two measured chlorogenic acid values.

According to Cásedas et al. (2016), chlorogenic acid is one of the main components of antioxidant capacity compounds in sour cherries and its determination is very important. Among the identified components, chlorogenic acid was the second most abundant, with values ranging from 0.0129 to 0.0838 mg mL<sup>-1</sup> (Fig. 5c). All three treatments showed a decreasing tendency during storage. Compared to the initial values (0.0719–0.0838 mg mL<sup>-1</sup>), the concentration of chlorogenic acid decreases until the 8<sup>th</sup> month (0.0129–0.0146 mg mL<sup>-1</sup>), and then maintains stable values during the rest of the storage period, regardless of the treatments.

On the whole, control sample showed the smallest change (81.5%), as did the cherry treated with acerola (81.9%), while the decrease in the ascorbic acid treated sample was 84.6%.

The statistical evaluation of the chlorogenic acid content of the sour cherry juice samples showed that there was no significant difference between the treatments (MAS–MC,  $P = 0.723$ ; MACE–MC,  $P = 1.000$ ; MACE–MAS,  $P = 0.723$ ). Storage time had a significant effect on the evolution of chlorogenic acid content for all treatments.

Papp (2014) investigated chlorogenic acid levels in 13 sour cherry cultivars. The results (3.8–40.6 mg 100 g<sup>-1</sup>) were similar to the results of the present study for several varieties. Papp's study showed that chlorogenic acid contributes significantly to the antioxidant capacity (FRAP). Also similar results were reported by Alrgei et al. (2016), chlorogenic acid ranging from 8.1 to 31.16 mg kg<sup>-1</sup> for sour cherry. Levaj et al. (2010) measured similar results for the 'Marasca' cherry variety (45.9 mg L<sup>-1</sup>), as well as for the 'Oblacinska' variety (28.3 mg L<sup>-1</sup>). Results from Bonerz et al. (2007) ranged from 119 to 268 mg 100 g<sup>-1</sup>, which is 2–3 times higher than our results.

Gallic acid content of the samples varied from 0.0591 to 0.0229 mg mL<sup>-1</sup>. A decreasing trend was observed as a function of storage time (Fig. 5d). The decrease is most significant during the first 4 months, after the values stagnate. The rate of decrease was the lowest for the ascorbic acid treated sample (32.6%) while the decrease was 54.6% for the control sample and 54.6% for the acerola treated juice.

The statistical evaluation of the antioxidant capacity of the sour cherry juice samples showed that there was no significant difference between the treatments (MAS–MC,  $P = 0.702$ ; MACE–MC,  $P = 0.888$ ; MACE–MAS,  $P = 0.418$ ). Storage time had a significant effect on the development of gallic acid content for all treatments.

## CONCLUSION

By the end of the storage period, the samples enriched with acerola had the highest quality parameters.



The amount of ascorbic acid generally used in industrial practice proved to be too high in the case of the 'Érdi bőtermő' variety, and did not ensure a sufficiently stable system for the protection of anthocyanins. The test results show that acerola could be a possible alternative to the currently used ascorbic acid to improve the quality of aseptic cherry juice during storage.

The amount of anthocyanin depends on the variety, so its stability during heat treatment and storage. It would be useful to optimize and determine the amount of ascorbic acid added per variety in order to maintain the content and quality parameters of the fruit juices rich in anthocyanins.

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