# Effect of ascorbic acid and acerola juice on some quality properties of aseptic filled apple juice

Á. Ribárszki<sup>1</sup>, D. Székely<sup>1</sup>, B. Szabó-Nótin<sup>1</sup>, B. Góczán<sup>1</sup>, L. Friedrich<sup>2</sup>, Q.D. Nguyen<sup>3\*</sup> <sup>1</sup> and M. Máté<sup>1</sup>

<sup>1</sup> Department of Fruit and Vegetables Processing Technology, Institute of Food Science and Technology, University of Agriculture and Life Sciences, Villányi út 29–43., Budapest H-1118, Hungary

<sup>2</sup> Department of Livestock and Food Preservation Technology, Institute of Food Science and Technology, University of Agriculture and Life Sciences, Ménesi út 45., Budapest H-1118, Hungary

<sup>3</sup> Department of Bioengineering and Alcoholic Drink Technology, Institute of Food Science and Technology, University of Agriculture and Life Sciences, Ménesi út 45., Budapest H-1118, Hungary

## SHORT COMMUNICATION

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#### ABSTRACT

Quality and storability are crucial factors in production of apple juice. The main goal of this study was investigation of the effects of ascorbic acid and acerola juice on the changes of some sensorial parameters and bioactive compounds of aseptically filled and industrial scale produced apple juice during storage for 12 months. While the viscosity and pH of apple juice did not change significantly, the  $\Delta E^*$  peaked (20–30) at month 6 of the storage period. The colour of apple juice was lighter than at the beginning of storage. Maximum total phenolic contents were 1,100, 1,400, and 1,250 mg L<sup>-1</sup> in the control, ascorbic acid, supplemented, and acerola added samples, respectively. Other parameters (antioxidant capacity, ascorbic acid, browning index, etc.) peaked in month 4. Acerola was a good alternative anti-browning and antioxidant agent for the treatment of apple juice in the processing. The antioxidant capacity of apple juice treated with acerola was higher than with ascorbic acid. The results were obtained with industrial samples, thus, they can serve as a very good base for the optimisation process and industrial production without the need for scale-up.



<sup>\*</sup> Corresponding author. E-mail: nguyen.duc.quang@uni-mate.hu

#### **KEYWORDS**

apple juice, aseptic filling technology, acerola, ascorbic acid, storage, phenols, antioxidant

## 1. INTRODUCTION

Worldwide, apple is one of the widely consumed fruits, both in fresh and processed forms. The potential health protection properties of apple and apple-based products were reported by many studies in the literature due to the abundance of numerous biologically active compounds such as vitamin C, vitamin E, ß-carotene, and polyphenols (Viškelis et al., 2019). Such compounds play a major role in the human immune system and help to protect against many diseases such as cardiovascular diseases, cancer, asthma, lung diseases, and diabetes (Zhu et al., 2021).

Apple juice, especially that is not from concentrate (NFC), is one of the most popular products (Pardo and Borges, 2020), but the quality of this product needs different preservation methods. Recently, thermal preservation methods are commonly applied techniques that guarantee the microbiological stability of juices (Zhu et al., 2021). However, this method usually causes sensorial issues (Zhu et al., 2021), thus, to maintain the quality of apple juice, gentle heat treatment methods are required. Recently, pasteurisation in combination with aseptic packaging is widely applied in the industrial production, because of its flexibility and cost efficiency (Bates et al., 2001). Additionally, this method provides good alternative for mitigation of the seasonality caused by the short harvest period of fruits.

In fruit processing, colour is an important sensorial property, thus, for certain fruits such as apple, pear, etc. an important step, namely colour blocking, must be inserted. It is mainly achieved by addition of ascorbic acid (Jiang et al., 2016), but because of the cost of ascorbic acid, manufacturers seek alternative natural sources. Acerola fruit that contains high amount of ascorbic acid (1,500–4,500 mg/100 g) and other bioactive compounds could be very promising antioxidant agent to replace the ascorbic acid (de Goes Carneiro et al., 2020). There is very few recent technological data available in the literature on acerola, thus, our study focuses on the effects of ascorbic acid and acerola juice on the quality of aseptically filled apple juice during storage.

## 2. MATERIALS AND METHODS

### 2.1. Materials

'Idared' apple variety originating from Kiskőrös (Hungary) was collected, harvested, and processed in the plant of Juice Products Plc. (Kiskőrös, Hungary). Acerola concentrated juice, cloudy, 65 °Bx (specification no: EUR-FO-K 201-014) was obtained from Sió-Eckes Ltd. (Siófok, Hungary). Ascorbic acid and acerola were added to the juices at the dose of 200 mg ascorbic acid per kg apple juice immediately after pressing. It means about 1.4 g acerola juice per kg apple juice. Then the juices were pasteurised at 86 °C for 60 s in the Della Toffola Priamo tubular pasteuriser and aseptically filled to 5-litre bags.



#### 2.2. Analytical methods

Colour of samples was determined by the Konica Minolta CR 400 device based on the CIELab system, and the total colour difference ( $\Delta E^*$ ) was calculated according to Eq. (1).

$$\Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}$$
(1)

where L\*, a\*, and b\* are colour parameters in CIELab.

Browning index (BI) was calculated based on Eq. (2).

$$BI = [100(x - 0.31)]/0.172$$
(2)

where:  $x = (a^* + 1.75L^*)/(5.645L^* + a^* - 0.3012b^*)$ 

Total polyphenol content as well as antioxidant capacity were determined using methods published by Tran et al. (2020). Ascorbic acid content was determined according to Székely et al. (2019).

The flow curves were recorded at 20 °C using a Physica MCR51 (Anton Paar Hungary Ltd.) rotational viscometer with a double-barrel concentric cylinder measuring system (DG 27).

Data were subjected to statistical evaluation using SPSS Statistics v27 software for the analysis of variance (ANOVA). As per the pairwise comparisons between the treatments, they were realised using Tukey's test with a 95% confidence level.

## 3. RESULTS AND DISCUSSION

#### 3.1. Viscosity and pH changes

The pH values before storage were 3.43, 3.40, and 3.42 for control (C), ascorbic acid added (AS), and acerola juice added (ACE) samples, respectively, and did not change significantly during storage (data are now shown). At the beginning, viscosity values ranged from 5.60 to 5.63 mPa.s, meaning no significant difference in viscosity was observed. It is explained by the processing technology (only crushing and pressing). The Herschel–Bulkley model with n = 1 was used to fit the experimental data, and the results showed that the apple juices behaved as Newtonian fluids, regardless of storage time and treatment. A similar result was reported by Salinas et al. (2019), who found that pressed apple juices behaves as Newtonian fluids even with the addition of 10% fibre. Kolniak-Ostek et al. (2014) studied juices from different apple varieties and reported that the viscosity of the varieties ranged from 1.9 to 5.1 mPa.s. During storage, viscosities decreased slightly until month 6 and then stagnated. The values were between 5.41 and 5.47 mPa.s. after 12 months. Dynamic changes of the viscosity of the control sample were stronger than of samples treated with acerola. At the end of storage, the viscosity of the control sample was the lowest (data are not shown). The results suggest that the change in viscosity is rather related to storage time and not to the treatment method.

#### 3.2. Change of colour

Generally, the colour (L<sup>\*</sup>, a<sup>\*</sup>, and b<sup>\*</sup>) of samples did not change in the first two months of storage (Fig. 1), then while the L<sup>\*</sup> values increased from around 30 to 55–60 (Fig. 1A) as well as b<sup>\*</sup> increased from 4–6 to 8–10 (Fig. 1C), the a<sup>\*</sup> decreased from 0.5 to -2 (Fig. 1B) by month 6





Fig. 1. Changes of colour parameters (L<sup>\*</sup>, a<sup>\*</sup>, b<sup>\*</sup>, and  $\Delta E^*$ ) during storage time. C: control; ACE: with accrola added; AS: with ascorbic acid added

of storage. It means that after 6 months the colour had turned light and yellowish, slightly greenish, and then it changed back. This trend was confirmed by the total colour difference  $(\Delta E^*)$  that also peaked (21–29) at month 6 (Fig. 1D). At month 12, the  $\Delta E^*$  values were 4.80, 4.42, and 3.97 for the control, the ascorbic acid-treated, and the acerola-treated sample, respectively. Despite the similarity of  $\Delta E^*$  values at the initial and end stages of storage, in the first two months fresh colour appearance was visually detected, whereas at the end, the juices became less favourable, oxidised and brownish.

The browning index (BI) as well as differences of pairwise  $\Delta E^*$  values were calculated to figure the effects of treatments and storage time on changes of colours during storage (Fig. 2). Both acerola and ascorbic acid helped to prevent the browning process during the processing and storage. BI of the sample treated with acerola was significantly lower than of the control sample. The effects of acerola and ascorbic acid were similar up to month 11–12, when only the sample treated with acerola had low BI value (Fig. 2A). The data from pairwise comparison of  $\Delta E^*$  values also confirm the effects of ascorbic acid and acerola treatments on colour (Fig. 2B). While the biggest differences between the treated samples and the control were detected at month 6, only a minimal difference between the two treatments (AS–ACE) was noticeable at month 12. O'Beirne (1986) studied the colour change of apple juice concentrates at different adjusted pH values and reported that the browning process was mostly due to the Maillard reaction, but in some cases, it was also due to non-enzymatic processes. Özoğlu and Bayındırlı





*Fig. 2.* Changes of browning index and comparison of  $\Delta E^*$  during storage time. C: control; ACE: with acerola added; AS: with ascorbic acid added

(2002) concluded that L-cysteine, cinnamic acid, and ascorbic acid presented the highest browning inhibitory effect.

#### 3.3. Change of total polyphenolic content

In the initial stage, the total polyphenolic contents (TPC) of control, ascorbic acid supplemented, and acerola added samples were 790, 847, and 951 mg L<sup>-1</sup>, respectively (Fig. 3). Vrhosek et al. (2004) reported the TPC of apple varieties ranging from 662 to 211 mg L<sup>-1</sup>, while Kahle et al. (2005) obtained around 154–970 mg L<sup>-1</sup>. Up to month 4, the TPC showed an increasing (40.5–48.5%) trend in all samples, reaching a maximum value. From month 6, the TPCs decreased back to the initial values and followed a stagnant trend thereafter. The lowest TPC (724.16 mg L<sup>-1</sup>) was observed in the case of the control at month 12, while the highest



*Fig.* 3. Changes of total polyphenol content of aseptic filled apple juice during storage. Superscript small case letters indicate significant differences by time per treatments. Presented values are means  $\pm$  SD. (Tukey's test, *P* < 0.05). C: control; ACE: with acerola added; AS: with ascorbic acid added



value  $(1,414 \text{ mg L}^{-1})$  belonged to the sample enriched with ascorbic acid at month 4. Similar results  $(1,000-2,250 \text{ mg L}^{-1})$  were published by Boyer and Liu (2004). Kolniak-Ostek et al. (2014) observed a decrease in TPC during the storage of "Idared" and "Shampion" apples. After 6 months of storage, the decrease amounted to 16-37% in the case of "Idared" juice. The reduction of polyphenols in "Shampion" juice fluctuated between 10 and 21%. In the whole storage period, the TPC of the ascorbic acid treated sample was the highest, while that of the control samples was the lowest. However, this difference gradually decreased towards the end of the storage period. In the case of the sample treated with acerola, 17.8% increase in TPC by the end of the storage was observed. This finding can be explained by transformation of polyphenols from one to other forms such as hydrolysis of glycosyl residues releasing different aglycons. Additionally, since the samples were heat treated at 86 °C that can inactivate many enzymes, the changes were clearly not due to only enzymatic processes.

The statistical evaluation of the TPC of the apple juice samples showed also a highly significant difference between the ascorbic acid-treated juice and the control sample (AS–C, P = 0.000) as well as between the acerola-treated and the control sample (ACE-C, P = 0.040). However, no significant difference was observed in the TPC of the two treatments (ACE–AS, P = 0.151). In conclusion, the change in TPC during the storage process depends not only on the storage time but also on the quality of agent used. Gliszczynska-Swiglo and Tyrakowska (2003) reported that decrease in the TPC under storage ranged between 5 and 21%. Martí et al. (2001) added ascorbic acid at higher concentrations (up to 330 mg L<sup>-1</sup>) and observed no positive benefit from it.

#### 3.4. Change of antioxidant capacity

The antioxidant capacities of ascorbic-treated, acerola-treated, and control samples were 537, 518, and 503  $\mu$ g mL<sup>-1</sup>, respectively, after processing (Fig. 4). Our results are similar to the ones



*Fig.* 4. Changes of antioxidant capacity (FRAP) of aseptically filled apple juice during storage. Presented values are means ± SD. C: control; ACE: with acerola added; AS: with ascorbic acid added

reported by Khanizadeh et al. (2008). They found that the antioxidant capacities of different apples varied between 323 and 1,246  $\mu$ g g<sup>-1</sup>. In case of the control sample and the one treated with ascorbic acid, antioxidant capacities showed an increasing trend until month 6 (from 503 to  $618 \,\mu g \,m L^{-1}$  and from 537 to  $826 \,\mu g \,m L^{-1}$ , respectively), then slightly decreased. The FRAP analysis indicated a slight increase (from 518 to 721  $\mu$ g mL<sup>-1</sup> at month 4 and 705  $\mu$ g mL<sup>-1</sup> at month 6) of the antioxidant capacity during the storage of apple juice with added acerola (ACE). In the control (C) sample, the maximum value was reached at the 6th month, and then it decreased, while in the acerola-treated (ACE) sample, the maximum value was reached at month 4, and then stagnated. All investigated samples showed increase in antioxidant capacities by month 12, with increase of 22.9, 20.1, and 34.6% for the control sample, the ascorbic acid-treated sample, and the acerola-treated sample, respectively. Acerola (ACE) was the best antioxidant agent. Ascorbic acid and other compounds in acerola affect antioxidant capacity (Belwal et al., 2018). These compounds together presumably followed different kinetics resulting in slow but steady increase over the 12 months. The transient increase in antioxidant capacity during storage is also reported by some scientific studies. Del Caro et al. (2004) studied the antioxidant capacity of citrus juices and showed significant increase in the antioxidant capacities of both grapefruit and orange juices.

Other researchers have demonstrated that while the antioxidant capacity increased during fruit storage, the amounts of phenolic compounds and vitamin C decreased. Such increase in the antioxidant capacity of stored fruit juices can be partly due to the formation of non-enzymatic reaction products (Pino-Garcia et al., 2012). According to Brudzynski and Miotto (2011), phenolic compounds take part in melanoid formation and produce melanoidins with antioxidant effects, but also induce changes in the browning index. As reported, Maillard reaction and the formation of melanoidins play a key role in the antibacterial and antioxidant activity of honey (Brudzynski and Miotto 2011). According to Piližota et al. (2012), the increase in antioxidant activity during storage can be attributed to a change in the structure of phenolic compounds, whereby new formations with antioxidant activity are generated because of cyclic compounds being split and rearranged.

The correlation between TPC and FRAP has been extensively studied. Some studies confirmed the existence of a strong correlation between the two metrics. Rentsendavaa (2021) monitored the changes of TPC and FRAP in sea buckthorn leaves during 14 months of storage, and they showed that the two parameters were highly correlated. However, no correlation between the two parameters was found by Ercisli and Orhan (2007) when they did studies on white, red, and black blackberry fruits.

#### 3.5. Change of ascorbic acid content

At the initial stage of storage, the control sample had the lowest ascorbic acid content (125 mg L<sup>-1</sup>), followed by the sample treated with acerola (258 mg L<sup>-1</sup>), whereas the ascorbic acid-enriched sample had the highest (326 mg L<sup>-1</sup>) value (Fig. 5). The lowest ascorbic acid content (98 mg L<sup>-1</sup>) was measured in the case of untreated sample at month 10, while the highest value (333 mg L<sup>-1</sup>) was detected in the ascorbic acid-treated sample at month 4. Our results are consistent with the experimental results presented by Lemmens et al. (2020), who reported the average ascorbic content of 79 Belgian apple cultivars as 129  $\pm$  6 mg kg<sup>-1</sup>, whilst Rasanu et al. (2005) recorded 126.8 mg kg<sup>-1</sup> in apple juice.





*Fig. 5.* Ascorbic acid content of aseptic filled apple juice during storage. Presented values are means  $\pm$  SD. C: control; ACE: with acerola added; AS: with ascorbic acid added

In the case of ascorbic acid treated sample, the ascorbic acid content stagnated in the range of 326 and 333 mg L<sup>-1</sup> for the whole storage period, while minor decrease was obtained in the case of the acerola-treated (258–219 mg L<sup>-1</sup>) and control (125–102 mg L<sup>-1</sup>) samples. Del Caro et al. (2004) analysed citrus juices and found that among the five studied samples only one showed decrease in ascorbic acid content during storage. Analysis of total antioxidant capacity, ascorbic acid content, and total polyphenol content of eight fruit drinks of grapes, oranges, and apricots during storage was done by Cilla et al. (2011). They found that while the antioxidant capacity of all beverages significantly increased (P < 0.05) by the end of storage (16.4 and 12.8%), the ascorbic acid contents remained stable. The statistical analysis of ascorbic acid content showed a highly significant difference between the control and ascorbic acid-treated samples (C-ACE), and no difference between the two treatments (ACE-AS). Storage time affected significantly ascorbic acid content, and only storage for up to 6 months is suggested.

## 4. CONCLUSIONS

Both ascorbic acid and acerola juice are good anti-browning agents in the production of apple juice with pasteurisation and aseptic filling technology. Addition of these agents provides good stability of apple juice during storage for even up to one year. Apple juice treated with acerola had very stable quality and bioactivity parameters, thus acerola can serve as an alternative anti-browning agent for storage of aseptic filled apple juice. Study on optimisation of concentration of acerola is needed for application at an industrial scale.



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