The Effect of Pasteurization Time on Phytochemical Composition and Antioxidant Capacity of Two Apple Cultivars Juices

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Abstract
The objective of this study was to characterize two varieties of apple (Florina and Liberty) grown in the North-West of Romania, with a focus on their volatile compounds, bioactive compounds and their antioxidant capacity. The content of bioactive compounds and antioxidant capacity was also tracked during pasteurization at different times (15, 20 and 25 minutes). Among bioactive compounds, the total phenol content was determined, as well as the total flavonoid and vitamin C content. The antioxidant capacity of fruits and pasteurized juice was evaluated by three different methods (DPPH, FRAP and ABTS). Of the 2 apple cultivars, Florina showed a remarkably higher content of total phenolic compounds (657.97 mg GAE/kg fw), flavonoids (122.07 mg QE/kg fw) and vitamin C (94.62 mg/kg), compared to the Liberty cultivar. Following pasteurization, the vitamin C contents decreased significantly relative to pasteurization time. However, in the case of total phenols content, only insignificant decreases were registered, compared to unpasteurized juice. The content of total flavonoids increased significantly after 15 minutes of pasteurization in apple cultivars juices. The apple varieties investigated are rich in bioactive compounds, and pasteurization treatment does not lead to drastic decreases in these compounds and in the antioxidant capacity of apple juices.

Keywords: antioxidant capacity, apple cultivars, flavonoids, total phenols, pasteurisation

Introduction
Apples are one of the most commonly consumed fruits in the world, and the consumption of apple juice has risen during the last 3 decades from 18 to 26% (Reyes-De-Corcuera et al., 2014).
Generally, apple production is located within the temperate regions of the world. China, the United States of America, Turkey, Poland and Iran are the largest apple producers (Reyes-De-Corcuera et al., 2014). In Romania, the production of apples reached 493,405.00 tons in 2013, in a cultivated area of around 56,942.00 ha (http://faostat3.fao.org/home/E).

Given the increasingly stressful lifestyle prevalent in modern society, apple juice comes as a solution to realize a normal balance between exogenic antioxidants and endogenic free radicals. On the Romanian market, there is a wide variety of apple juices. The beneficial effect of these fruits is well known, and it is due to their chemical composition, particularly to bioactive compounds like polyphenols, flavonoids and phenolic acids.
Numerous studies have revealed the antioxidant potential and beneficial effects of fruits and their juices on the organism (Furukawa et al., 2004; Ko et al., 2005; Åsgård et al., 2007). Reac-
tive oxygen species (ROS), which include hydrogen peroxide, hydroxyl radicals, superoxide anions are generated during human metabolism (Karav and Eksi, 2012). Oxidative stress, defined by the excess presence of ROSs is associated in humans with the deterioration of the DNA, proteins and biological membranes. The antioxidant mechanisms in the human body are insufficient to neutralize the effects generated by the severe oxidations produced by ROSs (Zujko and Witkowska, 2011). The presence of antioxidants, even in small quantities, significantly prevent the oxidation of a substrate (Bof et al., 2012). Antioxidants are represented by a series of enzymatic systems (superoxide dismutase, catalase and glutathione reductase), but also by non-enzymatic systems (vitamins, glutathione, melatonin, polyphenols etc.) (Gardner et al, 2000; Müller et al., 2010). Studies carried out suggest that a fruit-rich diet reduces the risk of certain chronic diseases (heart disease, diabetes, hepatic lesions, cancer and aging) (Ko, 2005). Apples exhibit very strong antioxidant activity (Eberhardt et al., 2000; Boyer and Liu, 2004) due to their high content of active biological compounds (phenols, flavonoids, carotenoids, quercetins, catechines, chlorogenic acid and phloretines). The largest share of total antioxidant activity from apple juice could be represented by chlorogenic acid and phloretines (Miller and Rice-Evans, 1997). The antioxidant capacity of fresh fruit and natural fruit juices is dependent on the cultivar, the harvesting time, storage conditions, applied processing technology etc. (Kevers, 2011). There is rising consumer interest towards natural fruit juices with high antioxidant content, such as vitamins and polyphenols.

Generally, in order to be able to commercialize fruit juices, they must undergo thermal treatment, the most common of which is pasteurization. This thermal treatment is necessary to prevent the development of microorganisms (yeast, molds and bacteria). Also, as a result of pasteurization, the enzyme polyphenol oxidase (which is the enzyme responsible for juice turning brown) becomes inactivated. In addition, thermal treatments can affect certain parameters which are considered quality descriptors of juice (5-hidroximetilfurfural, reductive sugars, ascorbic acid and titrable acidity) (Santini et. al., 2014; Goh, et. al., 2012). Some studies (Lemmens et al., 2009; Lemmens et al., 2010) have shown that modification of cellular membranes can occur during thermal treatments, which result in the release of bioactive compounds from the matrix of the fruit, into the cellular juice (medium), which renders them more bioavailable.

The most common apple cultivars all over the world (China, the United States of America, Turkey, Poland, and Iran) are Red Delicious, Golden Delicious, McIntosh, Rome, Beauty, Granny Smith, Fuji, and Braeburn (Reyes-De-Corcuera et al., 2014). New cultivars are being developed throughout the world, either for better adaptations to new climate conditions, or in order to improve their nutritional qualities.

Within this study, two apple varieties (Florina and Liberty), highly grown and produced in the North-Western of Romania were analyzed for their volatile profile, bioactive compounds (vitamin C, total polyphenols, and total flavonoids), and antioxidant capacity. The content of bioactive compounds and antioxidant capacity of juice obtained from fruits of these two varieties were also tracked, after pasteurization (80°C) for different periods of time.

**Materials and methods**

**Origin of fruit material**

The experiments were carried out using two apple cultivars (Florina and Liberty). Fruits were harvested from the orchard of SC Prototerra SRL company, at maturity consumption, in the year 2012. The orchard is situated on terrain with southern exposure, at 183 m altitude level. The average annual temperature was 8.75°C and total precipitation was 491.7 mm/m2/year. Soil type was classified as argillic. The climatic and geographic area of the land (47° 9’ 55” North, 21° 56’ 37” West) offers good conditions for the cultivation of thermophilic fruit tree species (apricot, peach, almond).

**Fruit quality indices**

Fruit weight (g), diameter (mm) of ten apples from three trees of each cultivar were measured. Soluble solid content (SSC) (%) was determined for the juice of each cultivar using a digital handheld refractometer (DR201-95). The pH of diluted fruit pulp (1:10, w/v) was measured using a pH-meter (WTW GmbH). Titrable acidity (TA) was determined by titrating 10 mL of diluted fruit-juice (1:10, w/v) with 0.1 M NaOH solution. The data were calculated as the average of the three determinations and expressed in g malic acid/100 g fresh weight (fw).
Measurement of bioactive compounds and determination of antioxidant capacity of apple fruits

**Extraction and determination of bioactive compounds from apple fruits**

To determine bioactive compounds (total polyphenols and flavonoids) from apple fruits, 10 grams of flesh from each replicate were homogenized with 100 mL distilled water, and then centrifuged (Hettich® Universal 320 centrifuge) at 15000 rpm for 15 minutes. After centrifugation, the supernatants were used for the analysis.

**Total polyphenols content (TPh)**

Total polyphenols content was determined using the Folin-Ciocalteu method (Singleton et al., 1999). Then the mixture of fruit (100 µL) was incubated with 1700 µL of distilled water, 200 µL of Folin-Ciocalteu reagent (dilution 1:10, v/v) and 1000 µL of 15% Na₂CO₃ solution, and the mixture was incubated at room temperature, in the dark, for 2 hours. The absorbance was measured at 765 nm using a spectrophotometer (Shimadzu 1240 mini UV-Vis). The results were expressed in mg gallic acid equivalents (GAE)/kg fresh weight (fw) and mg GAE/100 mL for apple fruits and juices respectively.

**Total flavonoid content**

The total flavonoids content was determined using the colorimetric method (Kim et al., 2003). Briefly, 1 mL of the apple supernatant was mixed with 4 mL of water and introduced in a volumetric flask (10 mL). Then, 3 mL of NaNO₂ (5%) solution was added, shaken up and left for 5 minutes. Secondly, 0.3 mL of the AlCl₃ (10%) solution was added to the volumetric flask, shaken, and was left to stand for 6 minutes. Finally, 2 mL of the NaOH (1M) solution was added to the volumetric flask, followed by addition of water to the scale, shaken, and left to stand for 15 minutes before determination. The absorbance was recorded at 510 nm using a spectrophotometer (Shimadzu 1240 mini UV-Vis) and the results were expressed in mg quercetin equivalent (QE)/kg fresh weight (fw) and mg QE/100 mL for apple fruits and juices, respectively.

**Vitamin C content in apple fruit**

Several methods are proposed for determining vitamin C in fruits (Bungau et al., 2003) but in this study, the titrimetric method with 2,6-dichloro-phenol-indophenol reagent was applied (Contreras-Calderón et al., 2011). 10 g of homogenized flesh apple was mixed with 20 mL solution of oxalic acid (2%) and diluted to 100 mL, with 2% oxalic acid solution, and filtered. 10 mL of filtered solution was titrated with 0.01% of 2,6-dichloro-phenol-indophenol solution, and the final point was considered when the solution had a pink color. The calibration curve was performed with 0.05% ascorbic acid solution. Results were expressed as mg of ascorbic acid /kg fw and mg ascorbic acid/100 mL for apple fruits and juices respectively.

**Antioxidant capacity assays**

**DPPH radical-scavenging activity**

The DPPH radical-scavenging activity was determined using the method proposed by (Singleton et al., 1999). Briefly, an aliquot of 100 µL sample was mixed with 1.4 mL of DPPH solution (80 µM) and 1 mL of ethanol. The homogenate was shaken vigorously and the decrease in the absorbance of the resulting solution was monitored at 515 nm for 5 min on a spectrophotometer (Shimadzu 1240 mini UV-Vis). The percentage of scavenging effect of different extracts against DPPH radicals, was calculated using the following equation:

\[
\text{DPPH scavenging effect(%) = \left( \frac{A_0 - A_s}{A_0} \right) \times 100 \}
\]

where, \(A_0\) is absorbance of the blank, and \(A_s\) is absorbance of the samples at 515 nm.

The results were expressed as mmols Trolox equivalents (TE)/kg fw, and TE/100 mL for apple fruits and juices respectively, using the calibration curve of Trolox.

**Ferric ion reducing antioxidant power (FRAP)**

The FRAP assay was used to performed antioxidant capacity of fruits. The assay was determined according to the method of Benzie and Strain (1996) with some modifications. The FRAP working solution included the mixtures of 300 mM acetate buffer (50 mL), 10mM TPTZ (2,4,6-tripyridyl-s-triazine) (5 mL) and 20 mM FeCl₃ · 6H₂O solution (5 mL). The FRAP working solution was freshly prepared. The apple extracts (100 µL) were allowed to react with 500 µL FRAP solution and 2 mL distilled water, for 1 hour, in dark conditions. The formation of the coloured product (ferrous tripyridyltriazine complex) at low pH was monitored by measuring the absorbance at 595 nm (Shimadzu 1240 mini UV-Vis). Results were expressed in mmols Trolox equivalents (TE)/kg fw and TE/100 mL for apple fruits and juices, respectively.
**TEAC assay**

Trolox Equivalents Antioxidant Capacity was determined using the method proposed by Arnao et al. (2011). Shortly, the ABTS⁺ cation radical was produced by reacting the ABTS⁺ (2,2’-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) solution (7 mM) with potassium persulfate (2.45 mM) solution, keeping the mixture in dark at room temperature for 16 h. The ABTS cation radical stock solution was diluted in order to obtain an absorbance of 0.70 ± 0.02 at 734 nm. After addition of 25 µl apple extract to 2.5 mL of diluted ABTS+, the mixture was mixed vigorously (using vortex) for 30 seconds and the interaction between the antioxidants and the ABTS⁺ was monitored spectrophotometrically at 734 nm, at 1 minute. The calibration curve was linear for the range of Trolox concentrations between 0.125 and 2 mmol/l, and the results were expressed in mmols Trolox equivalents (TE)/kg fw and TE/100 mL for apple fruits and juices, respectively.

**Analysis of volatile compounds of apple cultivars**

**Extraction of volatile compounds from fruits**

The extraction of volatile compounds from apple variety fruit samples was performed using in-tube extraction technique (ITEX). Around 100 g of sample were homogenized and then a 5g aliquot was placed in a 20 mL sealed headspace vial together with 0.6 g NaCl and incubated for 30 minutes at 60°C under continuous agitation. Sodium chloride was added to inhibit enzymatic reactions in order to protect the samples from oxidation. Also, sodium chloride has a salting out effect, promoting the efficiency of extraction process (Aprea et al., 2012). The volatile compounds from the headspace phase of the vial were trapped using an ITEX fibre (ITEX-2 TrapTXTA, (G23)-Siliconert 2000, Tenax TA 80/100 mesh, Switzerland) and subsequently thermally desorbed into the GC injector (250°C).

**GC-MS analysis**

The separation of the volatile compounds was achieved on a Shimadzu GC-MS QP-2010 (Shimadzu Scientific Instruments, Kyoto, Japan) model gas chromatograph-mass spectrometer equipped with a CombiPALOC-5000 autosampler (CTC Analytics, Zwingen, Switzerland). A ZB - 5ms column of 50m x 0.32 mm i.d. and 0.25 µm film thickness was used for the separation. The program for the column oven temperature was: 40°C (3 min) to 150°C at 5°C/min (hold for 2 min) and then to 220°C with 10°C/min (10 min). The carrier gas was helium 1.36 mL/min, the ion source and interface temperatures were set at 250°C and the MS detector was used in electron impact (EI) mode in a scan range of 40-350 m/z. The identification of volatile compounds was carried out by comparing the obtained mass spectra with NIST27 and NIST147 library information and verified by comparison with retention indices drawn from www.pherobase.com or www.flavornet.org (for columns with a similar stationary phase to the ZB-5ms column). All peaks found in at least one of the two total ion chromatograms (TIC) were taken into account when calculating the total area of peaks (100%) and the relative areas of the volatile compounds.

**The pasteurization treatment of apple juices**

The apple fruits were harvested at maturity consumption, washed and pressed using a commercial juice extractor. The raw apple juice (obtained from 20 apples) was placed in Erlenmeyer flask, covered with aluminum foil and heated in a water bath at 80°C for different time periods (15 (T15), 20 (T20) and 25 (T25) minutes), then cooled and centrifuged at 5000 rpm for 20 minutes at 4°C. The sample of the juice has not undergone pasteurization is denoted by T0. Supernatants were filled into plastic bottles and stored at -20°C before the analysis of the content of bioactive compounds (total polyphenols, flavonoids and vitamin C) and antioxidant capacity (by DPPH, FRAP and TEAC assay) described above.

**Statistical analysis**

For every apple cultivar, three replications were done (n=3), each repetition included 10 apples from 3 trees. The results were generated as mean and standard deviation (SD). The results are generated within the t test analysis (Unpaired t test) (P < 0.05), using GraphPad Prism.

**Results and discussion**

**The tested fruit quality parameters**

The fruit quality parameters tested were presented in Table1. Weight, diameter, SSC and titrable acidity showed considerable variations, while the pH values of fruits did not show statistical differences between the apple cultivars. The apple cultivars have a pH higher than 4.0 at maturity, and are considered non-acid (Abidi et al., 2011). The ripening indexes (RI=SSC/TA) were
similarly between the two cultivars, representing an important organoleptic quality point of the mature fruit.

Both cultivars showed high SSC values (18.09 and 16.48°Brix), with similar results obtained with other apple cultivars grown either in Romania (Campeanu et al., 2009) or in the eastern part of the Czech Republic (Rop et al., 2012).

**The content of bioactive compounds and antioxidant capacity of apple cultivars**

The bioactive compounds content (total phenols, flavonoids, and total vitamin C) from the two apple cultivars are presented in Table 2. Among apple cultivar tested, Florina variety was the richest in bioactive compounds.

Antioxidant capacity determined by three methods with different principles (DPPH, FRAP and ABTS) of two apple cultivars is presented in Table 3. The Florina apple cultivar stands out as having the highest antioxidant capacity, these results being directly correlated to its high bioactive compound content.

**Table 1.** Origin, fruit weight (g) and diameter (mm), soluble solid content (SSC, °Brix), pH, titrable acidity (TA, % malic acid) of two apple cultivars

<table>
<thead>
<tr>
<th>CULTIVAR</th>
<th>Origin</th>
<th>Weight (g)</th>
<th>Diameter (mm)</th>
<th>SSC (°Brix)</th>
<th>pH</th>
<th>TA (% malic acid)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Florina</td>
<td>RO</td>
<td>161.10 ± 9.99 a</td>
<td>60.60 ± 3.17 a</td>
<td>18.09 ± 0.12 a</td>
<td>4.44 ± 0.02 a</td>
<td>0.29 ± 0.03 a</td>
</tr>
<tr>
<td>Liberty</td>
<td>RO</td>
<td>180.00 ± 10.33 b</td>
<td>46.70 ± 1.89 b</td>
<td>16.48 ± 0.04 b</td>
<td>4.48 ± 0.08 a</td>
<td>0.22 ± 0.01 b</td>
</tr>
</tbody>
</table>

Data are means of three replicates with standard deviation (Mean ± SD, n=3). Values in the same column with different letters present statistical significance (P < 0.05).

**Table 2.** The content of phenols and total flavonoids, vitamin C in 2 apple cultivars (Florina and Liberty)

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>TPh (mg GAE/kg fw)</th>
<th>Flavonoid content (mg QE/kg fw)</th>
<th>Vitamin C (mg vitamin C/kg fw)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Florina</td>
<td>657.97 ± 7.17 a</td>
<td>122.07 ± 1.18 a</td>
<td>94.62 ± 1.05 c</td>
</tr>
<tr>
<td>Liberty</td>
<td>302.07 ± 8.95 b</td>
<td>69.33 ± 0.58 b</td>
<td>53.84 ± 1.38 b</td>
</tr>
</tbody>
</table>

Data are means of three replicates with standard deviation (Mean ± SD, n=3). Values in the same column with different letters present statistical significance (P < 0.05).

**Table 3.** The antioxidant capacity of 2 apple cultivars (Florina and Liberty) expressed as mmol TE/kg fw

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>DPPH</th>
<th>FRAP</th>
<th>ABTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Florina</td>
<td>400.37 ± 1.46 a</td>
<td>6.54 ± 0.12 a</td>
<td>0.37 ± 0.036 a</td>
</tr>
<tr>
<td>Liberty</td>
<td>389.73 ± 1.41 a</td>
<td>6.09 ± 0.17 a</td>
<td>0.33 ± 0.048 a</td>
</tr>
</tbody>
</table>

Data are means of three replicates with standard deviation (Mean ± SD, n=3). Values in the same column with different letters present statistical significance (P < 0.05).
structure of phenols rather than the amount of phenols.

Campeanu et al. (2009) showed that the content of vitamin C of apple-tree cultivars grown in Romania ranged between 7.19 and 7.89 mg/100 g fresh apple. Delicious, Mutzu and Jonathan cultivars showed the highest values.

Lachman et al. (2006) compared the content of total phenols and antioxidant capacity of fifteen apple cultivars growing on Experimental Station of Czech University, harvested in 2004. Total phenols content ranged between 760 and 1343 mg/kg of fresh weight.

The mineral substances, vitamin C, malic and citric acids in fifteen apple cultivars grown in Vâlcea Research Station, Romania, were analysed (Nour et al., 2010). One of the apple cultivars was Florina, which recorded the highest values of dry matter. Also, this apple cultivar contains the highest levels of potassium (160.85 mg/100 g).

**Volatile compounds present in apple cultivars**

Flavor is one of the most important features in consumers’ choice. Fruits’ flavor is determined by both taste and aroma. The organic acids and sugars are responsible for the fruits’ taste, whereas the volatile compounds are involved in the development of aroma (Espino-Diaz et al., 2016).

According to the review paper of El Hadi et al., (2013) more than 300 of volatile compounds have been reported as constituents of fresh apple, depending on the ripening stage and cultivar, but it is also affected by other factors before, during

| Table 4. Mean relative concentrations (expressed as % from total peak areas) of volatile compounds detected in apple cultivars samples analyzed by ITEX/GC-MS |
|----------------------------------|-----------------|-----------------|-----------------|
| **Volatile Compound** | **Florina** | **Liberty** | **Odour descriptors** |
| **Alcohols** | | | |
| 1-Butanol | 9.68±0.05 | 0.47±0.01 | wine |
| 2-Methyl-1-butanol | 46.47±0.45 | 0.00±0.00 | malt |
| 1-Pentanol | 0.41±0.02 | 0.00±0.00 | Sweet, balsamic, fruity |
| 4-Hexen-1-ol, (Z) | 1.14±0.11 | 1.98±0.80 | Green, herbal, fresh |
| 1-Hexanol | 18.58±0.28 | 7.73±0.09 | resin, flower, green |
| **Aldehydes** | | | |
| 2-methyl-butanal | 0.00±0.00 | 0.23±0.02 | Green, malty, butytery, cocoa, almond |
| Pentanal | 1.02±0.07 | 3.04±0.06 | Almond, malt, pungent |
| Hexanal | 13.01±0.44 | 65.06±0.12 | Green, grass |
| (E)-2-Hexenal | 1.82±0.08 | 20.61±0.13 | apple, green |
| Heptanal | 0.00±0.00 | 0.07±0.02 | citrus |
| Benzaldehyde | 0.22±0.06 | 0.17±0.08 | Almond, burnt sugar |
| **Ketones** | | | |
| 6-methyl-5-Hepten-2-one | 0.00±0.00 | 0.15±0.03 | Woody, Blackcurrant, Boiled fruit |
| Acetophenone | 0.25±0.04 | 0.13±0.05 | Sweet, flower, almond |
| **Esters** | | | |
| 2-Methylbutyl acetate | 1.22±0.06 | 0.00±0.00 | Fruit, sweet |
| Butyl acetate | 0.00±0.00 | 0.14±0.06 | pear |
| Butyl 2-methylbutanoate | 0.17±0.08 | 0.00±0.06 | Fresh, Sweet, Fruity |
| 2-methyl-, 2-methylbutyl butanoate | 0.51±0.10 | 0.00±0.00 | Fruity, apple, rum, berry |
| Hexyl butanoate | 0.20±0.09 | 0.25±0.08 | Apple peel, fruity |
| Hexyl 2-methylbutanoate | 5.36±0.18 | 0.00±0.00 | Fruity, apple, strawberry |
and after harvesting. Among them, only some are significantly contributing to the aroma, including esters, alcohols, aldehydes, ketones. The volatile compounds determined in the two apple cultivars taken under study are from the above-mentioned classes. Comparing the obtained results, it can be noticed that in the Liberty cultivar the aldehydes (hexanal and (E)-2-hexenal) are the major compounds, contributing to the specific green, apple aroma, although the content of aldehydes usually predominate in the immature apples (El Hadi et al., 2013). During ripening, the content in aldehydes decreases and the level of alcohols and esters increases. Nevertheless, when the determination of volatile compounds was performed, as in this case, in homogenized sample, high concentration of aldehydes may be produced. Similar results were observed by Aprea et al. (2012) when they determined the volatile profile of 18 apple cultivars by SPME/GC-MS using homogenized samples or by Both et al. (2014) when determining the volatile composition by HS/GC-MS of Royal Gala apple under different storage conditions. Moreover, (E)-2-Hexenal known as “leaf aldehyde” is a strong contributor to the herbaceous notes of the apple and together with esters it can contribute to the o “moscato grape” aroma (typical odour of a green aromatic grape) (Aprea et al., 2012).

Florina cultivar was characterized by a higher content of alcohols, especially 2-methyl-butanol and 1-hexanol, which impart green, malty flavor. Also, it has a higher amount of esters, hexyl 2-methylbutanoate and 2-methylbutyl acetate being the major ones, contributing to the specific fruity, apple aroma. Although the esters are of major importance, the perception of the aroma is influenced by the relative concentration of the compound but also by other factors such as the specific odor threshold concentration, interactions with other volatiles. The “sweet” aroma is associated with an increase in 2-methylbutyl acetate but it may be influenced by the presence of hexyl acetate and butanol (Both et al., 2014).

The effect of pasteurization on bioactive compounds and antioxidant capacity

Bioactive compounds (total phenols, TPh, total flavonoid and vitamin C) determined at different pasteurization times (T0, T15, T20 and T25, without pasteurization treatment, 15 20, 25 minutes, respectively) are shown in Figure 1 a,b,c. Figure 1, a, shows the changes in total phenols during pasteurisation of apple juices. Generally, the content in total phenols at the end of pasteurisation decreased from 77.95 to 75.54 mg GAE/100 mL and 74.05 to 72.34 mg GAE/100 mL for Florina and Liberty juices respectively. In figure 1, b, the changes in the content of total flavonoids during the pasteurisation apple juices are showed. In both apple juices, the content of flavonoids significantly increased after 15 minutes of pasteurisation treatment. Figure 1, c shows that ascorbic acid significantly decreased during the pasteurisation process, with the increase of storage time. The ascorbic acid in pasteurized juices at 25 minutes was decreased by 73.53% and 76.35% for Florina and Liberty juices respectively, compared to the apple juices without thermal treatment.

Antioxidant capacity determined at different pasteurization time is show in Figure 2. During the pasteurisation, the antioxidant capacity was not affected by time.

Conclusion

In this work it is shown that Florina and Liberty cultivars growing in the N-W part of Romania are rich in phenolic compounds with antioxidant capacity. The pasteurisation process at 80°C, for more than 15 minutes of apple juices increased the level of total phenols and flavonoid contents. Among the apple cultivars, Florina recorded the highest content of total phenols (657.97 mg GAE/kg fw) compared with Liberty cultivar (302.07 mg GAE/kg fw). Also, the Florina apple cultivar showed the highest antioxidant capacity due to the high content of bioactive compounds. From the point of view of volatile compounds, the Florina cultivar was characterized by a high level of alcohols, the main ones being 1-hexanol, while in the Liberty cultivar the aldehydes are the major compounds, namely hexanal and (E)-2-hexanal. It is important to establish the best conditions in order to obtain the apple juices with high level of bioactive compounds with antioxidant capacity.

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**Figure 1.** The effect of storage time (10, 20 and 25 minutes) on bioactive compounds: a) total phenols, b) total flavonoids and c) vitamin C, during pasteurisation of juices from 2 apple cultivars (Florina and Liberty). Data are means of three replicates with standard deviation (n=3).
Figure 2. The effect of storage time (10, 20 and 25 minutes) on antioxidant capacity during pasteurisation of juices from 2 apple cultivars (Florina and Liberty). Data are means of three replicates with standard deviation (n=3).
References


