Assessment of Polyphenols, Chlorophylls, and Carotenoids during Developmental Phases of Three Apple Varieties

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ELENA ANDRUTA MUREŞAN1, SEVASTIŢA MUSTE1,*, CRINA CARMEN MUREŞAN1, ELENA MUDURA1, ADRIANA PĂUCEAN1, LAURA STAN2, ROMINA ALINA VLAIC1, CONSTANTIN GHEORGHE CERBU3, VLAD MUREŞAN1

1Food Engineering Department, Faculty of Food Science and Technology, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania
2Food Science Department, Faculty of Food Science and Technology, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania
3Department of Infectious Diseases, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania

* Corresponding author e-mail: sevastiita.muste@usamvcluj.ro

Abstract

Variations of total polyphenol content, antioxidant capacity, chlorophyll pigments and carotenoid pigments during fruit development of Golden Delicious, Starkrimson, and Ionathan apple varieties were investigated at 7, 15, 35, 65, 107 and 144 days after full bloom (DAFB). For all varieties, the highest total polyphenol content was recorded for the apples harvested at 7 DAFB, decreasing while the fruit was growing. Antioxidant capacity reaches the maximum level at 15 DAFB and then declines until the apples reached technological maturity. Total chlorophyll, chlorophyll a and b recorded high values at 65 DAFB, when both the temperature and the light intensity are high (July). After this harvesting time, chlorophyll content decreased. The concentration of carotenoids accumulated in apple peel varied, however following a downward trend during fruit growth, regardless of the variety or the crown position. These results provide a preliminary foundation for the further use of apples from physiological falls in different foods and pharmaceuticals.

Keywords: Ionathan, Starkimson, Golden Delicious, spectrophotometry

1. Introduction

Clinical and epidemiological studies have established that a high consumption of fruits and vegetables may reduce chronic and degenerative diseases that are the leading causes of death in developed countries (HENRÍQUEZ & al. [1]). Researchers showed interest in the study of beneficial effects of pigments on human health as a response to food industry’s increasing demand for their use as food supplements and as food additives (LEE & al. [2]). Apples are a significant part of the human diet (WOLF & al. [3]) as a major source of antioxidants, mainly phenolic compounds (BOYER & al.[4]). The content of polyphenols and pigments found in apples is important because it contributes to the sensory quality of fresh fruits and products made from apples (KHANIZADEH & al. [5]). Several studies have reported that the apple peel contains higher levels of phenolic compounds than the ones found in apple pulp (KHANIZADEH & al. [5], WOLF & al. [6], CERBU & al. [7]). Polyphenol content and antioxidant capacity of apple fruits are mostly influenced by genetical factors such as the apple variety, the harvest quality or the fruit colour (red, yellow, green or bicolored shell), but also by apple pulp which can be more or less colourful and bright, by the fruit’s tissue and the variable content of sugars and acids (LEE & al. [8] or VRHOVSEK & al. [9] or DROGOUDI & al. [10]). Moreover, the polyphenol content and antioxidant capacity can thus be influenced by environmental factors and technological factors such as post-harvest
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factors, the harvest date, the fruit exposure in the crown of the tree (i.e., sunny or shady places) but also by its processing and storage (McGHIE & al. [11]). On the other hand, it is well known that genetics plays a major role in controlling the composition of polyphenols in apples and other fruits (KHANIZADEH & al.[12]). Chlorophyll is the most common pigment found in plants. The degradation of chlorophyll begins with fruit maturation due to the action of aging-related hormones (ethylene) (JACOB-WILK & al. [13]). The mechanism of chlorophyll disappearance is complex and still not fully understood because of the rapidity and the formation of catabolic color (VICENTINI & al.[14]). MUSSINA & al. [15] showed in Granny Smith variety a steady decline of chlorophyll during fruit development. The same pattern of chlorophyll decline was observed by GREER [16] for the Royal Gala and Braeburn variety. REAY & al. [17] showed that the total chlorophyll in Gala apples peaked at about 70-110 days after full bloom in two successive seasons, after which it decreased in the ripening fruit. The apple fruit carotenoid content varies depending on variety, stage of fruit development and environmental factors (temperature and light). A paramount aspect linked to apples which are due to be consumed is that the majority of carotenoid pigments are concentrated in the skin, contributing to the colour of the fruit, thus making it attractive; however, apple pulp carotenoid concentration is low. There are some apples varieties that are not intended for the consumers market, such as “Aotea” apples that have high concentrations of carotenoids in the pulp (AMPOMAH-DWAMENA & al. [18]).

The aim of the current study was to assess the total polyphenol, antioxidant capacity, chlorophyll and carotenoid pigments during fruit development phases for three apple varieties: Ionathan, Starkrimson and Golden Delicious. The results will help to select the best time to use the apples from physiological falls in different foods and pharmaceuticals.

2. Materials and Methods

Plant materials

A number of 80 apples from each studied cultivar (i.e., Starkrimson, Ionathan, Golden Delicious) were harvested from the same orchard’s position of Reghin region – Romania, at 7, 15 and 35 days after full bloom (DAFB) (each sample weighted between 50 and 1200 g depending on the apples size at the harvesting time). Following the same protocol, 40 apples from each studied cultivar were harvested at 65, 107 and 144 DAFB (each sample weighted between 1700 and 4000 g depending on the apple size at the harvesting time). The same samples were harvested from inside and periphery of the crown, respectively. Samples were preserved at -16°C until analysis.

Folin-Ciocalteu Method for Total Polyphenols Assessment

The total polyphenol content of the whole apple was determined according to the method described by CERBU & al. [7]. Absorbance was read at 750 nm with a Shimadzu UV-VIS 1700 spectrophotometer. The standard curve was carried out using concentrations of 0, 0.25, 0.50, 0.75, 1 mg/ml of gallic acid. Total polyphenol content in the whole apple was expressed in gallic acid equivalents, mg of GAE/100 g FW.

DPPH Method for Antioxidant Capacity Assessment

The antioxidant capacity was determined by assessing free radical scavenging effect over 1,1-diphenyl 1-2-picrylhydrazyl (DPPH) radical. This determination is based on the method proposed by ODRIOZOLA-SERRANO & al. [19]. An amount of 10 µl of the methanol extract from the analyzed samples, obtained according to the method described by BUNEA & al. [20], was mixed with 3.9 ml of DPPH (0.025g /l) and 90 µl of distilled water. The mixture was stirred and maintained properly in the dark for 30 min. The absorbance of the samples was measured at 515 nm (Shimadzu 1700 UV-VIS) against a methanol blank. Results were expressed as percent over standard DPPH absorbance.
\[
RSA [\%] = \frac{A_{\text{DPPH}} - A_p}{A_{\text{DPPH}}} \times 100
\]

RSA [%] – Radical Scavenging Activity; \(A_{\text{DPPH}}\) – DPPH absorbance; \(A_p\) – sample absorbance.

**Chlorophylls Assessment**

Apple peel chlorophyll extraction was performed according to the method described by LANCASTER & al. [21] with some modifications. Absorbance was measured at 645 and 663 nm. Chlorophyll content was calculated using ARNON [22] equations:

\[
\begin{align*}
Ca (\text{mg g}^{-1}) &= [(12.7 \times A_{663}) - (2.6 \times A_{645})] \times \text{ml acetone / mg sample} \\
Cb (\text{mg g}^{-1}) &= [(22.9 \times A_{645}) - (4.68 \times A_{663})] \times \text{ml acetone / mg sample} \\
CT &= Ca + Cb.
\end{align*}
\]

where: \(Ca\) – chlorophyll a; \(Cb\) – chlorophyll b; \(A_{663}\) – absorbance at 663 nm; \(A_{645}\) – absorbance at 645 nm; \(CT\) – total chlorophyll.

**Carotenoids Assessment**

Extraction was performed using the procedure described by BUNEA & al. [23] with some modifications. Apple skins were freeze-dried by using liquid N\(_2\). Carotenoids were extracted from the freeze-dried apple skins using as solvents: methanol, ethyl acetate, petroleum ether (1:1:1, v/v/v). Successive extractions were performed. The extracts were combined, filtered and washed with distilled water, diethyl ether and a saturated solution of NaOH. The ethereal phase was recovered and subjected to rotary evaporation at 35°C. The remaining extract was dissolved in a known volume of methanol and stored at -18°C until it was subjected to analysis.

Estimation of carotenoids was spectrophotometrically determined using Shimadzu UV-VIS 1700 set at 450 nm. Estimation of carotenoids content was calculated with the following formula:

\[
X (\text{mg of carotenoids}) = \frac{A \times V \times 10^3}{2500 \times V \times 100} \times 100
\]

\(A\) = absoption at \(\lambda_{\text{max}} = 450\) nm; \(V\) = sample volume (ml); 2500 = the molar absorption coefficient (E1%); \(l = 1\) cm – optical path length (BRITTON & al.[24]).

3. Results and Discussions

**Evolution of the total polyphenols content in apple fruit during development**

It was found that the highest total phenolic content was recorded seven DAFB. Apples total polyphenols content (TPC) was 1606.48 mg EAG/100g for Golden Delicious, 1192.58 mg EAG/100 g for Ionathan and 1094.47 mg EAG/100g for Starkrimson variety. TPC of analyzed apples, statistically significant (p<0.05) decreased while fruit growing and was not influenced by the fruit position in the tree crown (Figure 1). At the end of the experiment (144 DAFB), the polyphenol content in apple fruits decreased to 1.35 mg EAG/100g in Golden Delicious variety, 21.80 mg EAG/100g in Ionathan and 57.04 mg EAG/100g in Starkrimson variety.

At 65 DAFB there is a massive decrease in polyphenol content due to increased metabolic activity occurring in secondary metabolites biosynthesis and cell expansion phase. For Fuji variety, ZHENG & al. [25] observed a similar decrease of total phenolic compounds starting at early growth (25 DAFB). When comparing the three varieties studied, differences were found in TPC (Figure 1). These differences are due the distribution of phenolic compounds in fruit tissue, depending on the variety (ALONSO [26]). Moreover, previous research had shown that the position of the fruit in the tree crown, the tree’s position in the orchard, the microclimate and the cultivar play a major role in the polyphenols content of apple fruits (VOLZ & al. [27]). As discussed, our results confirmed that the variations in the polyphenol content are caused by fruit maturity, harvesting time, variety and fruit position in the tree crown (Figure 1).
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Antioxidant capacity of apple fruit during development

For each variety, regardless of position in the crown, the highest antioxidant capacity was recorded at 15 DAFB (Figure 2). Golden Delicious records statistically significant differences (p<0.05) starting 15 DAFB when antioxidant capacity reaches the highest level. During the growth and development of the fruit, antioxidant capacity decreased significantly (p<0.05) by the end of the experiment regardless of the position of the fruit in the tree crown. The same trend can be seen for the Ionathan and Starkrimson varieties (Figure 2). ZHENG & al. [25] reported a decline of the antioxidant capacity in the case of Fuji during the growth and the development of fruits similar to the decrease observed in our experiment.

Evolution of carotenoid content during fruit development

The concentration of carotenoids accumulated in the apple peel generally decreased depending on the time of harvest, regardless of the variety or the crown position (Figure 3). Comparing the three studied varieties by the crown position, Starkrimson variety accumulated the highest carotenoid content in both the apples harvested from inside of the crown and those harvested from the periphery of the crown.

Evolution of chlorophyll content during fruit growth

Ionathan variety registered statistically significant differences (p<0.05) starting 15 DAFB, when the amounts of CT, Ca and Cb were lower than the ones at 7 DAFB. These differences are observed both in the apples harvested from the periphery of the crown and from those harvested from inside of the crown. At 65 DAFB, when the temperature and the light intensity are high (July) there is a statistically significant increase in the CT, Ca and Cb content. On the other hand at 107 DAFB, CT, Ca and Cb decreased significantly until the end of the study. The same chlorophyll content pattern was observed for both Starkrimson and Golden Delicious varieties (Figure 4, 5, 6). Similar amounts of CT, Ca and Cb were reported by SUPARNA & al. [28] for Pink Lady variety, which accumulated, for fruits harvested at 60 DAFB, 200 µg/g CT, 120 µg /g Ca and 55 µg/g Cb. Regarding the content of Cb, for Ionathan variety values between 75.65 and 20.51 µg/g for apples harvested from inside of the crown and between 75.68 and 26.14 µg / g for the apples harvested from the periphery of the crown were recorded. Golden Delicious registered Cb values between 78.55 and 38.09 µg/g for the apples harvested from inside of the crown and between 93.67 and 51.52 µg / g for the apples harvested from the periphery of the crown. Starkrimson variety recorded Cb values between 103.66 and 52.36 µg/g for the apples harvested from inside of the crown and between 99.27 and 27.84 µg / g for the apples harvested from the periphery of the crown. At the end of apple fruit development statistically significant differences (p<0.05) were noticed; the amount of chlorophyll was influenced by the position in the crown and fruit variety. There is a range of important factors that may influence the absorption of chlorophyll in the apple peel: light, temperature, variety, fruit development, fruit position in the tree crown.
Effect of harvesting time, variety and position in the tree crown on the total polyphenols, antioxidant capacity, total chlorophyll, and carotenoids

For advanced interpretation of the obtained results, it was considered appropriate to calculate the proportion of the total variability of main factors effects and their interactions. This proportion was expressed by eta squared ($\eta^2$), being currently used as the estimate of effect size (MUREŞAN [29]).

Classical Eta squared value was calculated as follows:

$$\eta^2 = \frac{SS_{factor}}{SS_{total}}$$

where: $SS_{factor}$ was the variation attributed to the factor, and $SS_{total}$ represented total variance (PIERCE & al. [30]).

Three-factorial analysis of variance was performed to assess the influence of harvesting time, variety and fruit position in the tree crown over the total polyphenol content, antioxidant capacity, total chlorophyll and carotenoid. In the case total polyphenol main effects: Time, Variety and interaction between Time, Variety and Position indicated highly significant differences (***; $p<0.0001$), while position factor was statistically significant (*; $p<0.05$). Consequently, one is interested in debating the statistically significant interaction, in this case, the second degree interaction: Time*Variety*Position. The main effects: Harvesting time, Variety and Position in the crown explained 93.38%, 1.94%, 0.01% of the total variability of total polyphenols, indicating the importance of harvesting period (Table 1). Also, this statistical analysis showed the low influence of the variety (explains 1.94% of variance) and low importance of position (explaining 0.01% of variance). The greatest importance interaction was represented by Variety*Harvesting time that recorded 4.40% of the total polyphenols variability, while the remaining interactions gathered together less than 0.3%.

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F-value</th>
<th>P-value</th>
<th>Eta Squared</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variety</td>
<td>344282.37</td>
<td>172141.18</td>
<td>1133.90</td>
<td>3.086E-33***</td>
<td>0.0194</td>
</tr>
<tr>
<td>Position</td>
<td>1046.16</td>
<td>1046.16</td>
<td>6.891</td>
<td>0.013*</td>
<td>0.0001</td>
</tr>
<tr>
<td>Time</td>
<td>16584166.22</td>
<td>3316833.24</td>
<td>21848.163</td>
<td>1.326E-61***</td>
<td>0.9338</td>
</tr>
<tr>
<td>Int. Variety*Position</td>
<td>773.67</td>
<td>386.83</td>
<td>2.548</td>
<td>0.092 ns</td>
<td>0.0000</td>
</tr>
<tr>
<td>Int. Variety*Time</td>
<td>781329.71</td>
<td>78132.97</td>
<td>514.666</td>
<td>1.010E-35***</td>
<td>0.0440</td>
</tr>
<tr>
<td>Int. Position*Time</td>
<td>24873.08</td>
<td>4974.61</td>
<td>32.768</td>
<td>1.915E-12***</td>
<td>0.0014</td>
</tr>
<tr>
<td>Int.Variety<em>Position</em>Time</td>
<td>17966.01</td>
<td>1796.60</td>
<td>11.834</td>
<td>1.126E-08***</td>
<td>0.0010</td>
</tr>
<tr>
<td>Error</td>
<td>5465.26</td>
<td>151.81</td>
<td></td>
<td></td>
<td>0.0003</td>
</tr>
<tr>
<td>Total</td>
<td>17759902.51</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* significant ($p<0.05$); ** very significant ($p<0.01$); *** highly significant ($p<0.001$); ns - no statistical significance ($p>0.05$).
Three-factorial analysis of variance for the antioxidant capacity of apple samples under study, including the experimental factors: Harvesting time, Variety and Position in the crown, and the interactions between these, indicates highly significant significations (***, p<0.0001) for all experimental factors as well as their interactions. Relative sizes of the effects of the studied factors and their interactions over the antioxidant capacity are shown in Table 2. The main effects: Harvesting time, Variety, Position in the crown, explained 80.59%, 7.17% respectively 0.63% of the total variability in antioxidant capacity indicating the great importance of Harvesting time. The statistical analysis also showed the influence of the Variety (7.17% of variance explained) and the insignificance of Position (explaining 0.63% of variance).

The most important interaction is Variety*Harvesting time which recorded 10.31% from the variability of antioxidant capacity, while the remaining interactions gather together less than 2%.

**Table 2. Analysis of variance – apples antioxidant capacity**

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F-value</th>
<th>P-value</th>
<th>Eta Squared</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variety</td>
<td>260.39</td>
<td>130.19</td>
<td>4203.30</td>
<td>2.17E-43***</td>
<td>0.0717</td>
</tr>
<tr>
<td>Position</td>
<td>22.83</td>
<td>22.83</td>
<td>737.315</td>
<td>1.424E-25***</td>
<td>0.0063</td>
</tr>
<tr>
<td>Time</td>
<td>2927.30</td>
<td>585.46</td>
<td>18901.23</td>
<td>1.798E-60***</td>
<td>0.8059</td>
</tr>
<tr>
<td>Int. Variety*Position</td>
<td>13.09</td>
<td>6.54</td>
<td>211.416</td>
<td>1.270E-20***</td>
<td>0.0036</td>
</tr>
<tr>
<td>Int. Variety*Time</td>
<td>374.53</td>
<td>37.45</td>
<td>1209.171</td>
<td>2.316E-42***</td>
<td>0.1031</td>
</tr>
<tr>
<td>Int. Position*Time</td>
<td>4.99</td>
<td>0.99</td>
<td>32.222</td>
<td>2.443E-12***</td>
<td>0.0014</td>
</tr>
<tr>
<td>Int. Variety<em>Position</em>Time</td>
<td>28.23</td>
<td>2.82</td>
<td>91.167</td>
<td>1.714E-22***</td>
<td>0.0078</td>
</tr>
<tr>
<td>Error</td>
<td>1.11</td>
<td>0.03</td>
<td></td>
<td>0.0003</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>3632.51</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* significant (p<0.05); ** very significant (p<0.01); *** highly significant (p<0.001); ns - no statistical significance (p>0.05).

Analysis of variance for total chlorophyll values showed highly significant differences (***, p<0.0001) for the Harvesting time, Variety and very significant differences (**, p<0.01) for the Position in the crown. Second order interaction (Harvesting time*Variety*Position) was highly significant (***, p<0.0001) which explained why chlorophyll value depends on a combination between Harvesting time, Variety, and Position in the crown.

Relative sizes of the studied factors effects and their interactions regarding total chlorophyll content are shown in Table 3. Main effects, Harvesting time, Variety and Position in the crown are explaining 85.26%, 5.55% and 0.02% of total chlorophyll variability indicating the importance of Harvesting time. The most important interaction was the Variety*Harvesting time interaction, recording 5.30% of total chlorophyll variability while remaining interactions gathers together about 2%.

**Table 3. Analysis of variance – apples total chlorophyll content**

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F-value</th>
<th>P-value</th>
<th>Eta Squared</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variety</td>
<td>11999.67</td>
<td>5999.83</td>
<td>1045.888</td>
<td>1.291E-32***</td>
<td>0.0555</td>
</tr>
<tr>
<td>Position</td>
<td>39.03</td>
<td>39.03</td>
<td>6.804</td>
<td>1.316E-02**</td>
<td>0.0002</td>
</tr>
<tr>
<td>Time</td>
<td>184386.37</td>
<td>36877.27</td>
<td>6428.425</td>
<td>4.782E-52***</td>
<td>0.8526</td>
</tr>
<tr>
<td>Int. Variety*Position</td>
<td>788.01</td>
<td>394.00</td>
<td>68.683</td>
<td>5.153E-13***</td>
<td>0.0036</td>
</tr>
<tr>
<td>Int. Variety*Time</td>
<td>11471.47</td>
<td>1147.14</td>
<td>199.970</td>
<td>1.956E-28***</td>
<td>0.0530</td>
</tr>
<tr>
<td>Int. Position*Time</td>
<td>3237.94</td>
<td>647.58</td>
<td>112.887</td>
<td>5.833E-21***</td>
<td>0.0150</td>
</tr>
<tr>
<td>Int. Variety<em>Position</em>Time</td>
<td>4136.47</td>
<td>413.64</td>
<td>72.107</td>
<td>9.401E-21***</td>
<td>0.0191</td>
</tr>
<tr>
<td>Error</td>
<td>206.51</td>
<td>5.73</td>
<td></td>
<td>0.0010</td>
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</tr>
<tr>
<td>Total</td>
<td>216265.50</td>
<td></td>
<td></td>
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<td></td>
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</tbody>
</table>

* significant (p<0.05); ** very significant (p<0.01); *** highly significant (p<0.001); ns – no statistical significance (p>0.05).
Three-factorial analysis of variance for carotenoids extracted from the apple samples under study, including experimental factors Harvesting time, Variety and Position in the crown and the interactions between them, indicates highly significant differences (***, p<0.0001) for all experimental factors as well as for their interactions. Relative sizes of the studied factors effects and their interactions over carotenoids are shown in Table 4. The main effects Harvesting time, Variety and Position of the crown, explained 66.40%, 20.66% respectively 0.03% of the total variability carotenoids, indicating the importance of Harvesting time and Variety. However the statistical analysis showed the low influence of Position in the crown (explaining 0.03% of variance). The most important interaction is Position in the crown*Harvesting time which recorded 4.96% regarding carotenoids variability, while the remaining interactions gathers together less than 8%.

Table 4. Analysis of variance – apples carotenoids content

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F-value</th>
<th>P-value</th>
<th>Eta Squared</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variety</td>
<td>16623242.57</td>
<td>8311621.28</td>
<td>17962.367</td>
<td>1.020E-54***</td>
<td>0.2066</td>
</tr>
<tr>
<td>Position</td>
<td>22917.34</td>
<td>22917.34</td>
<td>49.527</td>
<td>2.931E-08***</td>
<td>0.0003</td>
</tr>
<tr>
<td>Time</td>
<td>254414864.11</td>
<td>23087.130</td>
<td>49.527</td>
<td>2.931E-08***</td>
<td>0.0003</td>
</tr>
<tr>
<td>Int. Variety*Position</td>
<td>867180.07</td>
<td>433590.03</td>
<td>937.038</td>
<td>9.006E-32***</td>
<td>0.0108</td>
</tr>
<tr>
<td>Int. Variety*Time</td>
<td>3845438.67</td>
<td>384543.86</td>
<td>831.043</td>
<td>1.921E-39***</td>
<td>0.0478</td>
</tr>
<tr>
<td>Int. Position*Time</td>
<td>3987258.01</td>
<td>797451.60</td>
<td>1723.384</td>
<td>8.808E-42***</td>
<td>0.0496</td>
</tr>
<tr>
<td>Int.Variety<em>Position</em>Time</td>
<td>1671010.80</td>
<td>167101.08</td>
<td>361.125</td>
<td>5.571E-33***</td>
<td>0.0208</td>
</tr>
<tr>
<td>Error</td>
<td>16658.07</td>
<td>462.72</td>
<td>0.0002</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>80448569.66</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* significant (p<0.05); ** very significant (p<0.01); *** highly significant (p<0.001); ns - no statistical significance (p>0.05).

4. Conclusions

For all varieties, the highest total polyphenol content was recorded for the apples harvested at 7 DAFB, decreasing while the fruit was growing. Antioxidant capacity reaches the highest level at 15 DAFB and then declines until the apples reached technological maturity. Total chlorophyll, chlorophyll a and b recorded high values at 65 DAFB, when both the temperature and the light intensity are high (July). After this harvesting time, chlorophyll content decreased. The concentration of carotenoids accumulated in apple peel varied, however following a downward trend during fruit growth, regardless of the variety or the crown position.

Following the results, the best time to use the apples from physiological falls in different foods and pharmaceuticals is when the total polyphenols, the antioxidant capacity or chlorophyll a and b record the highest values, depending on the way the product was designed and its main purpose.

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References

(1) C. HENRÍQUEZ, S. ALMONACID, I. FELLE, T. VALENZUELA, M. ARAYA, L. CABEZAS, R. SIMPSON, H. SPEISKY, Determination of antioxidant capacity, total phenolic content and mineral composition of different fruit tissue of five apple cultivars grown in Chile. *Chilean Journal of Agricultural Research*, 70(4), 523, 536 (2010).

12552 Romanian Biotechnological Letters, Vol. 22, No. 3, 2017
(30) C.A. PIERCE, R. BLOCK, H. AGUINIS, Cautionary note on reporting eta-squared values from multifactor ANOVA designs, Educational and Psychological Measurement, 64(6), 916, 924 (2004).