

The Changes of Polyphenols, Flavonoids, Anthocyanins and Chlorophyll Content in Plum Peels during Growth Phases: from Fructification to Ripening

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Abstract

Samples from ‘Stanley’, ‘Vânăt de Italia’ and ‘Tuleu Gras’ plum cultivars were collected from two different positions of the tree crown (i.e., inside, and periphery of the crown) at six different harvesting times, starting with the phase when plum fruits were the size of a bean until they reached full maturity at 21 days intervals. The main phytochemicals of plum skin during fruit development were analyzed. Total polyphenols from plum skin showed variations throughout the fruit growth (200.6 to 1244.5 mg GAE 100g⁻¹), a relatively ascending trend being noticed. For ‘Stanley’ DPPH-Scavenging Activity, statistically insignificant differences ($p > 0.05$), with values between 47.4% and 51.5%, were found, similar trends being observed for ‘Vânăt de Italia’ and ‘Tuleu Gras’. The total flavonoid content in the analyzed plum fruits statistically significantly decreased ($p < 0.05$) from fructification to ripening for ‘Stanley’ and ‘Tuleu Gras’. On the other hand, variations were registered for ‘Vânăt de Italia’ cultivar which had the highest flavonoid content on the sixth harvesting phase. Anthocyanin content accumulated in ‘Vânăt de Italia’ plum peel showed increasing values during fruit development, regardless of the harvesting phase, variety or position, while a slightly decreasing trend was found for ‘Stanley’ and ‘Tuleu Gras’ varieties. The largest quantity of total chlorophyll (287.4 $\mu\text{g g}^{-1}$) was found in the fructification phase, followed by a continuous decrease until ripening, whatever the variety or crown position.

Keywords: anthocyanins, chlorophyll, flavonoids, fruit development, plums phytochemicals, polyphenols

Introduction

Plums are considered a group of fruits rich in bioactive compounds or phytochemicals such as vitamins (A, C and E), anthocyanin and other phenols and carotenoids (Stacewicz-Sapuntzakis *et al.*, 2001). Plums are known for their high content of phytonutrients and are considered a rich source of natural antioxidants necessary in our daily nutrition. It has been proven that plums have a higher antioxidant capacity than most fruits that are predominant in human nutrition, such as apples, tomatoes, and peaches and similar to that of strawberries and blueberries (Valero

and Serrano, 2010). Díaz-Mula *et al.* (2009) reported significant differences in bioactive compounds of eight plum fruits cultivars, harvested on commercial ripening phase and then cold stored. Four cultivars (‘Blackamber’, ‘Larry Ann’, ‘Golden Globe’ and ‘Songold’) presented a typical climacteric ripening while other four (‘Golden Japan’, ‘Angeleno’, ‘Black Diamond’ and ‘TC Sun’) showed a suppressed-climacteric behaviour. At harvest, variations were reported among studied cultivars, overall, higher antioxidant activity and phytochemical concentration being found in fruits skin than in flesh.

Kayano *et al.* (2002) claimed that plums are rich sources of phenolic compounds, which, as expected, were correlated

with their antioxidant capacity, plums being ranked superior to apples, grapes, pears, watermelons, bananas. Cevallos-Casals *et al.* (2006) showed that plums from 14 cultivars harvested from USDA Fruit and Nut Research (Byron, U.S.) had three to four times higher phenolic concentration in skin as compared to pulp. The mixture, the content and the distribution of phenolic compounds of various cultivars of plums ('Beltsville Elite B70197', 'Cacak Best', 'French Damson', 'Long John', 'Stanley', 'Yugoslavian Elite T101') grown in New York State Agricultural Experiment Station orchard (Geneva, U.S.), were influenced by the fruit maturation, variety characteristics, geographic origins, cultural practices and, storage conditions (Kim *et al.*, 2003). Tomás-Barberán *et al.* (2001) reported a total amount of Flavan-3-ol between 662 and 1837 mg g⁻¹ (as a catechin equivalent) in the plum skin, while for plum pulp from 138 to 618 mg kg⁻¹, respectively; these compounds being responsible for the plums astringent taste. The same authors showed a progressive growth in the catechin concentration during fruits development, a decrease being reported when the fruits reached maturation. Díaz-Mula *et al.* (2009) noticed that anthocyanin level was between 20 to 40 times higher in the plum skin, than in the flesh with significant differences among studied varieties grown in Spain; the highest values were registered for fruit skin of 'Black Amber' cultivar (4370 mg kg⁻¹), while the lowest value was found in the skin of 'Black Diamant' plum (1310 mg kg⁻¹).

The chlorophyll is the molecule which absorbs the solar light and uses the energy to synthesize carbohydrates from CO₂ and water during the photosynthesis, a basis sustaining vital processes for all plants. Because animals and humans consume plants as part of their nutrition, photosynthesis may also be considered the well of our lives. Within the superior plants, *chlorophyll 'a'* (*Ca*) and '*b'*' (*Cb*) are representative (McGlasson *et al.*, 2007). The degradation of both chlorophyll types appears during the tissues' vegetative senescence, as well as during the fruit ripening. Therefore, chlorophyll content changes are used as a pattern of fruit ripening (Solovchenko *et al.*, 2005). Kader *et al.* (1999) observed a linear decrease of chlorophyll during the last development period of plum fruit, for both varieties researched, which is agreement with horticultural knowledge of fruits ripening. In another study, Abdi *et al.* (1997) reported a similar chlorophyll degradation pattern, the studied plum varieties changed the color from green to yellow.

To the best of our knowledge, there is a lack of information regarding the study of the biochemical characteristics during fruit development, for main plum varieties grown in Romania (i.e., 'Stanley'; 'Vânăț de Italia' and 'Tuleu Gras'). Therefore, this paper aims to assess the changes of polyphenols, flavonoids, chlorophyll and anthocyanin content in plum peels during fruit development – from fructification phase to ripe plums. The results obtained will facilitate the selection of optimal harvesting time for plum fruits based on their phytochemical content. In addition, different phytochemical extracts production is possible as a potential superior valorization of fruits physiological falls, which are underused in current horticultural production.

Materials and Methods

Materials

The plum varieties selected for the current study were 'Stanley', 'Vânăț de Italia' and 'Tuleu Gras', being widely known plums trees grown in Romania. The samples (between 30 and 60 plums, depending on the size) were harvested from the same trees, in 2013 season, from a farm located in Cluj-Napoca, Cluj Region, Romania. For each of the three varieties studied, the samples were collected from two different positions of the tree crown (i.e., inside, and periphery of the crown), at six different harvesting times, starting with the phase when plum fruits were the size of a bean (27.05.2013) until they reached full maturity (09.09.2013) at 21 days intervals. Consequently, based on the three experimental factors used and their levels (i.e., Variety [3], Position [2], and Harvesting phase [6]), the resulted research design (3×2×6) included 36 different samples. After each harvesting, the samples were individually vacuumed and stored at -18 °C, until further analysis.

Samples extraction

In order to assess the antioxidant capacity, the total phenolic, flavonoids, anthocyanin content, plum skin extractions were obtained as described by Abdel-Aal *et al.* (2002) with some minor modifications. For each extraction, the skin from 3 to 5 plums was used, chopped and mixed using a mortar. From each sample, 1 g was collected and homogenized with methanol containing 0.01% HCl in plastic tubes. The samples were centrifuged 5 minutes at 5000 rpm, the plant material being subjected to minimum three successive extractions, while all supernatants were collected. The obtained extracts were filtered and dried at 35 °C under reduced pressure (Heidolph Rotary Evaporator). Finally, the dried extracts were dissolved in a known volume (from 6 to 8 ml of methanol) and stored at -20 °C, until further analysis.

Determination of total polyphenol content by Folin-Ciocalteu method

The total polyphenols content was assessed using the Folin-Ciocalteu method (Singleton *et al.*, 1999), slightly modified. A quantity of 25 µl sample was mixed with 1.8 ml of distilled water and 120 µl Folin-Ciocalteu reagent in a glass vial. A Na₂CO₃ 7.5% solution in distilled water (340 µl), was added 5 minutes later, to assure basic conditions (pH 10) for the Redox reaction between the phenolic compounds and the Folin-Ciocalteu reagent. The samples were incubated for 90 minutes at room temperature. Methanol was used as a control sample. The absorbance at 750 nm was measured using a Shimadzu UV-VIS 1700 spectrophotometer. The calibration curve was plotted based on the 0.25, 0.50, 0.75, 1 mg ml⁻¹ concentration of gallic acid. The total polyphenol content of plum fruit skin was expressed for fresh weight (FW) in Gallic acid equivalents (GAE) - mg GAE 100g⁻¹.

Estimation of total flavonoid content

The total flavonoid content in the plum fruit skin extracts was assessed using the colorimeter method, as

described by Kim *et al.* (2003). The alcoholic extracts were diluted with distilled water up to 5 ml, adding 300 μ l of 5% NaNO₂ solution. After 5 minutes, the mixture was treated with 300 μ l AlCl₃ 10% solution and 6 minutes later, with 2 ml NaOH 1N solution. The absorbance was read at 500 nm using a UV-VIS 1700 Shimadzu spectrophotometer, the total flavonoid content, being expressed in mg quercetin equivalent for 100g of fresh sample.

Quantification of total anthocyanins

The total anthocyanins were extracted from plum fruit skin using the method described by Diaconeasa *et al.* (2015) and were determined using the pH differential method. The monomeric anthocyanin pigments change color reversible with the changing of the pH. At pH 1.0 a colored, oxonium compound is formed, while at pH 4.5 the transparent, hemiketal form is predominant. The pigment difference of absorption at 520 nm is proportional with the pigment concentration. The results were expressed using cyanidin-3-glucoside equivalent.

Two dilutions were prepared from the same sample, the first in potassium chloride buffer solution (0.025 M, pH 1.0), and the second in sodium acetate buffer solution (0.4 M, pH 4.5), the pH been adjusted using HCl. The samples were equilibrated at room temperature for 15 minutes. The absorbance was read at 520 and 700 nm, using the spectrophotometer Shimadzu UV-VIS 1700.

The total anthocyanins (mg cyanidin-3-glucoside equivalent L⁻¹) were calculated as follows:

Anthocyanins content (mg L⁻¹) = $(A \times MW \times DF \times 1000) / \epsilon \times L$, where: A = (A_{520 nm} - A_{700 nm} pH 1.0) - (A_{520 nm} - A_{700 nm} pH 4.5); MW = molecular weight for cyanidin-3-glucoside (cyn-3-glu), 449.2 g mol⁻¹; DF = dilution factor; 10³ = the transformation factor from g to mg; ϵ = 26 900 molar extinction coefficient, in L \times mol⁻¹ \times cm⁻¹ for cyn-3-glu; l = cell path length (1cm) (Giusti and Wrolstad, 2001).

DPPH-scavenging activity

The antioxidant capacity was determined by assessing the Free Radical Scavenging effect over 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) (Odriozola-Serrano *et al.*, 2008). The antioxidant activity was calculated as follows:

DPPH scavenging effect (%) = $[(A_0 - A_s) \times 100] / A_0$

where, A₀ is absorbance of the blank, and A_s is absorbance of the samples.

Assessment of chlorophyll content

The extraction of chlorophyll from the plum skin was performed according to the method described by Lancaster *et al.* (1994) with some modifications. Absorbance was measured at 645 and 663 nm. Chlorophyll content was calculated using the equations:

Ca (mg g⁻¹) = $[(12.7 \times A_{663}) - (2.6 \times A_{645})] \times \text{ml acetone} / \text{mg sample}$

Cb (mg g⁻¹) = $[(22.9 \times A_{645}) - (4.68 \times A_{663})] \times \text{ml acetone} / \text{mg sample}$

$CT = Ca + Cb$

where: Ca - chlorophyll a; Cb - chlorophyll b; A₆₆₃ - absorbance at 663 nm; A₆₄₅ - absorbance at 645 nm; CT - total chlorophyll (Arnon, 1949).

Statistical analysis

The analysis of variance was performed using Minitab 16.1.0, Tukey comparison tests being also included at a significance level $\alpha = 0,05$. Classical and relative eta-squared, used to compare the effects of different factors in the same design, were computed.

Results and Discussion

Changes of total polyphenol content (TPC)

Total polyphenols from plum skin showed variations throughout the fruit growth, as showed in Fig. 1 for 'Stanley' variety TPC recorded values between 200.6 mg GAE 100g⁻¹ and 568.3 mg GAE 100g⁻¹; for 'Vânăt de Italia' variety TPC recorded values were between 201.6 mg GAE 100g⁻¹ and 1244.5 mg GAE 100g⁻¹; in the case of 'Tuleu Gras' cultivar, TPC was between 201.2 mg GAE 100g⁻¹ and 378.4 mg GAE 100g⁻¹. It is interesting to notice - and somehow difficult to explain - the recorded TPC variations obtained for the plums skin of the three varieties during fruits development, which most probably could be related to climate conditions in 2013. Similar variations were also noticed by Chun *et al.* (2004) when analysing 13 different plum cultivars, TPC ranged from 138.1 \pm 2.9 mg GAE 100g⁻¹ to 833.6 \pm 4.8 mg GAE 100g⁻¹. Marinova *et al.* (2005) found a TPC of 303.6 mg GAE 100g⁻¹ in ripe plums harvested from Bulgaria.

It is generally well known that during fruit ripening, the phenolic concentration lowers, while the flavonoids concentration grows (Manach *et al.*, 2004). Amiot *et al.* (1995) investigated the influence of the maturity phase on the phenolic content of three pears varieties ('Williams', 'Harow Sweet' and 'Guyot') at three different times of the year, variations among cultivars being observed: TPC of 'Williams' variety tended to increase and subsequently decrease, whereas 'Harow Sweet' decreased continuously to ripening time. Conversely, 'Guyot' plums TPC increased during fruit development. The TPC variations observed in the current study are supported by Miletić *et al.* (2012), which attributed the lack of a clear trend concerning TPC to the variations in composition of compounds that fall within phenols group during ripening. In other words, the degradation of some phenols was faster or slower than the biosynthesis of other phenols. Moreover, a reversed correlation between the phenolic compounds concentration and air temperature was reported (Xu *et al.*, 2011), similar results being observed in the current study.

Changes of total flavonoid content

The highest flavonoid content was found in the skin of the 'Vânăt de Italia' plum variety, in the 6th harvesting phase, for the fruits collected from the periphery of the crown. The changes of total flavonoid content of plum skin during fruit development is presented in Fig. 2

The flavonoid content varied between 55.3 mg QE 100g⁻¹ and 190 mg QE 100g⁻¹ for the 'Stanley' variety, between 181.8 mg QE 100g⁻¹ and 383.9 mg QE 100g⁻¹ for 'Vânăt de Italia', whereas for 'Tuleu Gras' changed from 63.9 mg QE 100g⁻¹ to 133.5 mg QE 100g⁻¹. Similar results were obtained by Kim *et al.* (2003) for 11 plum varieties, flavonoid content varying between 64.8 mg QE 100g⁻¹ and

257.5 mg QE 100g⁻¹ FW. In another study, Miletić *et al.* (2013) reported a total flavonoid content of 207.9 mg QE 100g⁻¹ and 100.4 mg QE 100g⁻¹, for dried plums of the 'Valjevka' and 'Mildora' varieties, while emphasizing that the drying process did not influence the amount of flavonoids; Veličković *et al.* (2014) found a flavonoid content of 131 mg QE 100g⁻¹ for *Prunus spinosa* L. fruits methanolic extract. Several authors found similar oscillations regarding the amount of flavonoids during the maturation process of the plum fruit, for example Stöhr *et al.* (1975) showed that the flavonoids concentration decreases as the fruits were ripening. Tomás-Barberán *et al.* (2001) reported for 'Black Beau' and 'Angeleno' that the amount of flavonoids increased together with fruit maturation, whereas for the 'Wickson' variety, the amount of flavonoids decreased once the fruits reached maturation. Same authors indicated also great taste differences among studied fruits, while flavonoids were responsible for the plum stringency.

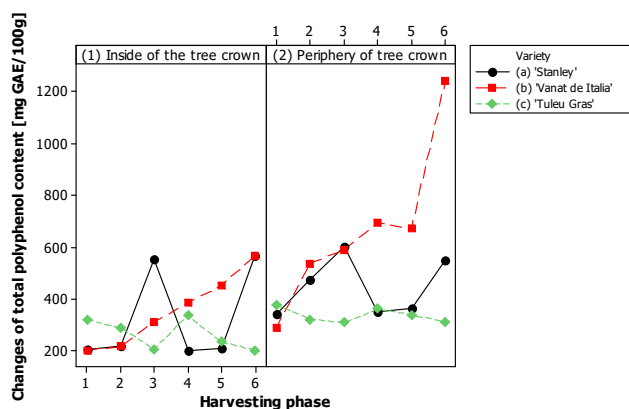


Fig. 1. Changes of total polyphenol content in the skin of plum fruits collected from different crown positions at six different harvesting times

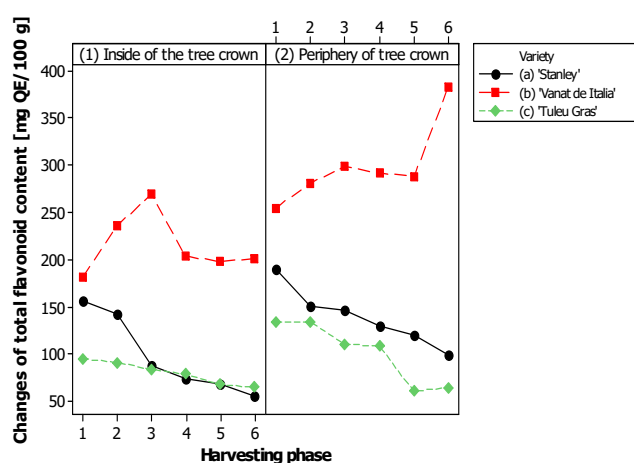


Fig. 2. Changes of total flavonoid content in the skin of plum fruits collected from different crown positions at six different harvesting times

Changes of anthocyanin content

The anthocyanin content in the plum skin of the three varieties studied ('Stanley', 'Vânăt de Italia' and 'Tuleu Gras') and harvested during fruit development from different positions in the tree crown (interior and periphery of the crown), was determined when the first purple color traces were noticed on the fruits surface (harvesting phases 4, 5 and 6), their trend being shown in Fig. 3. The anthocyanins accumulated in the plum skin registered increasing values irrespective of the harvest, variety or position. Thus, for the fruits harvested from inside of the tree crown, the 'Stanley' variety recorded values between 1.11 and 184.09 mg CE 100g⁻¹, 'Vânăt de Italia' between 1.34 and 194.80 mg CE 100g⁻¹, while for 'Tuleu Gras' the anthocyanins were increasing slowly from 6.24 to 45.96 mg CE 100g⁻¹. For the fruits harvested from the crown periphery, the anthocyanin content was between 25.56 - 261.93 mg CE 100g⁻¹ for 'Stanley', 6.87 - 306.98 mg CE 100g⁻¹ for 'Vânăt de Italia' and 20.45 - 58.45 mg CE 100g⁻¹ for 'Tuleu Gras'. The results obtained are in agreement with Vizzotto *et al.* (2007) study, where the anthocyanin content of 45 ripe plums and peaches cultivars with differently colored skin and pulp were determined. A similar trend of anthocyanin accumulation for 'Valor', 'Čačanska rodna', 'Čačanska najbolja', and 'Jojo' varieties, was found by Usenik *et al.* (2009) for fruits harvested in 6 different phases during development, all varieties showing the highest amount of anthocyanins either in the penultimate or last sampling time. Lancaster *et al.* (1997) observed that the distribution of anthocyanin in the skin and their concentration is mainly influenced by factors such as light, temperature, ethylene and cultural practices. Ripening of fruits is associated with the biochemical changing of the color, texture, taste and other quality characteristics; consequently it has an important effect over the anthocyanin changes of plum fruits. In the current study, it was found that the anthocyanin content showed different trends as influenced by plum variety (Fig. 3). Diaz-Mula *et al.* (2009) studied the amount of anthocyanin in 4 purple red varieties of plums, for both fruit's skin as well as their pulp, reporting differences of 20 up to 40 times higher anthocyanin content found in the skin, as compared with the pulp; similarly, Cevallos-Casals *et al.* (2006) reported differences from 7 up to 9 times higher, while Tomás-Barberán *et al.* (2001) identified differences up to 13 times higher anthocyanin content in plum skin as compared to the pulp.

DPPH-scavenging activity

The antioxidant activity of fruits is directly influenced by the phenolic compounds, and in lower amount to Vitamin C and carotenoids (Guorong *et al.*, 2009). The DPPH-Scavenging Activity of the plum skin for 'Stanley' variety recorded statistically insignificant differences ($p > 0.05$) with values between 49.1% and 51.5% when fruits were collected from inside the crown and between 47.4% and 50.2% for the periphery of the crown, samples being analyzed at different harvesting times. Similar trends were noticed for the other two plum cultivars studied 'Vânăt de Italia' and 'Tuleu Gras', as shown in Fig. 4. DPPH-Scavenging Activity variations were also reported by Miletić *et al.* (2012) for 'Stanley' plums during five harvesting

phases (7 days between sampling) in three consecutive years, no trend being noticed during fruit development. Same conclusions were drawn by Kristl *et al.* (2011) when analysing four plum varieties 'Valor', 'Stanley', 'Hanita' and 'Tophit', in the last weeks of ripening. Differences in antioxidant capacity depending on the variety during maturation of several plum varieties grown under the same conditions were reported by Díaz-Mula *et al.* (2009), confirming that the variety has a significant role for antioxidant capacity.

Chlorophyll content

Comparing the chlorophyll content of fruits skin for the three plum varieties studied, a decreasing trend was found, whatever the variety or the crown harvesting positions, as can be noticed in Fig. 5. Statistically significant differences ($p < 0.05$) were observed as a function of harvesting phase, the largest amount of *Ca*, *Cb* and CT was found in the first harvesting phase, while the lowest in the last. Kader *et al.* (1999) have observed a linear decrease in chlorophyll absorption in the last development of plum fruit on the tree for both varieties studied, which corresponds physiologically and horticulturally with the growth and maturation of the fruit. Abdi *et al.* (1997) stated that green-to-yellow color change for prunes in its research is associated with

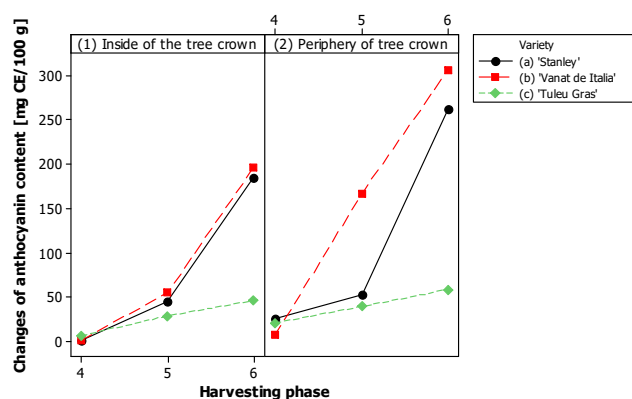


Fig. 3. Changes of antocyanin content in the skin of plum fruits collected from different crown positions at six different harvesting times

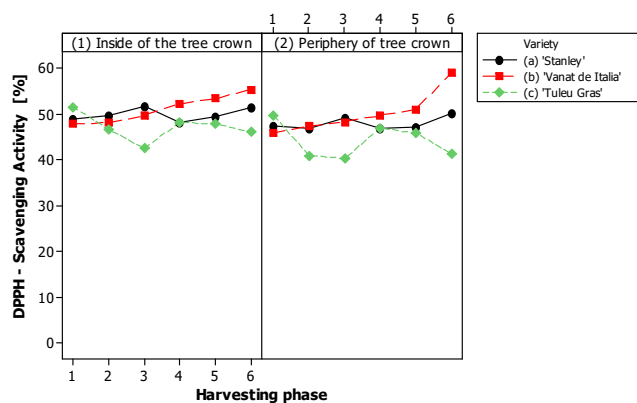


Fig. 4. Changes of DPPH-scavenging activity in the skin of plum fruits collected from different crown positions at six different harvesting times

chlorophyll degradation during fruit baking. A decrease of the chlorophyll content by 41% during 12 days of ripening, was also reported for the peel of 'Qingnai' plums (*Prunus salicina* Lindl.), once the fruits color changed to purple red (Luo *et al.*, 2009). The chlorophyll absorption might be a certain method to determine the real maturation stage of the fruits. High correlations between the chlorophyll absorption and the level of maturation were reported for several fruits, including plums (Ziosi *et al.*, 2008; Infante *et al.*, 2011). Moreover, the absorption of chlorophyll could be a reliable method to determine the actual ripeness state of the plum fruit (Infante *et al.*, 2011).

Regarding the position of the fruit in the crown of the tree for each variety, statistically significant differences ($p < 0.05$) for *Ca* and *Cb* were noticed. A higher amount of chlorophyll is found in the fruit harvested from the inside of the crown, compared to the fruits harvested at the crown's periphery. This could be attributed to the uneven distribution of light in the crown of the tree, as observed by Jacques *et al.* (2010).

The effect of variety, crown position and harvesting phase on the antioxidant activity, total polyphenol, flavonoids, anthocyanin and chlorophyll contents

The Analysis of variance revealed that the main effects, Variety, Crown Position and Harvesting phase indicated high significant differences (***, $p < 0.001$) for all studied phytochemicals characteristics, as shown in Fig. 6. First and second order interactions for total polyphenol, flavonoids and anthocyanin content showed also high significant differences (***, $p < 0.001$). Typically, the existence of a significant interaction exerts a null effect for subsequent interpretation of its inferior interactions and its principal effects. Consequently, essential is the discussion of the statistically significant second order interaction, the Variety * Position * Harvesting Phase for polyphenols, flavonoids and anthocyanins, as already performed in the previous section.

In order to emphasize the contribution of each factor or interaction, relative eta squared was computed as the proportion of total variation attributable to each factor or interaction. For each biochemical characteristic, a pie chart was used for displaying the proportion of total variance that corresponds to experimental factors and interactions (Fig. 6, sections A to E); the entire pie represents the total sum of squares, while each slice, expressed in percentages, is the effect size. For the antioxidant activity the first order interaction Variety * Harvesting phase showed high significant differences (***, $p < 0.001$) while the remaining interactions were statistically insignificant ($p > 0.05$) with a very low importance on the total variability. In the case of chlorophyll, the first order interactions Variety * Position and Position * Harvesting Phase, as well as the second order interaction of Variety * Position * Harvesting Phase showed very significant differences (***, $p < 0.001$), the interaction between Variety * Position being statistically insignificant ($p > 0.05$). The main effects Variety, Crown Position and Harvesting phase explained 19%, 18.6%, and 17.3% of the total variability of total polyphenol content in the skin of plum fruits (Fig. 6). One can also notice the weight of the Variety * Harvest Phase interaction which accounted for

29.8%, while the other interactions gather together 8.2%. The main effect Variety explained 75.1% of the total variability of flavonoid content in the plum skin, indicating the particular importance of the Variety for this characteristic, while the principal interaction was Variety * Harvesting phase with 7.1%. In the case of anthocyanins the main effect Harvesting Phase explained 55.2%, Variety 16.1% and Position only 5.1%, thus strengthening the significance of harvesting phase in the variability of the anthocyanins in the plum skin during fruits development. Among the most important interactions was the Variety * Harvesting phase, which registered 18.3% of the anthocyanin variability, while the remaining interactions altogether had less than 5%. For the antioxidant capacity the main effects: Variety, Harvesting Phase and Crown Position explain 29.5%, 12.2% and 6.5% respectively, indicating the particular meaning of the Variety. At the same time, this statistical analysis explained the very high influence (38.7%) of the Variety * Harvest Phase interaction of the antioxidant capacity variability in the plum skin, while the remaining interactions are less than 5.7%. The experimental factor Harvesting Phase explained 81.9% of the total chlorophyll variability, indicating, as expected, the high significance of the time of harvest on the chlorophyll content.

Concerning the chlorophyll content, the statistical tests performed, showed the influence of the Variety (explained 9.7% of the variability) and the low importance of the Crown position effect (1.7%). Among interactions the highest weight was recorded for the Variety * Harvesting phase (6% of the total variability), while the other interactions gather together less than 0.6%.

Conclusions

The total polyphenol content in the plums peel showed variations during the fruits growth for all three studied cultivars and crown positions used for harvesting the samples. As expected, a similar trend was highlighted in the case of antioxidant capacity. The total flavonoid content of the analyzed plums peel registered significantly decreasing values ($p < 0.05$) during fruits maturation; the exception was the plum peel of 'Vânăt de Italia' variety harvested from the periphery of the crown, which registered an unexpected high value ($383.9 \text{ mg QE } 100\text{g}^{-1}$) on the last harvesting phase. Anthocyanin content accumulated in 'Vânăt de Italia' plum peel showed increasing values during fruit development, regardless of the harvesting phase, variety or position, while a slightly decreasing trend was found for 'Stanley' and 'Tuleu Gras' varieties. The highest content of *Ca*, *Cb* and total chlorophyll was found in the first harvesting phase, while decreasing continuously to the last harvesting phase, for all studied varieties, regardless of crown positions. The present study recommends valorizing the plum fruit skin resulted as a by-product, as well as the fruits resulted from physiological falls for antioxidant extractions or natural dyes. The flavonoids and chlorophyll are more abundant during the immature stage of plum fruits, whereas the quantity of anthocyanin reaches the highest values during plum fruit ripening.

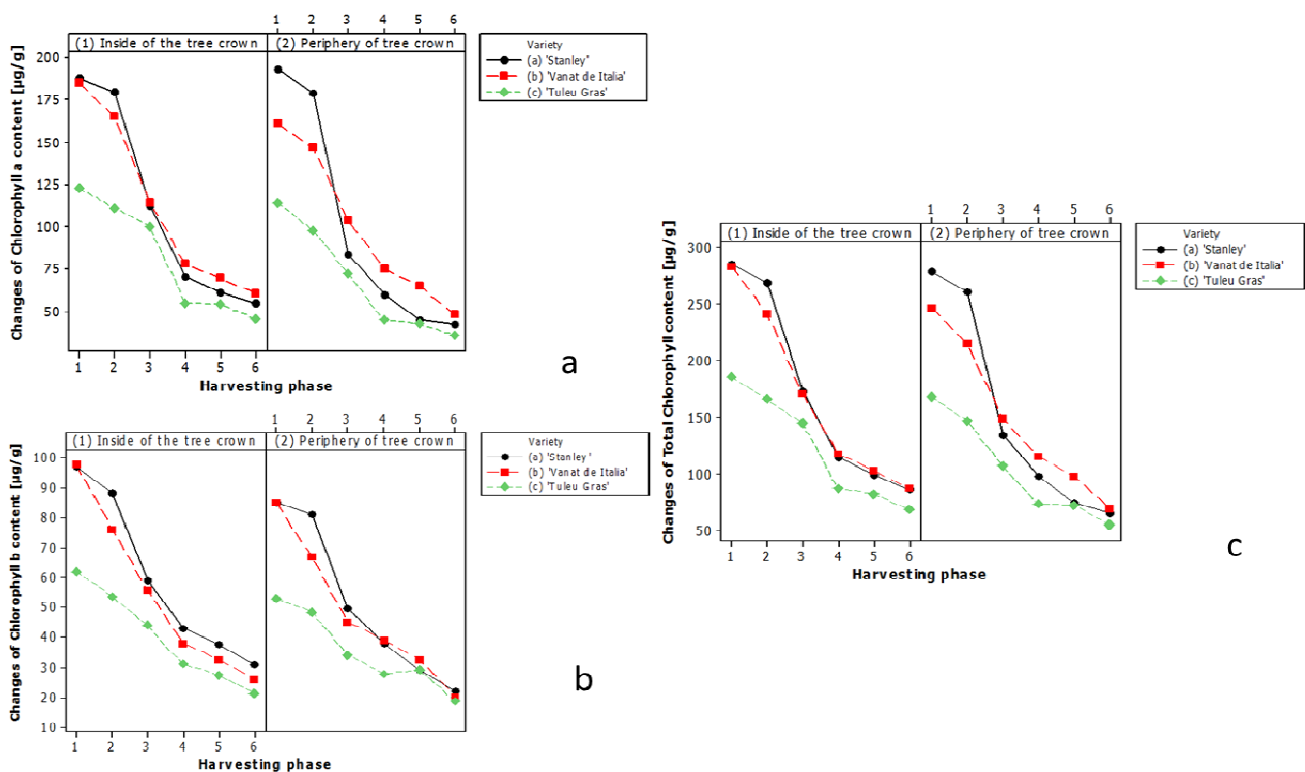


Fig. 5. Changes of *chlorophyll a* (A), *chlorophyll b* (B) and total chlorophyll (C) contents in the skin of plum fruits collected from different crown positions at six different harvesting times

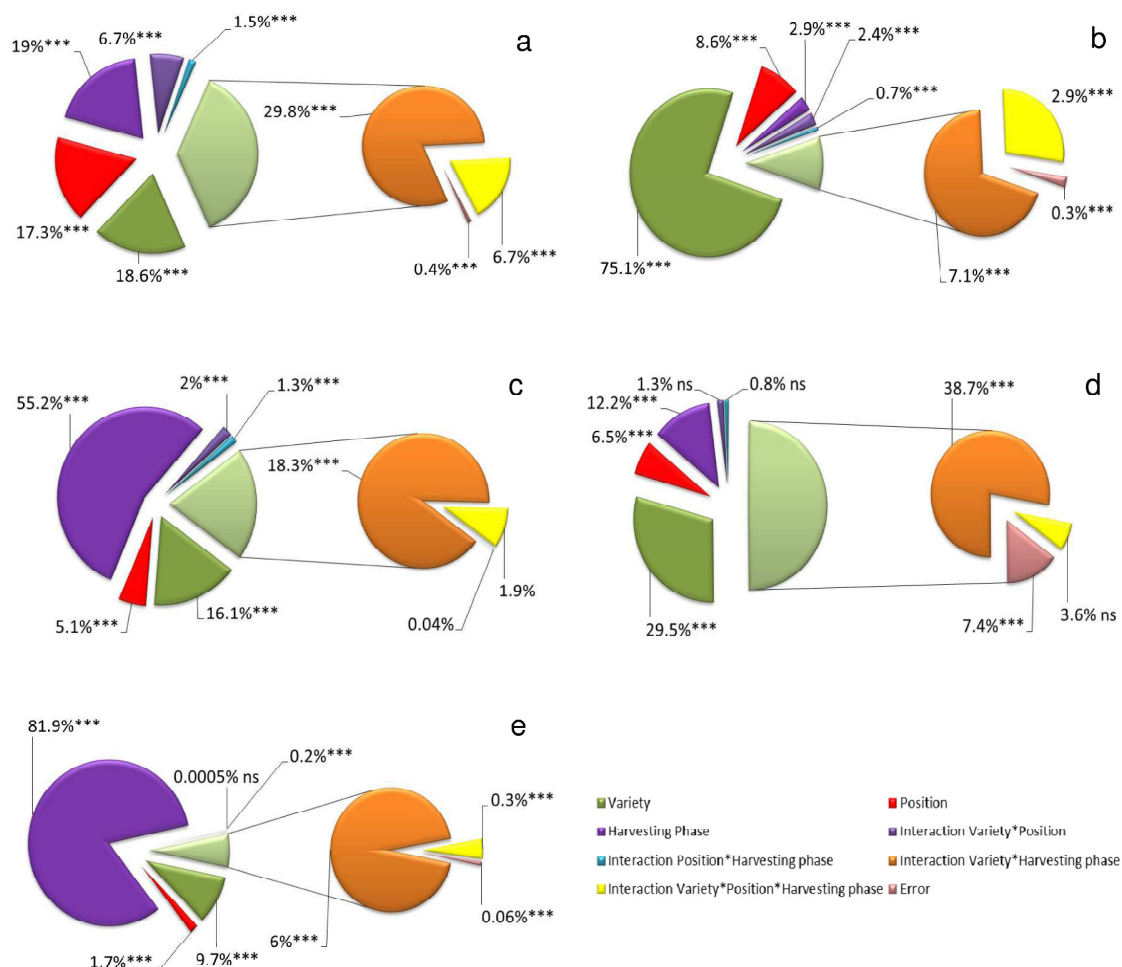


Fig. 6. The relative eta squared effect sizes on the total polyphenol content (A), total flavonoids content (B), anthocyanin content (C), antioxidant activity (D), chlorophyll content (E) of skin plums for the studied factors and their interactions (p - value: *significant ($p < 0.05$); **very significant ($p < 0.01$); ***high significant ($p < 0.001$); ns – insignificant ($p > 0.05$))

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