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Total phenolic content changes during apple growth as a function of variety and fruit position in the crown

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Abstract

Apples (*Malus domestica*) have been identified as one of the main food sources of phenolic compounds. The aim of this research was to evaluate total polyphenol content in apples peel of different apple varieties. Changes in total phenolic content (TPC) during apple growth were investigated from the 7th to 140th day after full bloom. Samples of three apple varieties (Starkrimson, Ionathan, Golden Delicious) were harvested from different position of the tree crown during apple growth. TPC was assayed by the spectrometric method using Folin-Ciocalteu reagent. The TPC varied from 154,12 –976,70 mg GAE /100g, for Starkrimson variety, between 101,12 – 925,04 mg GAE /100g for Ionathan variety and between 57,76–1224,40 mg GAE /100g for Golden Delicious variety. These results provide fundamental contributions to understand the dynamics of accumulation of phenolic compounds in apple peel.

Keywords: Folin-Ciocalteau assay, apple, polyphenols, different growth

1. Introduction

Apples are one of the most frequently consumed fruits in the world [16].World total annual apple production (2009) was around 71 million tonnes reaching the second place among fruits worldwide. Romanian annual apple production is around 0.5 million tonnes [4]. Increasing epidemiological studies and clinical trial have established that a high dietary intake of fruits is strongly associated with a reduced risk of developing some chronic diseases, such as various types of cancer, cardiovascular disease, type II diabetes and other degenerative or age-related diseases [1, 8]. Apples are an excellent source of phenolic compounds, these compounds being responsible for most of the antioxidant activity of the fruit [9,11]. Sun et al. (2002) found that apples had the highest soluble free phenolics when compared to 10 other commonly consumed fruits. In apples the phenolic content vary among different cultivars, within different tissues of the fruit, growing conditions, cultural practices, ripeness during harvest, post-harvest storage conditions, and processing [6, 2]. However, it is well established that the cultivar play a major role in controlling the polyphenol composition in apples [7, 10].

Considering the previous researches regarding the factors that determine the variation of total phenolic content from apples it was considered appropriate to assess the influence of fruit position in the apple tree crown on total phenolic content. Thus, this paper aimed to assess apples of three popular cultivars from Reghin region with emphasis on the influence of phenological moment and fruit position in the apple tree crown on total phenolic content.

2. Materials and methods

*Plant materials.*Apples of each studied cultivar (*Starkrimson, Ionathan, Golden Delicious*) were harvested from the same tree from an orchard of Reghin region - Romania, at 7th, 15th, 35th, 65th, 107th, 144th days after full bloom.

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Samples were harvested from different position of the tree crown during apple growth (inside and periphery of the crown). Samples were vacuumed and stored in a freezer at -20°C until analyzed.

Samples extraction. For sample extraction, 1g of apple peel was extracted on mortar with 5 ml of acidified methanol (MeOH:HCl 0.01%). The extract was separated and the residual tissue was re-extracted until the extraction solvents became colorless (the total solvent volume was between 100-200 ml). The filtrates were combined in a total extract, which was dried by vacuum rotary evaporator at 40°C. The dry residues were redisolved in 10 ml of methanol stored in a freezer at -20°C until analyzed [3].

Total phenolic content. The total phenolic content (TPC) was measured by the spectrometric method using modified Folin–Ciocalteu reagent [13,14,15]. Stock solution of sample extracts (25 µl each) were dissolved in methanol and further dilution were performed to obtain readings within the standard curve made with gallic acid (R=0.989). The extracts were oxidized by the Folin-Ciocalteu reagent (120 µl) and the neutralization was made with Na_2CO_3 (340 µl), after 5 minutes. The absorbance was measured at 750 nm. The results were expressed as milligram of gallic acid per 100 grams of sample [3].

Statistical analyses. Results were statistically analyzed using one way ANOVA statistical test and Tukey's multiple comparisons.

3. Results and discussion

The highest phenol contents were recorded for all varieties at seven days after full bloom. The total phenolic content (TPC) was 1224.40 mg GAE /100g fruit peel for Golden Delicious variety, 925.04 mg GAE /100g fruit peel for Jonathan variety, respectively 976.70 mg/100g fruit peel for Starkrimson variety. TPC of analyzed apples decreased significantly ($p<0.05$) with fruit grown regardless of studied variety or crown position (Figure 1). Phenol content decreased up to 57.76 mg GAE /100g fruit peel for Golden Delicious variety, 101.12 mg GAE /100g fruit peel for Ionathan variety and 154.12 mg GAE /100g fruit peel for Starkrimson variety.

For Ionathan variety statistically significant ($p<0.05$) differences started on 35th day after full bloom when higher TPC of apples from crown periphery (699.26 mg GAE / 100g fruit peel) compared to apples from inside the crown (614.54 mg GAE / 100g fruit peel) were obtained. Differences last till the end of experiment when apple peel from crown periphery has a TPC of 129.66 mg GAE/100g fruit peel and the ones harvested from inside the crown, 101.12 mg GAE/100g fruit peel, respectively.

For Starkrimson variety statistically significant ($p<0.05$) differences started to appear after 65 days after full bloom, when higher TPC of apples harvested from crown periphery (535.71 mg GAE /100g apple peel) compared to apples from inside the crown (471.94 mg GAE/100g apple peel) were recorded. This trend lasted till the end of experiment when apple peel from crown periphery has a TPC of 186.93 mg GAE/100g fruit peel and the ones harvested from inside the crown, 154.12 mg GAE/100g fruit peel, respectively.

For Golden Delicious variety on seventh day after full bloom TPC of apples harvested from the crown periphery (1224.40 mg GAE/100g fruit peel) was significantly ($p<0.05$) higher than TPC of apples harvested from inside the crown (973.74 mg/100g fruit peel). At 15 days after full bloom no statistically significant ($p>0.05$) differences appeared, but from 35 days after full bloom till the end of experiment statistically higher TPC were found in apples peels collected from inside the crown compared to crown periphery.

These results might be explained by different pigments classes of the studied apples varieties. Ionathan variety covering color, extended on almost all surface, is bright red bloody, and Starkrimson variety covering color is dark carmine red, showing thus that apples from these varieties are high in anthocyanin pigments which are part of phenolic compounds class. In the mean while Golden Delicious variety apples have a yellowish color little covered on sun exposed side with a light red orange wave, these fruits being characterized by carotenoids pigments predominance which are not belonging to phenolic compound class.

Similar findings were reported by [5] who attributed the different TPC of apples from Red Delicious, Granny Smith, Royal Gala and Pink Ladi varieties to the presence of the mixture of different cyanidin glycosides, of which cyanidin 3-galactoside is the main one, followed by cyanidin 3-glucoside, 3-arabinoside, 7- arabinoside.

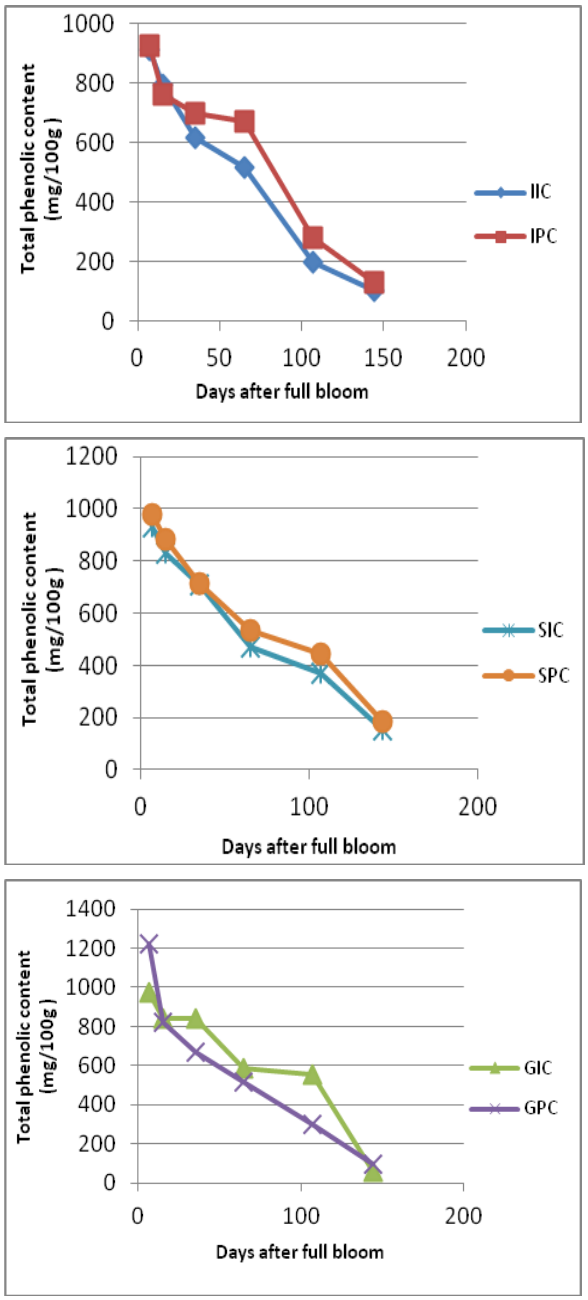

Figure 1. Total phenolic content (mg GAE/100g fruit peel) in the Golden Delicious, Ionathan and Starkrimson apples during fruits growth. IIC: Ionathan inside crown, IPC: Ionathan crown periphery, SIC: Starkrimson inside crown, SPC: Starkrimson crown periphery, GIC: Golden inside crown, GPC: Golden crown periphery

Comparing the three apple varieties studied as a function of fruit position in the crown (inside the crown and crown periphery), for apples harvested from inside the crown, statistically significant differences between studied cultivars started from 15 days after full bloom.

Golden Delicious variety recorded at this time 843.68 mg GAE/100g fruit peel, followed by Starkrimson variety with 827.90 mg GAE/100g fruit peel and Ionathan with 792.84 mg GAE/100g fruit peel, this trend being with little exception valuable till the end of the experiment.

When comparing the cultivars among apples harvested from crown periphery, a high TPC was obtained from the beginning of the experiment (7^{th} days after full bloom), 1224.40 mg GAE /100g fruit peel for Golden Delicious variety, 976.70 mg/100g fruit peel for Starkrimson variety, respectively, 925.04 mg GAE /100g fruit peel for Jonathan variety. At the end of experiment, i.e. 144 days after full bloom, this ranking changed and Starkrimson variety had the highest TPC 186.93 mg GAE/100g fruit peel, followed by Ionathan with 129.66 mg GAE/100g fruit peel and Golden Delicious with 95.40 mg GAE/100g fruit peel. These findings strengthen the fact that fruit position in the apple tree crown influence the TPC of apple peel. [12] reported also that TPC vary considerably as influenced by the cultivar and fruit tissue.

4. Conclusions

During this work it was found that total phenolic content of apples peel was influenced by cultivar, harvesting moment and fruit position in the crown. It is very important to know in which moment and from what position in the crown apples peel has the highest TPC in order to better valorize also the immature fruits.

At the beginning of this experiment Golden Delicious cultivar registered the highest TPC followed by Starkrimson and Ionathan. During the fruit growth the ranking of cultivars was changed and Starkrimson variety showed the highest TPC followed by Ionathan and Golden Delicious.

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