Total phenols and nutrients composition aspects of some apple cultivars and new studied breeding creations lines grown in Voinesti area – Romania

Received for publication, March 24, 2011
Accepted, June 5, 2011

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Abstract
In recent years, increased interest in human health, nutrition and disease prevention has enlarged consumers’ demand for functional foods including apple fruits; thus nutritional information is used increasingly by public agencies and agricultural agency to promote fresh products. Also, breeding for disease resistance is a promising way to control apple scab caused by the fungus Venturia inaequalis. The objective of this study was to determine some basic related constituents: total phenols and some macro and micronutrients in nineteen different apple cultivars and new studied breeding creations lines grown at Voinesti area-Romania. Research has carried out during the years 2006 and 2007, in the context of apples cultivars disease resistance and their internal quality. Leaves and fruits were picked up in June, and only fruits were analyzed at the end of August - beginning of September. Total phenol content was determined using Folin-Ciocalteau reaction and elemental composition was performed by an inductive coupled plasma emission spectroscopy. Despite the new creations contained comparably amounts of phenols and mineral elements as traditional apple cultivars, the influence of other factors such as climatic conditions had a significant influence. The obtained differences may be reflected not only in the degree of disease resistance, nutritional or health benefits for consumers, but also the nutritional balance has a great importance in maintaining post harvest quality.

Keywords: Malus domestica, biochemical composition, leaves, fruits

Introduction
Apples belong to the main fruit species, the most important fruit in the temperate zone and the main objectives of apple breeding are being pursued in a number of breeding stations, worldwide and also in Romania. These objectives include fruit quality, resistance to biotic stresses, environmental adaptability, changes in tree habit, fruiting characteristics and yield efficiency and constancy of production (N. BRANIŞTE) [1], (S. DELALIEUX & al.) [2], (K.W. LEE & al.) [3], (C. MASSONNET & al.) [4], (C.B OTEZ & al.) [5], (GH.CAMPEANU & al.) [6].

In the last years there was increased interest in human health, nutrition and disease prevention which has expanded requirement for functional food, including apples. Thus, the nutritional information is used increasingly over the agricultural and public agencies to promote fresh products. Also, improving the disease resistance is a promising way to control apple scab caused by the fungus Venturia inaequalis (Cooke) G. Winter.

Fruit quality in apple is generally determined by taste, aroma, color and shape, which characterize each particular cultivar. Nowadays, an increasing amount of work is being done to identify or develop special apple genotypes, because apples may be considered as well as by their potential high antioxidant capacity and their use for value-added food processing (F. FRATIANNI & al.) [7], (S. KHANIZADEH & al.) [8], due to their phenols and minerals content. One problem is apple scab, caused by the fungus Venturia inaequalis (Cooke). Wint., the most serious disease of apple (Malus* domestica Borkh.) and a limitation to apple
production in the world, despite years of research and development. So, from this point of view, one strategy is to make use of apple’s natural resistance to scab and the breeding and planting of scab resistant cultivars of apples should be encouraged, but cannot be relied on exclusively because the resistance will probably be overcome in time. Breeding programs around the world identified several sources of resistance and the most frequently used being Vf from Malus floribunda 821. Recently, J.M. SORIANO & al. [9] reported the identification and mapping a new apple scab resistance gene (Vd3) which provides resistance to the highly virulent EU-NL-24 strain race 7. Also, interesting results have been obtained by H. FLACHOWSK & al. [10] by transgenic apple plants overexpressing the Leaf Colour (Lc) gene from maize, as regard as strongly increased production of phenols and higher resistance against fire blight and apple scab.

Apple storability and quality is determined by the genotype of the cultivars, also some other factors may influence expression of this peculiarity (J. LANAAUSKAS & N. KVIKLIE) [11]. Nutrient uptake is influenced by many -inner factors (genetic factors) as well as external (such as condition of the habitat - soil, climate). Close correlations between mineral content of apple fruits and its shelf life and quality were found. The degree of influence of cultivar and date of sampling factors on variability of mineral content and (K+Mg)/Ca and Ca/Mg ratio parameters were calculated by M. KRIVOROTA & al. [12]. In order to improve quality of apple fruits and increase its resistance to storage decay, appropriate elemental composition is necessary. As regards V. inaequalis, although adopting a decision- based control strategy can result in significant reduction in fungicide input (A.M. BERRIE & X.-M. XA) [13], breeding cultivars with durable resistance remains the ultimate objective for effective scab control. Cultivar disease resistance and post harvest quality maintaining may be also associated with fruit mineral composition. As recent example are the results obtained by M.O. HAFEZ & H.E. HAGGAG [14] by pre-harvest application of each boric acid and calcium chloride alone or in combination on fruit firmness, total soluble solids, acidity, sugars, color and fruit decay.

So, in the development and implementation of sustainable agriculture techniques, resistant cultivars to the pathogens and especially to apple scab are of great importance in order to alleviate deterioration of natural and environmental pollution. New scab resistant cultivars are also in attention of the apple breeding program of Voinești Fruit Growing Research and Development Station, Romania. The aim of the present research was to determine some basic apple fruits constituents from the view point of apple resistance to the pathogens and human health: total phenols and some nutrients of selected apple cultivars and new breeding selections lines from Voinești area.

Material and methods

Samples. The apple (*Malus* domestica Borkh.) cultivars and apple selections (grafted on the rootstock MM 106 and planted in an experimental apple culture in 2002 at distances 4x2.5 m (1000 trees/ha) (cvs. Jonathan, Golden Delicious, Generos, Florina, Ciprian, Redix, Irisem; and twelve new studied breeding creations ) used in this study were grown at the Voinești Fruit Growing Experimental Research and Development Station, Romania (45° N, 25°15’ E, 400-600 m altitude), a village which is well known for excellent quality of its apples. Soil conditions were: moderate acid pH (5.7-5.9); medium humus content (2.0-2.9 %), slightly assuring of nitrogen and potassium. Samples were taken to measure total phenols content of leaves and fruits peel (June 2006 and 2007) and total (peel and flesh) some macro and microelements content at the end of August (2007), beginning of September (2006).
Analytical procedures. Total phenolic content. Total phenols were extracted in methanol 80% and determined by using the adjusted Folin-Ciocalteau assay, calorimetrically at 765 nm (V.L. SINGLETON & J.A. ROSSI) [15]. The standard curve was plotted using gallic acid (Sigma) as standard. Total phenols were expressed as mg gallic acid equivalents (GAE) 100 g⁻¹ fresh weight. Average results were obtained by two parallel determinations.

Mineral elements. The fresh vegetal material was dried for 24 hours at 105 °C, cooled in an exicator and after that it was weighed for the determination of the entire dried substance, then ash was resulted at 550°C. Elemental containing stock solutions were used for preparing reference solutions for the calibration curve and optimization of the analytical conditions. For acid digestions of the samples nitric acid 65 % (v/v) (Carlo Erba, Milan, Italy) of analytical grade was used. All aqueous solutions and dilutions were prepared with ultrapure water (18 MΩ cm⁻¹) obtained by using a Milli Q system (Millipore, Bedford, MA). The aqueous solution obtained was used to determine the mineral elements, using an Inductively Coupled Plasma Spectroscopy (ICP-OES IRIS Intrepid). The results were expressed in mg 100 g⁻¹ fresh weight. Average results were obtained by two parallel determinations.

Reagents. All the reagents (gallic acid and the Folin-Ciocalteau), solvents and standards that were used were of analytical quality (99% minimal purity) and were supply by Sigma-Aldrich (Madrid, Spain), Fluka (Buchs, Switzerland) and Merk (Darmstadt, Germany).

Using statgraphics software there were compared the obtained data.

Results and discussion

As regards the phenols, comparative to the apple leaves, content in fruits peel mean values for the two experimentally years were significantly lower, as can be observed in Figure 1. Because there were registered significantly differences from one year to another year we shortly discussed this aspect. The average values were 354.91 mg 100 g⁻¹ F.W. (June 2006) with the lowest value for Ciprian (159.1 mg 100 g⁻¹ F.W.) and the highest one for Redix (547.6 mg 100 g⁻¹ F.W.). In June 2007 the values range from 360.31 mg/100 g F.W. (Ciprian) to 2265.85 mg 100 g⁻¹ F.W.) (95/52).  So, it can be noticed the same trend as in the case of apple leaves (with significantly higher value in 2007), but values in the peel are about two times smaller than in leaves.

![Fig.1. Total phenols compounds in apple leaves and fruit peel (mean values: June, years 2006 and 2007)](image-url)

As regards the average content of gallic acid in apple peel, presented data emphasized that the total phenols was generally decreased in peel as against June (Figure 2). The lowest
The value was 215.10 mg 100 g⁻¹ F.W. (Generos, 2006) and the higher one 1020.00 (95/49 - 2006). In 2007, the values ranged from 26.89 mg 100 g⁻¹ F.W. (Generos) to 315.50 mg 100 g⁻¹ F.W. (95/52) with an average value of 177.92 mg 100 g⁻¹ F.W.

Our results emphasized that in apple fruits mezzocarp generally the phenols content was under 100 mg 100 g⁻¹ F.W., the lower values being registered for Ciprian (14.70 mg 100 g⁻¹ F.W.) and the higher for 98/72 (141.97 mg 100 g⁻¹ F.W.) (data are not shown).

Referring to the mineral composition (Figure 3), calcium fruits content varied between lines and cultivars from year to year, with values ranging from 6.15 mg 100 g⁻¹ F.W. (Irisem) to 26.68 mg 100 g⁻¹ F.W. (95/52), and a mean value of 13.71 mg 100 g⁻¹ F.W.

In the case of potassium level there was noticed the same trend, the richest was 95/15 (495.25 mg 100 g⁻¹ F.W.) and the lowest value was 185.71 mg 100 g⁻¹ F.W. (Irisem), while the mean value was 330.67 mg 100 g⁻¹ F.W. It can be remarked the large variation limits for potassium too, from apple line and cultivar.

Magnesium was situated around 16.59 mg 100 g⁻¹ F.W., ranging from 8.72 mg 100 g⁻¹ F.W. (V. H 1/26) up to 23.69 mg 100 g⁻¹ F.W. (95/15).

Ca/K, Ca/Mg, K/Ca and K/Mg ratios (Table 1) were near to the values reported earlier (VOICULESCU & al.), for Jonathan cv. – Voinești area (0.03; 0.72; 37.98 and 27.41).

As regard as micronutrients: iron accumulation ranged from 6.29 mg 100 g⁻¹ F.W. (H 1/26) up to 10.68 mg 100 g⁻¹ F.W. (95/15), while the mean value was 8.51 mg 100 g⁻¹ F.W. Natrium content ranged from 3.46 mg 100 g⁻¹ F.W. (H 1/8) up to 8.38 mg 100 g⁻¹ F.W. (95/15), while the mean value was 5.50 mg 100 g⁻¹ F.W. Mangan accumulation ranged from 1.86 mg 100 g⁻¹ F.W. (Ciprian), up to 2.87 mg 100 g⁻¹ F.W. (95/55) with a mean value of 2.37 mg 100 g⁻¹ F.W.
Following the literature review, it became clear that apple fruit quality is not only due to its appearance (form, color, etc.) but also by its internal quality. Internal quality can be due to the quantity and also quality of some constituents, including inorganic components, also by some organic compounds, including phenols. For both compounds, the importance derives from their benefits to people health, also for apple fruits resistance to the pathogens attack and preventing postharvest physiological disorders incidence. Taking into account the link between plants constitutive resistance to pathogens and the positive role as antioxidants in the cell metabolism and senescence, leaves and fruit phenols represent a potentially future characteristic in some breeding program. Treutter noticed the complexity pathways of these compounds (D. TREATUTTER) [16].

Also, recent results suggest that intracellular antioxidant level in the pathogen can be influenced by phenols present in host tissue and that changes in redox environment may influence gene expression and differentiation of structure associated with infection by the pathogen (M-H. LEE & R.M. BOSTOCK) [17].

Table 1. Some apple fruits macro elements ratios

<table>
<thead>
<tr>
<th>Variant</th>
<th>Ca/K</th>
<th>Ca/Mg</th>
<th>K/Ca</th>
<th>K/Mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jonathan</td>
<td>0.03</td>
<td>0.79</td>
<td>31.06</td>
<td>24.66</td>
</tr>
<tr>
<td>Golden Delicious</td>
<td>0.05</td>
<td>1.07</td>
<td>21.70</td>
<td>23.32</td>
</tr>
<tr>
<td>Generos</td>
<td>0.05</td>
<td>0.85</td>
<td>20.96</td>
<td>17.75</td>
</tr>
<tr>
<td>Ciprian</td>
<td>0.03</td>
<td>0.66</td>
<td>29.95</td>
<td>19.87</td>
</tr>
<tr>
<td>Redix</td>
<td>0.03</td>
<td>0.69</td>
<td>30.97</td>
<td>21.40</td>
</tr>
<tr>
<td>Irisem</td>
<td>0.03</td>
<td>0.64</td>
<td>30.19</td>
<td>19.38</td>
</tr>
<tr>
<td>Florina</td>
<td>0.04</td>
<td>0.91</td>
<td>27.66</td>
<td>25.25</td>
</tr>
<tr>
<td>V. H 1/26</td>
<td>0.04</td>
<td>1.04</td>
<td>22.39</td>
<td>23.34</td>
</tr>
<tr>
<td>V. H 9/78</td>
<td>0.04</td>
<td>0.67</td>
<td>27.34</td>
<td>18.20</td>
</tr>
<tr>
<td>V. H 1/8</td>
<td>0.04</td>
<td>0.82</td>
<td>27.56</td>
<td>22.63</td>
</tr>
<tr>
<td>95/12</td>
<td>0.03</td>
<td>0.63</td>
<td>35.80</td>
<td>22.39</td>
</tr>
<tr>
<td>95/15</td>
<td>0.04</td>
<td>0.93</td>
<td>22.60</td>
<td>20.91</td>
</tr>
<tr>
<td>95/49</td>
<td>0.08</td>
<td>1.24</td>
<td>12.23</td>
<td>14.14</td>
</tr>
<tr>
<td>95/52</td>
<td>0.07</td>
<td>1.28</td>
<td>14.08</td>
<td>18.06</td>
</tr>
<tr>
<td>95/55</td>
<td>0.05</td>
<td>0.97</td>
<td>21.17</td>
<td>20.46</td>
</tr>
<tr>
<td>95/230</td>
<td>0.03</td>
<td>0.63</td>
<td>28.63</td>
<td>18.00</td>
</tr>
<tr>
<td>95/272</td>
<td>0.03</td>
<td>0.57</td>
<td>35.07</td>
<td>20.08</td>
</tr>
<tr>
<td>97/192</td>
<td>0.04</td>
<td>0.65</td>
<td>25.23</td>
<td>16.42</td>
</tr>
<tr>
<td>98/72</td>
<td>0.03</td>
<td>0.50</td>
<td>36.82</td>
<td>18.33</td>
</tr>
</tbody>
</table>

As our results show, the content of gallic acid differs statistically significantly between cultivars and lines and changed during the growing period: it was the highest in June, while in August-September it declined. So, the apple peel phenols content was significantly lower in 2007, probably in relation with the environmental condition referring especially to the temperature effect (hot summer).

The accumulation of products was lower as the assimilation process was probably the same. It can be mentioned that the samples had not the same maturity degree, but of course the differences are due mainly in relation with their genetically specific characteristics, also by fruit development and solar radiation. Interesting facts are the results that showed that the contribution of the fruit pulp fraction to the total ORAC activity of fruit was less than 10% (J. LACHMAN & al.) [18].

Next to the benefic effect of phenols, it is necessary to consider the breeding programs according to people preferences, which are different: American/European dessert apples (‘Golden Delicious’); European refreshing apples (‘Granny Smith’); Asian dessert apples (‘Fuji’) or JFC, high quality apples, excellent combination of juicy firm and crispy flesh (JFC), sweetness and high acid content (Braeburn) (S. SANSAVINI & al.) [19].
Consumers prefer fruits with gentler taste which exhibit lower content of some groups of phenolics.

![Some micronutrients variation in apple fruits](image)

A new quality parameter is becoming more and more popular, namely the bioactivity of the fruit and its health promoting effect for the consumer. There is a conviction that the apple of the future may take on nutraceutical functions proper to foods that can improve health as well as prevent illness and even enhance cell metabolism by rapping free radicals so as to delay cell ageing.

A good level of polyphenols also helps to strengthen the plant self-defense system against pathogens (M. MIKULIC PETROVŠEK & al.) [20], although too high an expression results in an adverse change of taste. Cultivars with a high level of this polyphenol include ‘Granny Smith’ and ‘Braeburn’ (over 2400 mg kg⁻¹), as compared to ‘Golden Delicious’ (1200 mg kg⁻¹) (S. GUYOT & al.) [21].

Comparatively to the apple peel, phenols content of flesh was lower, with differences from cultivars to cultivar. Similar results were reported for 19 advanced apple lines and cultivars and antioxidant activity was correlated with phenol amount (S. KHANIZADEH & al.).

In general, rootstock can influence leaf mineral concentrations and fruit quality. Vigor of rootstock and crop load of the scion cultivar also plays a major role on uptake and translocation of minerals (E. FALLAI & al.) [22]. Also, mineral nutrient concentration varied from year to year and in function of the sampling time (in our study, 2006 – the end of August; 2007 – beginning of September).

Studies performed as regards the leaf and fruit macronutrient concentration during the growth period of apples emphasized that during maturation period nutrient concentration in apple flesh decreased. However, the differences between early and normally harvested fruits were not great and only mean K concentration was significantly higher in early harvested fruits (12.4 g kg⁻¹ D.M. (16-20 August) and 10.6 g kg⁻¹ D.M. (25 August-6 October sampling time) (R. DRIS & al.) [23].

In our biological material, in 2006, calcium content was generally at lower value as compared with data presented in the literature (N. VOICULESCU & al.) [24]. Therefore, the average value reported for Jonathan and Golden Delicious was 10.1 mg 100 g⁻¹ F.W., with a variation at Jonathan from 3 to 15 mg 100 g⁻¹ F.W. Potassium content varied between 58 mg 100 g⁻¹ F.W. to 204 mg 100 g⁻¹ F.W..

These results are difficult to interpret because cultivar selections and growing conditions varied widely and different methods of sampling and analysis were probably used for the investigations. Our obtained values are comparable with those reported for other apple
cultivars described in the literature such as Idared – fruit without bitter pit (total Ca: 8.1 mg 100 g⁻¹ F.W.; total potassium 88.8 mg 100 g⁻¹ F.W.) (N. PAVICIC & al.) [25].

Calcium content of apple fruit was found to be a heritable trait suggesting that selection and breeding of high Ca cultivars is a possibility for improving postharvest quality. The desirable Ca content may be different for various cultivars, but, in general, it is reported that a Ca content above 45-60 mg 100 g⁻¹ F.W. is satisfactory (N. VOICULESCU & al.) and when calcium fertilizers were applied, fruit calcium increased by 50-120 mg 100 g⁻¹ D.W. in comparison with the control.

Bitter pit is linked with critical level of Ca in the tissue, and for instance a Ca level of 5 to 6 mg 100 g⁻¹ F.W. has been suggested for Cox’s Orange Pippin apples as the critical concentration for susceptibility of fruits to this physiological disorder (R. DRIS & R. NISKANEN).

On the other hand, some authors suggested that K/Ca ratio gave a better indication on the susceptibility of fruits to bitter pit than the absolute K and Ca levels. As regard as iron apple content, comparing our results with data reported earlier (N. VOICULESCU & al.) the values are approximately near to the reported mean: 9.4 mg 100 g⁻¹ F.W., but higher than for Voinești area (2.9 mg 100 g⁻¹ F.W.) and lower than Fălticeni area (34.9 mg 100 g⁻¹ F.W.). In fact, the orchard management practices are the key factors in the production of high and qualitative yields of apples.

Taking into account the previous quality parameters, even if fruit consumption is reported to be beneficial to human health, nowadays attention must be focused also on other characteristic features, as for instance the aspect that apples can cause severe allergic reactions. In this context, recently there were cloned gene for apple allergens and determined their levels of expression as a function of genotype, fruit tissue and stage of development (G. PAGLIARANI & al.) [26].

As J.A. JURI & al. [27] previously mentioned, studies that contribute to the local information about the apple’s beneficial characteristics are important since the consumer is interested in knowing the composition of food in order to consume those that are more beneficial to their health.

Conclusions

There was registered a large variation of apple phenols and nutrient content, in relation with the biological material characteristics features, Voinești local area conditions, also year to year environmental conditions variability.

The obtained results represents not only an image of the possible degree of disease resistance, nutritional or health benefits for consumers, as a consequence of antioxidant activity and of the nutritional balance, but also a map of apple cultivars and new breeding creation characteristic feature in maintaining post harvest quality.

Acknowledgements

To National Authority for Scientifically Research (ANCS)

References


