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ACTUAL ASPECTS CONCERNING THE DETERMINATION OF THE ANTIOXIDANT PROPRIETIES IN SOME HORTICULTURAL PRODUCES

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ABSTRACT *- The horticultural produces contain numerous principles that have bioactive properties, among which a remarkable antioxidant activity. This property is due to a series of compounds having a more or less important level and a specific evolution, during the life of fruits and vegetables (flavones, phenolic acids, tocopherols, ascorbic acid, carotenoids, etc). The traditional antioxidants are vitamins (C, E, pro-vitamin A/ β carotene, lycopene, etc). Recently, they studied the antioxidant properties of flavones and other phenolic compounds. Gradually, there appeared the idea of evolution of these values, depending on the stage of maturity. At the maturity of the coloured fruits, studies were carried out on the contents of anthocyanines, flavonols proanthocyanidines, hydroxicinamates, β carotene, ascorbic acid and tocopherols. One may speak of an antioxidant activity specific to Pomaceae fruits, drupaceous fruits, bushes, citric fruits, grapes, and in vegetables, the crucifers from all the technological groups, followed by the radiculaceae and the bulbous ones. However, vegetables contain much lower levels of antioxidant compounds, among which we mention green peppers at their physiological maturity, Brussels sprouts and broccoli, besides the species rich in vitamins/provitamins with antioxidants properties.*

Key words: antioxidant properties, shrubs, peaches, apples, polyphenols, ascorbic acid

REZUMAT - Aspecte actuale privind determinarea proprietăţilor antioxidante la unele produse horticole. *Produsele horticole conţin numeroase principii, care au proprietăţi bioactive, între care şi o remarcabilă activitate antioxidantă. Această proprietate se datorează unei serii de compuşi, care au o prezenţă şi un nivel mai mult sau mai puţin importante, dar şi o evoluţie specifică pe parcursul vieţii legumelor şi fructelor (flavonoizi, acizi fenolici, tocoferoli, acid ascorbic, unii carotenoizi etc). Antioxidanţii tradiţional studiaţi au fost*

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vitaminele (C, E, provitamina A/ β carotenul, licopenul etc). Mai recent, au fost studiate şi proprietăţile antioxidante ale flavonoizilor şi ale altor compuşi fenolici. Treptat, s-a conturat ideea de evoluţie a acestor valori, în funcţie de stadiul de maturitate. La maturitatea de consum a fructelor colorate s-au realizat studii amănunţite privind conţinutul în antocianine, flavonoli, proantocianidine, hidroxicinamaţi, β caroten, acid ascorbic şi tocoferoli. Se poate vorbi despre o activitate antioxidantă tipică fructelor pomacee, drupacee, arbuşti, nucifere, citrice, struguri, iar la legume, cruciferele din toate grupele tehnologice, rădăcinoase, bulboase etc. Legumele conţin, totuşi, niveluri mult mai scăzute de compuşi antioxidanţi, remarcându-se, din acest punct de vedere, doar ardeii graşi la maturitatea fiziologică, varza de Bruxelles şi broccoli, alături de speciile bogate în vitaminele/provitaminele cu proprietăţi antioxidante.

Cuvinte cheie: proprietăţi antioxidante, arbuşti, piersici, mere, polifenoli, acid ascorbic

INTRODUCTION

The interest for the antioxidant activity of the horticultural produces is an actual trend that was gradually developed, due to the nutrition studies and food safety of the last decades (Dejica, 2001; Dumitrescu et al., 1991; Mişcalencu et al., 2005; Olinescu et al., 1990; Olinescu, 1994; Segal, 1998; Segal et al., 1983; Segal et al., 1986).

The researchers' concern focused on some groups of specific compounds or some species with significant antioxidant properties, some species more cultivated, respectively, and consequently, more available for the daily consumption (Banu et al., 1971; Banu et al., 2000; Barberan and Robins, 1997; Mişcalencu et al., 2005; Segal, 1998; Watson, 2001).

Florlani et al. (2003) underline the increasing interest given to the antioxidizing properties of fruits within their nutritive and even medicinal (nutriceutical) characteristics.

Scalzo et al. (2004) show that the main molecules, which may have antioxidizing action, are vitamin A (retinol), β carotene, vitamins of the B complex, vitamins C and E, coenzyme Q_{10} and the lipoic acid, besides some component minerals of the enzymes with antiradical action (manganese, molybdenum, copper selenium and zinc), some vegetal pigments, such as flavonols and chlorophyll, some amino acids, etc. The most important antioxidizing components from fruits and vegetables are vitamins C and E, phenolic substances, flavonols and anthocyans.

Antioxidants have a wide range and an unequal capacity to manifest these properties, depending on numerous factors (species, cultivars, technologies of production and preservation, capitalization channel, etc). TAC (total antioxidizing capacity) is a more and more studied characteristic.

Vrhovsek et al. (2004) notice that the occidental diet includes a daily consumption of about 1 g polyphenols, made of 2/3 flavonols and 1/3 phenolic acids. The polyphenols from apples are the most important antioxidant, exceeding vitamin C as importance. They assess that a regular consumption of apples may ensure a significant part of the necessary polyphenols, especially from the category of flavonols and glycoside flavonols. A recent study estimates that in the American diet, apples are the main source of pro-anthocyanins.

Andreotti et al. (2006) notice the impressing diversity of the phenolic compounds, associated to a considerable number of representatives (only the flavonols are estimated at 6000 molecular forms). Their interest does not refer only to the colouring properties (related to pigmentation), but also focuses on their dietetic and even medicinal features, since they reduce the incidence of some specific cancer forms or some cardiovascular diseases. Besides certain vitamins, fibres, phyto-estrogens, the polyphenols are among the most important fruit antioxidants.

RESULTS AND DISCUSSION

Florlani et al. (2003) studied the contents in substances with antioxidizing activity of peaches and nectarines, depending on the cultivar and the stage of maturation (time of harvesting and position of the fruit on the branch (7 days at 4 ^{0}C). They compared 21 cultivars (peaches –7 with white pulp and 8 with yellow pulp, nectarines - 2 with white pulp and 2 with yellow pulp, 2 breeds for industry, respectively) and they noticed a strong variability of the antioxidant content depending on origin.

In fresh state, the cultivars Manon (white pulp peach), Silvery and Caldesi 2000 (white pulp nectarines) and May Glo, respectively (yellow pulp nectarines) stood out positively since they presented more than 7 μg ascorbic acid /1 ml extract. The frigorific preservation determined a significant decrease for most cultivars. After preservation, only Caldesi 2000 registered more than 6 μg ascorbic acid /1 ml extract, beside May Glo (to the limit).

The time of harvesting and the position of the fruit on the tree have affected the activity of the hydrophilic antioxidants from fruits for all the five cultivars studied from this point of view. The most significant activity was signalled for the fresh fruits harvested at the first period, whereas the late harvesting and preservation diminished this property. The fruits harvested from the bottom of branches had a more reduced antioxidizing activity than those situated at the end of the branches and the increase was progressive. The hydrophilic antioxidants from peaches had an activity correlated to the firmness of fruits and to the value of the dry soluble substance, both in fresh fruits and in the refrigerated ones (Florlani et al., 2003).

Beceanu, Coşofreț et al. (2003) have published a series of analytical data referring to the chemical composition of the main wood fruits (seven species), harvested from the spontaneous flora of the Neamț County (Romania), where one might also find determinations tightly correlated to their antioxidant properties.

The total contents in phenolic compounds were evaluated by spectrophotometer at 280 nm and calculated based on a calibrating graph.

Table 1- Total contents in phenolic compounds for some species of wood fruits from the Neamț County (Romania)

Species	Time of harvesting	D 280	Total phenolic compounds (g/100 g fruits)
Bilberries	1-10.08	157.5	0.636
Blackberries	20-30.08	117.4	0.471
Wild cherries	20.06-10.07	107.5	0.435
Rose-hips	1-10.09	50.3	0.205
Raspberry	20.06-10.08	30.1	0.123
Sea-buckthorns	1-10.09	17.5	0.071

Table 1 shows that the limits of the contents in phenolic compounds of the analysed wood fruits ranged between the maximal values of 0.636 g/100 g analysed produce (bilberries) and 0.071 g/100 g analysed produce (sea-buckthorns). Important values were also signalled in blackberries (0.471 g/100 g produce) and wild cherries (0.435 g/100 g produce). A more important content in total phenolic compounds may be associated to the high values of the quantity of vitamin P (bioflavones or rutin) form the analysed produces.

The determination of anthocyans relied on their property to react in acid environment with the sulphurous acid, forming colourless sulphite combinations. The difference of optic density, read at 520 nm, between a non-discoloured sample and the same discoloured sample with sulphurous acid is proportional to the contents in anthocyans (g/100 g produce).

As for the contents in anthocyans (*Table 2*), expressed in g/100 g produce, the variation limits were ranged between 0.58 (bilberries) and 0.01 (sea-buckthorns). We have generally noticed the maintenance of the size order from the contents in total phenolic compounds. There was only one exception: the wild cherries exceed the blackberries, in terms of the contents in anthocyans, being found on the second place. The reason would be the higher proportion of the anthocyan-type phenolic substances, as compared to the blackberries.

By means of the chromatograms obtained on HPLC, we might identify some anthocyans, whereas by the method of discoloration with sulphurous acid we have determined a global content or a total quantity of anthocyans from the sample.

The chromatographic analysis on blackberries has shown that they largely contained a major pigment (cyanide 3 glycoside). This was identified for a retention time of 14.8 min. Calculating the area of the obtained peak, as compared to the area of the other accompanying peaks, we notice that this pigment is at proportion of 90% from the total of anthocyans, which is present in the fruit.

Table 2 -Total contents in anthocyans for some species of wood fruits from the Neamț County (Romania)

Species	Time of harvesting	Anthocyan content (g/100 g fruits)
Bilberries	1-10.08	0.58
Wild cherries	20.06-10.07	0.41
Blackberries	20-30.08	0.33
Rose-hips	1-10.09	0.12
Raspberry	20.06-10.08	0.09
Sea-buckthorns	1-10.09	0.01

For the wild cherries, the chromatogram indicates the presence of three peaks corresponding to the three major pigments, characteristic for this species, that is: cyanide 3 glycoside, peonidine 3 glycoside and peonidine 3 rutinoside. Peonidine 3 rutinoside is found at the highest proportion, followed by cyanide 3 glycoside, while peonidine 3 glycoside is found at the lowest quantity.

For raspberry, there was the possibility of chromatographic identification of the following pigments: cyanide 3 sophoroside (for the retention time of 18.3 minutes), cyanide 3 glycoside rutinoside (with the retention time of 19.8 minutes) and cyanide 3 rutinoside (with the retention time of 21 minutes). The major pigment is cyanide 3 sophoroside, at proportion of about 50%.

For bilberries, we may notice a great diversity of anthocyan pigments (more than 14 such pigments as different peaks). None of the existing pigments was major, noticing even a presence equal to these components. The peak from minute 14.47 was identified as belonging to the pigment cyanide - 3 glycoside (one of the most important), corresponding to a proportion of about 12% from the total contents in anthocyans (Beceanu et al., 2003).

For most of the species, the maximal and minimal values of the contents in vitamin C (*Table 3*) were relatively closed, except the sea-buckthorns samples, where a single variant registered double values as compared to the average. Wild cherries and, partially, raspberry have shown a decrease in the contents within one-month interval (Beceanu et al., 2003).

Scalzo et al. (2004) have also studied the antioxidizing capacity of some diverse types of peaches and nectarines. Their antioxidizing capacity was influenced in a polyvalent manner by the cultivar, the interaction with the father plant, the maturation time, the manner and the period of preservation. They showed that, although other fruits were more significant in terms of nutriceutical value, peaches and nectarines could still supply the daily sufficient quantity of antioxidizing substances.

The antioxidizing activity of peaches was determined by the method FRAP (Deighton – Benzie) and by the total contents in polyphenols TPH (Folin – Ciocâlteu). The antioxidizing capacity, expressed in μmol TE/g FW, was of 1 for Stark Satume (PG), 1.2 for Spring Belle (PG), 1.6 for Super Crimson Gold (NG) and Flaor Crest (PG), 2.1 for Ghiaccio 01 (PA), 2.4 for Caldesi 2000 (NA), 2.4 for Big Top (NG) and 4.1 for Maria Dorata (NG).

Table 3 -Total contents in ascorbic acid of some species of wood fruits from the Neamț County (Romania)

Species	Time of harvesting	Ascorbic acid (vitamin C) (mg/100 g fresh produce)
Bilberries	First decade/August	45.10 - 61.12
Raspberry	Third decade/ July	46.84 - 59.40
	First decade /August	38.92 - 51.75
Sea-buckthorns	First decade/ July	47.1- 111.53 average 58.60
Wild strawberry	Third decade/ July	55.13
Rose-hips	First decade/ July	51.07 - 53.69
Wild cherries	Third decade/ June	34.51 - 37.14
	First decade/ July	21.14 - 29.54
Blackberries	Third decade/ August	10 – 15

The total contents in polyphenols (μg GA /g FW) was 190 for Stark Satume (PG), 200 for Spring Belle (PG), 330 for Super Crimson Gold (NG), 360 for Flaor Crest (PG), 480 for Ghiaccio 01 (PA), 500 for Caldesi 2000 (NA), 600 for Big Top (NG) and 930 for Maria Dorata (NG). By calculation, they determined a correlation with R^2=0.9 between the antioxidizing capacity (FRAP) and the contents in polyphenols (Scalzo et al., 2004).

Radi et al. (2004) tried to characterize and identify some phenolic compounds from apricots. They attribute their sensitiveness to the enzymatic scald, due to the oxidization of the phenolic compounds, to the mechanical and thermal stress. The appearance of the unwanted brown colour also depreciates the sensorial/nutritive quality of the processed products.

Using some analytical methods, such as chromatography in this layer, the UV VIS detection, HPLC and mass spectrometry, they characterized and identified the main phenolic components of apricots: protocatechic acid (+ catechin), chlorogenic acid (- epi-catechin), naringine 7 glycoside/prunine, quercetin 3 glucoside, quercetin 3 rhamno-glucoside/rutin and campherol 3 rutinoside.

The results obtained have shown that the chlorogenic and neo-chlorogenic acids + catechin, - epi-catechin and rutin are major compounds among the apricot polyphenols. On the other hand, the protocatechic acid, prunine and pro-cyanides $B_{2,}$ B_3 and C_1 were for the first time identified in apricots (Radi et al., 2004).

Battino et al. (2004) have studied the exceptional nutritive value of strawberries. For determination, they used the TEAC method (Trolox equivalent antioxidant capacity) and the evaluation based on the total contents in polyphenols (TPH), determined by the Folin – Ciocâlteu method.

The authors wrote down the TEAC values (µmol TE/g FW) for nine fruit species cultivated in Italy. The shrub fruits stand out clearly as plus variants. Bilberries have the highest antioxidizing capacity (39 µmol TE/g FW), due to the high contents in anthocyanins. A remarkable antioxidizing capacity was also noticed in wild strawberries (33 µmol TE/g FW) and raspberry, respectively (23 µmol TE/g FW), followed, as an average value, by strawberries (15 µmol TE/g FW). A relatively reduced potential (under 5 µmol TE/g FW) was registered by quinces, kiwi, apples, apricots and peaches.

From the determinations effectuated, we may infer that the studied indicators represent a genetic character constituting sometimes an average of parentals for TPH (mg GAE/g), but there are also cases of positive transgression. As for TEAC (mol TE/g FW), in most cases, the values of the descending cultivars are superior to ascendants that served in melioration.

The authors have studied 16 cultivars of strawberries, representative for Europe, highlighting the variability manifest from case to case. The variants were placed in increasing order, from Queen Elisa (TPH minimum, about 1.8 mg GAE/g) and up to Sveva (TPH maximum, + about 3.1 mg GAE/g). We may not speak of a full concordance between the TPH values and the TEAC values, but the general trend indicates an approximate resemblance, at least for an important part of the variants. Thus, the reduced values of TEAC were registered by Queen Elisa, whereas important values were signalled in the last six cultivars having high TPH values. We appreciate that the deviation from an ideal increasing curve is ± 4 µmol TE/g FW (Battino et al., 2004).

Vrhovsek et al. (2004) investigated the quantity and the activity of the polyphenolic antioxidants from apples. A complete study consecrated to apple polyphenols seems to be more and more necessary. The papers already existing are consecrated only to some breeds and, sometimes, omit the study on the oligomer proanthocyanides. For the immature apples, most of flavonols are made of monomers (epicatechin and catechin), as well as of dimer and trimer proanthocyanides, whereas in the next phase, they have a more important polymerization degree (depending on the cultivar and tissue, between 5.7 and 7.1).

The study focuses on eight apple cultivars (more frequent in culture, old or recently introduced), cultivated in the area. They dosed 20 of the main monomers and oligomer proanthocyanides, the ascorbic acid as well as the antioxidizing capacity of extracts.

The total contents in polyphenols were on average of about 110 mg/100 g FW (212 for Renette and only 66 for Fuji). Flavonols (catechins, dimer and oligomer pro-anthocyanides) are the main class of apple poly-phenols. The oligomer proanthocyanides varied between about 38.8 mg/100 g for Fuji and 162.2 mg /100 g for Renette. Proanthocyanide B_2 oscillated between 5.6 and 19.3

mg/100 g, epicatechin between 5.2 and 18.4 mg/100 g and catechin between 0.5 and 4.3 mg/100 g.

The study also refers to the hydroxy-cynamic acids (Renette 38.4 mg /100 g and Granny Smith, only 4.5 mg/100 g), flavonols (between 8 mg /100 g for Braeburn and 3.5 mg /100 g for Renette), dehydro-chalcone (between 15 mg /100 g for Renette and about 2 mg /100 g for most of the variants), anthocyans, respectively (almost 4 mg /100 g for Imperatore, and absent for Renette, Granny Smith and Golden Delicious).

The ascorbic acid may be found in higher quantities at Golden Delicious and Braeburn (about 8 mg /100 g), and in very small quantities at Red Delicious and Royal Gala (0.5 ± 0.2 mg /100 g). The antioxidizing activity PRTE (Peroxyl Radical Trapping Efficiency) was determined by groups of cultivars (it is between 1.6 L/g for Renette and 0.5 L/g for Braeburn) and by groups of compounds (high effectiveness for quercetin and cyanide dilutions of 0.2 L/mg and low for the remainder of compounds, dilutions below 0.05 L/mg) (Vrhovsek et al., 2004).

Andreotti et al. (2006) also make a characterization of the phenolic profile for some peaches and nectarines. The studies on the phenolic composition of peaches originate in USA but refer only to a specific assortment. The authors have studied four technological groups of peaches and nectarines represented by cultivars typical to the region (peaches Redhaven yellow pulp, Fidelia white pulp, nectarines Stark Red Gold yellow pulp and Silver Rome white pulp) (*Table 4*).

Table 4 - Average contents in phenolic substances (monomer forms, mg/g DW) for peaches and nectarines from Bologna – Italy (Andreotti et al., 2006)

Specification	Part of the fruit	Stark Red Gold	Silver Rome	Redhaven	Fidelia
Catechin	skin	0.43	0.09	0.61	0.67
	pulp	0.11	0.19	0.18	0.2
Epicatechin	skin	0.15	0.39	0.15	0.1
	pulp	0.06	0.14	0.05	0.06
Pro-cyanide	skin	0.22	2.17	0.38	0.43
	pulp	0.06	1.01	0.15	0.16
Flavon 3-oli (total)	skin	0.8	2.66	1.13	1.19
	pulp	0.23	1.35	0.38	0.42

Qualitatively, the phenolic profile of the variants seems almost homogenous, the chromatograms registered at 280 nm having relatively similar peaks. The cynamic acids are represented by two compounds (chlorogenic acid and neo-chlorogenic acid, with a maximum absorbance at 320 nm). Catechin and epicatechin have a maximum absorbance at 280 nm. Glycoside flavonols (between 2 and 4 peaks) and cyanides (especially, cyanide 3 glucoside) specifically complete chromatograms.

Quantitatively, there is a similitude between the contents in total polyphenols from pulp (about 1 mg/g DW) and skin (about 3.5 mg/g DW) for three breeds (Stark Red Gold, Redhaven and Fidelia), whereas for Silver Rome the values are much higher (about 8 mg/g DW for skin and 3.5 mg/g DW for pulp). The studies have been extended on the contents in monomer forms and the contents in main phenolic compounds, respectively, for cultivars and parts of the fruit (skin and pulp) (*Table 5*).

Table 5 - Average contents in the main phenolic compounds (mg/g DW) for peaches and nectarines from Bologna – Italy (Andreotti et al., 2006)

Specification	Part of the fruit	Stark Red Gold	Silver Rome	Redhaven	Fidelia
Neo-chlorogenic acid	skin	0.32	1.03	0.31	0.17
	pulp	0.21	0.8	0.25	0.25
Chlorogenic acid	skin	0.95	2.86	1.02	0.84
	pulp	0.3	0.97	0.31	0.12
Total of cynamic acids	skin	1.27	3.88	1.33	1.01
	pulp	0.51	1.82	0.56	0.36
Cyanide 3 glucoside	skin	0.38	0.83	0.36	0.35
Quercetin 3 rutinoside	skin	0.41	0.13	0.18	0.27
Other quercetins	skin	0.19	0.11	0.14	0.06
Total flavonols	skin	0.6	0.24	0.32	0.34

They draw the attention on the dietetic and antioxidizing importance of pulp for peaches and nectarines (*Andreotti et al., 2006*).

Khanizadeh et al. (2007) determined the phenolic composition of 11 biotypes of apples, in epicarp and mesocarp, by HPLC, the phenolic contents by the method Folin – Ciocâlteu and the antioxidizing capacity by FRAP (ferric reducing antioxidant power). The HPLC analyses have identified and measured several groups of phenolic compounds: pro-cyanides, hydroxyl-cinamats, acids, anthocyanins, flavonols and di-hydroxo-chalcones. Pro-cyanides were the most present in pulp and skin, contributing by 52% and 44% to the total phenolic index (TPI). The quercetin glucosides were found almost exclusively in skin, whereas cyanide –3-galactoside was found only in the skin of the red apples.

The profile of the phenolic compounds for all the 11 genotypes presented differences and was more important in skin than pulp. Among the studied genotypes, *Reinette russet* registered the highest concentration in phenolic substances. The total phenolic contents (TPI/TPC) of extracts from pulp and skin, correlated well to the antioxidizing capacity estimated by FRAP. The low contents in chlorogenic acid and the lack of total flavonols in the pulp may be associated to the genotype with the weakest concentration in phenolic substances and with the lack of (enzymatic) scald, as compared to other cultivars (Khanizadeh et al., 2007).

Proust (2008) signals the increased antioxidizing capacity acquired by the grapes, kept for more than two months in the presence of an increased ozone concentration. The total quantity of polyphenols was superior in these bunches by about 23%, as compared to the quantity determined at harvesting. Our team led by Francisco Artes - Hernadez (Polytechnic University from Cartagena, Spain) attributes this accumulation of antioxidizing compounds to a reaction, which started in bunches before the biochemical aggression produced by ozone, through the induction at the biochemical level of several biochemical evolutions of antioxidizing defence (Proust, 2008).

CONCLUSIONS

The dietetic and the medicinal (nutriceutic) value of fruits is due to their antioxidant properties. The fruits from cultivated shrubs or from spontaneous flora, drupaceous fruits (peaches and apricots) or even apples bring a high quality contribution to the diet, by the compounds that avoid or neutralize the free radicals.

Nowadays, the chemical support of the antioxidant activity is very much studied, not only on the whole, but also as component substances or according to species, variety, growing technology and other means of differentiated estimate.

The present studies represent a permanence in the literature consecrated to the nutriceutic value of vegetables and fruits, containing valuable data that must be signalled, inclusively for other species or specific biochemical aspects.

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