

PHENOLIC COMPOUNDS AND ANTIOXIDANT CAPACITY OF PEAR AS AFFECTED BY ROOTSTOCK AND CULTIVAR

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During two consecutive years we investigated the influence of two traditional quince rootstocks and three commercial pear cultivars on the phenolic compounds content and the antioxidant capacity in whole fruit. Results showed that the impact of rootstocks was lesser than the impact of cultivars. Quince BA.29 showed the higher vitamin C content, total antioxidant capacity, condensed tannins and gallotannins, whereas quince MA induced better values of inhibitory activity against lipid peroxidation and metal chelating activity. 'Abbé Fétel' on both rootstocks was the cultivar with the highest vitamin C and total anthocyanin content (TAc), total phenolic (TPC) and total flavonoids contents (TFC), total antioxidant capacity (TAC), condensed tannins and gallotannins in comparison with 'Starking Delicious' and 'Beurré Bosc'. Values of DPPH scavenging activity, inhibitory activity against lipid peroxidation, metal chelating activity and hydroxyl radical scavenging activity were statistically similar and higher in both 'Starking Delicious' and 'Beurré Bosc' in relation to 'Abbé Fétel', i. e. they had a lower antioxidant capacity than 'Abbé Fétel'.

Keywords: anthocyanins, bioactive compounds, DPPH scavenging activity, fruit, *Pyrus communis* L., vitamin C

Einfluss von Unterlage und Sorte auf Phenolgehalte und antioxidative Kapazität von Birnen. In zwei aufeinanderfolgenden Jahren wurde der Einfluss von zwei traditionellen Quittenunterlagen und drei kommerziellen Birnensorten auf den Gehalt an Phenolverbindungen und die antioxidative Kapazität in ganzen Früchten untersucht. Die Ergebnisse zeigten, dass der Einfluss von Unterlagen geringer war als der von Sorten. Quitte BA.29 zeigte einen höheren Vitamin C-Gehalt, eine höhere antioxidative Gesamtkapazität, kondensierte Tannine und Gallotannine, während Quitte MA bessere Werte der Hemmaktivität gegen Lipidperoxidation und Metallchelatisierungsaktivität bewirkte. 'Abbé Fétel' auf beiden Unterlagen war die Sorte mit dem höchsten Gehalt an Vitamin C und Gesamt-Anthocyanin (TAc), Gesamtphenol (TPC) und Gesamtflavonoiden (TFC), Gesamtantioxidationskapazität (TAC), kondensierten Tanninen und Gallotanninen im Vergleich zu 'Starking Delicious' und 'Beurré Bosc'. Die Werte der DPPH-Fängerwirkung, der Hemmaktivität gegen Lipidperoxidation, der Metallchelatisierungsaktivität und der Hydroxylradikalfängeraktivität waren statistisch ähnlich und höher sowohl bei 'Starking Delicious' als auch bei 'Beurré Bosc' im Vergleich mit 'Abbé Fétel', d. h., diese Sorten hatten eine geringere antioxidative Kapazität als 'Abbé Fétel'.

Schlagwörter: Anthocyane, bioaktive Verbindungen, DPPH-Fängeraktivität, Früchte, *Pyrus communis* L., Vitamin C

The trees of pears (*Pyrus communis* L.) are grown worldwide in temperate zones and also in cooler climate with a total world production of around 23.7×10^6 t (FAO-STAT, 2020), and stand next to apples as the most frequently consumed fruit and the economically most important tree fruit. Pear fruit is very popular due to its sweetness, crispness, characteristic fragrance, and subtle aroma (CHEN et al., 2007). From this point of view, eating quality like aroma, flavour and texture are the most important traits that guide the consumer preferences for fruits. Beside fruit chemical composition, consumer acceptance highly depends on the physical and sensory attributes such as color, fruit size and shape, length/diameter ratio, flesh firmness, etc (KAPPEL et al., 1995; KADER, 1999). On this line, fruit quality is defined as the conjunction of physical and chemical properties which give good appearance and acceptability to the consumable product (KRAMER and TWIGG, 1966), and it is a human concept, which includes sensory properties, nutritional values, chemical compounds, mechanical and functional traits (ABBOTT, 1999). Pear fruits available on the world market belong either to *P. communis* L. and *P. pyrifolia* Nakai or hybrid groups of these two species – thus, the European and West Asian pears are relished for their buttery, juicy, fine texture and flavor, whereas East and North Asian pears for their crisp and sweet taste (AHMED et al., 2011).

Pear fruit are consumed fresh when fully matured, however, they are also suitable for processing industry in diverse groups of products such as syrup, fresh juice, canned and alcoholic beverages, jellies, jams, purées for use in nectars, yogurts, etc. (CHEN et al., 2007; BARROCA et al., 2011; WURM et al., 2011). The drying of pear fruit is a very ancient practice for food preservation, particularly for fruit of small dimension.

Many researchers reported that pear fruit is a rich source of carbohydrates, sugars such as fructose, sorbitol, sucrose, and, in lower levels, glucose and dietary fibre, but has low contents of lipids, proteins, organic acids, etc. (CHEN et al., 2007). For example, their fibre can be seen as a potential food additive (BARROCA et al., 2011). Recently, various studies have suggested that diets rich in fruits and vegetables can help in the prevention of diseases such as certain types of cancer, heart attack, various types of inflammation, allergic and other chronic diseases (SALTA et al., 2010). Namely, beside their richness in above phytochemicals, which had high nu-

tritional values, pear fruit also possess other substances such as minerals, vitamins and antioxidants as well as bioactive substances that are important sources of health-beneficial compounds. In general, the content of phenolic compounds in pears is positively correlated with their antioxidant capacity (AHMED et al., 2011; DADASHPOUR et al., 2012; LIAUDANSKAS et al., 2017). It is also well established that health benefits are, in part, attributed to the antioxidant capacity derived from the phenolic compounds present in edible fruit species such as pear (GALVIS-SANCHÉZ et al., 2003). These authors also reported that there is a great interest in determining relevant dietary sources of antioxidant phenolics. In addition, pear fruit show a significant antimicrobial activity (BARROCA et al., 2011).

As known the primary factor which influences fruit quality is the cultivar *per se* (genotype) (GALVIS-SANCHÉZ et al., 2003; SALTA et al., 2010). Additionally, other factors such as maturity stage, position and exposition of fruit in the canopy, cultivation practice and environmental conditions play a vital role in the formation of final fruit quality (TOMÁS-BARBERÁN and ESPÍN, 2001; ARZANI et al., 2008). Until now, these impacts have been more studied in pear cultivars than in rootstocks (GALVIS-SANCHÉZ et al., 2003; SALTA et al., 2010) and little is known about the effect of rootstocks on bioactive composition in general.

For these purposes, the major objective of this study was to test the effect of dwarf quince rootstocks on contents of bioactive compounds and antioxidant activity of 'Abbé Fétel', 'Starking Delicious' and 'Beurré Bosc', pear cultivars with important market potential when grown under western Serbian conditions. We hypothesized that a more detailed knowledge of the variability of these compounds of the cultivars on different rootstocks will be of benefit in the future selection of pear genotypes with improved consumer acceptance, nutritional quality and health potential. These results can also be supplied as new information for growers, breeders, consumers, nutritionists and other public.

MATERIALS AND METHODS

PLANT MATERIAL AND EXPERIMENTAL LAYOUT

'Starking Delicious', 'Abbé Fétel' and 'Beurré Bosc' pear

cultivars grafted on quinces MA and BA.29, respectively, were used as plant material in 2012 and 2013. Fruit of 'Starking Delicious' and 'Beurré Bosc' have yellowish-green ground skin color, and also 'Abbé Fétel', but with a blush on the side exposed to the sun. The orchard was established in 2008 (end of October) with 3.3 m x 1.2 m spacing (2,525 trees/ha) at a private orchard in Prislonica village (latitude 43°53' N, longitude 20°21' E, altitude 305 m) near Čačak city (Western Serbia). Treatments were distributed using the randomized complete block design with six trees for each rootstock/cultivar combination in four replicates (n = 24). Trees were trained as slender spindle and grown under common cultural practices (pruning, fertilization, pest and disease protection, weed control), except irrigation.

SOIL AND WEATHER CONDITIONS

The orchard had a clay-loam soil texture with 1.62 % organic matter and low soil pH (4.86) in 0 to 30 cm soil depth. Contents of total N, available P₂O₅ and K₂O, CaO and MgO are 0.16 %, 178 µg/g, 220 µg/g, 0.39 % and 6.2 µg/g on dry matter basis, respectively.

Weather data during the experimental period were characterized by the average annual temperature of 11.3 °C and total annual rainfall of 690.2 mm. The average air temperature during the vegetative cycle was 17.0 °C. During the experiment, no frost was registered. However, data presented in Table 1 show that in the period April to October in 2012 to 2013, mean monthly air temperatures were considerably higher than the long-term average, whereas rainfall had lower values in general, especially in July and August.

PREPARATION OF SAMPLES FOR ANALYSIS

Fruit for analysis were harvested at commercial maturity stage in both years ('Starking Delicious' at September, 2nd, and August, 24th, 'Abbé Fétel' at September, 23rd, and September, 17th, and 'Beurré Bosc' at September, 21st, and September, 14th, in 2012 and 2013, respectively) on both rootstocks. They were analysed at full maturity, i. e. approximately at 25 to 30 days after harvest. Full maturity was determined on the basis of color characteristics of each cultivar according to local procedure.

Ten randomly selected fruit in four replicates (n = 40) were taken for determination of amounts of bioactive compounds, antioxidant capacity and scavenging activities. Samples and sub-samples of whole fruit (peel and flesh) were prepared for chemical analyses according to laboratory procedures proposed by AOAC (2000).

Spectrophotometric measurements for TPC, TFC, TAC, as well as for TAC, condensed tannins and gal- lotannins and all other assays were performed on a UV-vis MA9523-SPEKOL 211 spectrometer (Iskra, Horjul, Slovenia). All measurements were made in triplicates. Results were presented as mean ± SE.

CHEMICALS

All chemicals and reagents were of analytical grade and were purchased from Sigma Chemical Co. (St Louis, USA), Aldrich Chemical Co. (Steinheim, Germany) and Alfa Aesar (Karlsruhe, Germany).

Table 1: Average monthly and yearly values of temperature (°C) and precipitation (mm) for the period of 1965 to 2010 and two studied years

Period	Air temperature (°C)							Precipitation (mm)						
	Apr	May	Jun	Jul	Aug	Sep	Mean	Apr	May	Jun	Jul	Aug	Sep	Mean
2012	12.6	15.9	22.3	25.5	24.5	21.0	20.3	70.3	106.8	11.8	45.1	0.0	7.8	241.8
2013	13.3	18.2	20.3	22.3	24.1	16.7	19.1	41.7	118.3	96.0	23.6	22.9	29.1	331.6
Normal	11.5	16.8	20.0	21.5	21.2	16.7	17.9	33.3	59.3	86.1	75.5	50.0	42.7	346.9

*Normal refers to the long-term average (45-year average, i. e. 1965 to 2010 period).

DETERMINATION OF VITAMIN C AND TOTAL ANTHOCYANIN CONTENT

The vitamin C content was determined by titration with Tillman's solution (0.02 % 2,6 dichloro-indophenol), as standardized by ARYA et al. (2000). Results were expressed as of fresh weight (mg/100 g FW).

Total anthocyanins content (TAc) in pear fruit was determined according to the method by GIUSTI and WROLSTAD (1996) based on the pH-differential method previously described by FULEKI and FRANCIS (1968). TAc was expressed as mg of cyanidin-3-glucoside equivalents per g of dry weight (mg C3GE/g DW).

DETERMINATION OF TOTAL PHENOLIC AND FLAVONOID CONTENT AND TOTAL ANTIOXIDANT CAPACITY

Total phenolics content (TPC) was determined spectrophotometrically by the Folin-Ciocalteu method (SINGLETON et al., 1999). The TPC was expressed as mg of gallic acid equivalents per g of DW (mg GAE/g DW).

Total flavonoids content (TFC) was determined according to the method described by BRIGHENTE et al (2007). The TFC was determined as mg of rutin equivalent (RUE) per g of dry weight (mg RUE/g DW).

Total antioxidant capacity (TAC) of the methanol extracts was evaluated by the phosphor-molybdenum method (PRIETO et al., 1999). Ascorbic acid (AA) was used as standard and the TAC was expressed as µg of AA per g of DW (µg AA/g DW).

DETERMINATION OF CONDENSED TANNINS AND GALLOTANNINS

The method for determination of condensed tannins relies on the precipitation of proanthocyanidins with formaldehyde (VERRMERIS and NICHOLSON, 2006). The content of condensed tannins was calculated as residuum of the total phenolic and unprecipitated phenol content, and expressed as mg GAE/g DW.

Gallotannins are hydrosoluble tannins containing a gallic acid residue esterified to a polyol. Gallotannins can be detected quantitatively by the potassium iodate assay. This assay is based on the reaction of potassium iodate (KIO₃) with galloyl esters (VERRMERIS and NICHOLSON, 2006), which will form a red intermediate

and ultimately a yellow compound. Gallotannin content was determined using gallic acid as standard and expressed as mg GAE/g DW.

DETERMINATION OF DPPH FREE RADICAL SCAVENGING ACTIVITY

The relative antioxidant capacity was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) in accordance with the method described by TAKAO et al. (1994) and slightly modified by KUMARASAMY et al. (2007). DPPH free radical scavenging activity (%) was calculated using the following equation

$$\% \text{inhibition} = \frac{Ac - As}{Ac} \cdot 100$$

where Ac is the absorbance of the DPPH solution and As is the absorbance of the sample.

The IC₅₀ value, defined as the concentration of the test material that leads to a 50 % reduction in the free radical concentration, was calculated as µg/ml through a sigmoidal dose-response curve.

DETERMINATION OF INHIBITORY ACTIVITY AGAINST LIPID PEROXIDATION

Antioxidant activity was determined by the thiocyanate method (HSU et al., 2008). Ascorbic acid, gallic acid, α-tocopherol and BHT (butylhydroxytoluene) were used as reference compounds. To eliminate the solvent effect, the control sample, which contained the same amount of solvent added to the linoleic acid emulsion in the test sample and reference compound, was used. Inhibition percentage of linoleic acid peroxidation was calculated using the formula for DPPH determination.

DETERMINATION OF HYDROXYL RADICAL SCAVENGING ACTIVITY

The ability of extracts to inhibit non site-specific hydroxyl radical-mediated peroxidation was carried out according to the method described by HINNEBURG et al. (2006). The percentage inhibition values were calculated from the absorbance of the control (Ac) and of the sample (As), where the controls contained all the reaction reagents except the extract or positive control substance.

DATA ANALYSIS

Since the differences of means between years were not significant, and the interaction effects of rootstocks, cultivars and years were also not significant, the differences between the rootstocks and between the cultivars were separately tested with the help of ANOVA. Post hoc tests for each variable were made using LSD comparisons. Significant differences for all statistical tests were evaluated at the level of $P \leq 0.05$. All data analyses were conducted with the Excel software (Microsoft Corp., Redmond, USA).

RESULTS AND DISCUSSION

VITAMIN C AND TOTAL ANTHOCYANIN CONTENTS

Vitamin C is a very important quality parameter because it acts as antioxidant and helps in maintaining good health. Fruits providing ample quality of this vitamin are always considered good for human health.

In the present study, vitamin C contents significantly varied between rootstocks and between cultivars (Table 2). Regarding rootstocks, there was an about 1.1-fold higher vitamin C content in fruit of cultivars grafted on BA.29 than on MA. This finding is consistent with results in our earlier work with apple (MILOŠEVIĆ et al., 2019a) in which certain rootstocks significantly improved the content of this compound.

Between cultivars, 'Abbé Fétel' on both BA.29 and MA rootstocks showed statistically higher amounts of vitamin C in comparison with the other two cultivars. Fruit of 'Starking Delicious' and 'Beurré Bosc' on MA had similar vitamin C contents, whereas on BA.29 'Beurré Bosc' had a higher amount of this compound than 'Starking Delicious'. Diversity among pears in vitamin C content was previously described by other researchers (CHEN et al., 2007; OZTURK et al., 2009; AHMED et al., 2011). Beside by cultivar, vitamin C can be influenced by various factors such as pre-harvest climatic factors, temperature levels during maturation and storage, soil quality, tree age and geographical region (LEE and KA-

DER, 2002; PLANCHON et al., 2004). Generally, vitamin C content in pears in our study was small and in the range previously described in literature. Thus, GALVIS-SANCHEZ et al. (2003) reported that the ranges of this compound were from 116 to 228 mg/kg in the peel and from 28 to 53 mg/kg in the flesh (GALVIS-SANCHEZ et al., 2003), whereas OZTURK et al. (2009) and HUSSAIN et al. (2013) reported values between 3.30 to 4.70 mg/100 g and 2.80 to 4.30 mg/100 g, respectively.

It has been long known that anthocyanins are responsible for most of the red, blue and purple colors of fruit, vegetables, flowers and other plant tissues or products (MILOŠEVIĆ et al., 2019a). In the present study, there were no significant differences between rootstocks regarding the total anthocyanin content in pears (Table 2). Similarly to vitamin C amount, this compound significantly varied between cultivars, being the highest in 'Abbé Fétel', intermediate in 'Beurré Bosc', and the lowest in 'Starking Delicious'. Several authors reported that peel contained much higher anthocyanin content as compared to flesh (GALVIS-SANCHEZ et al., 2003). Also, in their study more colored pear fruit contained higher vitamin C contents when compared with lesser colored fruit. In our study, we analyzed whole fruit (peel and flesh), and these results were expected because 'Abbé Fétel' has yellow skin ground color with a moderate amount of blush, 'Beurré Bosc' has yellowish-green skin ground color with a high amount of brown russets, and 'Starking Delicious' has yellow ground color without a blush. The selection of red-skinned pears is a breeding goal of many programs (ZHANG et al., 1997) due to their better contents of anthocyanins. Hence, cultivars rich in anthocyanins also contain high amounts of total phenolics since anthocyanins represent a large share of them (MILOŠEVIĆ et al., 2019b). Other authors also reported that anthocyanin content and its composition varies widely with genotype (DADASHPOUR et al., 2012).

Table 2: Vitamin C and total anthocyanin contents of three pear cultivars grafted on quinces MA and BA.29

Rootstock	Cultivar	Vitamin C (mg/100 g FW)	Total anthocyanin content (mg C3GE/g DW).
	Starking Delicious	3.34 ± 0.07 b	33.49 ± 0.77 c
Quince MA	Abbé Fétel	4.17 ± 0.15 a	42.43 ± 0.62 a
	Beurré Bosc	3.35 ± 0.19 b	39.33 ± 0.40 b
Mean		3.62 ± 0.14 B	38.42 ± 0.60 A
Quince BA.29	Starking Delicious	3.32 ± 0.14 c	33.45 ± 0.78 c
	Abbé Fétel	4.35 ± 0.11 a	43.36 ± 0.19 a
	Beurré Bosc	4.04 ± 0.08 b	38.31 ± 0.72 b
Mean		3.90 ± 0.11 A	38.37 ± 0.56 A

Values followed by the same small letter in a column were not statistically different at $P \leq 0.05$ by LSD test.

Values followed by the same capital letter in a column were not statistically different at $P \leq 0.05$ by LSD test.

PHENOLICS CONTENT AND TOTAL ANTIOXIDANT CAPACITY

Polyphenols are known to have strong antioxidant activities. As shown in Table 3, MA and BA.29 rootstocks were not different regarding TPC and TFC in pear fruit. BA.29 rootstocks induced significantly higher values in respect to TAC and significantly higher contents of condensed tannins and gallotannins in fruit as compared to MA rootstock. These results are similar to those obtained by REMORINI et al. (2008) who reported that some rootstocks for peach produced fruit having the highest TAC, probably due to its low-vigor characteristics. Significant differences in the content of

these compounds between cultivars were also detected, indicating that the cultivar *per se* (genotype) played an important role in the content of bioactive compounds which is in agreement with results of other researchers (AMIOT et al., 1995; MA et al., 2012; WURM et al., 2011; REZAEIRAD et al., 2013). In the present study, whole fruit of 'Abbé Fétel' on both rootstocks contained higher TPC and TFC in comparison with 'Beurré Bosc' and 'Starking Delicious' with no significant differences between them. TAC was the highest in 'Abbé Fétel', intermediate in 'Beurré Bosc' and the lowest in 'Starking

Table 3: Phenolic compounds of three pear cultivars grafted on quinces MA and BA.29

Rootstock	Cultivar	Total phenolic content (mg GAE/g DW)	Total flavonoids content (mg RUE/g DW)	Total antioxidant capacity (mg AA/g DW)	Condensed tannins (mg GAE/g DW)	Gallotannins (mg GAE/g DW)
Quince MA	Starking Delicious	78.71 ± 0.40 b	29.64 ± 0.70 b	76.45 ± 0.83 c	65.13 ± 0.65 c	31.74 ± 0.70 b
	Abbé Fétel	101.49 ± 0.51 a	40.54 ± 0.87 a	105.97 ± 0.39 a	78.28 ± 0.38 a	43.14 ± 0.89 a
	Beurré Bosc	79.57 ± 0.57 b	33.89 ± 1.20 b	79.61 ± 0.94 b	70.91 ± 0.62 b	34.58 ± 0.56 b
	Mean	86.59 ± 0.49 A	34.69 ± 0.92 A	87.34 ± 0.72 B	71.44 ± 0.55 B	36.49 ± 0.72 B
Quince BA.29	Starking Delicious	79.45 ± 0.59 b	31.49 ± 1.13 b	77.46 ± 0.72 c	68.47 ± 0.76 b	33.46 ± 1.20 c
	Abbé Fétel	103.23 ± 1.10 a	41.35 ± 0.57 a	107.48 ± 0.76 a	79.42 ± 0.46 a	45.77 ± 0.71 a
	Beurré Bosc	80.36 ± 0.31 b	34.45 ± 0.78 b	82.75 ± 0.62 b	71.63 ± 0.91 b	37.33 ± 0.34 b
	Mean	87.68 ± 0.67 A	35.76 ± 0.83 A	89.23 ± 0.70 A	73.17 ± 0.71 A	38.85 ± 0.75 A

Values followed by the same small letter in a column were not statistically different at $P \leq 0.05$ by LSD test.

Values followed by the same capital letter in a column were not statistically different at $P \leq 0.05$ by LSD test.

Delicious'. More authors also reported wider variations of secondary metabolite levels between cultivars (AMIOT et al., 1995; SALTA et al., 2010; HUSSAIN et al., 2013) and these quantitative differences are mainly due to the flavonol glycosides, as well as the high levels of chlorogenic acid, syringic acid, epicatechin, luteolin and quercetin in the peel (COLARIC et al., 2006; FU et al., 2011; MA et al., 2012; REZAEIRAD et al., 2013). Comparison of the TFC and TAC obtained here with those of other studies (MANZOOR et al., 2013; HUSSAIN et al., 2013) suggests similar results although differences in the units reported and spectrophotometric standards employed make a direct comparison difficult. However, previous work, carried out by MA et al. (2012), reported much higher TPC values than those obtained in our study. The differences between our results and those of MA et al. (2012) could be explained by differences of the group of cultivars studied and environmental conditions.

Condensed tannins (proanthocyanidins) and gallo-tannins (ellagitannins) are phenolic compounds which play a determinant role in the fruit quality and are key factors determining the degree of color and astringency in fruit and grapes (SIRAICHI et al., 2013). These compounds exert several pharmacological effects, including antioxidant and free radical scavenging activity as well as antimicrobial, anti-cancer, anti-nutritional and cardio-protective properties (SMERIGLIO et al., 2016). They also seem to exert beneficial effects on metabolic disorders and prevent the onset of several oxidative stress-related diseases.

Basically, 'Abbé Fétel' on both rootstocks had higher condensed tannin and gallotannin contents than the other two cultivars, whereas 'Starking Delicious' on both MA and BA.29 rootstocks had the lowest content of these compounds. When comparing the results of the present study with those previously reported in literature (MA et al., 2012) it is possible to see that there are some discrepancies in the phenolic composition, including tannins, of fresh fruit due to genetic, agronomic and environmental factors and also method and units used play an important role as previously reported (TOMÁS-BARBERÁN et al., 2001; ARZANI et al., 2008).

DPPH SCAVENGING ACTIVITY, INHIBITORY ACTIVITY AGAINST LIPID PEROXIDATION, METAL CHELATING ACTIVITY AND HYDROXYL RADICAL SCAVENGING ACTIVITY

The antioxidant power of foodstuff and food extracts, including fruits, cannot be evaluated by a single method, but at least two test systems have been recommended for the determination of antioxidant activity to establish authenticity (SCHLESIER et al., 2002). For this reason, numerous tests have been developed for measuring the antioxidant activity of fruits, but there is no universal method. In the present study, no significant differences between rootstocks were found for DPPH scavenging activity and hydroxyl radical scavenging activity (Table 4). On the other hand, MA induced better values of inhibitory activity against lipid peroxidation and metal chelating activity than BA.29. In this line, MA rootstock showed higher antioxidant activity, indicating that an increase in its cultivation might be a good solution for pear consumers.

Regarding cultivars, 'Starking Delicious' alongside with 'Beurré Bosc', had stronger DPPH scavenging and other related activities when compared with 'Abbé Fétel' (Table 4). Hence, 'Abbé Fétel' had better antioxidant potential than both 'Starking Delicious' and 'Beurré Bosc' cultivars. To our knowledge, there are very few studies on the antioxidant activity of pears. For this reason and due to the differences in the applied research methods it is difficult to compare our results with those obtained by other scientists. LIAUDANSKAS et al. (2017) reported that values of above assays in pear fruit were highly cultivar-dependent which supports our data. Since that all three pears grafted on two rootstocks in this study were grown at the same location using similar cultural practices, the variation in DPPH scavenging activity and other related activity demonstrates that the cultivar led to the differences in the biosynthesis of phenolic compounds in this fruit type. It was previously reported that pear cultivars had lower antioxidant activity compared to many fruit species (KARADENIZ et al., 2005). For example, RAUDONE et al. (2017) reported that the total reducing activity in apple was 3.2 to 5.0 times more potent than the reducing activity of pear fruit extract.

Table 4: DPPH scavenging activity, inhibitory activity against lipid peroxidation, metal chelating activity and hydroxyl radical scavenging activity of pear cultivars grafted on quinces MA and BA.29

Rootstock	Cultivar	^a IC ₅₀ (µg/mL)			
		DPPH scavenging activity	Inhibitory activity against lipid peroxidation	Metal chelating activity	Hydroxyl radical scavenging activity
Quince MA	Starking Delicious	35.45 ± 1.10 a	47.56 ± 0.82 a	58.91 ± 0.75 a	79.11 ± 0.45 a
	Abbé Fétel	19.42 ± 0.49 b	23.57 ± 0.70 c	44.23 ± 0.35 b	56.41 ± 0.62 c
	Beurré Bosc	30.34 ± 0.86 a	39.35 ± 0.66 b	57.14 ± 0.18 a	64.24 ± 0.32 b
	Mean	28.40 ± 0.82 A	36.83 ± 0.72 A	53.43 ± 0.43 A	66.59 ± 0.46 A
Quince BA.29	Starking Delicious	33.44 ± 1.23 a	42.38 ± 1.24 a	57.66 ± 0.42 a	78.26 ± 0.28 a
	Abbé Fétel	17.17 ± 0.46 c	21.56 ± 0.88 c	43.07 ± 0.89 b	53.55 ± 0.64 c
	Beurré Bosc	28.11 ± 0.85 b	32.73 ± 1.14 b	55.78 ± 0.40 a	64.12 ± 1.22 b
	Mean	26.24 ± 0.85 A	32.22 ± 1.09 B	52.17 ± 0.57 B	65.31 ± 0.71 A

Values followed by the same small letter in a column were not statistically different at $P \leq 0.05$ by LSD test.

Values followed by the same capital letter in a column were not statistically different at $P \leq 0.05$ by LSD test.

CONCLUSIONS

The results of the study verify that the rootstock and cultivar at the same growing area with hot and dry summer months (July and August) were the determinative factors during the cultivation, which influence the accumulation of phenolic compounds and antioxidant activity in pear fruit. Significant variations in phenolic constituents and antioxidant activity in the whole fruit were observed between quince BA.29 and MA rootstocks. In most cases, BA.29 showed better amounts of phytochemicals evaluated and antioxidant power in relation to MA, whereas MA induced higher DPPH scavenging and other activities. These results demonstrated that any quince rootstock type found to be the best choice for a particular pear cultivar can also be a poor choice for another cultivar. Whole fruit of more colored pears such as 'Abbé Fétel' were a much better source of phenolics and had higher antioxidant power when compared with lesser colored fruit such as 'Starking Delicious' and 'Beurré Bosc'. This status of 'Abbé Fétel' as cultivar with better antioxidant activity was confirmed by higher values of DPPH scavenging and other activities of 'Beurré Bosc', especially 'Starking Delicious'.

Results from this study can be of great importance to consumers, breeders and growers. Growers should consider very carefully the choice of pear scion/rootstock combination to achieve a better accumulation of secondary metabolites in the fruit and its higher antioxidant power. Moreover, the final decision should consider other parameters, which we have not touched upon, such as precocity, yield, pomological properties, plant resistance to pests and diseases, etc.

CONFLICT OF INTEREST

The authors declare no conflict of interest. The authors alone are responsible for the content and writing of this article.

REFERENCES

- ABBOTT, J.A. 1999: Quality measurement of fruits and vegetables. *Postharvest Biology and Technology* 15 (3): 207-225.
- AHMED, M., ANJUM, A.M., SHINWARI, Z.K., AWAN, M.S. AND RABBANI, M.A. 2011: Assessment of variability in fruit quality parameters of *Pyrus* germplasm collected from Azad Jammu and Kashmir (Pakistan). *Pakistan Journal of Botany* 43 (2): 971-981.
- AMIOT, M.J., TACCHINI, M., AUBERT, S.Y. AND OLESZEKZ, W. 1995: Influence of cultivar, maturity stage, and storage conditions on phenolic composition and enzymatic browning of pear fruits. *Journal of Agricultural and Food Chemistry* 43 (5): 1132-1137.
- AOAC 2000: Official methods of analysis of AOAC International, 17th ed. USA. Method No. 967.21.
- ARYA, S.P., MAHAJAN, M. AND JAIN, P. 2000: Non-spectrophotometric methods for the determination of vitamin C. *Analytica Chimica Acta* 417 (1): 1-14.
- ARZANI, K., KHOSHGHALB, H., MALAKOUTI, M.-J. AND BARZEGAR, M. 2009: Postharvest fruit physicochemical changes and properties of Asian (*Pyrus serotina* Rehd.) and European (*Pyrus communis* L.) pear cultivars. *Horticulturae, Environment and Biotechnology* 49 (4): 244-252.
- BARROCA, M.J. GUNÉ, R.P.F., PINTO, A., GONÇALVES, F.M. AND FERREIRA, D.M.S. 2006: Chemical and microbiological characterization of Portuguese varieties of pears. *Food and Bioprocess Processing* 84 (2): 109-113.
- BRIGHENTE, I.M.C., DIAS, M., VERDI, L.G. AND PIZZOLATTI, M.G. 2007: Antioxidant activity and total phenolic content of some Brazilian species. *Pharmaceutical Biology* 45 (2): 156-161.
- CHEN, J.Z., WANG, J., WU, J., WANG, Q. AND HU, X. 2007: Chemical compositional characterization of eight pear cultivars grown in China. *Food Chemistry* 104 (1): 268-275.

- COLARIC, M., STAMPAR, F., SOLAR, A. AND HUDINA, M. 2006: Influence of branch bending on sugar, organic acid and phenolic content in fruits of 'Williams' pears (*Pyrus communis* L.). *Journal of the Science of Food and Agriculture* 86 (14): 2463-2467.
- DADASHPOUR, A., AMOOIE, H.M., JOUKI, M. AND KHAZAEI, N. 2012: Evaluation of main anthocyanins in skin of forest Pear (*Pyrus pyraster*) (Sange Khouj) fruit in Guilan province of Iran. *Journal of Ornamental and Horticultural Plants* 2 (3): 183-190.
- FAOSTAT 2020: Available at: <http://faostat.fao.org>
- FU, L., XU, B.-T., XU, X.-R., GAN, R.-Y., ZHANG, Y., XIA, E.-Q. AND LI, H.-B. 2011: Antioxidant capacities and total phenolic contents of 62 fruits. *Food Chemistry* 129 (2): 345-350.
- FULEKI, T. AND FRANCIS, F. J. 1968: Quantitative methods for anthocyanins. 1. Extraction and determination of total anthocyanin in cranberries. *Journal of Food Science* 33 (1): 72-77.
- GALVIS-SANCHÉZ, A.C., GIL-IZQUIERDO, A. AND GIL, M.I. 2003: Comparative study of six pear cultivars in terms of their phenolic and vitamin C contents and antioxidant capacity. *Journal of the Science of Food and Agriculture* 83 (10): 995-1003.
- GIUSTI, M.M. AND WROLSTAD, R.E. 1996: Characterization of red radish anthocyanins. *Journal of Food Science* 61 (2): 322-326.
- HINNEBURG, I., DORMAN, H.J.D. AND HILTUNEN, R. 2006: Antioxidant activities of extracts from selected culinary herbs and spices. *Food Chemistry* 97 (1): 122-129.
- HSU, C.K., CHIANG, B.H., CHEN, Y.S., YANG, J.H. AND LIU, C.L. 2008: Improving the antioxidant activity of buckwheat (*Fagopyrum tataricum* Gaertn) sprout with trace element water. *Food Chemistry* 108 (2): 633-641.
- HUSSAIN, S., MASUD, T., ALI, S., BANO, R. AND ALI, A. 2013: Some physico-chemical attributes of pear (*Pyrus communis* L.) cultivars grown in Pakistan. *International Journal of Biosciences* 3 (12): 206-215.
- KADER, A.A. 1999: Fruit maturity, ripening, and quality relationship. *Acta Horticulturae* 485: 203-208.
- KAPPEL, F., FISHER-FLEMING, R. AND HOGUE, E.J. 1995: Ideal pear sensory attributes and fruit characteristics. *HortScience* 30 (5): 988-993.
- KARADENIZ, F., BURDURLU, H.S., KOCA, N. AND SOYER, Y. 2005: Antioxidant activity of selected fruits and vegetables grown in Turkey. *Turkish Journal of Agriculture and Forestry* 29 (4): 297-303.
- KRAMER, A. AND TWIGG, B.A. 1966: Fundamentals of quality control for the food industry, 2nd Eds., Avi Publishing Company, Inc., Westport, Connecticut.
- KUMARASAMY, Y., BYRES, M., COX, P.J., JASPARS, M., NAHAR, L. AND SARKER, S.D. 2007: Screening seeds of some Scottish plants for free-radical scavenging activity. *Phytotherapy Research* 21 (7): 615-621.
- LEE, S.K. AND KADER, A.A. 2000: Preharvest and postharvest factors influencing vitamin C content of horticultural crops. *Postharvest Biology and Technology* 20 (3): 207-220.
- LIAUDANSKAS, M., ZYMONĖ, K., JONAS VIŠKELIS, J., KLEVINSKAS, A. AND JANULIS, V. 2017: Determination of the phenolic composition and antioxidant activity of pear extracts. *Journal of Chemistry* 17: ID 7856521. doi: 10.1155/2017/7856521
- MA, J.-N., WANG, S.-L., ZHANG, K., WU, Z.-G., HATTORI, M., CHEN, G.-L. AND MA, C.-M. 2012: Chemical components and antioxidant activity of the peels of commercial apple-shaped pear (fruit of *Pyrus pyrifolia* cv. Pingguoli). *Journal of Food Science* 77 (10): 1097-1102.
- MANZOOR, M., ANWAR, F., BHATTI, I.A. AND JAMIL, A. 2013: Variation of phenolics and antioxidant activity between peel and pulp parts of pear (*Pyrus communis* L.) fruit. *Pakistan Journal of Botany* 45 (5): 1521-1525
- MILOŠEVIĆ, T., MILOŠEVIĆ, N. AND MLADENOVIĆ, J. 2019a: Role of rootstock and apple fruit tissue in antioxidant activity. *Acta Agriculturae Serbica* 24 (48): 97-106.

- MILOŠEVIĆ, T., MILOŠEVIĆ, N. AND MLADENOVIĆ, J. 2019b: Multivariate analysis as a reliable tool for segregation strawberry cultivars with the best antioxidant capacity. *Mitteilungen Klosterneuburg* 69 (4): 244-257.
- OZTURK, I., ERCISLI, S., KALKAN, F. AND DEMIR, B. 2009: Some chemical and physico-mechanical properties of pear cultivars. *African Journal of Biotechnology* 8 (4): 687-693.
- PLANCHON, V., LATEUR, M., DUPONT, P. AND LOGNAY, G. 2004: Ascorbic acid level of Belgian apple genetic resources. *Scientia Horticulturae* 100 (1-4): 51-61.
- PRIETO, P., PINEDA, M. AND AGUILAR, M. 1999: Spectrophotometric quantification of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application of vitamin E. *Analytical Biochemistry* 269 (2): 337-341.
- RAUDONE, L., RAUDONIS, R., LIAUDANSKAS, M., JANULIS, V. AND VISKELIS, P. 2017: Phenolic antioxidant profiles in the whole fruit, flesh and peel of apple cultivars grown in Lithuania. *Scientia Horticulturae* 216: 186-192.
- REMORINI, D., TAVARINI, S., DEGL'INNOCENTI, E., LORETI, F., MASSAI, R. AND GUIDI, L. 2008: Effect of rootstocks and harvesting time on the nutritional quality of peel and flesh of peach fruits. *Food Chemistry* 110 (2): 361-367.
- REZAEIRAD, D., BAKHSHI, D., GHASEMNEZHAD, M. AND LAHIJI, H.S. 2013: Evaluation of some quantitative and qualitative characteristics of local pears (*Pyrus* sp.) in the North of Iran. *International Journal of Agriculture and Crop Sciences* 5 (8): 882-887.
- SALTA, J., MARTINS, A., SANTOS, R.G., NENG, N.R., NOGUEIRA, J.M.F., JUSTINO, J. AND RAUTER, A.P. 2010: Phenolic composition and antioxidant activity of Rocha pear and other pear cultivars – A comparative study. *Journal of Functional Foods* 2 (2): 153-157.
- SCHLESIER, K., HARWAT, M., BOHM, V. AND BITSCH, R. 2002: Assessment of antioxidant activity by using different in vitro methods. *Free Radical Research* 36 (2): 177-187.
- SINGLETON, V., ORTHOFER, R. AND LAMUELA-RAVENTOS, R.A. 1999: Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology* 299: 152-175.
- SIRAICHI, J.T., FELIPE, D.F., BRAMBILLA, L.Z.S, GATTO, M.J., TERRA, V.A., CECCHINI, A.L., CORTEZ, L.E., RODRIGUES-FILHO, E. AND CORTEZ, D.A. 2013: Antioxidant capacity of the leaf extract obtained from *Arrabidaea chica* cultivated in Southern Brazil. *PLoS ONE* 8 (8): e72733.
- SMERIGLIO, A., BARRECA, D., BELLOCCO, E., TROMBETTA, D. 2016: Proanthocyanidins and hydrolysable tannins: occurrence, dietary intake and pharmacological effects. *British Journal of Pharmacology*. 174 (11): 1244-1262.
- TAKAO, T., WATANABE, N., YAGI, I. AND SAKATA, K. 1994: A simple screening method for antioxidants and isolation of several antioxidants produced by marine bacteria from fish and shellfish. *Bioscience, Biotechnology and Biochemistry* 58 (10): 1780-1783.
- TOMÁS-BARBERÁN, F.A. AND ESPÍN, J.C. 2001: Phenolic compounds and related enzymes as determinants of quality in fruits and vegetables. *Journal of the Science of Food and Agriculture* 81 (9): 853-876.
- VERRMERIS, W. AND NICHOLSON, R. 2006: Phenolic compound biochemistry. Springer, Netherlands.
- WURM, L., GÖSSINGER, M., WENDELIN, S. UND KORNTHEUER, K. 2011: Prüfung ausgewählter Mostbirnensorten als Spindelunter Bio-Produktionsbedingungen. *Mitteilungen Klosterneuburg* 61 (4): 236-246.
- ZHANG W.B., ZHANMG J.R., LI X.L. AND SHU Q. 1997: Yunnan red pear germplasm resources and their use. *China South Fruits* 26: 38-39.

Received May, 8th, 2020