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POMOLOGICAL PROPERTIES AND POLYPHENOL CONTENT OF CONVENTIONAL AND TRADITIONAL APPLE CULTIVARS FROM CROATIA

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Summary

Pomological properties and polyphenol content of six traditional and six conventional apple varieties from Croatia were studied. The highest fruit weight, height and width were measured in conventional apple cultivar, 'Red Delicious', and the lowest in traditional apple cultivar, 'Adamčica'. The highest content of soluble dry matter was measured in 'Zlatna Zimska Parmenka' (16.70%) and the lowest in 'Mutsu' (11.30%). The pH (3.90) value was highest in 'Red Delicious' and the lowest (3.12) in 'Zlatna Zimska Parmenka'. Total polyphenol content and antioxidant activity were on the highest level in traditional apple cultivar, 'Adamčica' 499.58 mg/100 g FW and 418.83 mmol trolox/L, respectively. Traditional apple varieties grown in local areas have so far been largely unexplored considering pomological and physicochemical properties. However, they might represent an important source of bioactive compounds and constitute the basis for further breeding. This research showed that traditional apple varieties are rich in polyphenols and have high antioxidant activity even higher than those found in conventional varieties. Due to the diversity of pomological properties and polyphenol content, it is important to preserve traditional apple varieties as a source of genetic variability as well as a factor of biodiversity of the area where they grow.

Keywords: traditional apple cultivars, pomological properties, polyphenols, antioxidant activity

Introduction

Apples have been grown for many centuries what is described in early legends, poems, and religious books. Current apple cultivars are developed from *Malus pumila* which originates from southwestern Asia (Cornille et al., 2012). From this cultivar, apple has been developed in many different varieties, with different sensory and organoleptic properties. Today the apple is one of the widely grown fruits with annual world production of 83 million metric tons in 2017 (FAOSTAT). Many apple cultiars have been developed over time but only some are grown for conventional use. The range of apple cultivars in the European market has been significantly reduced to no more than 12 cultivars (Jemrić et al., 2013). Planting only a small range of apple cultivars could endanger the biodiversity and lead to worldwide epidemics of certain pest and pathogens (Šavkin et al., 2014). Some of the most grown conventional apple cultivars are 'Idared', 'Jonagold', 'Golden Delicious', 'Red Delicious', 'Granny Smith' and 'Mutsu'. Traditional apple varieties are mostly cultivated in individual orchards, mainly in the marginal areas, and they show a good adaptability to the local environment and represent a valuable source for the crop genetic variability. Many traditional apple varieties carry genes for resistance to pests and diseases, drought tolerance, winter hardiness and unique fruit quality.

One of the first determinants of apples is pomological characterization. Defining pomological properties represent the basis for future scientific research work on the standardization of autochthonous genetic pool and creation of new cultivars adapted to given conditions with certain resistance and predisposition for the commercial and ecological production (Salkić et al., 2017). Traditional apples are not represented on the global market mainly because they usually do not meet some of the appearance standards (Šavkin et al., 2014). However, some of the earlier studies showed that traditional cultivars are more nutritious than newer cultivars (Jakobek et al., 2013; Balík et al., 2012; Donno et al., 2012; Iacopini et al., 2010). Apples are generally considered "healthy food", and like in other fruits and vegetables, polyphenols are one of the main compounds that are considered to have a positive impact on health. Polyphenols has several positive effects like their anticancerogenic properties, prevention of cardiovascular diseases and cancer, regulation of plasma cholesterol metabolism, antiviral properties, inhibition of *Helicobacter pylory* growth and staphylococcal enterotoxin A toxicity (Jakobek and Baron, 2016; Hyson, 2011; Valdenegro et al., 2010; Boyer and Liu, 2004). Studies showed that phytochemical composition of apples varies greatly between different apples varieties (Panzella et al., 2013), during the maturation and ripening (Kevers et al., 2011; Mainla et al., 2011) and even within different apple parts (Lončarić and Piližota, 2014;

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Lončarić et al., 2014). There are plenty of data on polyphenol content in apples; however they are often confined to a few cultivars. One of the more comprehensive evaluations of the polyphenol content and profile of 104 European apple varieties was conducted by Ceymann et al. (2012). Regarding the traditional apple cultivars in Balkan there are a few studies dealing with polyphenol content and antioxidant activity (Jakobek and Barron, 2016; Šavikin et al., 2014).

Due to the insufficient information in the literature, the aim of this study was to compare six conventional and six traditional apple varieties regarding their pomological properties, polyphenol content and antioxidant activity. New knowledge in traditional apple cultivars can help with diversification of the apple market, preventing potential disappearance of these cultivars and enabling the preservation of apple biodiversity.

Materials and methods

Apples Used for Experiment

Conventional apple cultivars, 'Idared', 'Jonagold', 'Golden Delicious', 'Red Delicious', 'Granny Smith' and 'Mutsu' were purchased from local market in Osijek and the traditional apple cultivars, 'Lijepocvjetka', 'Bobovec', 'Adamčica', 'Zlatna Zimska Parmenka', 'Božićnica' and 'Kanadska Reneta' were have been collected from OPG Horvatić, Cvetkovac, 48312 Rasinja. All studied apples are presented in Fig. 1.

Fig. 1. Conventional (first row) and traditional apple cultivars (second row)

Pomological Properties

The pomological determination has been carried out at Faculty of Agriculture of the University of Zagreb, where only healthy fruits were separated and then photographed and analyzed. The analysis of pomological properties (weight, height and width of fruit, number and mass of seeds) was performed on 5 fruits of each variety. The weight of the fruit was determined by a digital two-decimal laboratory scale (OHAUS Corporation, USA) and expressed in grams (g). The height (V) and the width (s) of the fruit are measured by the digital displacement meter (Somet, Czech Republic), and the values are expressed in millimeters (mm). From the height and width data obtained, the fruit shape index is represented by the ratio of height:width. The number of the healthy seeds from the cross-section of the apples was determined. The weight of the healthy seeds was determined by the four-decimal analytical scales (KERN® Analytical balance AES-C / AEJ-CM) expressed in miligrams (mg). The content of soluble solids of apples was measured with a table top Abbe refractometer and given in Brix (°Brix). pH was measured with table top pH meter (Mettler–Toledo GmbH, Giessen/D).

Determination of Total Polyphenol Content

The total phenols content was determined by the modified colorimetric Folin-Ciocalteu method (Lončarić et al., 2014). A 0.6 mL of apple extract was mixed with 3 mL of Folin-Ciocalteu reagent (1:10) and 2.4 mL of 7.5% of sodium carbonate (Na_2CO_3) solution in the test tubes. The colour was developed during 120 min, and the absorbance was read at 765 nm by spectrophotometer (Jenway 6300, Bibby Scientific, UK). The measurements were performed in triplicates for each sample and the average value was interpolated on a gallic acid calibration curve and expressed as mg of gallic acid per 100 g of sample equivalents, (mg GA/100 g) of fresh weight (FW) sample. Gallic acid calibration solutions were prepared in 5 point range from 0 to 500 mg of gallic acid per 100 g of solution.

Antioxidant Activity Determination

Antioxidant activity was measured by using DPPH method; 0.2 mL of the apple extract was diluted with methanol (2 mL), and 1 mL of DPPH solution (0.5 mM) was added. After 15 min the absorbance was measured at 517 nm. The results were expressed as mmol trolox equivalents/100 mL of sample. Additional dilution was needed if the measured DPPH value was over the linear range of the standard curve (Lončarić et al., 2014).

Statistical analysis

All measurements were done in at least triplicate and data were expressed as mean ± standard deviation. Normal distribution and homogeneity of cultivars for the experimental data were established with Shapiro-Wilkovim and Levenovim testom after which the experimental data were subjected to a one-way analysis of variance (ANOVA). Fisher's LSD was calculated to detect significant difference ($p \leq 0.05$) between the mean values within each group (traditional and conventional) separately. MS Excel (StatPlus, AnalystSoft Inc.) statistical program was used for statistical analysis. Pearson's correlation coefficient was calculated using Microsoft Excel 2016 (StatPlus, AnalystSoft Inc.) in order to determine correlation between total phenol content and antioxidant activity in conventional and traditional apple cultivars.

Results and discussion

Fruit quality

Market criterion for the first class fruit regarding the weight is from 160 to 180 g (Salkić et al., 2017). From the results presented in Table 1, it can be seen that all conventional apples belongs to the first class fruits. Regarding the weight of traditional apple cultivars, only 'Lijepocvijetka' and 'Kandaska Reneta' belongs to the first class fruits (Table 2). The fruit weight of traditional apple cultivars was in agreement with the results obtained by other studies (Jemrić et al., 2013; Balík et al., 2012; Mratinić and Fotirić-Akšić, 2012). The cultivars 'Red Delicious' and 'Gold Delicious' had highest fruit height 81.64 mm and 81.25 mm, respectively with no difference between those two cultivars (Table 1). 'Kanadska Reneta' had the highest fruit height (66.00 mm) regarding the traditional apple cultivars (Table 2). There was significant difference ($p < 0.005$) comparing the fruit height between conventional and traditional apple cultivars indicating that traditional varieties do not have an attractive appearance which puts them in the other plan at choice of customers.

Table 1. Pomological property of conventional apple cultivars

Apple cultivars	'Idared'	'Jonagold'	'Golden Delicious'	'Red Delicious'	'Granny Smith'	'Mutsu'	Average
Fruit weight (g)	212.53 ± 4.55^{b}	213.92 ± 14.81^{bc}	248.49 ± 17.38^{b}	352.85 ± 35.48^{a}	205.64 ± 6.96^{c}	246.68 ± 25.91^{b}	264.69 ± 54.58
Fruit height (mm)	68.40 ± 2.52^{b}	68.67 ± 0.80^{b}	81.25 ± 3.73^{a}	81.64 ± 6.02^{a}	70.18 ± 1.46^{b}	73.87 ± 5.55^{b}	74.00 ± 6.58
Fruit width (mm)	82.18 ± 2.36^{b}	79.33 ± 4.18^{b}	82.67 ± 3.42^{b}	97.51 ± 4.84^{a}	77.95 ± 2.50^{b}	79.83 ± 1.85^{b}	83.24 ± 7.34
Fruit shape index	0.83 ± 0.04^{c}	0.87 ± 0.04^{bc}	0.98 ± 0.05^{a}	0.84 ± 0.08^{bc}	0.90 ± 0.03^{abc}	0.92 ± 0.05^{ab}	0.89 ± 0.07
Total number of seeds	10.00 ± 1.00^{b}	7.00 ± 1.00^{c}	8.00 ± 1.00^{bc}	7.67 ± 0.58^{bc}	12.67 ± 2.52^{a}	7.00 ± 1.00^{c}	8.72 ± 2.37
Number of non-healthy seeds	4.67 ± 0.58^{ab}	4.00 ± 1.00^{bc}	5.67 ± 0.58^{a}	4.33 ± 1.15^{abc}	3.00 ± 1.00^{c}	4.33 ± 0.58^{abc}	4.33 ± 1.08
Number of healthy seeds	5.33^{b}	3.00^{c}	2.33^{c}	3.33^{bc}	9.67^{a}	2.67^{c}	4.39 ± 2.79
Mass of healthy seeds (mg)	350 ± 80^{ab}	160 ± 60^{b}	160 ± 40^{b}	220 ± 70^{ab}	550 ± 80^{a}	100 ± 40^{c}	260 ± 160
Mass of one seed (mg)	70 ± 00^{a}	50 ± 00^{c}	70 ± 10^{ab}	70 ± 10^{ab}	60 ± 0.00^{bc}	40 ± 10^{d}	60 ± 10

Each value is expressed as mean ± standard deviation (n = 3). Within the same row, means followed by different letters are significantly different at $p \leq 0.05$, (ANOVA, Fisher's LSD)

Table 2. Pomological property of traditional apple cultivars

Apple cultivar	'Lijepocvjetka'	'Bobovec'	'Adamčica'	'Zlatna Zimska Parmenka'	'Božićnica'	'Kanadska Reneta'	Average
Fruit weight (g)	179.13 ± 33.15^{b}	104.57 ± 1.90^{c}	95.60 ± 0.00^{c}	116.59 ± 45.64^{bc}	155.52 ± 46.73^{bc}	293.01 ± 13.81^{a}	157.64 ± 68.95
Fruit height (mm)	64.18 ± 3.45^{ab}	54.13 ± 0.06^{bc}	43.81 ± 0.00^{d}	52.18 ± 7.07^{cd}	52.33 ± 4.50^{cd}	66.00 ± 4.55^{a}	55.26 ± 8.74
Fruit width (mm)	75.51 ± 4.78^{bc}	63.37 ± 1.11^{c}	63.27 ± 0.01^{c}	64.98 ± 8.08^{c}	79.31 ± 7.90^{ab}	90.67 ± 0.34^{a}	72.99 ± 10.74
Fruit shape index	0.85 ± 0.01^{ab}	0.85 ± 0.01^{a}	0.69 ± 0.00^{cd}	0.80 ± 0.02^{b}	0.66 ± 0.00^{d}	0.73 ± 0.01^{c}	0.76 ± 0.07
Total number of seeds	8.00 ± 1.41^{b}	13.50 ± 2.12^{a}	8.00 ± 0.00^{b}	9.50 ± 0.71^{b}	8.00 ± 0.00^{b}	8.00 ± 0.00^{b}	9.85 ± 2.60
Number of non-healthy seeds	2.00 ± 0.00^{b}	4.00 ± 1.41^{a}	0.00^{c}	3.50 ± 0.54^{ab}	3.00 ± 2.82^{ab}	3.00 ± 2.83^{ab}	2.65 ± 2.12
Number of healthy seeds	6.00^{a}	9.50^{a}	8.00^{a}	6.00^{a}	5.00^{a}	5.00^{a}	7.18 ± 2.40^{a}
Mass of healthy seeds (mg)	380 ± 40^{a}	330 ± 40^{a}	350 ± 10^{a}	70 ± 40^{b}	250 ± 20^{a}	260 ± 110^{a}	334 ± 118
Mass of one seed (mg)	60 ± 10^{a}	30 ± 0.00^{b}	40 ± 00^{ab}	50 ± 20^{a}	50 ± 10^{a}	50 ± 10^{a}	48 ± 11

Each value is expressed as mean ± standard deviation (n = 3). Within the same row, means followed by different letters are significantly different at $p \leq 0.05$, (ANOVA, Fisher's LSD)

Fruit width as well as axis height is important from the agronomic and market point of view (Salkić et al., 2017). The largest fruit width had conventional apple cultivar 'Red Delicious' (97.51 mm) followed by traditional apple cultivar 'Kanadska Reneta' (90.67 mm). Generally traditional apple cultivars have smaller fruit width compared to the conventional apple cultivars. Based on fruit width and criteria in the European market (65-90 mm first class (Salkić et al., 2017) it can be concluded that most of the investigated cultivars, except 'Bobovec and 'Adamčica', belong to the first class fruits. The fruit shape index is represented by the ratio of height and width and it was used for detection of the fruit shape by the following scale; less than or equal to 0.90 - flattened fruit, from 0.90 - 1.00 elliptical fruit and 1.0 and higher elongated fruit (Salkić et al., 2017). In our study fruit index ranged from 0.66 – 0.90 for almost all studied apples whereby they can be classified as flattened fruit except 'Golden Delicious' (0.98) and 'Mutsu' (0.92) which have elliptical shape. Investigation of apple seeds showed that traditional apple cultivars had healthier seeds compared to the conventional apple cultivars (Tables 1 and 2). The highest total soluble solids (TSS) and lowest pH of all investigated cultivars had traditional apple cultivar 'Zlatna Zimska Parmenka'. Generally the traditional apple cultivars had higher total soluble solids 14.45% comparing to the conventional 12.92% and lower pH 3.19 compared to conventional apple cultivars 3.55 (Fig. 2). Both observed parameters are in the range of those reported for other conventional and traditional apple cultivars (Piegentini and Pirovani, 2017; Lončarić and Piližota, 2014; Jha et al., 201).

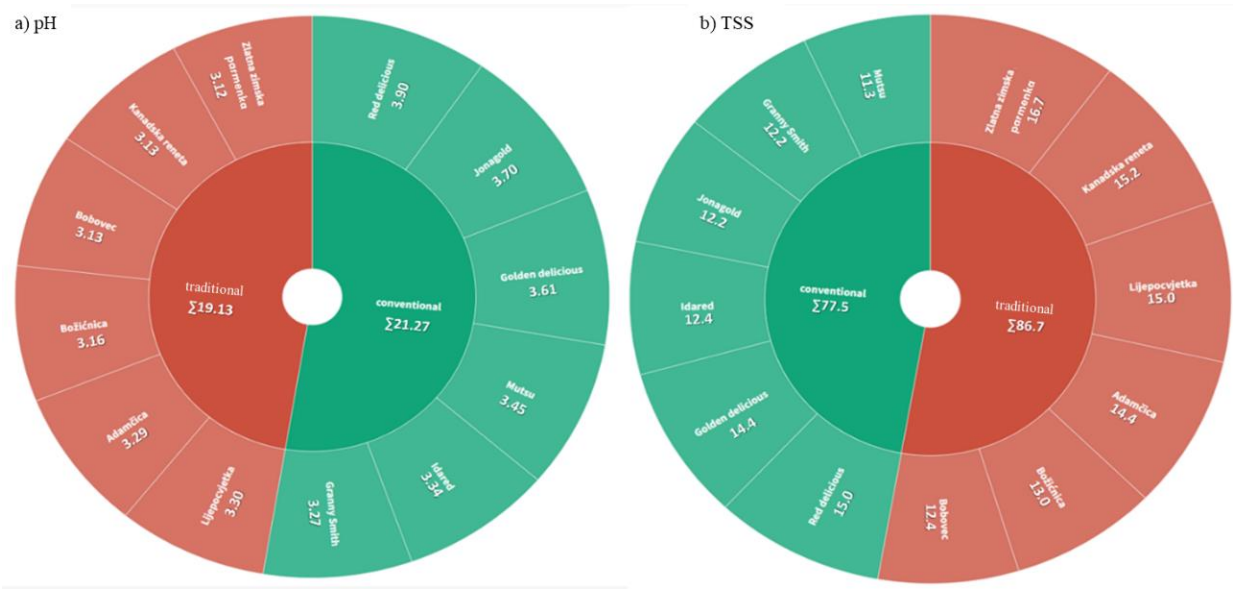

Fig. 2. Total soluble solids (b - TSS) and a - pH of activity of traditional and conventional apple cultivars

The total polyphenol content and antioxidant activity

The polyphenol content of fruits and vegetables depends on a number of intrinsic (genus, species, and cultivar) and extrinsic (agronomic, environmental, handling and storage) factors (Khanizadeh et al., 2007; Qing et al., 2007). Many studies pointed that intrinsic factors affects much more the polyphenol content of apples than extrinsic factors (Ceymann et al., 2012; Neveu et al., 2010; Wojdyło et al., 2008; Matthes and Schmitz-Eiberger, 2008; Łata, 2007; Łata and Tomala, 2007; McGhie et al., 2005; Kondo et al., 2002). The reasons for determination the total phenol content and antioxidant activity of traditional apple varieties was to enable the recognition of those varieties as a valuable source of polyphenols.

Table 3. Total phenol content and antioxidant activity of traditional and conventional apple cultivars

Apple cultivar	TPC (mg GA/100 g FW)	AOA (mmol trolox /100 mL)
Idared	160.59 ± 6.62^{cd}	384.22 ± 1.11^{b}
Jonagold	169.07 ± 1.83^{c}	398.74 ± 2.75^{a}
Golden Delicious	187.08 ± 5.50^{b}	370.91 ± 6.26^{b}
Red Delicious	252.75 ± 3.67^{a}	336.07 ± 13.74^{c}
Granny Smith	155.30 ± 8.41^{d}	398.98 ± 3.84^{a}
Mutsu	139.41 ± 3.18^{e}	373.09 ± 0.42^{b}
Lijepocvjetka	260.17 ± 0.00^{f}	402.37 ± 2.75^{b}
Bobovec	289.83 ± 3.67^{e}	368.01 ± 6.17^{d}
Adamčica	499.58 ± 8.00^{a}	418.83 ± 2.75^{a}
Zlatna Zimska Parmenka	387.29 ± 6.36^{c}	391.97 ± 1.51^{c}
Božićnica	402.12 ± 1.83^{b}	407.70 ± 8.08^{b}
Kanadska Reneta	330.08 ± 3.18^{d}	389.79 ± 3.27^{c}

Each value is expressed as mean ± standard deviation (n = 3). Within the same column, means followed by different letters are significantly different at $p \leq 0.05$, (ANOVA, Fisher's LSD)

Total phenol content (TPC) of convention apples were as in the previously reported range (Kschonsek et al., 2018; Piagentini et al., 2017; Ceymann et al., 2012; Drogoudi et al., 2008). The highest TPC have 'Red Delicious' followed by 'Golden Delicious', 'Jonagold', 'Idared', 'Granny Smith' and 'Mutsu', respectively (Table 3). Determination of total phenol content of traditional apple cultivars showed that all traditional apple cultivars have higher content of total polyphenols compared to conventional apple cultivars. The highest TPC of traditional apple cultivars have 'Adamčica' followed by 'Božićnica', 'Zlatna Zimska Parmenka', 'Kanadska Reneta', 'Bobovec' and 'Lijepocvjetka', respectively. The highest antioxidant activity was measured in traditional apple cultivar 'Adamčica' 418.83 mmol trolox /100 mL and the lowest in 'Red Delicious' 336.07 mmol trolox /100 mL. Person's correlation coefficient showed that there is no significant correlation between total phenol content and antioxidant activity in conventional apple cultivars (r = -0.806, $p < 0.05$). However, correlation between total phenol content and antioxidant activity was found in traditional apple cultivars (r = 0.667, $p < 0.05$).

Conclusion

The results of pomological properties showed that all conventional apple cultivars belong to the first class fruits. Regarding the traditional apple cultivars, results showed that they do not have satisfactory appearance except 'Kanadska Reneta'. Despite to that, traditional apple cultivars are rich in polyphenols and have high antioxidant activity; even higher than those found in conventional varieties. The results showed that due to the diversity of pomological characteristics and polyphenol content, traditional apple varieties represent a good source of genetic variability as well as a factor of biodiversity of the area where they are grown. With satisfactory appearance, high total polyphenol content and antioxidant activity 'Kanadska Reneta' might be suitable cultivar for selective growth in order to produce varieties with higher content of bioactive compounds and preservation of biodiversity of M. pulmila.

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