Short Communication

UDC: 665.766
DOI: 10.2478/contagri-2019-0010

A COMPARISON OF ANTIOXIDANT PROPERTIES OF TWO APPLE CULTIVARS

EMIR ŠAHINOVIĆ¹, SENAD MURTIĆ^{1*}

¹Faculty of Agriculture and Food Sciences, University of Sarajevo, Zmaja od Bosne 8, 71 000 Sarajevo, Bosnia and Herzegovina

*Corresponding author: murticsenad@hotmail.com

SUMMARY

The apple (*Malus domestica* Borkh.) possesses a number of health-promoting properties. The main beneficial effects of apple fruits on human health are associated with the antioxidant properties of components synthesising in apple fruits during their growth and development. Phenolic compounds and flavonoids are such components that contribute significantly to the antioxidant capacity of fruits, thus enhancing their health properties. The purpose of this study is to compare the antioxidant properties (namely antioxidant capacity, ascorbic acid, total phenolic and flavonoid contents) of the 'Idared' and 'Braeburn' apple cultivars, grown under optimal growing conditions in the Goražde area. Ascorbic acid contents were estimated using the 2,6-dichlorophenolindophenol titration method, whereas total phenolics were determined using the Folin-Ciocalteu method. Furthermore, total flavonoids were determined using the aluminium chloride colorimetric assay, whereas the ferric reducing/antioxidant power assay was used to determine the total antioxidant capacity of the apple fruits examined. The average values of the ascorbic acid, the total phenolic and flavonoids contents, and the total antioxidant capacity of the 'Idared' apples under consideration were 8.09 mg 100 g⁻¹, 34.56 mg gallic acid equivalents 100 g⁻¹, 12.62 mg catechin equivalents 100 g⁻¹ and 323.28 µmol Fe²⁺ 100 g⁻¹ of fresh weight, respectively. The average values of the total phenolic and flavonoids contents, as well as the antioxidant capacity of the 'Braeburn' apples examined were as follows: 8.89 mg 100 g⁻¹, 33.4 mg gallic acid equivalents 100 g⁻¹, 13.56 mg catechin equivalents 100 g⁻¹ and 367.01 µmol Fe²⁺ 100 g⁻¹ of fresh weight, respectively. The analyses performed indicate that the differences recorded in the parameter values between the apple cultivars 'Idared' and 'Braeburn' were statistically non-significant. A correlation data analysis confirmed that phenolic compounds are the carriers of the antioxidant activity of apples.

Key words: antioxidant capacity, health, phenolic compounds, flavonoids

Abbreviations: AA - ascorbic acid; TPC - total phenolic content; TFC - total flavonoid content; TAC - total antioxidant capacity, FW - fresh weight

INTRODUCTION

The health-enhancing properties of apples are mainly related to their chemical composition, primarily antioxidants (i.e. substances that prevent or delay oxidative degradation of active ingredients) (Hyson, 2011). Phenolic compounds are the most abundant antioxidants of apples and their antioxidant activity is associated with a large number of conjugated double bonds and hydroxyl groups in one or more phenolic rings of the annular structure of molecules (Del Rio et al., 2013). Owing to this structure, phenolic compounds have the ability to stabilize free radicals by delocalization of the unpaired electron on aromatic rings to create a stable resonance, i.e. low-energy radicals (Tsao, 2010).

Flavonoids comprise the largest group of phenolic compounds in apples, as well as in plants generally, and are considered the most effective antioxidants in plant cells (Arct & Pytkowska, 2008). Ascorbic acid is also an important antioxidant substance in plants. It reacts rapidly with reactive forms of oxygen which are generated during the aerobic metabolism or during the exposure of plants to stress conditions, thus participating in their neutralization or removal from plant cells (Li et al., 2014).

Numerous studies have found that the synthesis of antioxidant substances in apples is influenced by different factors such as agroecological conditions, harvest time, storage and cultivar selection (Kevers et al., 2011; Kschonsek et al., 2018). Over the last two decades, a number of relatively novel apple cultivars, such as 'Braeburn', have become more desirable among consumers in Bosnia and Herzegovina, resulting in a gradual increase in the presence of these cultivars in apple orchards. However, 'Idared' is still the predominant apple cultivar in the orchards in Bosnia and Herzegovina.

Considering that the most popular apple cultivars in the Goražde area are 'Braeburn' and 'Idared', as well as the fact that the current trend in apple production is enhancing the antioxidant properties of apples, the purpose of this study is to compare the antioxidant properties (namely antioxidant capacity, ascorbic acid, total phenolic and flavonoid contents) of the 'Idared' and 'Braeburn' apple cultivars, grown under optimal growing conditions in the Goražde area.

MATERIALS AND METHODS

The experiment was performed in an intensive apple orchard in the Goražde area (43°38'N 18°55'E; altitude of 455 m a.s.l.) in 2018. The experimental site features a humid continental climate, with an annual precipitation of about 1,137 mm, an average annual temperature of 8.7°C, and a sandy loam soil. The apple orchard was established in the autumn of 2002, covering about 1 ha, and half (50%) of the total production area was devoted to the 'Idared' and 'Braeburn' cultivars. The 'Idared' and 'Braeburn' apple trees on M9 rootstock were planted at a spacing of 1.3 × 3.5 m with an east-west row orientation. The experimental plot included a row of six apple trees for each cultivar and three such plots were the subject of study. Cultivation forms (slender spindle) were identical in all experimental plots. Moreover, all agricultural practices (fertilization, pruning, pest and disease management, irrigation) were applied identically to all the plots examined during the growing season.

'Idared' and 'Braeburn' apple fruits were collected at the stage of their commercial maturity. The 'Idared' cultivar produces medium-sized round apples with a firm body and green skin that is strongly streaked red especially on the sun-facing side. Furthermore, the 'Idared' apple has a white to creamy flesh with a sweet-tart flavour. The 'Braeburn' cultivar boasts a very firm, crisp, aromatic and exceptionally juicy flesh with a red skin, striped over a yellow background. For analysis purposes, three fruits were taken from the central zone of the crown of each experimental tree and transported to the laboratory in a portable refrigerator. In total, 54 fruits per cultivar were harvested for chemical analysis. The following chemical properties of fruits were determined: ascorbic acid content (AA), total phenolics (TPC) and flavonoids content (TFC), and total antioxidant capacity (TAC).

AA contents were estimated using the 2,6-dichlorophenolindophenol titration method (AOAC, 2006). A triplicate titration was conducted for all fruit juice samples (10 juice samples per cultivar), and the results obtained were expressed as mg of ascorbic acid per 100 g⁻¹ of fresh fruit (mg AA 100 g⁻¹ FW).

The extraction of phenolic compounds from the apple samples was performed using a 30% aqueous ethanol solution as follows: 1 g of dried and grinded apple sample was placed into a 100 ml glass bottle and mixed with 40 ml ethanol solution. Thereafter, the mixture was placed in an ultrasonic bath under the following conditions: a time of 30 min, a temperature of 35°C, and a power of 150 W (the mixture was vortexed thoroughly once every 5 min). After cooling down to room temperature, the mixture was filtered through Whatman filter paper, 11µm pore size) into a 50 ml flask and diluted to the mark with 30% aqueous ethanol solution. The prepared extract was used for the analysis of TPC, TFC and TAC.

TPC was determined according to the Folin-Ciocalteu method (Ough & Amerine, 1988) as follows: 0.25 ml of extract was placed into a 25 ml flask, then 15 ml distilled water, 1.25 ml Folin-Ciocalteu's phenol reagent, and 3.75 ml saturated sodium carbonate solution was added (Folin-Ciocalteu's reagent was diluted with distilled water in the 1:2 ratio before use). Thereafter, the flask was filled with 30% aqueous ethanol solution to the mark, and heated in a water bath at 50°C, for 30 min. After cooling to room temperature, absorbance was measured at 765 nm using the UV/VIS spectrophotometer (Schimadzu, Japan). TPC quantification was based on a standard curve of gallic acid (0 - 500 mg l⁻¹), and the results obtained were expressed as mg of gallic acid equivalent per 100 g of fresh weight (mg GAE 100 g⁻¹ FW).

TFC was determined according to the aluminium chloride colorimetric assay (Zhishen et al., 1999) as follows: 1 ml of extract was placed into a 10 ml flask, then 4 ml distilled water, 0.3 ml 5% NaNO₂ 0.3 ml 10% AlCl₃ and 2 ml 1 mol l⁻¹ NaOH was added. The flask was filled with distilled water to the mark and, after 15 minutes, absorbance was measured at 510 nm. TFC quantification was based on a standard curve of catechin (0 -100 mg l⁻¹), and the results obtained were expressed as mg of catechin equivalent per 100 g of fresh weight (mg CE 100 g⁻¹ FW).

Total antioxidant capacity (TAC) was determined according to the Ferric reducing ability power (FRAP) method (Benzie & Strain, 1996) as follows: 240 µl distilled water, 80 µl extract, and 2080 µl FRAP reagent (obtained by mixing 0.3 mol l⁻¹ acetate buffer (pH = 3.6), 10 mmol l⁻¹ TPTZ (2,4,6-tripyridyl-s-triazine) and 20 mmol l⁻¹ FeCl₃ × 6 H₂O in ratio 10:1:1) were added into a 10 ml flask and heated in a water bath at 37°C for 5 min. After cooling to room temperature, absorbance was measured at 595 nm. TAC quantification was based on a standard curve of FeSO₄ × 7 H₂O (0-2000 µmol l⁻¹), and the results obtained were expressed as µmol Fe²⁺ per 100 g of fresh weight (µmol Fe²⁺ 100 g⁻¹ FW).

The data processing was performed by the analysis of variance (ANOVA) using the Microsoft Excel 2010 software. Different means obtained between the treatments were tested using the least-significant difference (LSD) test at a 0.05 significance level of probability. All the experimental measurements were done in triplicate for each sample and the results were expressed as the average ± standard deviation. The data obtained were evaluated using Pearson's correlation coefficients to identify the relationships between phenolics, flavonoids, ascorbic acid and total antioxidant activity.

RESULTS AND DISCUSSION

Phenolic compounds are secondary metabolites that play an important role in the defence system of plants against reactive oxygen species. Therefore, high contents of phenolic compounds are highly desirable in apple fruits (Pandey & Rizvi, 2009). Different factors such as genetic, morphogenetic and environmental conditions can influence the biosynthesis and accumulation of phenolics. A change in any of the factors stated above may alter the content of phenolics even if other factors remain constant (Yang et al., 2018). In this study, no significant difference was found in the TPC values between the 'Idared' and 'Braeburn' apple cultivars. Furthermore, the AA and TFC values, as well as the TAC values, were found not to differ significantly between the cultivars under consideration (Tab. 1). Slight variations observed in the testing parameters are most likely implicated in the same growing conditions for both apple cultivars.

Table 1. Ascorbic acid, phenolics, flavonoids and antioxidant activity in the apple cultivars 'Idared' and 'Braeburn'

Cultivar	AA (mg AA 100 g ⁻¹ FW)	TPC (mg GAE 100 g ⁻¹ FW)	TFC (mg CE 100 g ⁻¹ FW)	TAC (µmol Fe ²⁺ 100 g ⁻¹ FW)
Idared	8.09 ± 0.83	34.56 ± 19.02	12.62 ± 5.76	323.28 ± 220.09
Braeburn	8.89 ± 2.50	33.4 ± 20.25	13.56 ± 2.86	367.01 ± 210.11
ANOVA	n. s.	n. s.	n. s.	n. s.

Legend: AA - ascorbic acid; TPC - total phenolic content; TFC - total flavonoid content; TAC - total antioxidant capacity; FW - fresh weight; n. s. - no significant

For most plants, external variables of environmental factors (drought, salinity, light, temperature, nutrition etc.) can significantly affect some process associated with secondary metabolism, leading to an increase in both phenolic compounds and antioxidant activity (Verma & Shukla, 2015). Several studies have demonstrated that the phenolic content in apple fruits may differ significantly, depending on the growth conditions (Zupan et al., 2014; Mditshwa et al., 2015). Veberic et al. (2005) noted that genetic factors, i.e. cultivars, also have an important role in the biosynthesis of phenolic compounds and, consequently, in the antioxidant activity of apples. Our study is not consistent with this hypothesis. As only two apple cultivars were involved in the present study, a valid conclusion cannot be drawn with irrefutable certainty regarding the effect of cultivars on the synthesis and accumulation of phenolic compounds in apples.

This study unequivocally confirmed a very strong relationship between the TPC/TFC and TAC of apples, regardless of the cultivar (Fig. 1). These results indicate that the apple TAC may be principally caused by the phenolic compounds. A significant correlation between the content of phenolic/flavonoid compounds and the total antioxidant activity of apples has been observed in a number of other studies (Lee et al., 2003; Wojdylo et al., 2008; Stracke et al., 2009).

Figure 1. Correlation coefficients between ascorbic acid content (AA), total phenolics (TPC), flavonoids content (TFC), and total antioxidant capacity (TAC) (FRAP) in the apple cultivars 'Idared' and 'Braeburn'

In both the 'Idared' and 'Braeburn' apple cultivars, the correlations between the AA and TAC contents proved high, but the strength of these correlations was slightly lower than the correlation between TPC/TFC and TAC.

According to Huang et al. (2005), the antioxidant capacity can be monitored by a wide variety of assays with different mechanisms such as the hydrogen atom transfer (HAT) reaction and the electron transfer (ET) reaction-based assays. The choice of assays depends on the reaction between free radicals and antioxidants. HAT-based assays measure the potential of an antioxidant to neutralize free radicals via hydrogen donation, whereas the ET-based assays measure the potential of an antioxidant to transfer one electron in order to neutralize free radicals. All the reactions stated above are followed by a colour change, which is the base for the antioxidant capacity calculation (Apak et al., 2007).

Considering that all the assays argued in the present study share the same mechanism, it was expected that the correlations between phenolics/flavonoids and the total antioxidant capacity of apples would be high, which was ultimately confirmed by the results obtained.

CONCLUSION

The results obtained in the present study show that differences in the antioxidant properties (TAC, AA, TPC and TFC) of the 'Idared' and 'Braeburn' apple cultivars, grown under the same conditions, are not statistically significant. This study also revealed that phenolic compounds significantly participate in the antioxidant capacity of apples.

REFERENCES

- Apak R., Güçlü K., Demirata B., Özyürek M., Çelik S.E., Bektaşoğlu B., Berker K.I., Özyurt D. (2007): Comparative Evaluation of Various Total Antioxidant Capacity Assays Applied to Phenolic Compounds with the CUPRAC Assay. *Molecules*, 12: 1496-1547.
- Arct J. & Pytkowska K. (2008): Flavonoids as components of biologically active cosmeceuticals. *Clinics in dermatology*, 26: 347-357, 2008.
- AOAC (2006): Official Methods of Analysis: Vitamin C in juices and vitamin preparations, method 967.21. 18th edition. Arlington.
- Benzie I.F. & Strain J.J. (1996): Ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. *Analytical Biochemistry*, 239: 70-76.
- Del Rio D., Rodriguez-Mateos A., Spencer J.P., Tognolini M., Borges G., Crozier A. (2013): Dietary (poly)phenolics in human health: Structures, bioavailability, and evidence of protective effects against chronic diseases. *Antioxidants & Redox Signaling*, 18: 1818-1892.
- Huang D., Ou B., Prior RL. (2005): The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry*, 53(6): 1841-1856.
- Hyson D.A. (2011): A comprehensive review of apples and apple components and their relationship to human health. *Advances in Nutrition*, 2(5): 408-420.

- Kevers C., Pincemail J., Tabart J., Defraigne J.O., Dommès J. (2011): Influence of cultivar, harvest time, storage conditions, and peeling on the antioxidant capacity and phenolic and ascorbic acid contents of apples and pears. *Journal of Agricultural and Food Chemistry*, 59(11): 6165-6171.
- Kschonsek J., Wolfram T., Stöckl A., Böhm V. (2018): Polyphenolic Compounds Analysis of Old and New Apple Cultivars and Contribution of Polyphenolic Profile to the In Vitro Antioxidant Capacity. *Antioxidants*, 7(1): 20.
- Lee K.W., Kim Y.J., Kim D., Lee H.J., Lee C.Y. (2003): Major phenolics in apple and their contribution to the total antioxidant capacity. *Journal of Agricultural and Food Chemistry*, 51: 6516-6520.
- Li Z., Zhang H., Yang O., Komla M.G., Zhang X., Zhu S. (2014): Ascorbic Acid Enhances Oxidative Stress Tolerance and Biological Control Efficacy of *Pichia caribbica* against Postharvest Blue Mold Decay of Apples. *Journal of Agricultural and Food Chemistry*, 62(30): 7612-7621.
- Mditshwa A., Vries F., Van der Merwe K., Crouch E., Opara U.L. (2015): Antioxidant content and phytochemical properties of apple 'Granny Smith' at different harvest times. *South African Journal of Plant and Soil*, 32(4): 221-226.
- Ough C.S. & Amerine M.A. (1988): *Phenolic compounds*. In: Methods for analysis of must and wines (Wiley, Ed.). New York, pp. 50-70.
- Pandey K.B. & Rizvi S.I. (2009): Plant polyphenols as dietary antioxidants in human health and disease. *Oxidative Medicine and Cellular Longevity*, 22(5): 270-278.
- Stracke A.B., Rüfer C.E., Bub A., Weibel F.P., Kunz C., Watzl B. (2009): Three-year comparison of the polyphenol contents and antioxidant capacities in organically and conventionally produced apples (*Malus domestica* Bork. cultivar Golden Delicious). *Journal of Agricultural and Food Chemistry*, 57: 4598-4605.
- Tsao R. (2010): Chemistry and biochemistry of dietary polyphenols. *Nutrients*, 2(12): 1231-1246.
- Veberic R., Trobec M., Herbinger K., Hofer M., Grill D., Stampar F. (2005): Phenolic compounds in some apple (*Malus domestica* Borkh.) cultivars of organic and integrated production. *Journal of the Science of Food and Agriculture*, 85: 1687-1694.
- Verma N. & Shukla S. (2015): Impact of various factors responsible for fluctuation in plant secondary metabolites. *Journal of Applied Research on Medicinal and Aromatic Plants*, 2: 105-113.
- Wojdylo A., Oszmianański J., Laskowski P. (2008): Polyphenolic compounds and antioxidant activity of new and old apple varieties. *Journal of Agricultural and Food Chemistry*, 56: 6520-6530.
- Yang L., Wen K.S., Ruan X., Zhao Y.X., Wei F., Wang Q. (2018): Response of Plant Secondary Metabolites to Environmental Factors. *Molecules*, 23: 762.
- Zhishen J., Mengcheng T., Jianming W. (1999): The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry*, 64(4): 555-559.
- Zupan A., Mikulic-Petkovsek M., Slatnar A., Stampar F., Veberic R. (2014): Individual phenolic response and peroxidase activity in peel of differently sun-exposed apples in the period favourable for sunburn occurrence. *Journal of Plant Physiology*, 171: 1706-1712.

Submitted: 12.4.2019.

Accepted: 4.10.2019.