Pol. J. Environ. Stud. Vol. 22, No. 2 (2013), 475-480

Original Research

The Content of Some Antioxidants in Apple Depends on the Type of Fertilization

Senad Murtić[1]*, Hamdija Čivić[1], Milena Đurić[2], Gorica Paunović[2], Gordana Šekularac[2], Fikreta Behmen[1], Milorad Krsmanović[2]

[1]Faculty of Agriculture and Food science, University of Sarajevo, Bosnia and Herzegovina
[2]Faculty of Agriculture Čačak, University of Kragujevac, Serbia

Received: 21 February 2012
Accepted: 2 October 2012

Abstract

Different agro-technical measures are used in growing apples and fruit crops in general in an attempt to increase the rate of photosynthesis and, hence, both the quantitative and qualitative value of the fruit. Very rapid and effective results in terms of an increase in the above parameters can be achieved through adequate fertilization adapted to plant requirements. Fertilization can substantially alter and disrupt the course of plant development if fertilizers are inadequately applied, regardless of the adequately pre-determined plant nutrient requirements.

The objective of this study was to evaluate the effect of four different types of fertilization on the pigment and antioxidant content of apple cv. Idared (*Malus domestica* cv. Idared) grown in orchards in the municipality of Gradačac.

The results obtained show that fertigation and foliar fertilization induced the statistically highest increase in the test parameters. These types of fertilization, if adequately employed, provide nutrition to plants according to their requirements at a specific phenostage, leading to increased yields and enhanced fruit quality, and indirectly reducing nutrient leaching from soils – a frequent accompanying manifestation of other fertilization types.

Keywords: fertigation, foliar application, pigments, vitamin C, phenols

Introduction

The current trend in apple production and agriculture in general is to enhance the quality of products by increasing the amount of antioxidant substances in edible parts of plants. Antioxidants are substances that have a very important role in strengthening the human immune system, particularly as regards neutralization of the adverse effects of free radicals [1]. Among the antioxidant substances found in apple fruits, β-carotene, vitamin C, and phenolic components occur in significant amounts [2]. The synthesis of these antioxidant substances in the plant is closely related to the photosynthetic process; therefore, different agro-technical measures are used in apple cultivation to increase the rate of photosynthesis. Fertilization can significantly increase the rate of photosynthesis provided that all quality fertilization conditions are satisfied, including not only an adequate choice of fertilizer and formulation and fertilizer amount, but also proper fertilizer according to plant requirements during a certain stage of development [3].

The objective of this study conducted in an apple cv. Idared orchard located in the area of Gradačac was to evaluate the effect of four different types of fertilization on the pigment and antioxidant contents in apple, determine their mutual correlations according to plant development stages, and recommend the most suitable type of fertilization for

*e-mail: murticsenad@hotmail.com

use in apple cultivation at the test location. To this end, determination was made of the content of pigments that have the most important role during photosynthesis: chlorophyll *a*, chlorophyll *b*, β-carotene; and the content of antioxidants that substantially improve the functional and nutritive value of apple fruit: vitamin C and phenols.

Material and Methods

Four different types of fertilization were used in this study. The types of fertilization differed only during growing season whereas basal (autumn) fertilization was identical in all applied types at a particular location. During the growing season, the following variants of fertilization were involved: fertigation, foliar fertilization, conventional fertilization applied in two different variants, and a control variant without the use of fertilizers during the growing season, respectively.

The location used for the research was the municipality of Gradačac, one of the most favorable regions for apple cultivation in Bosnia and Herzegovina according to its agro-environmental characteristics. The present research involved apple cv. Idared for a number of reasons: this cultivar is highly adaptable to different agro-climatic conditions, and it is suitable for intensive cultivation, high-yielding, good-quality, finely colored fruits [4]. These characteristics have made Idared the most commonly grown cultivar in apple plantations in Bosnia and Herzegovina. Therefore, attempts at improving its production are of importance both for producers and consumers.

The first stage of the study was conducted in autumn 2009 involving basal (autumn) fertilization with manure and mineral fertilizers at rates determined after soil chemical analysis. The soil chemical analysis covered determination of soil acidity, available potassium and phosphorus levels in the soil, and humus content. Soil acidity was determined by a pH meter [5], potassium and phosphorus levels by AL-method [6], and humus content by colorimetric method [7]. Results on soil analysis at the test location are given in Table 1.

Soil chemical analysis at the test site showed that the soil was acid to very acid. The humus content and available phosphorus levels in the soil were within reference values required for apple cultivation [8], whereas potassium content was exceptionally high. Based on the results obtained, basal fertilization was performed (involving liming for reduced acidity of the soil), followed by phosphorus fertilization, with potassium fertilization being completely excluded. Basal fertilization was identical throughout the orchard, with no differences between the test orchard plots. The trial plot included a row of fruit trees, each containing five trees of apple cv. Idared grafted onto the same rootstock (M_9). The age of the apple trees was seven years, the training system was a slender spindle, and the spacing between trees was 3.3×1.2 m (2,525 trees per ha).

The second stage of the study was conducted in 2010, involving evaluation of the effect of spring/summer fertilization on the test parameters in leaves and fruits of apple. The trial was set up as a randomized block design with five variants and three replications. The fertilization variants most widely used in apple nutrition in Bosnia and Herzegovina were employed in this study, including fertigation, foliar fertilization, two conventional fertilization variants, and an unfertilized control. All fertilizer rates within particular fertilization types were defined according to soil nutrient status and fruit requirements during growing, and calculated according to plot size. The rates, types, and dates of fertilization used in spring/summer variants are outlined in Table 2.

Table 1. Results on soil chemical analysis at the test location.

Parameters	Soil	Reference values for apple
pH (H_2O)	6.0	6.2-7.7
pH (KCl)	4.5	5.5-7.2
mg P_2O_5/100 g soil	15.6	12-16
mg K_2O/100 g soil	78.5	20-30
% humus	3.2	3-5

Excepting fertilization, all other agro-cultural measures vital for successful apple development (pruning, pest and disease management, irrigation) were identical in all trial plots.

Leaf sampling for analysis of the test parameters was carried out at the beginning, in the middle and at the end of the growing season, and fruit sampling was performed at commercial maturity. Leaf samples were analyzed for content of pigment, and fruit samples were assessed for total phenols and vitamin C. Content of pigment in apple leaves was determined by spectrophotometry using the Eijckelhoff-Dekker method [9], total phenols in apple fruit by UV/VIS spectrophotometry [10], and vitamin C content by titration method using 2,6-p-dichlorophenol-indophenol [11]. The data obtained were subjected to analysis of variance (F test), and differences between the treatments were determined by LSD-test using standard computer programs (Excel, SAS Program).

Results and Discussion

In order to obtain a more thorough insight into the pigment content dynamics in apple leaves throughout the growing season as dependent upon the fertilization type used, the results are presented separately for each pigment tested.

The results presented in Table 3 suggest that all types of fertilization, except conventional variant A, had a statistically significant effect in terms of increasing the chlorophyll *a* pigment in apple leaves as compared to the unfertilized control. These results comply with those of many authors who determined the positive effect of fertigation on chlorophyll *a* pigment content [12, 13]. The present results,

Table 2. Types, rates, and dates of fertilization in the test treatments.

Treatment	Phenostage	Fertilizer type and formulation	Fertilizer rate	Fertilization date
Fertigation	Onset of growing season (12 Apr-9 May)	Master 13-40-13	75 kg/ha (30 g/tree~ 4 m^2)	3 × at 7-day intervals (10 g/tree)
	Mid growing season (25 May-18 July)	Master 20-20-20	150 kg/ ha (60 g/tree~ 4 m^2)	6 × at 7-day intervals (10 g/tree)
	Fruit maturation (1 Aug-17 Sep)	Master 15-5-30	150 kg/ ha (60 g/tree~ 4 m^2)	6 × at 7-day intervals (10 g/tree)
Foliar fertilization	Onset of growing season (12 Apr-9 May)	Plantafol 10-54-10	0.2% (20 g in 10 l/plot)	3 × at 7-day intervals
	Mid growing season (25 May-18 July)	Plantafol 20-20-20	0.2% (20 g in 10 l/plot)	6 × at 7-day intervals
	Fruit maturation (1 Aug-17 Sep)	Plantafol 5-15-45	0.2% (20 g in 10 l/plot)	6 × at 7-day intervals
Conventional fertilization Variant A	Onset of growing season (12 April)	KAN 27%	187.5 kg/ha (75 g/tree)	single application
Conventional fertilization Variant B	Onset of growing season (12 April)	KAN 27%	375 kg/ha (150 g/tree)	single application
Control fertilization	without fertilization during the growing season			

Table 3. Chlorophyll *a* pigment content in leaves of apple cv. Idared depending the fertilization type.

Variant of fertilization	Average values of chlorophyll *a* pigment (mg/g fresh leaf)						
	Growing season			Average	Differences against control	%	Sign.
	Beginning	Mid	End				
Fertigation	4.36±1.12	4.44±0.81	4.33±0.87	4.38	1.72	64	**
Foliar fertilization	4.06±0.62	3.96±0.66	3.84±0.69	3.95	1.29	48	**
Conventional A	3.22±0.82	2.91±0.73	2.79±0.77	2.97	0.31	11	ns
Conventional B	3.39±0.56	3.41±0.59	3.11±0.64	3.30	0.64	24	*
Control	2.78±0.66	2.82±0.64	2.31±0.55	2.66	0.00	0	
$LSD_{0.05}$ = 0.58, $LSD_{0.01}$ = 0.81							

**high significant, *significant, ns – non significant

outlined in Table 3, also show a significant decrease in chlorophyll *a* pigment content in the leaf of apple cv. Idared throughout the development stages of apple from mid- to the end of the growing season, regardless of fertilization treatment. This can be explained by the fact that during this stage, nutrients go mostly to fruits, and leaves are left without enough nutrients necessary for pigment synthesis. An additional reason for the decrease in their leaf content is leaf ageing, which involves gradual inhibition of processes oriented toward synthesis stimulation, as well as intensification of processes focused on pigment degradation [14].

Interestingly, as compared to the results of other authors, chlorophyll *a* content in Idared leaves was extremely high, regardless of fertilization treatment. Munteanu et al. found a considerably lower leaf chlorophyll *a* concentration in Idared leaves, being as low as 0.891 mg/g fresh matter [15]. Some higher concentration of chlorophyll *a* in leaves of certain apple cultivars, ranging from 1-2.3 mg/g fresh matter, were reported by Dulf et al. [16]. These differences in chlorophyll *a* content in leaves between apple cultivars, even within the same cultivar, can occur for a number of different reasons, including the effect of environmental factors, genetic potential of a particular cultivar, date and method of sampling, pigment determination method, and type of agricultural practices used (with fertilization being one).

The highest statistically significant effect on content of chlorophyll *b* in apple leaves was exhibited by fertigation. These results are consistent with results of Neilsen et al., who reported much higher values of chlorophyll *b* content in apple leaves as stimulated by fertigation [17]. The present results, outlined in Table 4, also show that chlorophyll *b* content was lowest at the beginning of the growing season, regardless of the fertilization type employed. The value

Table 4. Chlorophyll *b* pigment content in leaves of apple cv. Idared as dependent on fertilization type.

Variant of fertilization	Average values of chlorophyll *b* pigment (mg/g fresh leaf)						
	Growing season			Average	Differences against control	%	Sign.
	Beginning	Mid	End				
Fertigation	1.24±0.44	1.57±0.36	1.47±0.41	1.43	0.56	64	**
Foliar fertilization	1.16±0.27	1.24±0.23	1.21±0.30	1.24	0.37	43	**
Conventional A	0.90±0.33	1.04±0.27	1.01±0.29	0.99	0.12	13	ns
Conventional B	0.99±0.18	1.22±0.19	1.03±0.21	1.08	0.21	24	*
Control	0.76±0.29	1.03±0.24	0.82 ±0.25	0.87	0.00	0.00	
$LSD_{0.05}$ = 0.21, $LSD_{0.01}$ = 0.33							

**high significant, *significant, ns – non significant

Table 5. β-carotene pigment content in leaves of apple cv. Idared as dependent on fertilization type.

Variant of fertiliza-tion	Average values of chlorophyll *b* pigment (mg/g fresh leaf)						
	Growing season			Average	Differences against control	%	Sign.
	Beginning	Mid	End				
Fertigation	0.78±0.33	0.72±0.31	0.71±0.37	0.73	0.22	43	**
Foliar fertilization	0.80±0.18	0.62±0.24	0.64±0.23	0.69	0.18	35	*
Conventional A	0.61±0.21	0.42±0.22	0.51±0.28	0.52	0.01	1	ns
Conventional B	0.61±0.10	0.49±0.14	0.61±0.10	0.57	0.06	11	ns
Control	0.59±0.20	0.49±0.18	0.44±0.11	0.51	0.00	0.00	
$LSD_{0.05}$ = 0.11 $LSD_{0.01}$ = 0.20							

**high significant, *significant, ns – non significant

suddenly increased toward mid growing season, reaching its maximum, but starting to decrease thereafter toward the end of the growing season – which corresponds to chlorophyll *a* pigment dynamics during the growing season.

The results given in Table 5 reveal that fertigation and foliar fertilization induced the most significant increase in β-carotene content in apple leaves.

There are rather scarce data in the available scientific literature regarding β-carotene content in Idared leaves. The reason that β-carotene content in apple leaves is rarely dealt with is the assumption that it has only a subsidiary role in photosynthesis. However, laboratory experiments with algae have shown that the removal of β-carotene and carotenoids in general from the chloroplast results in a sudden decline in chlorophyll concentration, suggesting that carotenoids have an important role in photosynthesis, primarily in chlorophyll protection from high light intensities, through prevention of their oxidative degradation [18].

The analysis of the dynamics of β-carotene in certain stages of the growing season reveals that β-carotene, as opposed to chlorophyll, does not show a tendency to decrease from mid to the end of the growing season, regardless of the fertilization type used. This also is seen during leaf observation in autumn, when leaves turn from green to reddish-yellow, depending on the prevalence of either carotene or xanthophyll. The longer persistence of β-carotene in leaves, relative to chlorophylls, is not attributed to the increase in β-carotene synthesis at the end of the growing season, but rather to the earlier degradation of chlorophyll.

The results on the effect of type of fertilization on total phenolic content in apple fruit are presented in Table 6.

The results in Table 6. show that all fertilization types induced a statistically significant increase in total phenolic

Table 6. Average phenolic content in fruit of apple cv. Idared (g/l apple juice) as dependent on fertilization type.

Variant of fertilization	Phenolic content (g/l)	Sign.
Fertigation	0.92±0.03	**
Foliar fertilization	0.85±0.04	**
Conventional variant A	0.73±0.04	**
Conventional variant B	0.79±0.03	**
Control	0.61±0.08	
$LSD_{0.05}$ = 0.079, $LSD_{0.01}$ = 0.112		

**highly significant

Table 7. Average vitamin C content in fruits of apple cv. Idared (mg/100g fruit) as dependent on fertilization type.

Variant of fertilization	Vitamin C content (mg/100 g fruit)	Sign.
Fertigation	5.07±0.11	**
Foliar fertilization	4.80±0.14	**
Conventional variant A	4.17±0.12	**
Conventional variant B	4.40±0.07	**
Control	3.40±0.05	
$LSD_{0.05}$ = 0.159 $LSD_{0.01}$ = 0.265		

**high significant

content in apple fruit as compared to the unfertilized control. Total phenolic content of apple fruit was found to be highest in the treatment involving fertigation and lowest in the control, as expected. Apart from fertigation, a statistically very significant effect on total phenols in apple fruits of cv. Idared was exhibited by foliar fertilization. The information serves to support the thesis that foliar nutrition can substantially increase phenolic levels in apple fruits [19].

There are numerous data in the related scientific literature on the phenolic content of apples. Nogueira et al. determined high differences in total phenolic content in apple fruit between apple cultivars, ranging from 188.4-2,776 mg/l [20]. High differences in the phenolic content of fruits of the same cultivar are presented in a study by Hecke et al. [21]. Quite interesting data on the phenolic content of apple fruit were reported by a group of authors affiliated with the University of Colorado. They evaluated the fruit phenolic content of some uncultivated apple species. The values obtained were even up to 10-fold higher than those of domestic apple cultivars [22]. The very high values of total phenolics were the result of a high tannin content in uncultivated apple species. From the above it can be inferred that for the higher quality and health benefits of apples, information about the individual phenolic components of apple is more important than information on total phenolic content. However, the information on total phenols in apples is highly indicative when analyzing the qualitative composition of fruits of apple cultivar.

The results on the effect of fertilization type on vitamin C content in apples are given in Table 7.

The results presented in Table 7 show that the highest average content of vitamin C in apples was obtained in the fertigation and foliar fertilization, and lowest in control. The positive effect of the fertigation model on vitamin C content in apples also was determined by Tahir and Gustavsson [23], and that of foliar fertilization on the same parameter by Bochis et al. [24].

Scientific literature reports substantial variations in vitamin C values between certain apple cultivars. V. Nour et al. mentioned data on vitamin C fruit content in a dozen leading commercial cultivars in Romania [25], with the average values ranging from 2.6 mg/100 g fruit in cv. Patul and 11.4 mg/100 g in cv. Idared, to 18.7 mg/100 in cv. Red Boskoop. Brown and Maloney found an even higher range of values for the vitamin C content of apples, varying from 5-10 mg/100 g fruit, depending on the cultivar [26]. The differences observed in the vitamin C content of apple cultivars reported by researchers can be most likely associated with the chemical properties of vitamin C. Namely, vitamin C is a very unstable compound susceptible to photooxidation and subject to rapid degradation and loss in high light intensities; this should be considered when determining the vitamin C content of apple fruits as well as when storing fruits to prevent a substantial loss of quality [27].

The results obtained in this study suggest that fertigation and foliar fertilization induced the statistically highest increase in all test parameters. These types of fertilization, if adequately employed, provide nutrition to plants according to their requirements at a specific phenostage, leading to increased yields and enhanced fruit quality, and indirectly reducing nutrient leaching from soils, a frequent accompanying manifestation of conventional types of fertilization.

References

1. SAVATOVIĆ S., ĆETKOVIĆ G., ĐILAS S., TUMBAS V., ČANDANOVIĆ-BRUNET V., ČETOJEVIĆ-SIMIN D., MANDIĆ A. Antioxidant and antiproliferative activity of Granny Smith apple pomace. Acta Periodica technologica, **39**, 201, **2008**.
2. WOLFE K., WU X., LIU R. H. Antioxidant activity of Apple Peels. J. Agr. Food Chem. **51**, (3), 609, **2003**.
3. NEILSEN G. H., NEILSEN D., PERYEA F. Response of soil and irrigated fruit trees to fertigation or broadcast application of nitrogen, phosphorus and potassium. Hort Technology, **9**, (3), 393, **1999**.
4. MIŠIĆ P. Jabuka. Nolit, Beograd, **1994**.
5. ISO 10390. Soil Quality – Determination of pH. International Organization for Standardization, Geneve, Switzerland, **5**, **1994**.
6. EGNER H., RIEHM H. DOMINGO W. R. Studies on the chemical soil analysis as a basis for the assessment of the ground. Nahrstoffzustandes II. K. LantbrHogsk. Ann., **26**, 199, **1960**.
7. ČIVIĆ H., ŠAĆIRAGIĆ B., ELEZI DŽ. Agrochemistry and plant nutrition. Graforad, Travnik, **2004**.
8. HANIĆ E., ČIVIĆ H., MURTIĆ S. Basics of plant nutrition with practicum. University Džemal Bijedić in Mostar. Bemust, Sarajevo, **2009**.
9. EIJCKELHOFF C., DEKKER J. P. A routine method to determine the chlorophyll *a*, pheophytin a and β-carotene contents of isolated Photosystem II reaction center complexes. Photosynth. Res. **52**, 69, **1997**.
10. OUGH C. S., AMERINE M. A. Phenolic compounds, In: Methods for analysis of musts and wines, John Wiley & Sons, Inc, New York, USA, **1988**.
11. AOAC. Official methods of analysis (17th ed.). Washington, DC. Association of Oficial Analytical Chemists, **2002**.
12. ČMELIK Z., TOJNKO S. Influence of fertirigation with nitrogen on iron, copper and boron content in leaf and cropping of 'Jonagold' and 'Golden Deliciuos' apple trees. Agri. Cons. Sc., **66**, (4), 187, **2001**.

13. NEILSEN G. H., NEILSEN D., HeERBERT L. C. Nitrogen Fertigation Concentration and Timing of Application Affect Nitrogen Nutrition, Yield, Firmness, and Color of Apples Grown at High Density. HortScience, **44**, (5), 1425, **2009**.
14. HENSEL L. L., GRBIĆ V., BAUMGARTNER D. A., BLEECKER A. B. Developmental and age-related processes that influence the longevity and senescence of photosynthetic tissues in Arabidopsis. Plant Cell, **5**, 553, **1993**.
15. MUNTEANU N., IUREA D., ISTRATE M., CHINTEA P. Research on the pigment content in various apple tree varieties, treated with Capsicode. Cercetari Agronomice in Moldova, **3**, 65, **2008**.
16. DULF F., ANDREI S., MATEA C., BUNEA A., PINTEA A. Fatty Acids, Phytosterols and Chlorophylls in Leaves of Apple Cultivars (*Malus Domestica* Borkh). Agriculture, **67**, (1), 107, **2010**.
17. NEILSEN G. H., PARCHOMCHUK P., WOLK W. D., LAU O. L. Growth and mineral composition of newly planted apple trees following fertigation with N and P. Journal Amer. Soc. Hort. Sci. **118**, 50, **1993**.
18. NEŠKOVIĆ M., KONJEVIĆ R., ĆULAFIĆ LJ. Fiziologija biljaka. NNK-International, Beograd, **2003**.
19. SHANIN M. F. M., FAWZI M. I. F., KANDIL E. A. Influence of Foliar Application of some Nutrient (Fertifol Misr) and Gibberellic Acid on Fruit Set, Yield, Fruit Quality and Leaf Composition of "Anna" Apple Trees Grown in Sandy Soil. Journal of American Science, **6**, 202, **2010**.
20. NOGUEIRA A., GUYOT S., MARNET N., LEQUERE J. M., DRILLEAU J. F., WOSIACKI G. Effect of alcoholic fermentation in the content of phenolic compounds in cider processing. Braz. arch. biol. technol., **51**, (5), 1025, **2008**.
21. HECKE K., HERBINGER K., VEBERIĆ R., TROBEC M., TOPLAK H., ŠTAMPAR F., KEPPEL H., GRILL D. Sugar, acid- and phenol contents in apple cultivars from organic and integrated fruit cultivation. Eur. J. Clin. Nutr., **60**, 1136, **2006**.
22. STUSHNOFF C., McSAY A. E., FORSLINE P. L., LUBY J. J. Diversity of Phenolic Antioxidant Content and Radical Scavenging Capacity in the USDA Apple Germplasm Core Collection. ACTA Horticulturae **623**, 305, **2003**.
23. TAHIR I., GUSTAVSSON K. E. Improving Quality and Storability of Apples by a Combination of Aluminum Reflective Mulch, Summer Pruning and Controlled Nitrogen Fertilizatin. Proceedings of the VIth international postharvest conference, Antalya, Turquie, **3**, 245, **2009**.
24. BOCHIS C., ROPAN G., ROMAN I., POP A., MITRE V., MITRE I., BALEA G. The effect of foliar fertilization upon photosynthesis process at five apple cultivars. Horticulture, **65**, 507, **2008**.
25. NOUR V., TRANDAFIR I., IONICA M. E. Compositional Characteristics of Fruits of several Apple (*Malus domestica* Borkh.) Cultivars. Not. Bot. Hort. Agrobot. **38**, (3), 228, **2010**.
26. BROWN S., MALONEY K. Recent Advantages in Apple Breeding, Genetics and New Cultivars. New York Fruit **13**, (1), 2, **2005**.
27. HICKEY S., ROBERTS H. The science of vitamin C. Napa California: Lulu Press, **2004**.