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EVALUATION OF THE BIOCHEMICAL QUALITY OF *ARONIA MELANOCARPA* FRUITS IN THE CONDITIONS OF SOUTHERN ROMANIA, UNDER THE INFLUENCE OF FERTILIZATION

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Abstract

The aim of the present study is to evaluate the chemical composition of chokeberry fruits (Aronia melanocarpa (Michx.) Elliott, variety 'Nero'), specifically total anthocyanins, total polyphenols, vitamin C and malic acid under the influence of fertilization applied to soil, foliar, and combined (soil + foliar). In the 'Nero' variety, soil fertilization and foliar fertilization were applied, respectively, with the graduations: a1 - mineral fertilization in the soil with the quantities recommended by the SMART program! Fertilizer Management software, a2 - application of Biozyme foliar fertilizer in a concentration of 0.1% and a3- soil mineral fertilizers in combination with foliar fertilizer (graduations a1 + a2). Between 2017 and 2019, in the experimental plot, the following doses and forms of mineral fertilizers were applied to the soil, adapted to the species requirements: 74.10 kg/ha MAP (ammonium monophosphate), 125.7 kg/ha Magnisal (magnesium nitrate), 122.7 kg/ha ammonium nitrate and 196.5 kg/ha Multi K (potassium nitrate). Chemical analyzes show a high content of antioxidants in chokeberry fruits. The high anthocyanin content (3535 mg kg fresh fruit, as well as total polyphenols (83128 mg/kg fresh fruit), vitamin C (over 97 mg/100 g fresh fruit), and malic acid (0.72 g/100 g fruit) emphasizes the pharmaceutical properties of the chokeberry species.

***Key words**: polyphenols, anthocyanins, antioxidant, vitamin C, organic acids.*

INTRODUCTION

Aronia, known as chokeberry, is native to North America, from where it has spread throughout the world. The genus includes the species *Aronia arbutifolia* (L.) Pers (red chocheberry), *Aronia melanocarpa* (Michx.) Elliott (black chokeberry) and *Aronia prunifolia* (Marshall) Rehder (purple chokeberry). In Europe it is cultivated on large areas as an important industrial crop (Hardin, 1973; Seidemann, 1993; Strigl et al., 1995). In recent years, the potential of chokeberry fruits has been recognized as a source of natural food and valuable phytonutrients (Slimestad et al., 2005; Nicola et al., 2012). In the future it is possible that the natural dyes extracted from *Aronia* will replace synthetic coloring, azo-types that are presently used (Snebergrova, 2014). *A. melanocarpa* is among the richest sources of polyphenols in the plant kingdom (Denev et al., 2012; Denev et al., 2013). Numerous scientific studies show the chemical composition of chokeberry fruits (Kulling & Rawel, 2008; Denev et al., 2012), their clinical efficacy and their use for various diseases (Park et al., 2013; Daskalova et al., 2015; Borowska, 2016). According to Kulling & Rawel (2008), the chemical composition of chokeberry fruits is characterized by high nutritional and biological values and depends on several factors: genotype, climate, date of harvest and use of fertilizer (Jeppsson, 2000; Skupien et al., 2007). Substances with antioxidant effect (polyphenols, organic acids, vitamins, anthocyanins, carbohydrates and proteins, etc.) are involved in inhibiting the free radical propagation reactions produced *in vivo* by reactive oxygen species, nitrogen species, and lipid peroxidation in food (Cevallos-Casals & Cisneros-Zevallos, 2004).

Research in the field of phenols extracted from plants (Schaffer et al., 2005) shows that phenols depend quantitatively and qualitatively on genetic information (species, variety), environment and geographical conditions. Climate, season, light, temperature, maturation period strongly influence the synthesis of phenols in plants (Aherne & O'Brian, 2002).

Because oxidative damage is involved in the development of various diseases, vitamin C could have a preventive or even therapeutic effect. Vitamin C thus acts only together with other biochemically active compounds from fruits and vegetables (Dimitrović, 2006).

The hydroxyl radical, one of the strongest free radicals known, can initiate lipid peroxidation, can break DNA strands, and oxidize virtually any organic molecule (Burkitt & Duncan, 2000; McCord, 2000).

A possible mechanism for neutralizing free radicals by the ascorbic ion (Dimitrović, 2006; Halliwell & Gutteridge, 1990) is presented below:

$AscH^- + O_2 \rightarrow AscH^{\bullet -} + O_2^{\bullet -}$

$AscH^- + O_2^{\bullet -} \rightarrow AscH^{\bullet -} + H_2O_2$

or hydroxyl radical $OH^{\cdot}$) (Halliwell & Gutteridge, 1990):

$Fe(III) + AscH^- \rightarrow Fe(II) + Asc^{\cdot -}$

$Fe(II) + H_2O_2 \rightarrow Fe(III) + OH^{\cdot} + OH^-$

In the literature the observations on the technology of chokeberry cultivation are contradictory. Mineral fertilizer, among other agronomic practices, can influence the nutritional value and content of biochemically active compounds in fruits. Cultivation methods can be used to improve phenol content and fruit pigmentation, although information on this topic is very rare and often contradictory (Tomás-Barberán & Espin, 2001). Carbonaro et al. (2002), observed an improvement of the plant's antioxidant defense system (peach and pear) as a possible consequence of agronomic cultivation practices. On the other hand, Häkkinen & Törrönen (2000), found a similar content of polyphenols in three conventionally grown and organically grown strawberry crops.

In this study we estimate whether mineral soil fertilizer, foliar fertilizer and combined fertilizer (soil and foliar) influence the content of basic nutrients and improve sensory attributes by (increasing sugar content) and therefore the nutritional value of chokeberry fruits.

MATERIALS AND METHODS

The present study was carried out at the Research Institute for Fruit Growing Pitesti, Mărăcineni, in the experimental field of shrubs (44° 51′ 30" N, 24° 52" E), in an *Aronia melanocarpa* plantation, year 5, Romania. The experiment was organized on a soil from the class of wet phreatic aluviosol protisols, formed on fluvial deposits, with a loam-sandy granulometric composition. The field was located in a meadow terrace of the Argeș River. The soil, is characterized by a strongly moderate acid reaction, a low humus content and a low assimilable phosphorus content in the arable layer. The biological material chosen was the chokeberry variety, 'Nero', (planting distance being 3.0 m between rows and 1.5 m between plants per row). In the 'Nero' variety, soil fertilizer and foliar fertilizer were applied, respectively, with the graduations: a_1 - mineral fertilizer in the soil, with the quantities recommended by the SMART program! Fertilizer Management software (smart-fertilizer.com), a_2 – application of Biozyme foliar fertilizer in a concentration of 0.1% and a_3- application of mineral fertilizers to the soil in combination with foliar fertilizer (a_1 + a_2 graduations). During the vegetation period, from 2017-2019 the following doses and forms of mineral fertilizers were applied to the soil, in the experimental plot, adapted to the species requirements for an expected harvest of 10 t/ha: 74.10 kg/ha MAP (ammonium monophosphate), 125.7 kg/ ha, Magnisal (magnesium nitrate) 122.7 kg/ha, ammonium nitrate and 196.5 kg/ha, Multi K (potassium nitrate). The fruit samples were harvested between 2017-2019, in three replications, at the technical maturity of harvesting.

Laboratory chemical determinations consisted of the determination of total sugar content (%), using the Fehling-Soxlet volumetric method (Singleton & Rosi, 1965), titratable acidity expressed as total malic acid %, using the volumetric method with 0,1N sodium hydroxide, vitamin C (mg/100 g fresh fruit), which was dosed using the iodometric method by solvent extraction using ethyl alcohol-hydrochloric acid, and total polyphenols expressed as mg galic acid/kg fresh fruit using Folin - Ciocalteu method (Singleton & Rosi, 1965). All analytical determinations were performed on three replications, and the data were subjected to analysis of variance (ANOVA). The influence of experimental factors was analyzed by the Duncan test, with a significance level of P≤0.05. Correlations were also made between the biochemical quality indicators of the fruits. Statistical data analysis was performed using SPSS 14.0 for Windows software.

RESULTS AND DISCUSSIONS

Anthocyanins

On average, over the three years of experimentation, soil fertilizer combined with foliar fertilizer led to a significant increase in anthocyanin content up to 3252 mg/kg fresh fruit, compared to foliar fertilizer (2690 mg/kg fresh fruit) and root fertilizer (2280 mg/kg fresh fruit) (Figure 1). Both in 2017 and 2019 the anthocyanin content was higher in the case of fertilization with both soil and foliar fertilizer, compared with the application of only one type of fertilizer. However, in 2018, the difference between the three types of fertilizer applications was insignificant. It is obvious that agronomic practices influence the biosynthesis (metabolism) of anthocyanins. Some hormones (yasminic acid, abscisic acid, etc.) can increase the content of anthocyanin pigments and the biochemical quality of fruits (McClure, 1975; Lee et al., 1996). In the version of combined soil and foliar fertilizer , the product used Biozyme 1% contains cytokinins and auxins which accelerate the metabolism of the plant. Dixon & Paiva (1995) show that these nutrients actually affect the activity of the enzyme phenylalanine ammonialyase responsible for the increase in the substrate of anthocyanin biosynthesis (Figure 1).

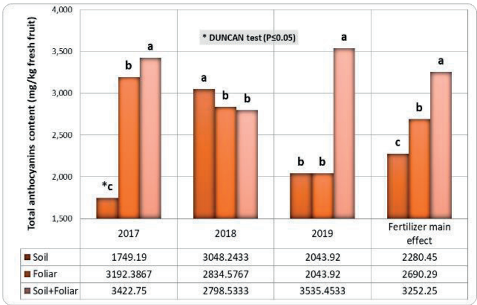

Figure 1. Influence of fertilizer on the anthocyanin content of fruits depending to the study year, in the 'Nero' variety

However, there were deviations from this trend, a1 graduation (soil fertilizer) in 2018 registering the highest content in anthocyanins; in the a2 graduation in 2018 was registered the lowest anthocyanin content (Figure 2).

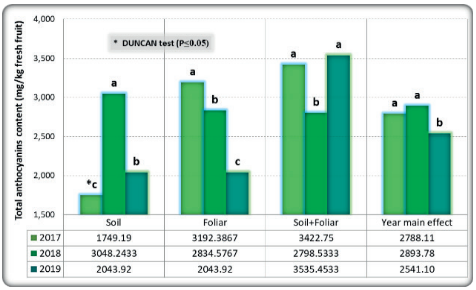

Figure 2. Influence of the study year on the anthocyanin content of fruits depending on the fertilizer, in the 'Nero' variety

Polyphenols

Overall, the polyphenol content decreased significantly in the third year of fertilizer application (Figure 3).

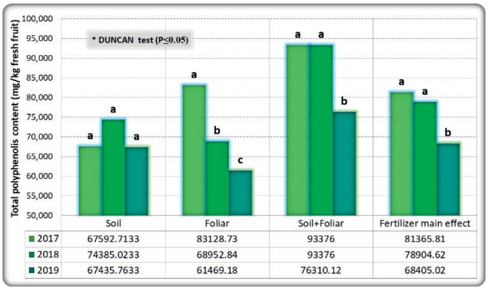

Figure 3. Influence of the study year on the content of polyphenols in fruits depending on the fertilizer, in the 'Nero' variety

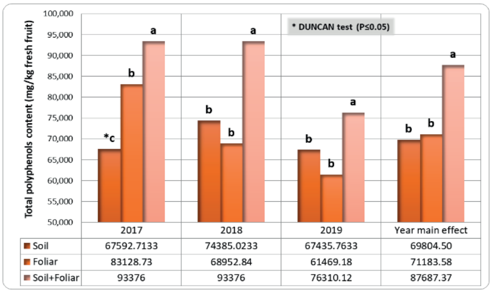

Figure 4. Influence of fertilizer on fruit polyphenol content depending to the study year, in the 'Nero' variety

On average, over the three years of the study, the polyphenol content was significantly higher in the a3 graduation (soil + foliar fertilizer) (87,687.37 mg/kg fresh fruit), compared to the other two graduations, the effect being maintained with small oscillations, in all years of experimentation (Figure 4).

Agronomic practices (McClure, 1975, McGarry et al., 1996) such as fertilization (Misra et al., 1991; McNabnay et al., 1999) influence the biochemical quality of fruits in the sense of increasing the content of polyphenols in response to stress (fertilization with insufficient doses of potassium or high doses of potassium, high doses of phosphorus, etc.).

Sanchez et al. (2000) confirm the control of the enzyme phenylalanine ammonialyase on the biosynthesis of anthocyanins and polyphenols, during the stress period of plants by inducing nitrogen toxicity to green beans.

In this case the content of polyphenols decreases due to the inhibition of the mentioned enzyme (Figure 4).

Vitamin C

On average, depending on the graduation of fertilizer applied, the vitamin C content was significantly higher in 2018 (94.01 mg/fresh fruit), compared to 2017 (64.26 mg/fresh fruit) and compared to 2019 (58.28 mg/fresh fruit). The trend was maintained on each fertilizer variant (Figure 5).

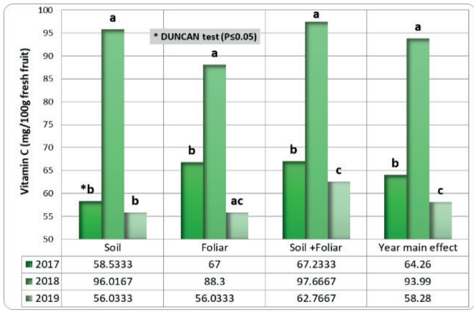

Figure 5. Influence of the study year on the vitamin C content of fruits depending on the fertilizer, in the 'Nero' variety

Over the three years of the study, the application of the third graduation, a3 (soil combined with foliar fertilizer), stimulated the increase of vitamin C content (75.89 mg/ 100 g fruit pulp), compared to the a1 (soil fertilizer only) (70.19 mg/100 g fruit pulp) or a2 (foliar fertilizer only) (70.44 mg/100 g fruit pulp). The average trend was generally maintained throughout the years of experimentation (Figure 6).

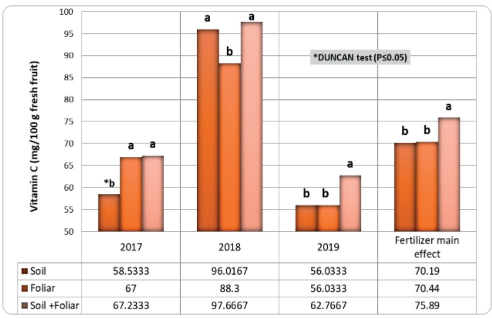

Figure 6. The influence of the fertilizer on the vitamin C content of the fruits depending to the study year, in the 'Nero' variety

Malic acid

The malic acid average content in 2018 was higher (0.64%), compared to other years. Unlike the average trend, in the a1 graduation (soil fertilizer only), the malic acid content decreased, whereas in the a3 graduation (combined soil and foliar fertilizer), the malic acid content increased over time (Figure 7).

Looking at the 3 years of study, the third graduastion, a3 (combined soil and foliar fertilizers) determined an increase in the percentage of malic acid in fruits (0.66%), compared to the other two graduations. Only in 2017, the first year of fertilization, the soil only fertilization (a1) exceeded the other two graduations in terms of the percentages of malic acid found in the fruit (Figure 8).

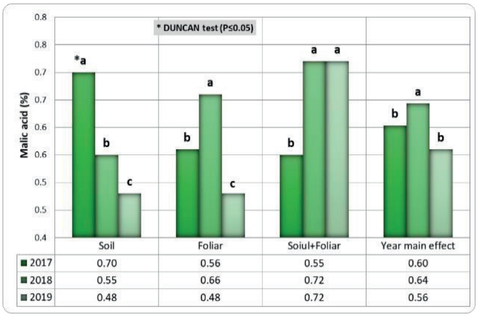

Figure 7. Influence of the study year on the malic acid content of fruits depending on the fertilizer, in the 'Nero' variety

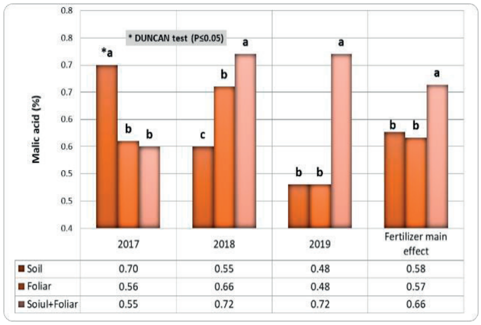

Figure 8. Influence of fertilizer on fruit malic acid depending on the study year, on the 'Nero' variety

Table 1. Matrix of correlation (Pearson "r" correlation coefficients "r") of the main physical and biochemical indicators studied in 'Nero' variety).

Indicators		Total sugar content (%)	Malic acid (%)	Total anthocyanins content (mg/kg fresh fruit)	Total polyphenol content (mg/kg fresh fruit)	Vitamin C (mg/kg fresh pulp)
Total sugar content (%)	Pearson Correlation	1.000	0.019	0.104	-0.183	0.081
	Sig. (2-tailed)		0.921	0.591	0.342	0.675
Malic acid (%)	Pearson Correlation	0.019	1.000	0.300	-0.664(**)	0.276
	Sig. (2-tailed)	0.921		0.114	0.000	0.147
Total anthocyanins content (mg/kg fresh fruit)	Pearson Correlation	0.104	0.300	1.000	-0.288	0.306
	Sig. (2-tailed)	0.591	0,114		0.130	0.106
Total polyphenol content (mg/kg fresh fruit)	Pearson Correlation	-0.183	-0.664(**)	-0.288	1.000	-0.428(*)
	Sig. (2-tailed)	0.342	0.000	0.130		0.021
Vitamin C (mg/kg fresh pulp)	Pearson Correlation	0.081	0.276	0.306	-0.428(*)	1.000
	Sig. (2-tailed)	0.675	0.147	0.106	0.021	

**Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed).

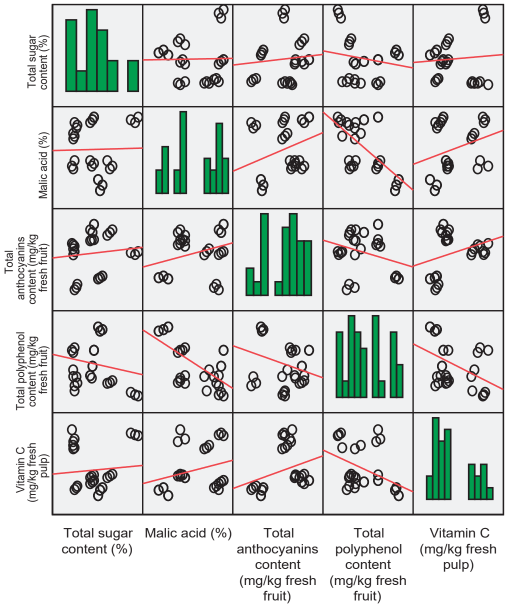

Figure 9. Chart of correlations between biometric and biochemical indicators studied in 'Nero' variety

Analyzing the values obtained (Table 1, Figure 9) a distinctly significant negative correlation is observed between the malic acid content and the polyphenol content. The phenomenon is explicable, if we consider the ripening period of the fruits. Polyphenols accumulate in fruits when day/night temperature differences are large, in response to plant stress. Also, a distinctly significant negative correlation can be observed between the content of vitamin C and the content of total polyphenols in chokeberry fruits.

CONCLUSIONS

In the end of the present study, the conclusions are that in the chokeberry culture from Research Institute for Fruit Growing Pitești Mărăcineni, under the influence of soil mineral fertilization, foliar fertilization and combined fertilization, the content of organic acids, expressed as total malic acid, accumulates in the fruit to the detriment of polyphenols biosynthesis.

Similarly, under the influence of the aforementioned fertilization, the content of vitamin C decreased as well in the fruit during the polyphenols biosyntesis.

Although still preliminary, these results have provided evidence that mineral fertilization may influence the chemical composition of chockeberry fruits. Other studies show that fertilizer application with increased doses of NPK have as result a higher yield whereas pigment content and total acidity decrease (Jeppsson, 2000).

The increase of the contents of biochemically active compounds was obtained using combined (soil and foliar) fertilization. Therefore, we recommend for the chokeberry culture as optimal the third graduation (a3), combined soil mineral fertilization and foliar fertilization with 0,1% Biozyme .

In the future, we intend to study an experimental model of fertilization with optimal doses of mineral fertilizers with NPK and microelements administered in the soil, combined with foliar administration of growth biostimulators.

REFERENCES

Aherne, S.A.&O'Brien, N.M. (2002). Dietary flavonols: chemistry, food content, and metabolism. *Nutrition,* 18, 75-81.

Borowska, S., Brzoska, M.M. (2016). Chokeberries (*Aronia melanocarpa*) and their products as a possible means for the prevention and treatment of noncommunicable diseases and unfavorable health effects due to exposure to xenobiotics. *Compr. Rev. Food. Sci. Food Saf*, 15, 982-1017.

Burkitt, M.J.& Duncan, J. (2000). Effects of trans-Resveratrol on Copper-Dependent Hydroxyl-Radical Formation and DNA Damage: Evidence for Hydroxyl-Radical Scavenging and a Novel, Glutathione-Sparing Mechanism of Action Arch. *Biochem Biophys*, 381 (2), 253-63.

Carbonaro, M., Mattera, M., Nicoli, S., Bergamo, P., Cappelloni, M. (2002). Modulation of Antioxidant Compounds in Organic vs Conventional Fruit (peach, Prunus persica L., and Pear, Pyrus communis L.). *Journal of Agricultural and Food Chemistry,* 50(19), 5458-5462.

Cevalos-Casalss, B.A.&Cisneros-Zevallos, L. (2004). Stability of anthocyanin-based aqueos extracts of Andean purple corn and red-fleshed sweet-potato compared to syntetic and natural colorants. *Food Chemistry*, 86, 97-77.

Daskalova, E., Delchev, S., Peeva, J., Vladimirova-Kitova, I., Kratchanova, M., Kratchanov, C., Denev, P. (2015). Antiatherogenic and cardioprotective effects of black chokeberry (*Aronia melanocarpa*) juice in aging rats. *Evid. Based. Compl. Alternat. Med*., 1-10.

Dixon, R.A., Paiva N.L. (1995). Stress phenylpropanoid metabolism. *Plant Cell*, 7, 1085-1097.

Dimitrovic, R. (2006). Vitamin C in disease prevention and therapy. *Biochemia Medica Jurnal*, 16(2), 89-228.

Denev, P.N., Kratchano C., Ciz M., Lojek A., Kratchanova M. (2012). Bioavailability and antioxidant activity of black chokeberry (*Aronia melanocarpa*) polyphenols: in vitro and in vivo evidences and possible mechanisms of action: a review. *Comprehensive Reviews in Food Science and Food Safety*, 11(5), 471-489.

Denev, P.N., Lojek, A., Ciz, M., Kratchanova, M. (2013). Antioxidant activity and polyphenol content of Bulgarian fruits, *Bulgarian Journal of Agricultural Science*, 19(1), 22-27.

Hakkinen, S.H&Torronen, A.R. (2000). Content of flavonols and selected phenolic acids in strawberries and *Vaccinium* species: Influence of cultivar, cultivation site and technique. *Food Research International*, 33, 517-524.

Halliwell, B, Gutteridge, J.M.C. (1990). Role of free radicals and catalytic metalions in human disease: an overview. *Methods Enzymol*.,186, 1-8.

Hardin, J.W. (1973). The enigmatic chokeberries (*Aronia*, *Rosaceae*), *Bulletin of the Torrey Botanical Club*, 100(3), 178-184

Jeppson, N. (2000). The effects of fertilizer rate on vegetative growth, yield and fruit quality, with special respect to pigments, in black chokeberry (*Aronia melanocarpa*) cv. `Viking'. *Scientia Horticulturae*, 83, 127-137.

Kulling, S.E.&Rawel, H.M. (2008). Chokeberry (*Aronia melanocarpa*) - A review on the characteristic components and potential health effects. *Planta Med.*, 74(13), 1625-34. DOI: 10.1055/s-0028-1088306. Epub 2008 Oct 20.

Lee,Y., Howard, L.R., Villalon, B. (1995). Flavonoid and ascorbic acid content and antioxidant activity of fresh pepper (*Capsicum annuum*) cultivars, J. Food. Sci, 60, 473-476.

McClure, J.W. (1975). Physiological functions of flavonoids, in Harnborne J.B., Mabry T. J.&Mabry, H. (Eds), *The flavonoids*, London, Chapman&Hall, 970-1055.

Misra, J. B., Sukumaran, N. P., Verma S., C. (1991). Reduction of cresolase and catecholase activities in tubers of some Indian potato varieties by the application of potash fertilisers. *J. Sci. Food. Agric.* 54, 339-345.

McCord, M. (2000). The evolution of free radicals and oxidative stress. *Am J Med*, 108(8), 652-9.

McGarry, A., Hole, C.C., Drew, R.L.K., Parsons, N. (1996). Internal damage in potato tubers: a critical review. *Postharv. Biol. Technol*, 8, 239-258.

McNabnay, M., Dean, B.B., Bajema, R.W., Hyde G.M. (1999). The effect of potassium deficiency on chemical, biochemical and physical factors commonly associated with blackspot development in potato tubers. *Am. J. Potato Res.,* 76, 53-60.

Nicola, C., Chiţu, E., Mladin, P., Ancu, I., Florea, A. C-tina (2012). Evaluation of fruit quality in some black currant cultivars under conditions of meadow arges *Scientific Papers of the Research Institute for Fruit Growing Pitesti, Romania*, XXVIII, 38-42.

Park, S., Kim J.I., Lee, I. (2013). *Aronia melanocarpa* and its components demonstrate antiviral activity against influenza viruses. *Biochemical and Biophysical Research Communications*, 440(1), 14-19.

Seidemann, J. (1993). Chokeberries a fruit little-known till now, *Deutsche Lebensmittel-Rundschau,* 89, 149-151.

Strigl, A.W., Leitner E., Pfannhauser W. (1995). Die schwarze Apfelbeere (*Aronia melanocarpa*) als naturliche farbstoffquelle, *Deutsche Lebensmittel-Rundschau,* 91: 177-180.

Sanchez, E., Soto, J.M., Garcia, P.C., Lopez-Lefebre, L.R., Rivero, R.M., Ruiz, J.M., Romero L. (2000). Phenolic compounds and oxidative metabolism in green bean plants under nitrogen toxicity. *Aust.J. Plant Physiol*., 27, 973-979.

Schaffer, S., Schmitt-Schillig, S., Muller, W.E.&Eckart, G.P. (2005). Antioxidant properties of Mediteranean food plant extracts: geographical differences. *Journal of Physiology and Pharmacology,* 56, 115-124.

Singleton, V.L.&Rosi, J.A. (1965). Colorimetry of total phenolics with phosphomolibdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.*, 16, 144-158.

Skupien, K., Oszmianski, J., Ochmian I., Grajkowski J. (2007). Characterization of selected physico-chemical features of blue honeysuckle fruit cultivar 'Zielona' *Polish J. Nat. Sci. Suppl*., 4, 101-107.

Slimestad, R., Torskongerpoll, K., Nateland, H.S., Johannssen, T., Giske, N.H. (2005). *J. Food Compos Anal*, 18, 61-68.

Snebergova, J. (2014). Variability of characteristic components of aronia. *Czech journal of food Sciences* (Czech Republic) ISSN: 1212-1800.

Tomas-Barberan, F.& Espin, C.J. (2001). Phenolic compounds and related enzymes as determinants of quality in fruits and vegetables. *J. Sci. Food Agric*., 81, 853-876. DOI: 10.1002/jsfa.885.