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EXTRACTION TIME INFLUENCE ON THE PHENOLIC AND CAROTENOID LEVEL, AND THE DYNAMICS OF ANTIOXIDANT ACTION OF CHOKEBERRY DRY RESIDUE

Ivona Cristina Enescu (Mazilu) [1, 2], Sina Cosmulescu [3, *], Daniela Giosanu [4], Loredana Elena Vijan [4]

[1]Research Institute for Fruit Growing Marului 402, 117450, Maracineni, Arges Romania
[2]University of Craiova, Doctoral School of Plant and Animal Resources Engineering Horticulture Faculty, Alexandru Ioan Cuza 13, 200585, Craiova, Romania
[3]University of Craiova, Horticulture Faculty, Department of Horticulture and Food Science, Craiova, Alexandru Ioan Cuza 13, 200585, Craiova, Romania
[4]University of Pitesti, Targu din Vale 1, 110040, Pitesti, Romania

Abstract
This study aimed to analyze the extraction time influences on some phenolics and carotenoids determined in the chokeberry dehydrated residue. In addition, the dynamics of the DPPH· radical reduction under the influence of the different amounts of chokeberry extract and reaction time were registered. For this purpose, fruits belonging to the 'Melrom' and 'Nero' cultivars grown in the experimental plots of the Research Institute for Fruit Growing Maracineni-Arges were harvested at full maturity, in the middle of August 2021 and pressed for juice extraction. The resulting pomace was subjected to convective dehydration at 45°C. Values of 12286.11-16560.88 mg GAE/100g DW for TPC, 6567.96-9428.90 mg GAE/100 g DW for TTC, and 3293.74-5109.63 mg CE/100 g DW for TFC were registered. Lycopene and β-carotene ranged between 0.78-1.43 mg/100 g DW and 0.21-0.37 mg/100 g DW. Longer ultrasound treatment led to higher TPC and TFC, while TTC decreased after 60 minutes. Raising the extraction time by at least 24 hours resulted in higher amounts of carotenoids. The lowest remanent radical activity (A=20.55%) was determined for the 50 and 60 µL extract doses. In this case, A% was minimal after 20 minutes of the DPPH reduction reaction (12.62-12.94 %), but not significantly different compared to the values determined after the first 8 minutes of the reaction (17.59-19.11 %).

Keywords: Aronia melanocarpa, flavonoids, lycopene, polyphenols, tannins, β-carotene

1. INTRODUCTION

Plant chemical constituents focused attention in the very early days of modern science since plants were used for many purposes besides food, such as medical treatment and cloth dying.
Anthocyanins were first investigated in 1664, while plant alkaloids were first isolated in crystalline form between1817-1820. Still, the large world of plant chemicals was brought to be known after de Second World War, through the development of new elaborated analysis techniques (Harborne, 1999). Recently many studies regarding plants' biochemical components and their biological activities were conducted and their results showed that an increased intake of food rich in natural antioxidants is associated with lower risks of degenerative diseases, particularly cardiovascular diseases and cancer (Pérez-Jiménez et al., 2008).

*Corresponding author, E-mail address: sinacosmulescu@hotmail.com

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According to Harborne (1999), among plant chemicals, classified as primary or secondary constituents, the first category includes common sugars, the protein amino acids, lipids, the purines and pyrimidines of nucleic acids, the chlorophylls, and so on. Secondary constituents comprise remaining plant chemicals from alkaloids to terpenoids and acetogenins to phenolic and represent substances that do not appear to have an essential role in metabolism and vary in their distribution from plant to plant. Secondary metabolites have a key role in protecting the plant from environmental pressures or in controlling plant growth: plant growth substances, floral pigments, odors, antiherbivore agents, and antifungal agents (Harborne, 1999). These compounds protect not only plants, fruits, and vegetables from oxidative damage but have been used as antioxidants and several disease therapy by humans.

Phenolic compounds, cyclic derivatives of benzene with one or more hydroxyl groups associated with the aromatic ring, account for one of the largest and most widely distributed groups of phytochemicals (Andjelkovic et al., 2006 cited by Tan et al., 2013). They may exhibit a wide range of physiological properties, such as anti-allergenic, anti-atherogenic, anti-inflammatory, anti-microbial, anti-viral, cardioprotective, and vasodilatory effects (Balasundram et al., 2005; Tabart et al., 2007 cited by Tan et al., 2013). The beneficial effects derived from phenolic compounds in human life have been attributed to their antioxidant activity mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators, singlet oxygen quenchers, and metal-chelators (Rice-Evans et al., 1996 cited by Tan et al., 2013).

Phenolic phytochemicals are the largest category of phytochemicals and the most widely distributed in the plant kingdom and the 3 most important groups of dietary phenolics are flavonoids, phenolic acids, and polyphenols (King and Young, 1999).

Flavonoids are the largest group of plant phenols, of low molecular weight that usually occur bound to sugar molecules. Flavonoids are grouped into anthocyanins and anthoxanthins. Anthocyanins are molecules of red, blue, and purple pigment. Anthoxanthins, which include flavonols, flavones, flavonols, and isoflavones, are colorless or white to yellow molecules (King and Young, 1999).

Tannins are compounds of high molecular weight that react with mouth proteins, thereby causing the taste sensation we know as astringency. Chemists divide tannins into 2 groups: condensed and hydrolyzable. Condensed tannins are polymers of catechins or epicatechins and are found mainly in fruit, grains, and legumes. Condensed tannins usually accumulate in the outer layers of plants. Their content is associated with a dark color of the vegetal material (King and Young, 1999).

Carotenoids are natural pigments that are found in plants, algae, fungi, birds, and fish flesh cuticles of crustaceans or insects. They are referred to as pigments, because of their characteristic colors that range from the yellow to red spectrum (Kiokias et al., 2016; Langi et al., 2018). Carotenoids can be classified into two groups according to their function: xanthophylls, including lutein and zeaxanthin, and carotenes, such as α-carotene, β-carotene, and lycopene (Saini et al., 2015; Langi et al., 2018). Their importance is that they cannot be synthesized in vivo by humans or animals, and they are consumed only through diet (Langi et al., 2018). Carotenoids were mentioned to possess antioxidant, anti-inflammatory, and anticancer activity, thus preventing degenerative and cardiovascular diseases (Eggersdorfer and Wyss,2018; Hussain et al., 2022; Saini et al., 2022).

The extraction and study of plants' bioactive compounds have been possible through some techniques, such as distillation, conventional solvent extraction (maceration, digestion, infusion, decoction, percolation, and hot continuous extraction), microwave-assisted, ultrasound-assisted and supercritical fluid extraction, counter-current extraction, cold compression, and non-conventional methods, namely supercritical fluid extraction, vortical (turbo) extraction, extraction by electrical

*Corresponding author, E-mail address: sinacosmulescu@hotmail.com

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energy and ultrasound-assisted extraction, among which solvent extraction (solid-liquid and liquid-liquid extraction techniques) is the most commonly used (Vinatoru, 2001; Handa et al., 2008; Tan et al., 2013; Dranca and Oroian, 2016). According to Dranca and Oroianu (2016), compared to traditional extraction methods, ultrasound-assisted extraction offers many advantages including shorter extraction time, less amount of solvent, and higher extraction yields (Ma et al., 2008) through the cavitation phenomena and mechanical fixing effect (Ince et al., 2014). Ultrasounds exert a mechanical effect, causing a disruption of plant cell walls when the cavitation bubbles collapse at the surface of the solid matrix. Thereby, mass transfer is enhanced, and the contact surface area between solvent and plant material increases. Moreover, a marked increase in the very local temperature enhances the solubility of the analytes in the solvent and eases their diffusion from the sample matrix to the outer region (Esclapez et al., 2011). In addition, the type of solvent or solvent mixture, temperature, extraction time, and even the vegetal matrix may influence the extraction efficiency (Deng et al., 2015; Brglez Mojzer et al., 2016). However, there are reports regarding the noninfluence of the particle size in the phenolics extraction process when ultrasounds are involved presented in Jovanović et al. (2017) review. Also, the optimal polyphenols yield extracted through an ultrasound-assisted process resulted in alcohol/water solvent, compared to pure alcohol in Chizzola et al. (2008), Fecka and Turek (2008), Paz et al. (2015), Jovanovic´ et al. (2017) studies. Undesired results can occur, i.e., damage to the extracted natural antioxidants and degradation of extract quality, if ultrasound treatment takes too long due to higher temperature and free radicals produced by the ultrasound waves (Horžic et al., 2012; Jovanovic´ et al., 2017).
The present paper studied the extraction time influence on the TPC, TTC, TFC, and two carotenoid content, lycopene and β-carotene on Aronia melanocarpa dry pomace. A second study of antioxidant activity dynamics of the methanolic extract of the same vegetal material was also performed to establish a protocol to be followed for the next determinations.

2. MATERIALS AND METHODS

Samples Preparation

Chokeberry fruits (from ´Melrom´ and ´Nero´ cultivars) were hand-picked at full maturity stage, in the middle of August 2021 from the experimental plot of the Research Institute for Fruit Growing Pitesti-Maracineni, Arges County, Romania. Fresh fruits were juiced in the laboratory with a domestic fruit press and the resulting pomace was convectively dehydrated until constant weight in the Memmert laboratory oven. The dehydrated pomace was kept in sealed plastic bags, in the dark, at 4ºC until the analysis was performed. Right before analysis pomace was powdered using mortar and pestle.

Chemical substances

Gallic acid, catechin, Folin-Ciocalteu reagent, methanol, sodium hydroxide, sodium carbonate, sodium nitrite, aluminum chloride, hexane, ethanol, and acetone were purchased from Redox Bucharest - Sigma Aldrich, Dako, Epp. Romania.

Extraction of Bioactives

Total phenolic and flavonoid extraction. 0.5 g of sample (dry pomace powder) was added to 10 mL of solvent (80% methanol) and vortexed well for 2 minutes. Each of these samples was incubated in an ultrasonic water bath (ULTR-2L0-001) at room temperature for 30 (T1), 60 (T2), 90 (T3), 120 (T4), 180 (T5), and 240 minutes (T6). Finally, the extracts were collected by using Whatman filter paper (No. 4) (Hossain et al., 2020).

*Corresponding author, E-mail address: sinacosmulescu@hotmail.com

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Total tannins extraction. Pomace powders (0.5 g) were suspended in 10 ml ultrapure water and vortexed for 2 minutes. Similar to total phenolic and flavonoid content analysis, samples were incubated in an ultrasonic water bath (ULTR-2L0-001) at 80 ºC temperature for 30 (T1), 60 (T2), and 90 minutes (T3). The extracts were obtained after filtering the powder: water mixture.
Carotenoids extraction. For carotenoids extraction 2 g pomace powder was suspended in 25 ml hexane: ethanol: acetone (in a 2:1:1 volume ratio) solvents mixture and stirred for 30 minutes at 1500 rpm, then 10 ml ultrapure water was added and stirred for another 10 minutes. After 15 minutes of rest, the phases were separated and the carotenoids dosing was performed in the upper layer. Three extraction times were studied, i.e., T1-the carotenoids were determined after the separation of the layers, T2 - the powder was added to the solvent mixture, stirred according to the protocol, and determinations were performed the second day, and T3-similar to T2, but determinations were performed the third day.
Antioxidant activity dynamics. The methanolic extract for antioxidant activity dynamics study was obtained by incubating 0.5 g powder chokeberry pomace in 10 ml methanol (99.8%) for 20 minutes in an ultrasonic bath (40 kHz), followed by 15 minutes centrifuge (3000 rpm) and the filtration through a Buchner funnel.

Chemical analysis and equipment

Quantitative determination of bioactive compounds was performed by a spectrophotometric method using a UV-Vis spectrophotometer PerkinElmer Lambda25.
Determination of DPPH Radical Activity. The Brand-Williams (1995) method with slight modification was utilized to perform the DPPH radical scavenging assay of chokeberry pomace methanolic extract. Different volumes (20-60 µL) of methanolic extract were added to 1940-1980 µL DPPH solution in a test tube, vortexed well, and kept in dark conditions for 0-20 min. The absorbance reading of the mixture was registered using a UV-Vis spectrophotometer at 515 nm. The results were expressed in percentages.
The following formula was used to calculate the residual radical activity (A%) of the samples:

$$A\% = (\text{Absorbance of the sample at } t_i \text{ moment/ Absorbance of the sample at } t_o \text{ moment}) \times 100,$$

i=2, 4, 6, 8, …, 20 minutes

Dosing the components with antiradical potential. TPC determinations were performed according to Cosmulescu et al. (2014) and for TTC, TFC, and carotenoids Tudor-Radu et al. (2016) protocols were followed.

Statistical Analysis. The data were analyzed using two-way ANOVA with IBM SPSS 28 (trial version) software and Excel 2010. Duncan's test was used to determine the significant difference ($P<0.05$). Data were expressed as means of three independent measurements.

3. RESULTS AND DISCUSSIONS

It is known that the extraction procedure (ultrasound, ethanolic, or high-pressure extraction) influences the TPC, TFC, TTC, and antioxidant and antimicrobial activity of vegetal extracts.
Determination of total phenolic content for each of the two cultivars analyzed (´Melrom´ and ´Nero´) was performed through the Folin Ciocalteu method. As Table 1 presents, TPC averaged 14422.06 mg GAE/100 g DW and was significantly influenced only by the extraction time ($p=0.000$). The longer the extraction time the higher the TPC found in the extract. On average, TPC varied from 12414.05 (T1) to 16283.57 mg GAE/100 g DW (T6) and its maximal increase reached 32.04% for ´Nero´ cultivar (from 12541.98 in T1 to 16560.88 mg GAE/100 g DW in T6) while a

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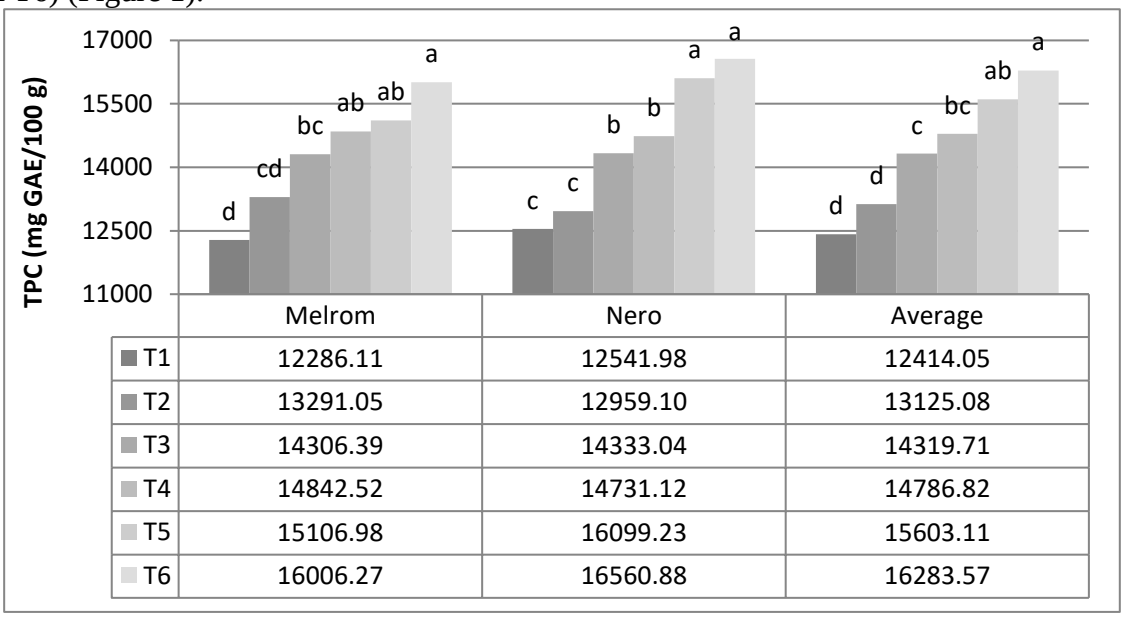
30.28% increase was registered for ´Melrom´ (from 12286.11 in T1 to 16006.27 mg GAE/100 g DW in T6) (Figure 1).



	Melrom	Nero	Average
T1	12286.11	12541.98	12414.05
T2	13291.05	12959.10	13125.08
T3	14306.39	14333.04	14319.71
T4	14842.52	14731.12	14786.82
T5	15106.98	16099.23	15603.11
T6	16006.27	16560.88	16283.57

Figure 1. Effect of ultrasound time treatment on TPC in chokeberry dehydrated pomace methanolic extract

Carrera et al. (2012), in an experiment on fresh grapes, compared the classical extraction method (60 minutes) to an ultrasound-assisted method and obtained better results when ultrasound treatment was applied for only 6 minutes.

Londoño-Londoño et al. (2010) extracted TPC from dry and wet citrus peels the highest yield was obtained with dry material and 30 minutes of extraction, while at 30 minutes with wet material the lowest yield was obtained. Authors explain this behavior by presuming that the dry material has more porosity and the solvent diffusion rate could be higher.

Extraction of TPC from dried berries of *A. melanocarpa* performed by D'Alessandro et al. (2012) was improved when ultrasound treatment temperature and time were higher. A 60ºC working temperature produced triple TPC yield compared to 20 ºC and TPC yields were almost constant after the first 1 h of extraction. Higher extraction temperature (51.45-41.53ºC) than our study and shorter time (25.67-27.86 minutes) produced the highest TPC yield from frozen pumpkin and peach samples (Altemimi et al., 2016).

The variation of the extraction time in the TFC protocol was similar to the TPC. Similar to TPC, TFC extraction was improved as the extraction protocol takes more time. Though, because of the very high flavonoids content determined in T6 samples (1.8–3.3 times higher than T5 value, data not presented) we chose to not consider T6 data, considering the possibility of extracting other compounds, in addition to flavonoids, in the 240 minutes of extraction.

TFC varied between 3975.06 mg CE/100 g DW for ´Melrom´ and 4801.91 mg CE/100 g DW for ´Nero´ (p=0.000), under the cultivar effect, from 3923.62 (T1) to 5050.09 mg CE/100 g DW (T5) (p=0,000), under extraction time effect, while a variation between 3293.74 (´Melrom´, T1) and 5109.63 mg CE/100 g DW (´Melrom´, T6) (p=0.012) was registered under the cultivar × extraction time combined effect (Figure 2). As can be seen in the graphical representation, longer ultrasound treatment led to a 55.16% higher TFC after 150 minutes compared to 30 minutes for ´Melrom´, although a nonsignificant TFC increase was registered for ´Nero´.

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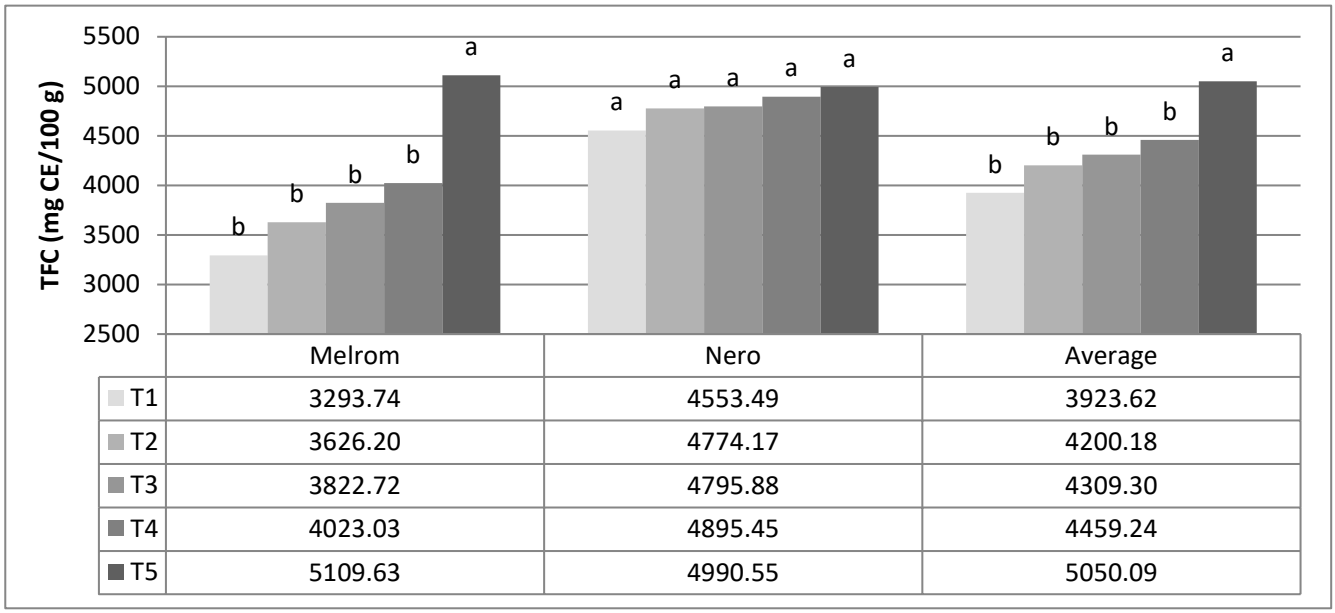

	Melrom	Nero	Average
T1	3293.74	4553.49	3923.62
T2	3626.20	4774.17	4200.18
T3	3822.72	4795.88	4309.30
T4	4023.03	4895.45	4459.24
T5	5109.63	4990.55	5050.09

Figure 2. Effect of ultrasound time treatment on TFC in chokeberry dehydrated pomace methanolic extract

Huang et al., 2009 dried *F. eucommiae* and ground it into powder (0.2-0.5 mm particle diameter). Samples of 5 g were soaked with ethanol (varying ethanol concentration from 30 to 50%, v/v; varying solid-to-liquid ratio from 1/50 to 1/70, w/v) for 2.5 h, and then placed in an ultrasonic bath and sonicated at 59 kHz for a certain time (varying extraction time from 45 to 75 minutes) at 55 °C. Unlike our experiment, the extraction time was varied from 45 to 75 minutes, as the flavonoids extraction ratio appeared to reduce beyond 75 minutes or below 45 minutes depending on ethanol concentration or solid-to-liquid ratio: the maximum level of flavonoids was achieved at the optimum point (42% ethanol concentration and 70 minutes extraction time) and when the extraction time increased from 30 to 70 minutes while solid to liquid ratio decreased from 1/50 to 1/60.

In another experiment (Yang et al., 2010), the dried flower of *C. aurantium* L. was ground to pass through an 80-mesh sieve to obtain fine powder and extracted through an ultrasound-assisted technique. The flavonoid yield increased by delaying the extraction time until 50 minutes or raising the process temperature to 70°C and declined right after. Still, the optimal extraction conditions reported were: extraction temperature of 72.11°C, time of 51.89 minutes, the ethanol concentration of 51.19%, and liquid/solid ratio of 40:10.

Longer extraction time led to higher total phenols and flavonoids yield in aqueous stevia extracts obtained by ultrasound-assisted extraction in Žlabur et al., 2015 study and was also reported by Li et al., 2015 for orange peel ethanolic extract. A decline in flavonoids level was registered after 120 minutes of ultrasound treatment as Li et al., 2015 reported.

For total tannins extraction, the above-presented protocol was followed, except for the ultrasound treatment which lasted 30-120 minutes. Based on the fact that after T2 TTC decreased in all samples, only the T1 - T4 time interval was analyzed. TTC varied significantly between cultivars: 7822.35 (´Melrom´) and 8237.29 mg GAE/ 100 g DW (´Nero´), p=0.009. A significant variation was also found between the extraction times: from 7049.92 (T1) to 9102.81 mg GAE/100 g (T2), p=0.000 (Figure 3).

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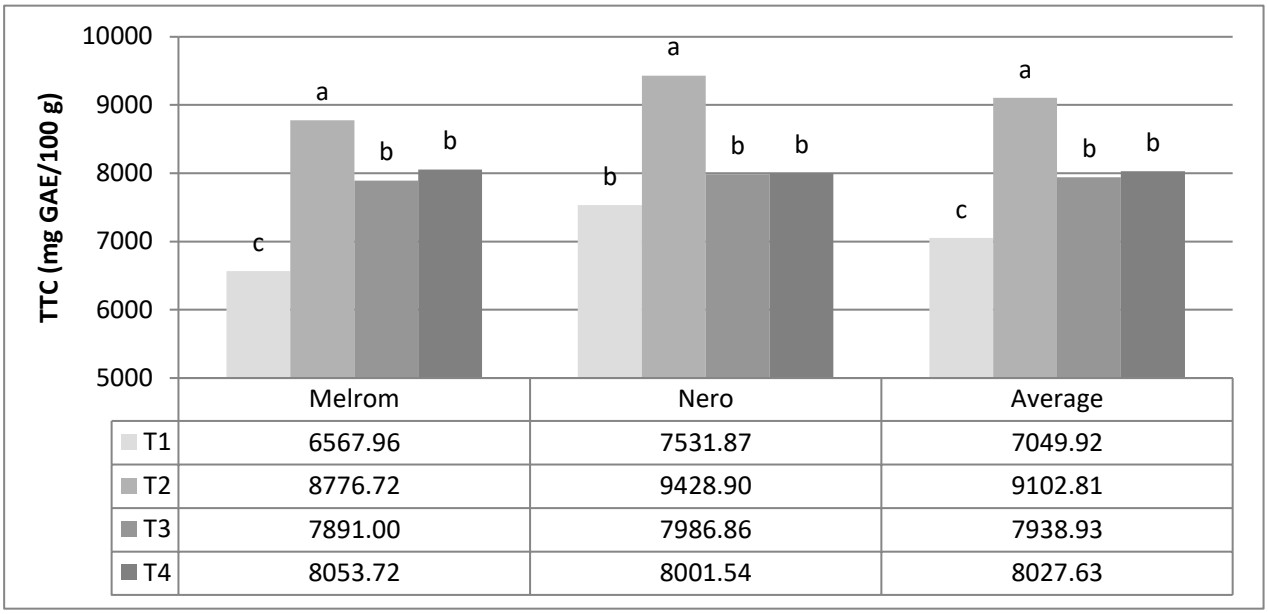

	Melrom	Nero	Average
T1	6567.96	7531.87	7049.92
T2	8776.72	9428.90	9102.81
T3	7891.00	7986.86	7938.93
T4	8053.72	8001.54	8027.63

Figure 3. Effect of ultrasound time treatment on TTC in chokeberry dehydrated pomace water extract

On average, total tannin content increase significantly by almost 29% after 60 minutes of ultrasound treatment. Still, longer ultrasonication leads to a significant TTC decrease of 14.66% (in T3) and 13.39% (in T4).

A shorter ultrasonic time (2.5 minutes) led to the maximum tannins yield extracted at 45.0ºC in an enzyme-based ultrasound-assisted method used for the extraction of tannins from acorns Luo et al., 2019, while a longer ultrasound treatment was followed by tannins yield decrease. As the extraction time is raised (over 30 minutes or 1 hour), the extraction efficiency tends to decrease, as reported in De Hoyos-Martinez et al., 2019 review. The authors explained that this result was attributed to the possible degradation of the proanthocyanidins due to the increased temperature of the water bath, provided by the prolonged sonication time (Dalzell and Kerven, 1998, cited by De Hoyos-Martinez et al., 2019).

In a study on leaf and bark extracts of *Solidago canadensis* L., Deng et al., 2015 obtained higher TPC in ultrasound-treated samples of leaves or barks, TTC mostly in ethanolic extracts (leaves and barks) and higher TFC resulted following high-pressure extraction. The higher TPC yield resulting from ultrasound treatment was longer was attributed by Şahin and Şamlı (2013) cited by Medina-Torres et al. (2017) to a two main stages mechanism: the first one called the "washing" step and the second, called "slow extraction". During the first 10-20 minutes of extraction ("washing" step), the dissolution of the soluble components on the surfaces of the matrix occurs and up to 90% of the recovery of the total content of the phenolic compounds can be achieved thus indicating a considerably rapid extraction rate (Tao et al., 2014). During the second step ("slow extraction"), a diffusion process takes place, when the mass transfer of the solute from the matrix into the solvent occurs and the time this process can last from 60 to 100 minutes (Şahin and Şamlı, 2013).

As Figure 4 presents, the lycopene content of the samples varied under the cultivar influence from 0.85 (´Melrom´) to 1.10 mg/ 100 g DW (´Nero´), p=0.002. On average, raising the extraction time significantly increased lycopene content from 0.84 (T1) to 1.19 (T3). Comparing to T1, the lycopene content raised in T3 by almost 23.08% for ´Melrom´ and 60.67% for ´Nero´.

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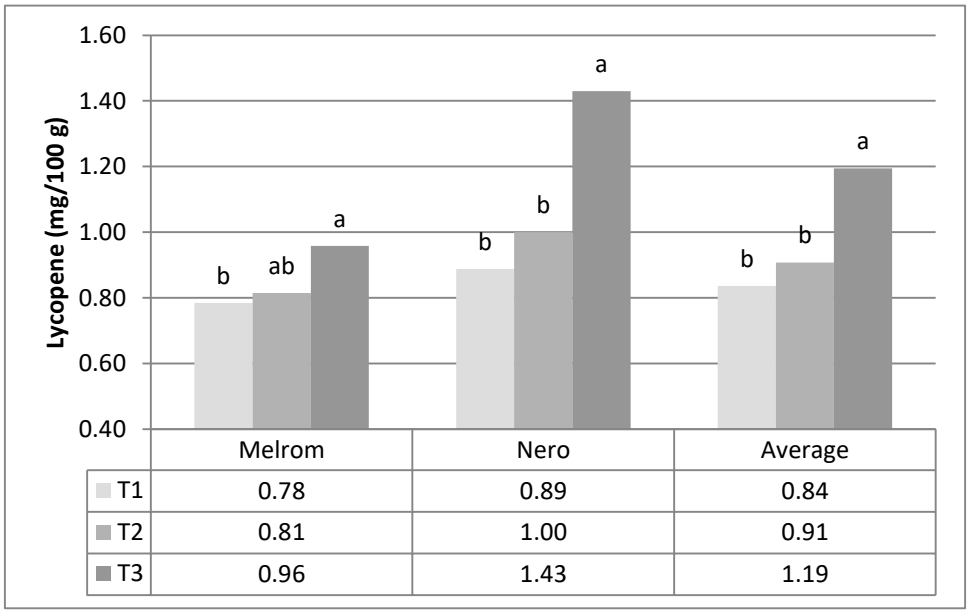
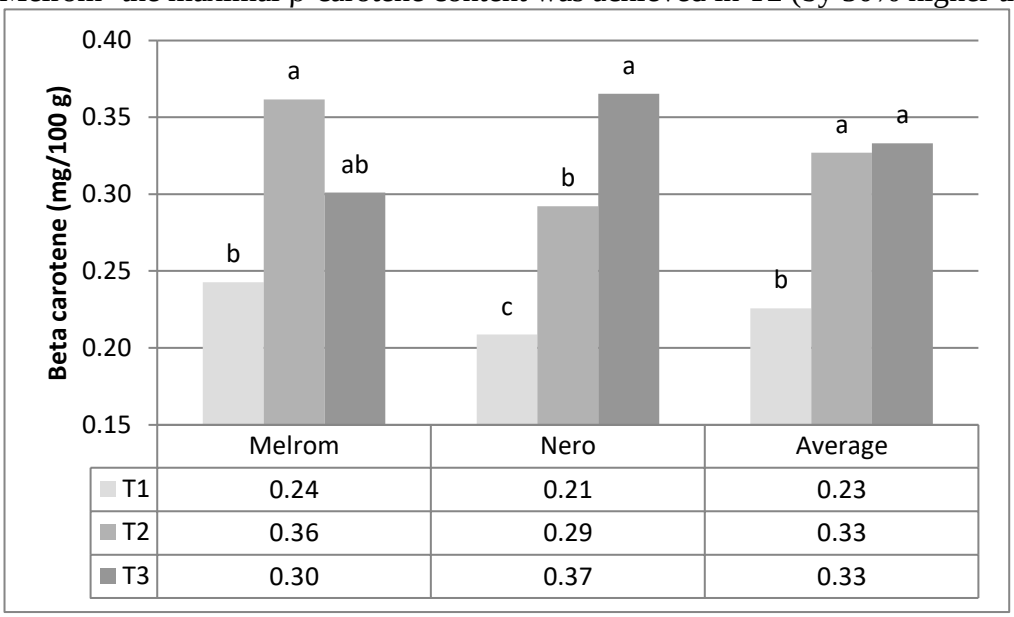


	Melrom	Nero	Average
T1	0.78	0.89	0.84
T2	0.81	1.00	0.91
T3	0.96	1.43	1.19

Figure 4. Effect of time extraction on lycopene in chokeberry dehydrated pomace extract

Applying the same extraction protocol for the β-carotene resulted in significant variation between T1 and T3 (p=0.000) and also a significant cultivar × extraction time influence was observed (p=0.005). Therefore, on average the β-carotene content increased by 44.8% from T1 to T2 and by 47.6% from T1 to T3. No significant differences were noted between T2 and T3. Overall, the highest β-carotene content was determined for the ´Nero´ cultivar, in T3 (76.19% higher than T1), while for ´Melrom´ the maximal β-carotene content was achieved in T2 (by 50% higher than T1).



	Melrom	Nero	Average
T1	0.24	0.21	0.23
T2	0.36	0.29	0.33
T3	0.30	0.37	0.33

Figure 5. Effect of time extraction on lycopene in chokeberry dehydrated pomace extract

The maximum ammount of total carotenoids, expressed as lycopene, extracted from tomato waste was obtained using ethyl lactate for three successive extractions of 30 minutes, at 70ºC (Strati and

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Oreopoulou, 2011). Minimum lycopene yield was obtained from dried tomato wastes at the lowest extraction temperature (20 °C), the lowest liquid-solid ratio (20:1 v/w) for 10 minutes extraction duration in a mixture of solvents hexane: methanol: acetone (2:1:1 v/v). The maximum lycopene yield was obtained at the highest extraction temperature (60°C), and the highest liquid-solid ratio (50:1 v/w) for the longest run time (40 minutes). The lycopene yield increased with time in the beginning and did not vary significantly with time from 30 to 40 minutes because of the osmotic balance mechanism (Kumcuoglu et al., 2014). The amount of conventionally extracted lycopene and β-carotene in dried tomato waste samples increased as extraction time increased but decreased after 30 minutes in Yilmaz et al. (2017) experiment.

Testing the intensity of the correlation between biologically active compounds determined in the study (Table 1) showed that higher TPC was positively significantly correlated to TFC, lycopene, and β-carotene. A similar positive significant correlation was established between TFC and both TTC and lycopene, while TTC was only correlated to β-carotene. Also, higher lycopene content was found when higher β-carotene was determined.

Table 1. Correlation intensity between total phenolic, total tannins, total flavonoid content, lycopene and β-carotene

		TFC	TTC	Lycopene	β-carotene
TPC	Pearson Correlation	**.549*****	0.237	**.497****	**.554*****
	Sig. (2-tailed)	0.000	0.105	0.002	0.000
TFC	Pearson Correlation	1	**.388****	**.632*****	0.161
	Sig. (2-tailed)		0.007	0.000	0.348
TTC	Pearson Correlation		1	0.262	**.503****
	Sig. (2-tailed)			0.123	0.002
Lycopene	Pearson Correlation			1	**.578*****
	Sig. (2-tailed)				0.000

The DPPH assay tested the remanent radical activity (A%) of five doses of methanolic extract per each cultivar (20, 30, 40, 50, and 60 µL) added to 2980, 2970, 2960, 2950, and 2940 µL methanolic DPPH solution (1.16×10^{-4} mol/L). The dynamics of the DPPH activity decrease was assessed through the determination of DPPH remanent activity every two minutes during the reaction between the methanolic extract and methanolic DPPH solution per dose. Therefore, a bifactorial A×B experiment was conducted with five levels of pomace methanolic extract dose, A (a_1= 20 µL, a_2= 30 µL, and so, with a_5=60 µL) and 10 levels of reaction time, B (b_1= reaction debut (T_0), b_2= 2 minutes after the T_0, b_3= 4 minutes after the T_0 and so, with b_{10}= 20 minutes after T_0).

Figure 6 presents the extract dose effect on remanent radical activity (main effect and its interaction with the reaction time). As can be seen, between the five tested doses of the methanolic extracts, the most efficient were 60 (20.55%) and 50 µL (21.89%). As the reaction of DPPH reduction progresses the differences between methanolic extract doses diminish. Therefore, there were no significant differences between methanolic extracts doses registered after 14 minutes, except for 20 µL. Even at the lower dose of 30 µL (A=15.65-20.09%), the methanolic extract of chokeberry pomace exerts a reducing action similar to 60 µL dose (A=12.62-13.68%).

*Corresponding author, E-mail address: sinacosmulescu@hotmail.com

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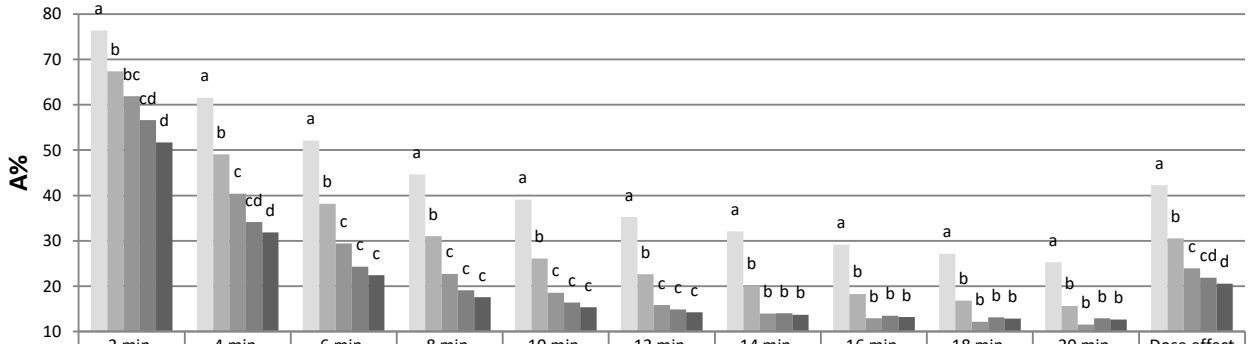
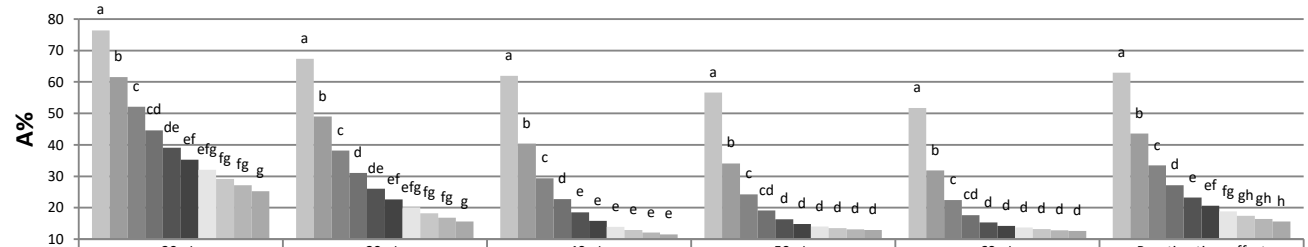


	2 min.	4 min.	6 min.	8 min.	10 min.	12 min.	14 min.	16 min.	18 min.	20 min.	Dose effect
20 µL	76.40	61.58	52.12	44.64	39.09	35.25	32.04	29.18	27.15	25.28	42.27
30 µL	67.40	49.05	38.19	31.04	26.10	22.64	20.09	18.28	16.82	15.65	30.53
40 µL	61.91	40.38	29.41	22.69	18.54	15.85	13.95	12.88	12.13	11.55	23.93
50 µL	56.60	34.12	24.28	19.11	16.38	14.84	14.00	13.49	13.15	12.94	21.89
60 µL	51.72	31.82	22.45	17.59	15.33	14.25	13.68	13.20	12.87	12.62	20.55

Figure 6. Effect of methanolic extract dose on remanent radical activity (A%)

The effect of the reaction time is presented in Figure 7. As can be observed, the highest antiradical activity (i.e., the lowest A%) was registered after 16 minutes (15.65-17.47%). Comparing the influence of reaction time depending on the extract dose, it can be observed that, the reducing power of the chokeberry extract added in 40 µL dose reached maximum antiradical activity after 10 minutes. The reaction time was shortened to 8 minutes for the next 50 and 60 µL samples.



	20 µL	30 µL	40 µL	50 µL	60 µL	Reaction time effect
2 min.	76.40	67.40	61.91	56.60	51.72	62.97
4 min.	61.58	49.05	40.38	34.12	31.82	43.56
6 min.	52.12	38.19	29.41	24.28	22.45	33.44
8 min.	44.64	31.04	22.69	19.11	17.59	27.15
10 min.	39.09	26.10	18.54	16.38	15.33	23.20
12 min.	35.25	22.64	15.85	14.84	14.25	20.66
14 min.	32.04	20.09	13.95	14.00	13.68	18.82
16 min.	29.18	18.28	12.88	13.49	13.20	17.47
18 min.	27.15	16.82	12.13	13.15	12.87	16.47
20 min.	25.28	15.65	11.55	12.94	12.62	15.65

Figure 7. Effect of reaction time on remanent radical activity (A%)

4. CONCLUSIONS

As the present study showed, chokeberry powdered pomace contains high amounts of antioxidants: 12,286.11-16,560.88 mg GAE/100g DW TPC, 6567.96-9428.90 mg GAE/100 g DW TTC, 3293.74-5109.63 mg EC/100 g DW TFC. Lycopene ranged between 0.78-1.43 mg/100 g DW and β-carotene ranged between 0.21-0.37 mg/100g DW. Therefore, chokeberry dehydrated residue

*Corresponding author, E-mail address: sinacosmulescu@hotmail.com

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represents a valuable by-product with multiple uses. Ultrasound-assisted extraction is a low-cost and fast method for biological compound extraction and adapting it to different vegetal matrices represents one step to perfecting this technique. Moreover, establishing the proper extract dose and knowing the time until the maximum antioxidant power is achieved can be helpful for future DPPH assays.

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