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Serviceberry, a berry fruit with growing interest of industry: Physicochemical and quali-quantitative health-related compound characterisation

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ABSTRACT

Amelanchier canadensis (L.) Medik., commonly called serviceberry, is a potential functional food that is also used for its medicinal purposes. This work evaluated the potential of a cultivated serviceberry species as a functional food by characterising its physicochemical characteristics, antioxidant capacity, vitamin C, phenolics and other phytochemicals selected as health-promoting biomarkers, using high-performance liquid chromatography. The most important compound class identified was polyphenols (62.10%), followed by organic acids (22.63%), monoterpenes (7.95%), and vitamins (7.32%). Results showed that serviceberry fruits could be good sources of phenolic constituents, as catechins (343.46 ± 29.46 mg/100 g_{FW}), anthocyanins (220.66 ± 17.43 mg/100 g_{FW}), and tannins (209.29 ± 7.81 mg/100 g_{FW}) (FW = fresh weight). These results highlight the potential role of *A. canadensis* fruits as a functional food. Further studies are needed to identify several genotypes for breeding to get suitable cultivars for fresh consumption and processing.

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1. Introduction

New demands are constantly directed to food producers by consumers. Rising consumer incomes, changes in lifestyle and demographics, and shifting preferences due to advanced knowledge about the relationship between food and health contribute to new demands for foods (Dolgoplova, Teuber, & Bruschi, 2015). Today, foods are intended to not only satisfy hunger and provide necessary nutrients, but also to prevent nutrition-related diseases and improve physical and mental well-being (Annunziata & Vecchio, 2011): functional foods could play an important role in human health. Underutilised fruits, as serviceberry, could be an important source of health promoting

phytonutrients with medicinal properties. Furthermore, these fruits have often high pigment contents, which could represent an alternative to synthetic dyes (Rymbai et al., 2016).

The genus *Amelanchier* (family Rosaceae) is represented by approximately 25 species widespread in North America and in parts of Eurasia (Michalczyk & Macura, 2010). Serviceberry is native to North America from Alaska, across western Canada, and in the western and north-central USA (Lim, 2012). It is less known and rarely grown in Europe (except Scandinavian countries) in spite of its high frost resistance, decorative value as a shrub, and edible fruits (Bakowska-Barczak, Marianchuk, & Kolodziejczyk, 2007). In its native environment, serviceberry is found in thickets, woodland margins, banks of streams, canyons, and hillsides, from sea level to 3000 m altitude. It prefers a rich,

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well-drained loamy soil but it can also grow in any sandy or clayey soil that is not waterlogged or too dry. It is relatively drought and salt tolerant and thrives in a sunny or semi-shade position (Lim, 2012).

A. alnifolia, *A. arborea* and *A. canadensis* are the best known species. *A. alnifolia* (Saskatoon serviceberry or Alder-leaved serviceberry) is a western North American species. It has a variable habitus but it is usually found as a multi-stemmed shrub ranging from 2–3 m in height: some genotypes are cultivated for commercial fruit production in western USA and Canada. Its fruits are approximately 1 cm in diameter, blue-purple and ripen in July (Hu, Kwok, & Kitts, 2005). *A. arborea* (Downy serviceberry) is an eastern North American large shrub or small tree 8–10 m high and is crossed with *A. laevis* to produce cultivars for the landscape industry in the USA. The fruits of *A. arborea* are purple-black, slightly sweet and ripen in late June (Adhikari, Francis, Schutzki, Chandra, & Nair, 2005). *A. canadensis* (Shadblow serviceberry) is another eastern North American species. It ripens in early June and it is used in the landscape trade. It is a stoloniferous shrub or small tree reaching 8 m in height with a fastigiate crown and smooth ash-grey bark. Twigs are slender, reddish-brown becoming glabrous during flowering, while leaves are alternate, simple, oval-obovate to nearly round. The maroon-purple fruits are similar to *A. arborea* ones (pome, 7–15 mm across, glabrous, wax-coated) (Lim, 2012).

North American indigenous people uses different parts of the serviceberry plant for several medicinal purposes: in Canada, the fruits are used as juice for treating stomach ailments and as a laxative. Eye- and ear-drops are also prepared from ripe serviceberries (Kershaw, 2000). The boiled bark is used as a disinfectant, while the root infusion is used to prevent miscarriage after an injury (Lim, 2012). Native American communities prepare a tea from the twigs and stem and administer it to women just after childbirth. Moreover, a tonic from the bark is given to women after delivery to hasten discharge of the placenta (Turner, 1997).

Amelanchier spp. can also be used as a windbreak plant. The wood can be used for tool handles, canes, canoe crossbars, and small implements, because it is hard, strong, and fine-grained, while the young stems are used to make basket rims, handles, arrows, combs, digging sticks, salmon spreaders, and pipes. Fruits provide a purple dye (Lim, 2012).

The ripe fruit of *Amelanchier* spp. is sweet with a hint of apple, and there is growing interest in using it in the food industry (fresh, pies, pastries, preserves, jams, jellies, spreads cereals, and snack food). The fruits are also been added into cider, wine, beer, or tea (Adhikari et al., 2005; Bakowska-Barczak & Kolodziejczyk, 2008). The native people and early settlers of the North American prairies used serviceberry as one of their main food sources, but its use was limited because of its natural distribution area in the wild. In the last two decades, however, there has been growing interest in the industrial cultivation and utilisation of this fruit in Canada and USA (Michigan) (Adhikari et al., 2005; Kershaw, 2000). Fruit processing has an important role in exploiting the raw material because serviceberry has a relatively short harvest period (Michalczyk & Macura, 2010). Innovative methods of processing, freezing, and packaging have greatly increased the uses of this fruit. Moreover, growers are promoting it as a potential functional food (Jamin, 2009), alongside other fruit species, as *Ribes nigrum*

(Donno et al., 2013b), *Morus nigra* (Donno, Cerutti, Prgomet, Mellano, & Beccaro, 2015c) and *Lycium* spp. (Donno, Beccaro, Mellano, Cerutti, & Bounous, 2015a).

The composition of fruits considerably varies depending on genotype, ripening stage at harvest and growing conditions (Michalczyk & Macura, 2010). There are few reports concerning the chemical composition of *Amelanchier* spp.: phytochemical studies on *A. canadensis* are rarely found in the literature. However, the available literature usually emphasises its important health benefits: serviceberry appears to be an excellent source of manganese, magnesium, and iron, and a relatively good source of calcium, potassium, copper, and carotenoids (e.g. lutein). Moreover, the fruit is rich in nutraceuticals, particularly phenolic compounds, as anthocyanins, chlorogenic acid, catechins and rutin (Bakowska-Barczak & Kolodziejczyk, 2008; Bakowska-Barczak et al., 2007). In addition, *Amelanchier* spp. seed oil may serve as a potential dietary source of tocopherols, sterols, and unsaturated fatty acids (Lim, 2012).

Several cultivars of *Amelanchier* spp. were found to possess free radical scavenging activity in a concentration-dependent manner related to their relatively high anthocyanin content (Hu et al., 2005), and antiviral activity against enteric coronavirus. Moreover, serviceberry fruits show antidiabetic properties (as aldose reductase inhibitor activity), and exhibit the ability to regulate lipid metabolism and energy expenditure in a manner consistent with improving metabolic syndrome (Burns Kraft et al., 2008).

The identification and quantification of bioactive compounds in fruits and the evaluation of their biological activities are important to gauge their efficacy as dietary interventions (Donno et al., 2012; Fu et al., 2011). Chromatographic fingerprinting could be considered an easy and reliable technique to characterise and differentiate *Amelanchier* spp. checking the fruit quality and safety (Donno et al., 2015c). The technique shows a relatively complete picture of fruit extracts and provides insight into the synergistic and additive biological effects of the bioactive constituents to total phytochemical complex (Donno, Beccaro, Mellano, Cerutti, & Bounous, 2014).

This work aimed to evaluate the potential of a cultivated serviceberry species (*A. canadensis* (L.) Medik.) as a functional food by characterising its physicochemical characteristics, and antioxidant capacity. Furthermore, the characterisation and quantification of several phytochemicals, selected as health-promoting biomarkers, was performed, using high-performance liquid chromatography-diode array detection (HPLC-DAD).

2. Materials and methods

2.1. Plant material, field description, and climate data

Fully ripened fruits (0.5 kg for each replication) were collected from a cultivated genotype of *A. canadensis* (L.) Medik in the middle of June 2015, in Chieri (45°1'0"N, 7°49'0"E, at 305 m A.S.L.), Piedmont (north-western Italy), in the fruit tree germplasm collection of the Department of Agricultural, Forest and Food Sciences, University of Turin. The climate of the area is temperate, with rains in spring and autumn, and a rainfall of approximately 810 mm/year; the soil is loam-clay.

Immediately after harvest, samples were protected from light to avoid loss of antioxidant components, as reported by [Cazares-Franco et al. \(2014\)](#), and sent directly to the laboratory where they were divided into two equal portions. One portion was used to determine their physicochemical parameters, on the same day of harvest. The second portion was stored at 4 °C and 95% relative humidity (RH), until extraction and nutraceutical analysis.

2.2. Solvents and chemicals

Sodium carbonate, Folin–Ciocalteu phenol reagent, sodium acetate, citric acid, potassium chloride, hydrochloric acid, iron(III) chloride hexahydrate, 2,4,6-tripyridyl-S-triazine, 1,2-phenylenediamine dihydrochloride (OPDA), all polyphenolic and terpenic standards, potassium dihydrogen phosphate, phosphoric acid and HPLC-grade methanol and acetonitrile were purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetic acid, ethanol, organic acids and HPLC-grade formic acid were purchased from Fluka BioChemika, Buchs, Switzerland. Ethylenediaminetetraacetic acid disodium salt was purchased from AMRESKO (Solon, OH, USA). Sodium fluoride was purchased from Riedel-de Haen (Seelze, Germany). Cetyltrimethylammonium bromide (cetrimide), ascorbic acid (AA) and dehydroascorbic acid (DHAA) were purchased from Extrasynthèse (Genay, France). Milli-Q ultrapure water was produced by Sartorius Stedim Biotech mod. Arium (Sartorius, Göttingen, Germany).

2.3. Determination of quality properties

Approximately 10% of the ripe fruits were washed, drained, and dried with paper towels. Their width and length were measured using a 0.01 mm sensitive digital calliper (Traceable Digital Caliper-6", VWR International, Milano, Italy) and their weight determined to the nearest 0.01 g (Mettler, Greifensee, Switzerland). The fruit was then homogenised in a blender and centrifuged (4000 rpm, 10 min), and pH, total soluble solids (TSS), and titratable acidity (TA) were evaluated. The remaining fresh fruits were immediately stored at 4 °C and 95% RH, until further analysis.

The TA ($\text{meq} \cdot \text{L}^{-1}$) was determined in a mixture of 10 mL serviceberry juice diluted in 90 mL Milli-Q water, by titration with 0.2 M NaOH using an automatic titrator (Crison, Alella, Spain) to an end-point of pH 8.2. The pH of the fruit juice was measured directly. The TSS was measured directly in serviceberry juice with a digital refractometer (Tsingtao Unicomp Optics Instruments, Laixi, China), and the results were expressed as °Brix.

2.4. Spectrophotometric analysis

2.4.1. Total polyphenolic compounds

The total polyphenol content (TPC) was determined following the Folin–Ciocalteu colorimetric method ([Slinkard & Singleton, 1977](#)), and the results were expressed as mg of gallic acid equivalents (GAE) per 100 g of fresh weight (FW). Gallic acid standard solutions were prepared at 0.02–0.10 $\text{mg} \cdot \text{mL}^{-1}$.

2.4.2. Total anthocyanins

The total anthocyanin content (TAC) in the extracts was determined using the pH-differential method ([Giusti & Wrolstad, 2001](#); [Lee, Durst, & Wrolstad, 2005](#)), and expressed as milligrams of cyanidin-3-O-glucoside (C3G) per 100 grams of fresh weight ($\text{mg}_{\text{C3G}}/100 \text{ g}_{\text{FW}}$).

2.4.3. Antioxidant activity

The antioxidant activity was evaluated by the ferric reducing antioxidant power (FRAP) assay ([Benzie & Strain, 1999](#)), and the results were expressed as millimoles of ferrous iron (Fe^{2+}) equivalents per kilogram (solid food) of FW. The standard curve was obtained using $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ at 100–1000 $\mu\text{mol} \cdot \text{L}^{-1}$.

2.5. Chromatographic analysis

2.5.1. Sample preparation protocols for HPLC analysis

Samples were filtered with circular pre-injection filters (0.45 μm , polytetrafluoroethylene membrane) prior to HPLC-DAD analysis. In the case of vitamin C analysis, a C_{18} cartridge for solid phase extraction (Sep-Pak® C-18, Waters, Milford, MA, USA) was used to absorb the polyphenolic fraction. Then, 250 μL of OPDA solution ($18.8 \text{ mmol} \cdot \text{L}^{-1}$) was added to 750 μL of each sample for DHAA derivatisation into the fluorophore 3-(1,2-dihydroxyethyl)furo(3,4-b)quinoxaline-1-one. After 37 min in the dark, these samples were analysed using HPLC-DAD ([Gonzalez-Molina, Moreno, & Garcia-Viguera, 2008](#)).

2.5.2. Standard calibration

External standard calibration method was used for quantitative determinations. Three manual injections of each standard (20 μL) at the concentrations listed in [Table 1](#) were performed. The calibration curves were obtained by plotting the peak area (y) of the compound at each concentration level versus the sample concentration (x).

2.5.3. Apparatus and chromatographic conditions

An Agilent 1200 High-Performance Liquid Chromatograph coupled to an Agilent UV-Vis diode array detector (Agilent Technologies, Santa Clara, CA, USA) was used for the chromatographic analysis. Five chromatographic methods were used to separate the biomolecules on a Kinetex C18 column (4.6 \times 150 mm, 5 μm , Phenomenex, Torrance, CA, USA), as listed in [Table 2](#). Several mobile phases were used for biomarker identification and UV spectra were recorded at different wavelengths, based on HPLC methods previously tested and validated for herbal medicines ([Donno et al., 2015b](#)).

2.5.4. Identification and quantification of bioactive compounds in the extracts

All the samples were analysed in triplicate, and standard deviations are given in order to assess the repeatability of the used methods. Total bioactive compound content (TBCC) was determined as the sum of selected biomarkers having a positive role in human health ("multi-marker approach") ([Mok & Chau, 2006](#)). Five polyphenolic classes were considered: benzoic acids, catechins, cinnamic acids, flavonols, and tannins. Monoterpenes, organic acids, and vitamin C (as the sum of ascorbic and dehydroascorbic acids) were also considered to

Table 1 – Main chromatographic parameters of the methods used for calibration.

Chromatographic method	Class	Standard	Identification code	Retention time (t_R)	Wavelength	Calibration curve equation	R^2	Calibration curve range	LOD	LOQ
				(min)	(nm)			(mg L^{-1})	(mg L^{-1})	(mg L^{-1})
A	Cinnamic acids	Caffeic acid	1	4.54	330	$y = 59.046x + 200.6$	0.996	111–500	0.305	1.016
		Chlorogenic acid	2	3.89	330	$y = 13.583x + 760.05$	0.984	111–500	0.940	3.134
		Coumaric acid	3	6.74	330	$y = 8.9342x + 217.4$	0.997	111–500	2.907	9.690
		Ferulic acid	4	7.99	330	$y = 3.3963x - 4.9524$	1.000	111–500	1.245	4.150
	Flavonols	Hyperoside	5	10.89	330	$y = 7.1322x - 4.583$	0.999	111–500	3.372	11.241
		Isoquercitrin	6	11.24	330	$y = 8.3078x + 26.621$	0.999	111–500	0.252	0.840
		Quercetin	7	17.67	330	$y = 3.4095x - 98.307$	0.998	111–500	4.055	13.518
		Quercitrin	8	13.28	330	$y = 2.7413x + 5.6367$	0.998	111–500	5.456	18.187
		Rutin	9	12.95	330	$y = 6.5808x + 30.831$	0.999	111–500	2.937	9.790
B	Benzoic acids	Ellagic acid	10	18.65	280	$y = 29.954x + 184.52$	0.998	62.5–250	0.611	2.035
		Gallic acid	11	4.26	280	$y = 44.996x + 261.86$	0.999	62.5–250	0.435	1.451
	Catechins	Catechin	12	10.31	280	$y = 8.9197x + 66.952$	1.000	62.5–250	2.343	7.809
		Epicatechin	13	14.30	280	$y = 12.88x - 43.816$	0.999	62.5–250	0.763	2.543
	Tannins	Castalagin	14	16.35	280	$y = 4.236x - 8.535$	1.000	62.5–250	1.009	3.363
		Vescalagin	15	17.25	280	$y = 4.939x - 1.232$	1.000	62.5–250	0.603	2.010
C	Monoterpenes	Limonene	16	3.35	250	$y = 0.1894x - 5.420$	0.999	125–1000	8.654	28.847
		Phellandrene	17	3.57	210	$y = 8.783x - 145.3$	0.998	125–1000	0.562	1.874
		Sabinene	18	3.45	220	$y = 18.14x - 1004$	0.998	125–1000	0.094	0.314
		γ -Terpinene	19	3.28	235	$y = 0.4886x - 23.02$	0.999	125–1000	17.577	58.590
		Terpinolene	20	4.83	220	$y = 26.52x + 876.8$	0.999	125–1000	0.241	0.804
D	Organic acids	Citric acid	21	5.30	214	$y = 1.0603x - 22.092$	1.000	167–1000	18.805	62.682
		Malic acid	22	4.03	214	$y = 1.415x - 80.254$	0.996	167–1000	15.721	52.404
		Oxalic acid	23	7.85	214	$y = 6.4502x + 6.1503$	0.998	167–1000	0.550	1.835
		Quinic acid	24	3.21	214	$y = 0.8087x - 38.021$	0.998	167–1000	26.106	87.021
		Succinic acid	25	3.46	214	$y = 0.9236x - 8.0823$	0.995	167–1000	7.135	23.783
		Tartaric acid	26	5.69	214	$y = 1.8427x + 15.796$	1.000	167–1000	8.520	28.401
E	Vitamins	Ascorbic acid	27	4.14	261	$y = 42.71x + 27.969$	0.999	100–1000	0.836	2.786
		Dehydroascorbic acid	28	3.41	348	$y = 4.1628x + 140.01$	0.999	30–300	1.095	3.649

Table 2 – Chromatographic conditions of the methods used to characterise the nutritional parameters of serviceberry (*A. canadensis* (L.) Medik).

Method	Compounds of interest	Stationary phase	Mobile phase	Flow	Time of analysis	Gradient ^b	Wavelength
				(mL min ⁻¹)	(min)		(nm)
A	Cinnamic acids, flavonols	KINETEX – C18 column (4.6 × 150 mm, 5 μm)	A: 10 mM KH ₂ PO ₄ /H ₃ PO ₄ , pH = 2.8 B: CH ₃ CN	1.5	20 + 2 (CT ^a)	Yes	330
B	Benzoic acids, catechins, tannins	KINETEX – C18 column (4.6 × 150 mm, 5 μm)	A: H ₂ O/CH ₃ OH/HCOOH (5:95:0.1 v/v/v), pH = 2.5 B: CH ₃ OH/HCOOH (100:0.1 v/v)	0.6	23 + 2 (CT ^a)	Yes	280
C	Monoterpenes	KINETEX – C18 column (4.6 × 150 mm, 5 μm)	A: H ₂ O B: CH ₃ CN	1.0	17 + 3 (CT ^a)	Yes	210, 220, 235, 250
D	Organic acids	KINETEX – C18 column (4.6 × 150 mm, 5 μm)	A: 10 mM KH ₂ PO ₄ /H ₃ PO ₄ , pH = 2.8 B: CH ₃ CN	0.6	13 + 2 (CT ^a)	No	214
E	Vitamins	KINETEX – C18 column (4.6 × 150 mm, 5 μm)	A: 5 mM C ₁₆ H ₃₃ N(CH ₃) ₃ Br/ 50 mM KH ₂ PO ₄ , pH = 2.5 B: CH ₃ OH	0.9	10 + 5 (CT ^a)	No	261, 348

^a CT = conditioning time.
^b Elution conditions.
Method A gradient: 5%B to 21%B in 17 min + 21%B in 3 min.
Method B gradient: 3%B to 85%B in 22 min + 85%B in 1 min.
Method C ratio of phase A and B: 95:5.
Method D ratio of phase A and B: 95:5.

obtain a complete analytical fingerprint. All the results were expressed as mg/100 g of FW.

2.6. Statistical analysis

All samples were prepared and analysed in triplicate. Results were subjected to analysis of variance (ANOVA) for mean comparison (SPSS 22.0 Software) followed by HSD Tukey multiple range test ($P < 0.05$).

3. Results and discussion

The present study characterises the morphological properties, quality parameters and phytochemical composition of *A. canadensis* fruits. The results are discussed in relation to the potential of serviceberry as a functional food.

3.1. Morphological and quality parameters

Table 3 presents the fruit weight and size, TSS, pH, and TA of the analysed serviceberry genotype.

Results showed that the fresh fruit are small and spheroidal (length: 9.94 ± 0.22 mm; width: 8.76 ± 0.22 mm), with a mean weight of 0.65 ± 0.03 g. The cultivated serviceberry fruit size is on average greater than the size of the fruit harvested in the wild, as reported in the previous studies (Bakowska-Barczak & Kolodziejczyk, 2008; Cazares-Franco et al., 2014). The results (Table 3) corroborated with Stushnoff (1991), who reported that cultivated serviceberry showed up to 25% larger fruit size compared with wild fruit one. Quality analysis reported a mean value of TSS 21.90 ± 2.89 °Brix, TA 68.82 ± 7.37 meq · L⁻¹ and pH

3.42 ± 0.02 (Table 3). The TSS in various serviceberry genotypes ranged from 20% to 30% FW, with 16–23% sucrose and 8–12% reducing sugars (Mazza, 1982). Wolfe and Wood (1971) found that the sugar content slowly increased as the fruit matured and then considerably accelerated before ripening: fructose content greatly decreased (–25%) after the fruit ripened while the glucose content remained unaltered. The pH of wild fruits, as reported in previous studies (Cazares-Franco et al., 2014; Lim, 2012), is often lower than the pH of cultivated fruits: the higher pH indicates that the cultivated fruit is a low-acid food, a positive sensory feature, as confirmed by TA values reported in this study. Moreover, a lower TSS/TA ratio than other

Table 3 – Morphological and quality parameters of serviceberry (*A. canadensis* (L.) Medik).

	Mean value	SD
Weight ^a	0.65	0.03
Width ^b	8.76	0.22
Length ^c	9.94	0.22
TSS ^d	21.90	2.89
TA ^e	68.82	7.37
pH ^f	3.42	0.02

Mean value and standard deviation (SD) of each sample is given (N = 3).

Results were expressed as:

^a g.

^b mm.

^c mm.

^d °Brix.

^e meq · L⁻¹.

^f pH-units.

berry fruits, mainly due to the low fruit acidity, provides a longer shelf life (Galletta, Ballinger, Monroe, & Kushman, 1971).

3.2. Nutraceutical properties

Studies by Hosseinian and Beta (2007) showed that serviceberry had high potential value for the fresh market and food-derived manufacturers, because of their high polyphenolic content, in particular, anthocyanins. The TPC (539.24 ± 29.20 mg_{GAE}/100g_{FW}) and TAC (220.66 ± 17.43 mg_{C3G}/100g_{FW}) values obtained from the analysed extracts are reported in Table 4. Anthocyanins and total phenolics in serviceberries increase with fruit ripening (Lim, 2012). The phenolic compounds found in fruits and vegetables have attracted much interest due to their beneficial properties (Faller & Fialho, 2010). Based on the TPC, serviceberry fruits are excellent polyphenolic sources compared with commonly consumed fruits. For example, TPC reported for some berries (e.g. raspberry, blueberry, strawberry, and blackcurrant) (Donno et al., 2013b, 2015c) were markedly lower than TPC reported in this study for serviceberry. Moreover, a high positive correlation between TPC and antioxidant capacity has been established (Donno et al., 2012; Faller & Fialho, 2010): therefore, *A. canadensis* is a very attractive fruit species from a functional point of view. In other studies (Lim, 2012), anthocyanin content was correlated with TPC, TA, pH, and sugar : acid ratio. The results in Tables 3 and 4 suggested that high content of anthocyanins in serviceberries could be associated with high TPC and low pH and sugar : acid ratio. Hellström, Sinkkonen, Karonen, and Mattila (2007) found that fruits of different serviceberry genotypes contained proanthocyanidins from dimers to heptamers and higher polymers. In serviceberry, proanthocyanidins were generally of the procyanidin type, mainly consisting of epicatechin units linked by B-type bonds. Bakowska-Barczak et al. (2007) found serviceberries contained the following anthocyanidins: cyanidin, delphinidin, pelargonidin, petunidin, peonidin, and malvidin. In the current research, only TAC was considered.

The FRAP assay was used to evaluate antioxidant capacity of *A. canadensis* fruits. The FRAP assay is based on the ability of antioxidants to reduce ferric(III) ions to ferrous(II) ions: it is a simple and widely used method to evaluate antioxidant capacity (Donno et al., 2015a; Fu et al., 2011). In this study, fresh fruits showed a mean FRAP value of 25.07 ± 0.48 mmol Fe²⁺ · kg⁻¹ (Table 4). Thus, serviceberry presented a higher FRAP value compared to some common fruit, as black mulberry, goji, raspberry,

and apple (Donno et al., 2015c). Antioxidant activity values of the above mentioned common fruits are reported in other studies (Cazares-Franco et al., 2014; Michalczyk & Macura, 2010). On the basis of TAC and TPC measurements, it would appear that the antioxidant properties of serviceberry fruits are mainly affected by anthocyanins (Pearson correlation index for TAC/antioxidant activity presented a value of 0.73).

3.3. Phytochemical composition

The chemical fingerprint of *A. canadensis* (L.) Medik is reported in Tables 5 and 6 (single compound composition). The TBCC was calculated as the sum of the main biomarkers selected for their healthy properties and detected in the extracts. Results showed a mean TBCC value of 1549.36 ± 22.77 mg/100 g_{FW}. These biomarkers were selected for the fingerprint because they have been described as important health-effective molecules in humans (de Cassia da Silveira e Sa, Andrade, & de Sousa, 2013).

The phytochemical fingerprint of serviceberry fruit has been described: 20 bioactive compounds were identified by HPLC-DAD (Mazza, 1982; Ozga, Saeed, Wismer, & Reinecke, 2007). In this study, *A. canadensis* fruits showed the following bioactive compound composition: four cinnamic acids (caffeic, chlorogenic, coumaric, and ferulic acids), five flavonols (hyperoside, isoquercitrin, quercetin, quercitrin, rutin), one benzoic acid (ellagic acid), one catechin (epicatechin), two tannins (castalagin, vescalagin), two monoterpenes (limonene, γ -terpinene), three organic acids (oxalic, succinic, and tartaric acids), and vitamin C (expressed as the sum of ascorbic acid and dehydroascorbic acid). Catechin, phellandrene, sabinene, terpinolene, and citric, malic, gallic and quinic acids were not detected, in agreement with serviceberry fruit phytochemical fingerprints reported in similar studies (Juríková et al., 2013; Michalczyk & Macura, 2010). Other peaks were detected in the obtained chromatograms, but they did not match any of the biomarkers used in the present work. Further studies, adding other biomarkers

Table 4 – Total polyphenol content (TPC), total anthocyanin content (TAC) and antioxidant activity data of serviceberry (*A. canadensis* (L.) Medik) extracts.

	Mean value	SD
TPC ^a	539.24	29.20
Antioxidant activity ^b	25.07	0.48
TAC ^c	220.66	17.43

Mean value and standard deviation (SD) of each sample is given (N = 3).

Results were expressed as:

^a mg_{GAE}/100g_{FW}.

^b mmol Fe²⁺/kg.

^c mg_{C3G}/100g_{FW}.

Table 5 – Phytochemical fingerprint of the polyphenolic compounds found in serviceberry (*A. canadensis* (L.) Medik).

		Mean value	SD
Cinnamic acids	Caffeic acid	1.17	0.89
	Chlorogenic acid	60.17	9.01
	Coumaric acid	43.13	15.96
	Ferulic acid	9.04	1.33
Flavonols	Hyperoside	3.43	0.15
	Isoquercitrin	15.15	0.96
	Quercetin	15.48	4.04
	Quercitrin	25.67	0.25
	Rutin	2.82	0.20
Benzoic acids	Ellagic acid	12.70	1.80
	Gallic acid	n.d.	/
Catechins	Catechin	n.d.	/
	Epicatechin	343.46	29.46
Tannins	Castalagin	192.13	5.41
	Vescalagin	17.16	4.34

Mean value and standard deviation (SD) of each sample is given (N = 3). Results are expressed as mg/100g_{FW} (FW = fresh weight). n.d., not detected.

Table 6 – Phytochemical fingerprint (vitamins and other health-promoting molecules) of serviceberry (*A. canadensis* (L.) Medik).

		Mean value	SD
Monoterpenes	Limonene	91.96	11.19
	Phellandrene	n.d.	/
	Sabinene	n.d.	/
	γ -Terpinene	31.20	1.67
	Terpinolene	n.d.	/
Organic acids	Citric acid	n.d.	/
	Malic acid	n.d.	/
	Oxalic acid	18.65	1.14
	Quinic acid	n.d.	/
	Succinic acid	36.41	9.34
	Tartaric acid	295.57	9.63
Vitamins	Ascorbic acid	3.00	0.18
	Dehydroascorbic acid	110.39	11.00

Mean value and standard deviation (SD) of each sample is given (N = 3). Results are expressed as mg/100g_{FW} (FW = fresh weight). n.d., not detected.

with demonstrated biological activity, are needed for a complete identification of the chromatogram.

The identified health-promoting agents were grouped into classes to evaluate the single contribution of each class to total fruit phytochemical composition (Fig. 1). The phytochemical fingerprint showed the prevalence of polyphenols (as the sum of anthocyanins, cinnamic acids, flavonols, benzoic acids, catechins, and tannins) and organic acids in all the analysed samples (mean values were considered). Indeed, the most important class was polyphenols ($62.12 \pm 1.68\%$), followed by

organic acids ($22.62 \pm 0.75\%$), monoterpenes ($7.94 \pm 0.57\%$), and vitamins ($7.31 \pm 0.63\%$).

Results showed that serviceberry fruits could be a good source of phenolic constituents and deserved special attention focused on studying their phytochemical profile: the main phenolic groups were catechins (343.46 ± 29.46 mg/100 g_{FW}) > anthocyanins (220.66 ± 17.43 mg/100 g_{FW}) > tannins (209.29 ± 7.81 mg/100 g_{FW}) > cinnamic acids (113.52 ± 6.26 mg/100 g_{FW}) > flavonols (62.56 ± 2.86 mg/100 g_{FW}) > benzoic acids (12.70 ± 1.80 mg/100 g_{FW}). The phenolic compounds detected in the present work were similar to those reported in other studies on different serviceberry genotypes (Bakowska-Barczak & Kolodziejczyk, 2008; Ozga et al., 2007). The most important polyphenolic classes, in relation to the TPC, were catechins ($35.67 \pm 2.62\%$) > anthocyanins ($22.95 \pm 2.04\%$) > tannins ($21.76 \pm 1.07\%$) > cinnamic acids ($11.80 \pm 0.58\%$) > flavonols ($6.50 \pm 0.23\%$) > benzoic acids ($1.32 \pm 0.20\%$) (Fig. 2). Results showed few differences in phenolic constituents compared to other serviceberry genotypes: the variation of phenolic compounds in berries, as well as in other fruits, depends on many factors, as ripening stage, genetic differences, and environmental conditions during fruit development (Sánchez-Salcedo, Mena, García-Viguera, Martínez, & Hernández, 2015).

These results highlight the potential of *A. canadensis* fruits as a functional food due to their biological and pharmacological effects associated with the detected phytochemicals.

3.3.1. Polyphenols

Plants containing flavonoids are often used to treat diabetes in several natural medicines; these compounds are reported to have glucose-lowering effects and repress hepatic glucose production in animals (Wolfram et al., 2006). The presence of

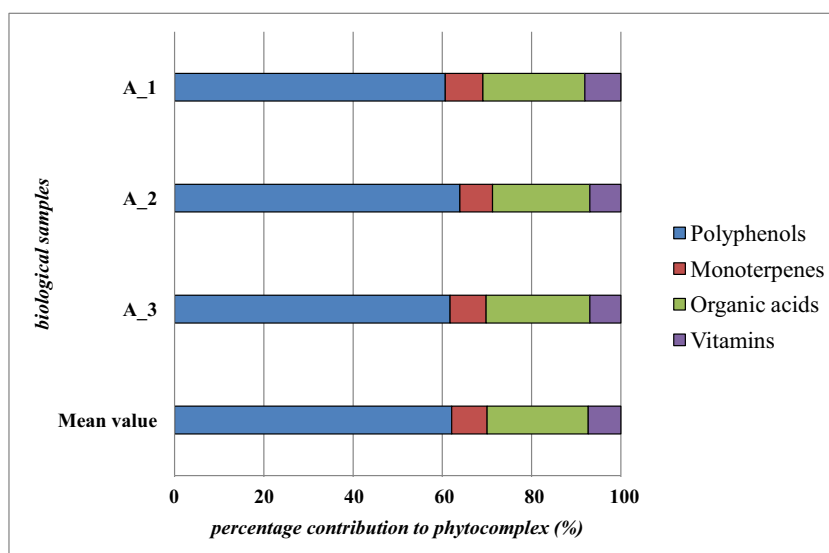


Fig. 1 – Phytochemical representation of serviceberry (*A. canadensis* (L.) Medik) extracts. The mean value of each analysed sample is given (N = 3). A1, A2, and A3 represent mean values of different biological samples (three replications for each biological sample) of the same genotype. *A. canadensis* fruits showed the following bioactive compound composition: four cinnamic acids (caffeic acid, chlorogenic acid, coumaric acid, ferulic acid), five flavonols (hyperoside, isoquercitrin, quercetin, quercitrin, rutin), one benzoic acid (ellagic acid), one catechin (epicatechin), two tannins (castalagin, vesicalagin), two monoterpenes (limonene, γ -terpinene), three organic acids (oxalic acid, succinic acid, tartaric acid), and vitamin C (expressed as the sum of ascorbic acid and dehydroascorbic acid).

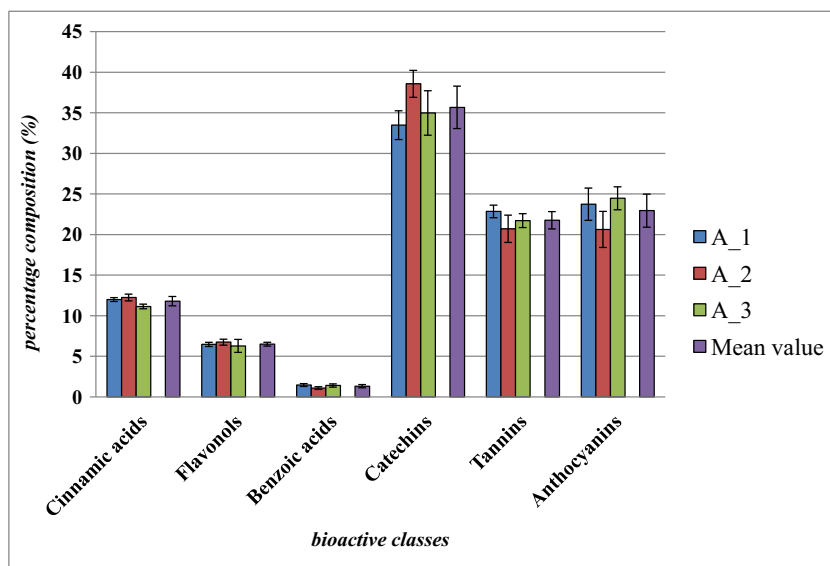


Fig. 2 – Contribution of each polyphenolic class to the total phenolics in serviceberry (*A. canadensis* (L.) Medik). The mean value of each analysed sample is given (N = 3). A1, A2, and A3 represent mean values of different biological samples (three replications for each biological sample) of the same genotype. Error bars represent the standard deviation of the replicates. HPLC fingerprint showed this phenolic composition by multi-marker approach: caffeic acid, chlorogenic acid, coumaric acid, ferulic acid (cinnamic acids), hyperoside, isoquercitrin, quercetin, quercitrin, rutin (flavonols), ellagic acid (benzoic acids), epicatechin (catechins), castalagin, vescalagin (tannins). Anthocyanins were expressed as cyanidin-3-O-glucoside.

ellagic acid (12.70 ± 1.80 mg/100 g_{FW}) in serviceberry makes this fruit an excellent candidate to evaluate several effects *in vivo*.

The concentration of cinnamic acids, in particular chlorogenic acid (60.17 ± 9.01 mg/100 g_{FW}), may be important when fruits are processed, as these compounds are considered a preferential substrate for the catecholase activity of polyphenol oxidase (Wojdyło, Oszmiański, & Bielicki, 2013). The relative concentration of cinnamic acids could also influence the oxidation and colour development processes occurring during technological processing (Sánchez-Salcedo et al., 2015). Moreover, chlorogenic acid has been recognised for its extensive biological properties (Radojkovic, Zekovic, Vidovic, Kocar, & Maskovic, 2012).

Anthocyanin pigments are of prominent importance in *A. canadensis* fruits because of their dual value. First, they constitute an integral part of the sensory attributes, as their levels and various forms directly pertain to the colouration of the final product. On the other hand, they have been claimed to possess several biological properties and therefore they are considered as secondary metabolites with potential nutraceutical value (Chen, Xin, Yuan, Su, & Liu, 2014). The high content of tannins in serviceberry fruits is interesting, thanks to their well-known association with human health, as anticarcinogenic and antimutagenic agents important in protecting cells by oxidative damages (Chung, Wong, Wei, Huang, & Lin, 1998). Although flavonols are not the main phenolic components of serviceberry fruits, they should be considered in the phytochemical profile of *Amelanchier* spp. fruits, since they may exert critical beneficial features related to human health (Del Rio et al. 2013).

3.3.2. Organic acids and monoterpenes

Organic acids constituted an important component of the *A. canadensis* fruits (350.63 ± 13.18 mg/100 g_{FW}). These compounds

affect fruit quality properties, as stability, colour, and flavour. They are mainly used to determine fruit maturity stage, identify adulteration in fruit juices, indicate the spoilage of fruit products, and function as food acidifiers (Soyer, Koca, & Karadeniz, 2003). In addition, organic acids are antioxidants with multi-purpose uses in pharmacology (Eyduran et al., 2015).

Although monoterpenes only represent a small fraction of the total phytocomplex of serviceberry fruits (123.16 ± 8.60 mg/100 g_{FW}), they could be an important bioactive class: monoterpenes are increasingly being referred to as a potential pharmacological group that act as anti-inflammatory drugs (de Cassia da Silveira e Sa et al., 2013).

3.3.3. Vitamin C

Dehydroascorbic acid shows biological activity and can easily be converted to ascorbic acid by humans (Corral-Aguayo, Yahia, Carrillo-Lopez, & Gonzalez-Aguilar, 2008). Thus, the sum of ascorbic and dehydroascorbic acids was considered as vitamin C, according to previous similar studies (Cazares-Franco et al., 2014; Donno et al., 2013a). If compared on a fresh weight basis, the vitamin C content of serviceberry (113.39 ± 9.10 mg/100 g_{FW}) was higher than that found in most common fruits, as kiwifruit (74.56 ± 9.84 mg/100 g_{FW}), orange (71.12 ± 1.96 mg/100 g_{FW}), strawberry (57.95 ± 2.60 mg/100 g_{FW}), blackberry (45.07 ± 5.82 mg/100 g_{FW}), blueberry (12.60 ± 2.79 mg/100 g_{FW}), apple (3.91 ± 0.48 mg/100 g_{FW}), and black mulberry (2.97 ± 0.23 mg/100 g_{FW}) as previously reported (Donno et al., 2015a, 2015c). The recommended daily intake of vitamin C for adults is 60–90 mg (Monsen, 2000) and, therefore, a portion of 100 g of ripe serviceberry fruits could contribute more than 100% of the recommended daily intake of vitamin C.

4. Conclusions

Evidence continues to emerge suggesting that many commonly consumed fruits and vegetables are beneficial to health in relation to the prevention of several diseases. For this reason, there is a growing interest in novel or less well-known foods that offer an opportunity for health maintenance. This study demonstrated that serviceberry fruits present a plethora of phytochemicals with the capacity to promote health and protect against chronic diseases. The results of this study provided a detailed assessment of the quality, chemical characteristics, and potential of a cultivated edible serviceberry as a functional fresh fruit. Substantial amounts of simple phenolics, as chlorogenic, coumaric and ferulic acids, and flavonols are present in this fruit. Some of these simple phenolics, together with complex polyphenols, monoterpenes and other phytochemicals (organic acids and vitamin C) make serviceberry an alternative fruit that could contribute to the prevention of chronic diseases resulting from oxidative stress. Finally, as the opportunity for medium-scale production on marginal lands, further studies are needed to investigate this genotype by environment interactions and identify several genotypes suitable for fresh consumption, processing, or breeding research.

Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.jff.2016.07.014.

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