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Structure, health benefits and identification of chokeberry polyphenols: A comprehensive review

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ABSTRACT

Polyphenols receive special attention from consumers and researchers due to their proven health benefits. They are present in different plants, and chokeberries are one of the significant polyphenol sources. Polyphenols found in chokeberries belong to the groups of phenolic acids, anthocyanins, proanthocyanidins, flavonols and flavanols. The highest concentration found in chokeberries belongs to proanthocyanidins followed by anthocyanins. The analysis of polyphenols is very demanding due to the great diversity and presence of conjugated forms. So, development of sensitive, rapid, and accurate analytical methods for analysis of polyphenols is very important. Liquid chromatography is a promising technique for polyphenols analysis, especially one equipped with mass spectrometry that gives additional information about molecular mass and structure. In this review, relevant studies conducted on the analysis of chokeberry polyphenols are presented.

KEY CONTRIBUTION
Chokeberries are a rich source of polyphenols.
Chokeberry polyphenols are phenolic acids, anthocyanins, proanthocyanidins, flavonols and flavanols.
The combination of liquid chromatography and mass spectrometry is suitable for the analysis of complex food samples.

Introduction

A chronic inflammatory state presents a main reason for the development of different chronic pathologies such as cancers, infections, obesity, cardiovascular and neurodegenerative diseases. The results of epidemiological studies showed the connection between a diet rich in fruits and vegetables with reduced incidence of inflammatory disorders (Kasprzak-Drozd et al., 2021). The use of plants for healing is an old approach that has persisted for years, and nowadays it receives special attention from consumers and researchers (Jurendić and Ščetar, 2021). Polyphenols are considered important natural

antioxidants. Antioxidants are low molecular weight compounds that are of natural or synthetic origin and prevent or inhibit cell damage. They have the ability to scavenge free radicals that cause cell damage in the human body. Oxidative stress as a consequence of the action of free radicals leads to the development of different chronic diseases (Jurikova et al., 2017; Apak et al., 2016).

Black chokeberry (*Aronia melanocarpa*) is a rich source of bioactive compounds with many functional activities that are mainly due to antioxidant activity of polyphenols. Chokeberry fruits have higher quantities of polyphenols than blackberries, blueberries, raspberries and others (Jakobek et al., 2007a). Chokeberry is very abundant in proanthocyanidins that are responsible for astringency and high antioxidant activity. Besides proanthocyanidins, anthocyanins, phenolic acids, flavonols and flavanols are present in chokeberries. For many years anthocyanins were used as natural food colorants. Nowadays, the focus of their application is as supplements and medicines. Anthocyanins in chokeberry are generally cyanidin glycosides. These found in significant concentrations are galactoside, glucoside, arabinoside, and xyloside. From the group of hydroxycinnamic acids, chlorogenic and neochlorogenic acids are present in the highest concentration. Also, quercetin derivatives are widely present (Willemse et al., 2013; Zhang et al., 2021; Zielińska et al., 2020).

The analysis of polyphenols is very challenging due to the great diversity and presence of conjugated forms. For the science community and food technology, the development of rapid, sensitive and accurate methods for polyphenols analysis is very important (Pyrzynska and Sentkowska, 2019). The high-performance liquid chromatography method was introduced in the 1970s and since then has been continuously used for the analysis of food compounds. The combination of liquid chromatography and mass spectrometry is suitable for the analysis of food samples with the advantages of separation chromatography and mass spectrometry as an identification tool . Mass spectrometry data can give information about type, molecular weight and number of glycosides (Motilva et al., 2013; Zhu et al., 2021).

This review addresses the main polyphenols present in chokeberries as well as their beneficial effects on human health. Also, the application of liquid chromatography and ultra-high liquid chromatography coupled with mass spectrometry or tandem mass spectrometry for qualitative and quantitative analysis of polyphenols are presented.

The main characteristics of chokeberries

Black chokeberry (*Aronia melanocarpa*) belongs to the Rosaceae family native to North America. Besides *Aronia melanocarpa* there is also *Aronia arbutifolia* (red chokeberry) and a hybrid of black and red chokeberry, purple chokeberry (*Aronia prunifolia*) (Jurendić and Sčetar, 2021). At the beginning of the 20th century, chokeberry was first mentioned in Northern, Eastern and Central Europe. Almost 90% of global chokeberries production belongs to Poland (Platonova et al., 2021).

Chokeberry shrubs grow up to 2-3 meters in height and have umbels of white flowers from which fruits ripen. They are very resistant to cold (they tolerate temperatures below -35 °C), so they can be grown in different climatic conditions (Jurendić and Sčetar, 2021). Chokeberry fruits primarily ripen in August, although they can begin to ripen as early as mid-July. It turned out that, for maximum fruit mass and anthocyanin content, it would be the most appropriate to harvest them at the beginning of September (Jurikova et al., 2017). The black chokeberry fruits have sweet, astringent and sour taste. Due to the unattractive taste of chokeberries, the food industry uses it for processing into juices, syrups, jams, jellies, fruit desserts, tea, puree, concentrates, fruit wine or natural food colorants (Esatbeyoglu et al., 2017; Sidor and Gramza-Michałowska, 2019). The chemical composition of chokeberries is connected

with climate conditions, soil composition, harvest methods, maturity of berries and storage conditions (Jurendić and Ščetar, 2021). In Table 1., chemical composition of chokeberry fruits, juice and pomace are presented.

Table 1. Chemical composition of chokeberry fruits, pomace and juice (Jurendić and Ščetar, 2021)

Chemical composition	Fruit (g/kg)	Pomace (g/kg)	Juice (g/L)
Total sugars	68-158	84	110-143
Fibers	56	630-780	3
Fats	1.4	29-139	<1
Proteins	7	49-241	2
Minerals	4-6	14-39	5
Total polyphenols	79	31-63	48-64

Chokeberry polyphenols

Polyphenols are a large class of phytochemicals (over 8000 different compounds) widely represented in the plant kingdom (Ištuk et al., 2020; Pyrzynska and Sentkowska, 2019). The chemical structure of polyphenols is characterized by at least one aromatic ring with one or more hydroxyl groups attached (Motilva et al., 2013). They are present in plants seeds, leaves, fruits, roots and stems and are involved in the plant's defense system (Pyrzynska and Sentkowska, 2019). They are natural antioxidants and serve as UV screens, signaling molecules and antimicrobial agents. Polyphenols are mainly located in the outer layers of plant tissue and in seeds (Weber and Passon, 2019). Kaloudi et al. (2022) found out that around 30% of polyphenols of *Aronia melanocarpa* are located in the peel while the rest is present in the flesh and seeds. Generally, polyphenols are divided into subgroups such as simple polyphenols, phenolic acids, flavonoids, coumarins, stilbenes, condensed and hydrolysable tannins. Differences in the structure are the result of hydroxylation, acylation, alkylation and glycosylation reactions that change the basic molecule (Pyrzynska and Sentkowska, 2019). The most represented group of polyphenols in nature are flavonoids (over 4000 known flavonoids). Flavonoids are made of two aromatic rings connected by three carbon bridges (Ištuk et al., 2020; López-Fernández et al., 2020). According to the degree of polymerization and hydroxylation they can be divided into several subgroups: flavonols, flavanones, flavones, isoflavones, flavan-3-ols, anthocyanins and chalcones (López-Fernández et al., 2020). There is a diversity of plants that are abundant in flavonoids, such as vegetables, cereals, fruits, spices and herbs (López-Fernández et al., 2020). Polyphenols that are found in chokeberries belong to the groups of phenolic acids, anthocyanins, proanthocyanidins, flavonols and flavanols (Sidor and Gramza-Michałowska, 2019). The chemical structure of the main polyphenols identified in chokeberries is shown in Figure 1.

Phenolic acids
Phenolic acids are made of a phenol ring with one or more hydroxyl groups attached and are divided into derivates of hydroxybenzoic acids and derivates of hydroxycinnamic acids (Rocha et al., 2012). Hydroxybenzoic acids derivates such as gallic, vanillic, syringic and salicylic acids are usually present in insoluble bound forms and make more complex compounds such as tannins and ligands. Their

oncentration in fruits and vegetables are very low compared to hydroxycinnamic acids (Capriotti et al., 2014). Hydroxycinnamic acids have a simple C6-C3 chemical structure, and the main representatives are *p*-coumaric, ferulic, caffeic and sinapic acids. Although chemical structures are fairly similar, they have an impact on polyphenols properties and biological activity due to presence of hydroxyl groups on the aromatic ring. Hydroxycinnamic acids are rarely found in free form and more often as glycosylated derivatives of quinic, tartaric and shikimic acids (Coman and Vodnar, 2020). Chlorogenic acids are formed by esterification of cinnamic acids with quinic acid (Zielińska et al., 2020). Neochlorogenic acid is a chlorogenic acid isomer made by ester binding of caffeic and D-(-)-quinic acids. In chokeberries, the most abundant are chlorogenic acid (3-O-caffeoylquinic acid) and neochlorogenic acid (5-O-caffeoylquinic acid) (Zielińska et al., 2020).
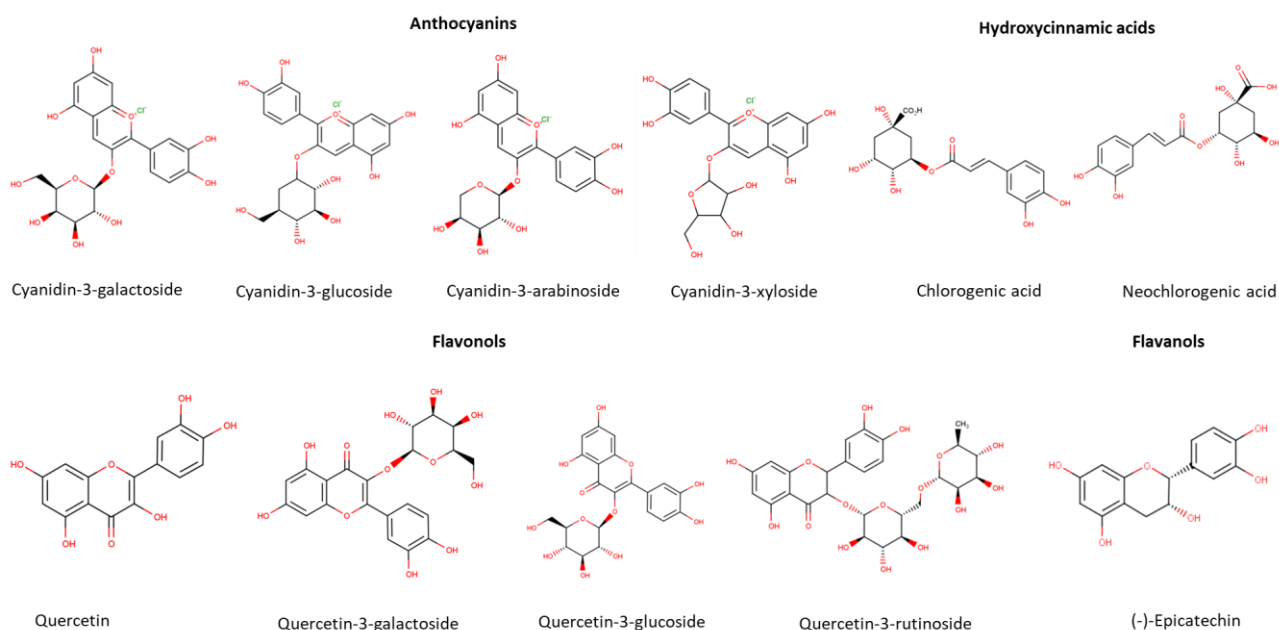

Figure 1. Chemical structure of the main polyphenols in chokeberries

Flavanols

Flavanols are the main subgroup of flavonoids present in black chokeberry including mainly (+)catechins and (-)epicatechins as well as proanthocyanidins condensed from them (Gao et al., 2023). Concentrations of proanthocyanidins in juice is very low, because they are abundant in pomace (Sójka et al., 2013). With the increase in the degree of polymerization, proanthocyanidins' functional and antioxidant activities decrease. Also, polymeric proanthocyanidins are poorly absorbed by the human body and the reason is in large molecular size and gut barrier. In this sense, the main contribution of polymeric proanthocyanidins to human health is reflected in the digestive tract, such as inhibition of amylase and lipase (Gao et al., 2023). Polymeric procyanidins represent 66% of chokeberry fruits polyphenols (Jurendić and Ščetar, 2021).

Anthocyanins

Anthocyanins are known as natural, widely spread water-soluble pigments. Due to their attractive color (red, blue, or purple), they are often used in food, pharmaceutical and cosmetics products (Płatosz et

al., 2020). Anthocyanins are anhocyanidins (cyanidin, deplhinidin, malvidin, petunidin and peonidin) glycosylated derivates (Topić Božič et al., 2019). They are the second largest group of chokeberry polyphenols (Zhang et al., 2021) and are present in higher concentrations in pomace than in juice (Gao et al., 2023). Chokeberry anthocyanins are generally cyanidin glycosides (galactoside, glucoside, arabinoside and xyloside). Cyanidin-3-galactoside is the main anthocyanin in chokeberries. Galactoside and arabinoside make up more than 75% of total anthocyanins in chokeberries (Zhang et al., 2021; Wilkes et al., 2013) or more than 90% according to some authors (Klisurova et al., 2018). Cyanidin-3-galactoside and cyanidin-3-glucoside have very similar structures with the same molecular weight and the only difference is in the position of one OH group in the hexose ring. The same is for cyanidin-3-xyloside and cyanidin-3-arabinoside with the difference in OH position in the pentose ring (Esatbeyoglu et al., 2017).

Flavonols

Although flavonols are represented in small concentrations in chokeberry, they are widely studied due to their strong biological activity. Main flavonols in chokeberries are quercetin glycosides such as quercetin-3-O-rutinoside, quercetin-3-O-galactoside and quercetin-3-O-glucoside. Also, small concentrations of kaempferol, isorhamnetin, quercetin-3-O-robinobioside, eriodictyol-glucuronide have been found in the black chokeberry (Oszmiański and Lachowicz, 2016; Slimestad et al., 2005).

Health benefits of chokeberry

The high antioxidant capacity of chokeberry, i.e., its bioactive compounds, has made it the subject of numerous studies in the treatment of various chronic diseases as well as the prevention of their development. The results obtained *in vitro* in cells or cell lines, as well as in vivo on humans and animals, showed that chokeberry has a potential for the positive effect on the treatment of diabetes, cardiovascular diseases, and cancer, as well as antibacterial, gastroprotective, hepatoprotective and anti-inflammatory effects (Jurikova et al., 2017).

Inflammation is the response of the immune system to some undesirable stimuli in the body. Inflammatory reactions are accompanied by producing cytokines and they act as signals between immune cells (Kasprzak-Drozd et al., 2021). Chokeberry showed the possibility of inhibiting the release of cytokinome IL-6, IL-8 and TNF-α in the monocytes of humans (Appel et al., 2015; Jurikova et al., 2017; Kasprzak-Drozd et al., 2021). The study of Yamane et al. (2023) showed neuroprotective function of chokeberry juice on male 5XFAD Alzheimer's disease model mice. Fraction that contained three quercetin glycosides reduced δ-secretase activity in SH-SY5Y cells (Yamane et al., 2023). Furthermore, chokeberry extract showed significant ability to reduce tissue damage in the hippocampus in mice (Lee et al., 2018). The reduction of monocytes and granulocytes levels, which cause inflammation in diabetic rats, as well as increase of lymphocytes, which inhibit atherosclerotic plaques, by chokeberry extract, is one of the proofs of the positive impact on cardiovascular diseases (Sidor et al., 2019). Some studies showed positive effects of chokeberries on hyperlipidemia and hypercholesterolemia. For example, chokeberry juice consumption caused a reduction of the high level of triacylglycerols in women (Nowak et al., 2016), while juice with glucomannan decreased the high-density lipoprotein levels and systolic blood pressure from 127.6 to 116.4 mmHg in obese women (Kardum et al., 2014). A positive impact on the lipid profile of chokeberries was noticed in obese patients and those with metabolic syndrome (Sidor et al., 2019). Laboratory studies on animals showed a reduction in glucose levels after chokeberry feeds in merino lambs (control group 3.38 mmol/L; 150 and 300 g chokeberry/kg feeds 2.42 and 1.55 mmol/L,

respectively) (Lipińska and Jóźwik, 2017). Anthocyanins and their metabolites that are absorbed by the human body may have an impact on organs with the flow of blood. The unabsorbed anthocyanins have an impact on digestive tract on enzymes, intestinal flora and epidermal cells (Gao et al., 2023). Given that cyanidin glycosides are poorly adsorbed in the human body, the influence of chokeberry anthocyanins is mainly based on the intestinal microflora (Yu et al., 2021; Gao et al., 2023). On the other hand, Płatosz et al. (2020) found out that chokeberry anthocyanins adsorb and appear in the body fluids of sheep, but over 70% as metabolites. Anthocyanin levels in cerebrospinal fluid ranged from 0.04-0.32 µmol/L, but future studies need to explain mechanism of anthocyanins transfer to the brain and if these levels may have biological activity. Previous research showed that anthocyanins from chokeberry may be beneficial in decreasing blood glucose levels through α-glucosidase inhibition, relieving oxidative stress (Bräunlich et al., 2013) and have hypolipidemic effects (Kim et al., 2013). Cyanidin-3-arabinoside has high inhibitory activity toward α-glucosidase and together with proanthocyanidins may be responsible for diabetes prevention (Kaloudi et al., 2022). Also, black chokeberries possess the potential to inhibit the development of leukemia, intestinal cancer and breast cancer (Sidor et al., 2019).

There are a lot of comprehensive reviews of chokeberry health benefits (Kasprzak-Drozd et al., 2021; Hawkins et al., 2020). Gao et al. (2023) published a review of chokeberry polyphenols and their impact on hypertension, hyperlipidemia, obesity, hyperglycemia and inflammation. Also, a comprehensive review by Sidor et al. (2019) describes health promoting effects of chokeberry and its products.

Liquid chromatography for polyphenols analysis

Sample preparation

Before the analysis, adequate sample preparation is obligatory. For liquid samples, filtration or centrifugation before analysis is enough. For solid samples, several types of pretreatments (sieving, milling, grinding, and homogenization) are necessary (Pyrzynska and Sentkowska, 2019). Studies showed that grinding samples before solvent extraction resulted in better polyphenols accessibility than ungrounded samples (Pyrzynska and Sentkowska, 2019; Becker et al., 2016). It has been shown that dry, freeze-dried, or frozen samples, that are in powder form, are better than fresh material due to possible degradation in the presence of enzymes, especially when long-term solvent extractions are performed. Since most of the polyphenols are linked to the cell wall, for effective extraction it is necessary to disrupt the structure and allow the release of those compounds (Capriotti et al., 2014).

For liquid chromatography-based methods, solvent extraction methods are necessary (Capriotti et al., 2014). Solvent extraction of the analytes depends on the sample nature and chemical properties of polyphenols such as molecular structure and polarity (Mojzer et al., 2016). Usually used solvents are methanol, acetone, ethanol, ethyl acetate, or their mixture with water. A certain amount of water in the mixture is needed due to the high polarity of some polyphenols such as phenolic acids (Capriotti et al., 2014). Since anthocyanins are more stable at low pH, aqueous-organic solvents with acidic pH are usually used. It should be noted that there is a lack of solvents that can fully extract all compounds of interest (Pyrzynska and Sentkowska, 2019). Hydrolysis treatment of bounded polyphenols to the cell wall (polysaccharides, lignins, or proteins) or polyphenols trapped in cores is often. Hydrolysis can be acid, alkaline, or enzymatic and is a very challenging procedure. To avoid the oxidation of polyphenols, an inert atmosphere for hydrolysis is used (Capriotti et al., 2014). For polyphenols extraction, conventional methods, ultrasound-assisted and microwave-assisted extraction methods are usually used (Rajbhar et al., 2014).

High-performance liquid chromatography (HPLC), ultra-high performance liquid chromatography (UHPLC) and comprehensive two-dimensional liquid chromatography (LCxLC)

The analysis of polyphenols is very demanding due to the great diversity and presence of conjugated forms. Previously used methods for polyphenols purification and identification were thin-layer chromatography and column chromatography (Ajila et al., 2010). For the science community and food technology, the development of rapid, sensitive and accurate methods for polyphenols analysis is very important (Pyrzynska and Sentkowska, 2019). The commonly used technique for polyphenols determination is UV-Vis spectroscopy. The disadvantage of this method is an inability to identify individual polyphenols (López-Fernández et al., 2020). To overcome that issue, high-pressure liquid chromatography (HPLC), usually equipped with a diode array detector (DAD), is used for the separation of these compounds.

The limitation of this detector is that the identification of compounds is achieved only by UV-Vis spectra and retention time (Pyrzynska and Sentkowska, 2019; López-Fernández et al., 2020). Despite that, the HPLC method is preferred for the identification and quantification of polyphenols. Some of the commonly used HPLC method requirements are a reverse phase C18 column, a UV-Vis diode array detector, and a binary solvent system where the solvents are usually acidified water and a polar organic solvent (Ignat et al., 2011). Since HPLC analysis requires long analysis time and high resolution for complex samples and speed is an important factor in laboratories, ultra-high performance liquid chromatography (UHPLC) has been developed. With this kind of improvement, it became possible to achieve 5-to-10-fold faster separation with a small detection volume. The use of shorter columns with smaller particle sizes minimizes the injection volume and increases mobile phase linear velocity. So, the advantages of UHPLC over HPLC are improved resolution, faster separation, higher peak efficiency, and reduced solvent consumption (Motilva et al., 2013). Polyphenols are generally small molecules and for their separation, it is necessary to use columns with small pores to avoid the leak of molecules and ensure interaction with analytes (Tong et al., 2021).

Together with columns with small particles, UHPLC systems with up to 1300 bar working pressure ensure improved separation speed and peak efficiency (Capriotti et al., 2014). Due to the rapid separation of UHPLC, the mass spectrometer (MS) must be connected to ensure an equally fast-duty cycle (Motilva et al., 2013). Comprehensive two-dimensional liquid chromatography (LCxLC) ensures better resolution in determination of polyphenols present in a complex matrix. In this technique, the on-line transferring of all fractions from the first to the second dimension occurs (Motilva et al., 2013; Motilva et al., 2013). Compounds separated in the first dimension stay separated in the second dimension with the preservation of elution profiles of both dimensions (Dugo et al., 2008).

Detectors

For the identification of polyphenols, different detectors have been used together with HPLC separation such as UV-Vis and DAD, fluorescence, chemiluminescence, or mass spectrometry detectors. Nowadays, MS and diode array detectors are used mostly. Liquid chromatography, coupled to tandem mass spectrometry (LC-MS/MS), is an advanced technique used nowadays to overcome all the shortcomings of the previously mentioned techniques (Pyrzynska and Sentkowska, 2019; López-Fernández et al., 2020). The combination of LC and MS is suitable for the analysis of food samples with the advantages of separation chromatography and MS as an identification tool (Motilva et al., 2013). Mass spectrometry data can give information about type, molecular weight and number of glycosides (Zhu et al., 2021). The occurrence of chromatographic columns packed with sub-2 μm particles and mass spectrometry with high resolution made the analyses of polyphenols from complex matrix much easier (Motilva et al.,

2013). For the analysis with MS detector, several types of ionization in the ion source may be used. The most common are electrospray ionization and atmospheric pressure chemical ionization (Pyrzynska and Sentkowska, 2019). Some other MS systems are fast atom bombardment mass spectrometry, MS-MS, matrix-assisted laser desorption ionization time-of-flight mass spectrometry and electron impact mass spectrometry (Ajila et al., 2010).

Electrospray ionization is very favorable for the identification of polar compounds that are separated by liquid chromatography. This is a gentle ionization method that generates deprotonated molecules monitored in positive or negative mode (Ajila et al., 2010; Pyrzynska and Sentkowska, 2019). Negative mode is very useful for the compounds in the matrix due to higher selectivity (Pyrzynska and Sentkowska, 2019). Positive mode is generally used for anthocyanins (Karaaslan and Yaman, 2016). A group of authors proposed negative ionization as an effective way for anthocyanins identification. They pointed out the problem of interfering with some flavonol glycosides and anthocyanins ions and fragmentation patterns in positive ionization. In negative mode, doublet ions of $[M-2H]^-$ and $[M-2H+H_2O]^-$ are only for anthocyanins (Sun et al., 2012).

Time-of-flight mass spectrometry provides a measurement of the isotopic pattern, helping determine the elemental composition of the compounds. MS-MS analysis provides information of aglycone moiety, carbohydrate types or present substituents, the sequence of glycan part, stereochemical assignment of terminal monosaccharide units, interglycosidic linkages and the attachment points of the substituent to the aglycans (Ajila et al., 2010; Cuyckens and Claeys, 2004).

Liquid-chromatography for identification and quantification of chokeberry polyphenols

Anthocyanins have the ability to absorb in the visible range (about 500-550 nm), which makes them selectively detected and differentiates them from other groups of flavonoids. Liquid chromatography coupled with the photodiode-array detector, has limitations in the identification of individual anthocyanins because it does not provide enough structural information (Willemse et al., 2013). Reverse phase liquid chromatography with mass spectrometry gives information on molecular mass and structure and makes easier the identification of anthocyanins. Furthermore, tandem mass spectrometry (MS/MS) provides additional information due to selective fragmentation.

Limitations of LC-MS of anthocyanins identification in mixtures occur due to identical molecular ions and fragments between glycosidic isomers such as galactosides and glucosides. For these issues chromatographic separation is crucial. To overcome low chromatographic efficiency, it is necessary to use the gradient program that lasts for a longer period of time (Willemse et al., 2013). Furthermore, increasing the temperature during analysis improves chromatographic separation through the reduction in band broadening and better peak shape and resolution is achieved. It was observed that temperatures up to 50 °C do not cause thermal degradation of anthocyanins for analysis less than one hour (De Villiers et al., 2009).

Meng et al. (2019) studied anthocyanins from *Aronia melanocarpa* from China. They perform purification of anthocyanins with absorbent resin and identified seven anthocyanins (cyanidin-3,5-dihexoside, dimer of cyanidin-hexoside, cyanidin-3-galactoside, cyanidin-3-glucoside, cyanidin-3-arabinoside, cyanidin-3-xyloside, and delphidinin-3-rutinoside) by HPLC and mass spectrometry. Cyanidin-3,5-dihexoside and dimer of cyanidin-hexoside were found for the first time in *Aronia melanocarpa* (Meng et al., 2019). Furthermore, group of authors developed a HPLC-MS/MS method for identification and quantification of chokeberry anthocyanins (cyanidin-3-galactoside, cyanidin-3-glucoside, cyandind-3-arabinoside, and cyanidin-3-xyloside) as well as their metabolites in rat plasm,

urine and feces. For chromatographic separation they used Poroshell EC-C18 column and for elution formic acid (0.1%) in methanol and formic acid (0.1%) in water. The optimized flow rate was 0.3 mL/min and temperature of 30 °C.

Mass spectra were obtained by scanning a mass range of *m/z* 100-2000 Da (Tong et al., 2021). Romani et al. (2016) investigated polyphenols and volatile compounds in commercially available chokeberry products (compote, juice and dried fruit). Using HPLC-TOF analysis authors identified, among other known polyphenols, a 4-*O*-caffeoyl-quinoic acid based on retention time in comparison with isomers and fragments in negative ionization mode. The main fragment ion was at *m/z* 353 and minor ion fragments were at *m/z* 191 and *m/z* 173. The ion at *m/z* 173 ("dehydrate" quinic acid moiety) is typical for a substituted isomer in position 4. Table 2 gives molecular ions and fragment ions of polyphenols commonly present in chokeberries. By reviewing the literature, the compounds listed in Table 2 were mostly identified and quantified in chokeberry fruits, juice, or pomace. Table 3 provides an overview of several researches conducted on chokeberry polyphenols as well as details of used methods, extraction techniques and main observations.

Table 2. LC-MS data of main polyphenols present in chokeberries (Rodríguez-Werner et al., 2019)

Polyphenol	[M+H]$^+$	[M-H]$^-$	Fragment ion (*m/z*)
Cyanidin-3-galactoside	449		287
Cyanidin-3-glucoside	449		287
Cyanidin-3-arabinoside	419		287
Cyanidin-3-xyloside	419		287
Neochlorogenic acid		353	191, 179, 135
Chlorogenic acid		353	191, 179, 161
Quercetin-3-galactoside		463	301
Quercetin-3-glucoside		463	301
Quercetin-3-vicianoside		595	301
Quercetin-3-rutinoside		609	301
Quercetin		301	
(-)-Epicatechin		289	245

Table 3. Selected studies of chokeberry polyphenols analyzed by liquid chromatography

Chokeberry fruit		Reference
Extraction technique and conditions	Maceration – optimized conditions: 0.75 mm – fruit size; 50% ethanol – solvent; 1:20 – solvent:solid ratio; 90 min – extraction time.	Ćujić et al., 2016
	Ultrasound-assisted extraction: 35 W – bath power, 40 kHz – frequency; 30 and 60 min – extraction time; 50% ethanol – solvent; 1:20 – solvent:solid ratio; 0.75 mm - fruit size.	
Chromatographic technique and conditions	HPLC analysis; reverse phase Lichrospher RP-18 column; DAD detector.	
	For anthocyanins: mobile phase A formic acid (10%) in water and B acetonitrile; gradient elution: 1% B 0–0.5 min; 1–7% B 0.5–1 min; 7% B 1–4 min; 7–10% B 4–7.5 min; 10–14% B 7.5–	

11.5 min; 14–25% B 11.5–15.5 min; 25–40% B 15.5–18.5 min; 40–75% B 18.5–22 min; 75% B 22–25 min; flow rate 1 mL/min; detection wavelengths 290 nm, 350 nm and 520 nm.

For flavonoids: mobile phase A 1% orthophosphoric acid in water and B acetonitrile; gradient elution: 90-80% A 0–5 min; 80% A 5–20 min; 80-40% A 20–30 min; 40–0% A 30–35 min; flow rate 1 mL/min; detection wavelengths 260 nm and 350 nm.

Polyphenols	Cyanidin-3-galactoside, cyanidin-3-glucoside, cyanidin-3-arabinose, quercetin-3-rutinoside, quercetin-3-galactoside, quercetin-3-glucoside.
The main observations	Maceration resulted in higher polyphenol amounts than ultrasound-assisted extraction. Cyanidin-3-galactoside was the anthocyanin with the highest concentration in fruit (0.81 mg/g). From the group of flavonols, quercetin-3-rutinoside was the most abundant (0.42 mg/g).

Chokeberry fruit

Extraction technique and conditions	Fresh chokeberries (100 g) were extracted with water (300 mL) for 1 h at 60 °C. Crude liquid extract was freeze-dried to obtain crude extract.	
Chromatographic technique and conditions	HPLC analysis; TC-C18 column; UV-Vis detector; mobile phase A 0.5% acetic acid and B acetonitrile; gradient elution: 14% B 0-6 min, 14-25% B 6-30 min, 25-50% B 30-40; flow rate 0.8 mL/min; detection wavelength 280 nm.	Denev et al., 2019
Polyphenols	Neochlorogenic acid, chlorogenic acid, epicatechin, quercetin 3-rutinoside, quercetin-3-glucoside, quercetin.	
The main observations	The most abundant compound found in crude extract was neochlorogenic acid (10.2 mg/g), chlorogenic acid (8.3 mg/g) and quercetin 3-rutinoside (7.8 mg/g).	

Chokeberry juice

Extraction technique and conditions	1.5 mL of juice was vortexed with 3 mL of 1% formic acid in 80% methanol for 1 min and extracted for 15 min in an ultrasonic bath (40 kHz frequency) at 50 °C. Extraction solvent was added up to 5 mL, then the mixture was centrifuged, and the supernatant was filtered.	
Chromatographic technique and conditions	HPLC analysis; Zorbax Eclipse-XDB-C18 column; UV/Vis-Photo Diode Array detector; mobile phase A 3% of formic acid in water and B 3% of formic acid in 80% acetonitrile; elution: 0% B 0-28 min, 25% B 28-35 min, 50% B 35-40 min, 80% B 40-45 min, 0% B 45-55 min; flow rate 0.8 mL/min; detection wavelengths 320 nm (hydroxycinnamic acids), 510 nm (anthocyanins) and 360 nm (flavonols).	Bursać Kovačević et al., 2016
Polyphenols	Neochlorogenic acid, chlorogenic acid, caffeic acid, quercetin-3-vicianoside, quercetin-3-rutinoside, quercetin-3-galactoside, quercetin-3-glucoside, cyanidin-3-galactoside, cyanidin-3-glucoside, cyanidin-3-arabinoside, cyanidin-3-xyloside.	
The main observations	From the group of hydroxycinnamic acids, neochlorogenic acid had the highest concentration (53.10 mg/L) in fresh juice while from the group of flavonols the quercetin-3-galactoside had the highest concentration (10.10 mg/L). Cyanidin-3-galactoside had the highest concentration in the juice from the group of anthocyanins (122.60 mg/L).	

Chokeberry juice

Extraction technique and conditions	Hot maceration was performed at 80 °C for 5 min followed by chokeberry pulp cooling at 40 °C and pectin hydrolysis.	Wilkowska et al., 2017

Chromatographic technique and conditions	HPLC analysis; Acclaim C18 column; mobile phase A formic acid:water (1:20) and B acetonitrile:water (20:1); elution: 5% B 0-5 min, 5-50% B 5-45 min, 50-75% B 45-55 min, 75-100% B 55-65 min; detection wavelengths: 320 nm and 280 nm for conjugated forms of hydroxycinnamic acids and 510 nm for anthocyanins.	
Polyphenols	Neochlorogenic acid, chlorogenic acid, gallic acid, cyanidin-3-galactoside, cyanidin-3-glucoside, cyanidin-3-arabinoside.	
The main observations	Cyanidin-3-galactoside was detected in highest concentration in chokeberry juice (3.16 g/L) followed by cyanidin-3-arabinoside (1.44 g/L) and neochlorogenic acid (1.18 g/L).	
Chokeberry pomace		
Extraction technique and conditions	In aliquots of chokeberry pomace powder samples aqueous formic acid was added then the samples were rehydrated in an ice bath in the dark for 5 min and homogenized.	
Chromatographic technique and conditions	HPLC-DAD; Kinetex C18 column; mobile phase A acetonitrile:formic acid (90:10, *v/v*) and B 10% formic acid; elution: 96.7-86.2% A 0-8 min, 86.2-75.8% A 8-14 min, 75.8-72.2% A 14-16 min, 72.2-49.7% A 16-18 min; flow rate 1 mL/min; detection wavelengths: 520 nm (anthocyanins), 325 nm (phenolic acids) and 360 nm (flavonols).	Schmid et al., 2020
Polyphenols	Not defined individual polyphenol.	
The main observations	Anthocyanins were present in the highest concentrations (1.80 g/100 g), followed by phenolic acids (0.31 g/100 g) and flavonols (0.18 g/100 g).	
Chokeberry fruit		
Extraction technique and conditions	The homogenized fruit samples were extracted in the ethanolic solution acidified with 1% hydrochloric acid for 24h and then evaporated at 35 °C.	
Chromatographic technique and conditions	HPLC; OmniSpher C18 column; ProStar 330 PDA detector. For flavonols and phenolic acids: mobile phase A 0.1% phosphoric acid and B 100% methanol; elution: 5-80% B 0-30 min, 80% B 30-33 min, 80-5% B 33-35 min; flow rate 0.8 mL/min; injection volume 20 μL. For anthocyanins: mobile phase A 0.5% phosphoric acid and B 100% methanol; elution: 3-65% B 0-38 min, 65% B 38-45 min; flow rate 1 mL/min; injection volume 20 μL.	Jakobek et al., 2012
Polyphenols	Neochlorogenic acid, chlorogenic acid, quercetin-3-rutinoside, quercetin, cyanidin-3-galactoside, cyanidin-3-glucoside, cyanidin-3-arabinoside, cyanidin-3-xyloside.	
The main observations	All tested samples contained the same polyphenols. The cultivars "Nero" and "Viking" had the highest polyphenols content. Anthocyanins were the main group of polyphenols identified and quantified in samples, followed by phenolic acids and the end flavonols.	
Chokeberry fruit		
Extraction technique and conditions	Five samples of chokeberries (frozen, blanched, enzyme-threated, mash and presscake) were homogenized with methanol/water/acetic acid (60:37:3, *v/v/v*) for anthocyanins, flavonols and hydroxycinnamic acids and with methanol/water/acetic acid (70:29.5:0.5, *v/v/v*) for	Wilkes et al., 2013

proanthocyanidins extraction. After that the mixtures were filtered through MiraCloth and extraction was repeated 2 more times. The juice samples required no extraction.

Chromatographic technique and conditions	HPLC analysis of anthocyanins and hydroxycinnamic acids: dried extracts were resuspended in 1 mL of 3% formic acid in water and filtered through PTFE syringe filters (0.45 µm); reverse phase HPLC; Symmetry C18 column; mobile phase A 5% formic acid and B methanol; elution: 2-60% B 0-60 min; flow rate 1 mL/min; detection wavelengths: 510 nm (anthocyanins), 320 nm (hydroxycinnamic acids), 280 nm (protocatechuic acid and phloroglucinaldehyde).
	HPLC and HPLC-MS analysis of flavonols: dried extracts were resuspended in 1 mL of 50% methanol in water and filtered through PTFE syringe filters (0.45 µm); reverse phase HPLC with Aqua C18 column for separation and LC-MS ion trap mass spectrometer for identification; mobile phase A 20 g/kg acetic acid and B 5 g/kg acetic acid in water and acetonitrile (50:50 *v/v*); elution: 10-55% B 0-50 min; 55-100% B 50-60 min; flow rate 1 mL/min; negative ion electrospray mode was used for mass spectral analysis; flavonols were monitored at 360 nm. Proanthocyanidins were analyzed spectrophotometrically.
Polyphenols	Cyanidin 3-galactoside, cyanidin 3-glucoside, cyanidin 3-arabinoside, cyanidin 3-xyloside, neochlorogenic acid, chlorogenic acid, quercetin 3-dihexoside, quercetin 3-dihexoside, quercetin 3-vicianoside, quercetin 3-robinobioside, quercetin, 3-rutinoside, quercetin, 3-galactoside, quercetin 3-glucoside, quercetin.
The main observations	The authors found out that the processing of chokeberry juice had a more significant impact on polyphenol losses than juice storage. Anthocyanins were more sensitive and prone to losses during processing than flavonols, proanthocyanidins and hydroxycinnamic acids.

Chokeberry fruit, pomace and juice

Extraction technique and conditions	Lyophilized pomace and fruits were extracted with acidified methanol (0.1% HCl in methanol) using sonification for 20 min. Diluted juice and supernatant of extracted pomace and fruits were filtered before analysis through a 0.45 µm syringe filter.	
	Additionally, enzymatic hydrolysis of anthocyanin and flavonol glycosides was performed using citrate buffer solution (pH 5) and specific enzymes (β-glucosidase, β-galactosidase, β-xylosidase and β-hesperidinase).	
	For proanthocyanidins analysis, thiolysis with acidified methanol (3.3% *v/v*) and toluene α-thiol (5% in methanol) was performed.	Oszmiański and Wojdylo, 2005
Chromatographic technique and conditions	HPLC analysis of (-)epicatechin, anthocyanins, flavonols, hydroxycinnamic acid using diode array detector and Synergi Fusion RP-80A column; mobile phase A 2.5% acetic acid and mobile phase B acetonitrile; elution: 0-25% B 0-36 min; flow rate 1 mL/min; detection wavelengths 280 nm ((-)epicatechin), 320 nm (hydroxycinnamic acids), 510 nm (anthocyanins) and 360 nm (flavonols).	
	HPLC analysis of proanthocyanidins with DAD and scanning fluorescence detectors and Merch Purospher RO 18 end-capped column; mobile phase A 2.5% acetic acid and mobile phase B acetonitrile; elution: 9% B 0-5 min, 16% B 5-15 min, 50% B 15-45 min; flow rate 1 mL/min; excitation wabelenght 278 nm and emission wavelength 360 nm.	

Polyphenols	Cyanidin 3-galactoside, cyanidin 3-glucoside, cyanidin 3-arabinoside, cyanidin 3-xyloside, neochlorogenic acid, chlorogenic acid, quercetin 3-rutinoside, quercetin 3-galactoside, quercetin 3-glucoside, quercetin derivates, (-)epicatechin, polymeric procyanidins.
The main observations	Chokeberry pomace contains the highest content of total polyphenols (10583.27 mg/100 g). Polymeric procyanidins take a huge part of polyphenols ranging from 5181.60 mg/100 g (fruits) to 8191.58 mg/100 g (pomace). Concentrations of chlorogenic and neochlorogenic acid were the highest in the juice (415.86 and 393.10 mg/100 g, respectively). Cyanidin 3-galactoside was the main anthocyanin in samples with the highest concentration in the fruits (1282.41 mg/100 g)

Chokeberry juice

Extraction technique and conditions	Juice was prepared by pressing the chokeberry fruits and filtering the obtained mass. Diluted juice was filtered before analysis through a 0.20 μm syringe filter.	
Chromatographic technique and conditions	HPLC analysis; Poroshell 120 EC C-18 column; UV/Vis-Photo Diode Array detector; mobile phase A 0.1% of orthophosphoric acid in water and B 100% of methanol; elution :5% B 0 min, 30% B 3 min, 35% B 15 min, 37% B 22 min, 41% B 30 min, 45% B 32 min, 49% B 40 min, 80% B 45 min, 80% B 48 min, 5% B 50 min, 5% B 53 min; flow rate 1 mL/min; detection wavelengths 320 nm (phenolic acids), 520 nm (anthocyanins) and 360 nm (flavonols).	Ćorković et al., 2021
Polyphenols	Cyanidin 3-galactoside, cyanidin 3-arabinoside, cyanidin 3-xyloside, neochlorogenic acid, chlorogenic acid, quercetin, quercetin 3-rutinoside, quercetin 3-galactoside.	
The main observations	Chokeberry juice that the authors further used for microencapsulation had the highest content of neochlorogenic acid (1410 mg/L) followed by chlorogenic acid (172 mg/L). From the group of anthocyanins and flavonols, cyanidin-3-galactoside (42 mg/L) and quercetin 3-rutinoside (26 mg/L) were present in the highest concentrations, respectively.	

Chokeberry fruit, juice and compote

Extraction technique and conditions	Juice (50 mL) was mixed with 50 mL of 70% ethanol and pH was adjusted to pH 2 (with formic acid). Dried fruit and compote were extracted overnight with 70% ethanol and pH was adjusted to pH 2 (with formic acid) followed by filtration.	
Chromatographic technique and conditions	HPLC analysis; Diode Array detector, Phenomenex C18 column; mobile phase A water (adjusted to pH 3.2 with formic acid) and B 100% of acetonitrile; gradient elution from 95% A to 100% B in 53 min; flow rate 0.6 mL/min; detection wavelengths 330 nm (phenolic acids) and 350 nm (flavonols). HPLC-TOF analysis; electrospray ionization (ESI) source; mass range m/z 100-1500 in both positive and negative modes; ESI conditions (drying gas N_2, drying gas temperature 350 °C, drying gas flow rate 6 L/min, nebulizer 20 psi, capillary voltage 4000 V, fragmentation 80-150 V, skimmer 60 V).	Romani et al., 2016
Polyphenols	Neochlorogenic acid, chlorogenic acid, 4-*O*-caffeoyl-quinic acid, quercetin 3-galactoside, quercetin 3-glucoside, quercetin 3-rutinoside, quercetin 3-rhamnosylgalactoside.	
The main observations	From the group of phenolic acids, chlorogenic acid was present in the highest concentrations in dried fruit (2.33 mg/g), juice (0.51 mg/g) and compote (0.37 mg/g). 4-*O*-Caffeoyl-quinic acid was present in low concentrations in samples (0.03-0.17 mg/g). Quercetin 3-rutinoside and	

quercetin 3-rhamnosylgalactoside could not be quantified individually but as a sum, and their concentration was the highest in the dried fruit (0.23 mg/g) while in juice (0.04 mg/g) and compote (0.03 mg/g) were in low concentrations. From the group of flavonols, quercetin 3-galactoside was present ih the highest concentration in dried fruit (0.31 mg/g), juice (0.05 mg/g) and compote (0.06 mg/g).

Chokeberry fruit		
Extraction technique and conditions	Anthocyanins from samples were extracted at room temperature with acidified methanol (1 mL 30% HCl per 100 mL MeOH). Flavonoids and phenolic acids were extracted with methanol under a reflux condenser for 2h.	
Chromatographic technique and conditions	LC-DAD-ESI-MS analysis of anthocyanins; Supelcosil LC-18 column; mobile phase A 0.1% trifluoroacetic acid in water and B acetonitrile/0.1% trifluoroacetic acid in water 50:50 (*v/v*); gradient elution: 15% B 0 min, 30% B 60 min, 15% B 80 min, 15% B 85 min, 15% B 85.01 min; flow rate 0.5 mL/min; detection wavelength 520 nm; electrospray ionization (ESI) source; mass spectrometric secected detection in positive ion mode (*m/z* 449, 419 and 287). LC-DAD analysis of flavonoids and phenolic acids; RP-18e column; mobile phase A methanol:0.5% acetic acid (1:4 *v/v*) and B methanol; gradient elution: 0% B 0-20 min, 0-20% B 20-35 min, 20-30% B 35-45 min, 30-40% B 45-55 min, 40-50% B 55-60 min 50-70% B 60-65 min, 75-100% B 65-70 min; flow rate 1 mL/min; detection wavelength 254 nm.	Szopa et al., 2017
Polyphenols	Cyanidin 3-galactoside, cyanidin 3-arabinoside, cyanidin 3-glucoside, quercetin, neochlorogenic acid, chlorogenic acid, 3,4-dihydroxyphenylacetic acid, protocatechuic acid, rosmarinic acid.	
The main observations	In all tested samples, from the group of anthocyanins, cyanidin 3-galactoside was present in the highest concentrations (19.4-323.2 mg/100 g DW (dry weight)) followed by cyanidin 3-arabinoside (7.2-140.9 mg/100 g DW) and cyanidin 3-glucoside (0.3-10.8 mg/100 g DW). Quercetin was only flavonol detected in samples (12.2-44.3 mg/100 g DW). Neochlorogenic and chlorogenic acid were the most abundant phenolic acids in samples (92.3-212.6 mg/100 g DW and 16.3-276.9 mg/100 g DW, respectively).	
Chokeberry juice, powder, capsule, fruit and tea		
Extraction technique and conditions	Extraction was performed with 50% methanol and 1.2 M HCl in water bath for 16 h at 35 °C under nitrogen.	
Chromatographic technique and conditions	LC method, Diode Array detector, Zorbax Eclipse XDB-C18 column; mobile phase A 1% of acetic acid in water and B 100% of methanol; gradient elution: 5-80% B 0-30 min, 80% B 30-33 min, 80-5% B 33-35 min; column temperature – 20 °C; flow rate 0.8 mL/min.	Tolić et al., 2018
Polyphenols	Quercetin, kaempferol, gallic acid, chlorogenic acid, caffeic acid, *p*-coumaric acid, ellagic acid.	
The main observations	Quercetin was found in all analyzed samples with concentration range of 11.80-73.60 mg/L in juices. Kaempferol was detected in one sample of juice (0.90 mg/L) and in powders and capsules. Gallic acid was found in several juices in small concentrations (0.60-5.51 mg/L). Chlorogenic, caffeic, *p*-coumaric and ellagic acids were present in all samples and their	

concentrations in juice were in range of 46.11-111.19 mg/L, 0.89-3.57 mg/L, 99.28-284.55mg/L and 5.71-23.03 mg/L, respectively.

Chokeberry fruit	
Extraction technique and conditions	Anthocyanins were extracted with 70% ethanol in water acidified with 0.1 N HCl by sonication at room temperature for 15 min. Flavonols and hydroxycinnamic acids were extracted with 60% methanol in water in a boiling-water bath for 1h.
Chromatographic technique and conditions	HPLC-DAD-MS analysis; Phenomenex Luna C18(2) column. Anhocyanins analysis: mobile phase A 1% formic acid in water and B 1% formic acid in acetonitrile; gradient elution: 10% B 0 min, 12% B 10 min, 15% B 20 min, 30% B 30-32 min, 10% B 33-45; flow rate 0.5 mL/min; column temperature 40 °C; detection wavelength 520 nm. Flavonols analysis: mobile phase A 0.1% formic acid in water and B 0.1% formic acid in acetonitrile; gradient elution: 15% B 0 min, 60% B 35-40 min, 15% B 41-50 min; flow rate 0.5 mL/min; column temperature 30 °C; detection wavelength 370 nm and 350 nm. Hydroxycinnamic acids analysis: mobile phase A 0.1% formic acid in water and B 0.1% formic acid in acetonitrile; gradient elution: 10% B 0 min, 25% B 18 min, 40% B 30 min), 60% B 35 min, 10% B 36-45 min; flow rate 0.5 mL/min; column temperature 30 °C; detection wavelength 330 nm and 275 nm. Ionization source: heated electrospray ionization; mode positive and negative; capillary voltage 3500 V and 2500 V; ion source temperature 350 °C (anthocyanins), 325 °C (flavonols) and 275 °C (hydroxycinnamic acids); ion transfer tube temperature 325 °C (anthocyanins), 300 °C (flavonols) and 275 °C (hydroxycinnamic acids); sheath gas flow rate 5.6 L/min; auxiliary gas flow rate 8 L/min; sweep gas flow rate 1.5 L/min; MS scanning *m/z* range: 150-1500 (anthocyanins and flavonols) and 100-1000 (hydroxycinnamic acids).	Gerasimov et al., 2023
Polyphenols	Cyanidin 3-hexoside-epicatechin, cyanidin 3-pentoside-epicatechin, cyanidin 3-hexoside-epicatechin-epicatechin, cyanidin 3-galactoside, cyanidin 3-glucoside, cyanidin 3-arabinoside, cyanidin 3-xyloside, 5-carboxypyranocyanidin-3-pentoside; 5-carboxypyranocyanidin-3-hexoside, quercetin 3-dihexoside, quercetin 3-vicianoside, quercetin 3-rutinoside, quercetin 3-galactoside, ispoquercitrin, quercetin, neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid.
The main observations	From the group of anthocyanins, cyanidin 3-galactoside was present in the highest concentrations in all samples (around 68 mg/100 g FW (fresh weight)). Quercetin 3-galactoside and quercetin 3-rutinoside were the most abundant flavonols in samples (5.7-19.5 mg/100 g FW and 7-15.8 mg/100 g FW, respectively). Chlorogenic acid was the main phenolic acid in samples (21.3-81.2 mg/100 g FW).
Chokeberry peel, flesh, fruit and juice	
Extraction technique and conditions	Samples were extracted with methanol acidifed with trifluoracetic acid (0.5%) in ultrasonic bath at 30 °C for 40 min.	Kaloudi et al., 2022
Chromatographic	HPLC-DAD analysis; ZORBAX Eclipse XDB-C18 column; mobile phase A water, B methanol and C acetonitrile each containing 0.2% trifluoracetic acid; elution: 90% A, 6% B, 4% C 0 min, 0% A,

technique and conditions	85% B, 15% C 5 min, 71% A, 17.4% B, 11.6% C 30 min, 0% A, 85% B, 15% C 60 min; flow rate 1 mL/min; detection wavelengths 280 nm (universal wavelenght), 360 nm (flavonols), 320 nm (phenolic acids) and 520 nm (anthocyanins).	
Polyphenols	Cyanidin 3-galactoside, cyanidin 3-glucoside, cyanidin 3-arabinoside, cyanidin 3-xyloside, quercetin 3-vicianoside, quercetin 3-rutinoside, quercetin 3-glucoside, neochlorogenic acid, chlorogenic acid.	
The main observations	Cyanidin 3-galactoside was present in the highest concentrations in the peel, flesh and fruit (1.3-65 mg/g). Concentrations of chlorogenic and neochlorogenic acids in peel, flesh and fruit were in range of 0.5-2.4 mg/g and 0.24-1.2 mg/g, respectively. From the group of flavonols, quercetin 3-glucoside was present in the highest concentrations in peel, flesh and fruit (0.15-2.48 mg/g). Total anthocyanins, flavonols and phenolic acids were in higher concentrations in the juice produced in laboratory (1395 mg/L, 269 mg/L and 1116 mg/L, respectively) than the commercial one (58.2 mg/L, 27.9 mg/L and 654 mg/L, respectively).	
Chokeberry fruit		
Extraction technique and conditions	For anthocyanins analysis, samples were extracted two times with methanol acidified with HCl (0.1%) for 60 min. Extraction and hydrolysis of flavonols were performed with the addition of fruit samle (5 g), methanol (25 mL) and 6 M HCl (10 mL) into ascorbic acid solution (80 mg in 5 mL of water). Final volumen was adjusted at 50 mL and HCl concentration of 1.2 M. This solution was refluxed on water bath at 85 °C for 2h followed by filtration, evaporation and dissolution in 2 mL MeOH.	
Chromatographic technique and conditions	HPLC analysis; UV-Vis detector and Photodiode Array detector; OmniSpher C18 column. Anthocyanins analysis: mobile phase A 0.5% phosphoric acid in water and B 100% methanol; elution: 3-65% B 0-38 min, 65% B 38-45 min; flow rate 1 mL/min; detection wawelenght 520 nm. Flavonols analysis: mobile phase A 0.1% phosphoric acid in water and B 100% methanol; elution: 5-80% B 0-30 min, 80% B 30-33 min, 80-5% B 33-35 min; flow rate 0.8 mL/min; column temperature 20 °C; detection wawelenght 360 nm.	Jakobek et al., 2007b
Polyphenols	Cyanidin 3-galactoside, cyanidin 3-glucoside, cyanidin 3-arabinoside, cyanidin 3-xyloside, quercetin, kaempferol.	
The main observations	Total anthocyanin concentration was 4056 mg/kg with cyanidin 3-galactoside (2795 mg/g) as the main anthocyanin present in sample. Quercetin was present in concentration of 71 mg/kg while concentration of kaempferol was only 5.30 mg/kg.	

Conclusions

Polyphenols are known as antioxidants that effectively remove free radicals and have an impact on prevention and treatment of chronic diseases. Extensive research has shown that some polyphenols have a direct effect on individual human tissues and/or organs and can thus have a potential to positively influence the course of disease development. Chokeberries are abundant in polyphenols, what makes them the subject of many studies. Liquid chromatography coupled with mass spectrometry is the most powerful technique for identification and quantification of polyphenols. With the information that this review provides, the scientist can easily get insight into the main parameters for chokeberry polyphenols

analysis, such as solvents for chromatographic elution, preferred columns for separation, detectors, as well as polyphenols profile of chokeberries.

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