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Fractionation of black chokeberry pomace into functional ingredients using high pressure extraction methods and evaluation of their antioxidant capacity and chemical composition

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ABSTRACT

Black chokeberry is well-known for its high anthocyanin content and strong antioxidant capacity; however, due to their undesirable flavour, chokeberry fruits are processed into various products such as juice and fruit wine. Pressing juice results in high amounts of pomace, which is frequently discarded as waste. This study aimed at valorizing chokeberry pomace as a source of valuable functional ingredients. Conventional and high pressure extraction methods were applied; the yields of extracts were in the range of 3–48%. Some extracts possessed high antioxidant potential as determined by oxygen radical absorbance capacity (ORAC) which was in the range of 0.21–15 mmol Trolox equivalents per gram; ethanol extract rendered the strongest antioxidant effect. Twenty-nine constituents were identified in pomace extracts by chromatography/mass spectrometry with high concentrations of chlorogenic acid, cyanidin derivatives and quinic acid. Thus, chokeberry pomace is a good source of bioactive constituents that might be used for various applications, particularly functional food ingredients and nutraceuticals.

Abbreviations: DPPH•, 2,2-diphenyl-1-picrylhydrazyl free radical; GA, gallic acid; AAPH, 2,2′-azobis (2-amidinopropane) dihydrochloride; FL, fluorescein; ABTS•+, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid); HPLC, high performance liquid chromatography; T, tocopherol; PLE, pressurized liquid extraction; SFE, supercritical fluid extraction; FCR, Folin–Ciocalteu reagent; GAE, gallic acid equivalents; PBS, phosphate buffered saline; RSC, radical scavenging capacity; TEAC, Trolox equivalent antioxidant capacity; ORAC, oxygen radical absorbance capacity; AUC, area under curve; TE, Trolox equivalent; UPLC, ultra performance liquid chromatography; Q-TOF, quadrupole-time of flight; MS, mass spectrometry; ESI, electro-spray ionization; CID, collision induced dissociation; SIR, selected ion recording; H-ORAC, hydroxyl radical antioxidant capacity

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Chemical compounds: 1-Caffeoylquinic acid (PubChem CID: 6451212); 3-Caffeoylquinic acid (PubChem CID: 1794427); 4-Caffeoylquinic acid (PubChem CID: 9798666); 5-Caffeoylquinic acid (PubChem CID: 5280633); Hyperoside (PubChem CID: 5281643); Rutin (PubChem CID: 5280805).

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1. Introduction

Berries are among the richest sources of polyphenolics such as anthocyanins, flavonols, flavan-3-ols, procyanidins, ellagitannins, and hydroxycinnamates, which have been the focus of numerous studies with regard to their putative impact on human health (Chiou et al., 2014; Norberto et al., 2013; Shahidi & Ambigaipalan, 2015a; Stewart et al., 2007; Szajdek & Borowska, 2008). Epidemiological evidence indicates that cardiovascular health benefits of diets rich in berries are related to their (poly)phenol content (Basu, Rhone, & Lyons, 2010; Rodriguez-Mateos, Heiss, Borges, & Crozier, 2014; Shahidi, 2004). A sustained pro-inflammatory state is regarded as a major contributing factor in chronic disease development, progression, and complication, and berry polyphenols purport to have an anti-inflammatory activity (Joseph, Edirisinghe, & Burton-Freeman, 2014).

Many berry species may be consumed fresh, but the fruits of some berry plants are not suitable for eating because their flavour is less favourable or even unacceptable by the consumers (Kraujalytė, Leitner, & Venskutonis, 2013). Consequently, all harvests of such berries have to be processed into other products. Black chokeberry (*Aronia melanocarpa*) belongs to the latter group of berries. It is a distinctive berry with a high content of polyphenols, particularly anthocyanins and proanthocyanidins (Wangensteen et al., 2014), and possessing the highest *in vitro* antioxidant activity among many fruits. There is a strong evidence for health benefits of chokeberry, including antidiabetic, cardioprotective, hepatoprotective, antimutagenic, and anticarcinogenic effects. For instance, positive impact of regular chokeberry juice consumption on cellular oxidative damage suggests its putative role in the protection against oxidative stress (Kardum et al., 2014). Anti-inflammatory activity of chokeberry extracts was reported in murine splenocytes (Martin et al., 2014), while chokeberry juice inhibited mouse embryonal carcinoma stem cell proliferation, induced cell cycle arrest in S phase and triggered apoptosis (Sharif et al., 2013). In general, the products derived from chokeberry are claimed to be beneficial in attenuating disorders associated with oxidative stress: bioavailability and antioxidant activity of chokeberry polyphenols, possible mechanisms of action *in vivo* in the prevention and treatment of oxidative stress-related diseases (Denev, Kratchanov, Ciz, Lojek, & Kratchanova, 2012). Clinical effectiveness of chokeberry (Chrubasik, Li, & Chrubasik, 2010) as well as its characteristic components and their potential health effects (Kulling & Rawel, 2008) have been reviewed.

Chokeberries are used for making fruit wine; they are also dried for herbal teas, used for flavouring and colouring beverages or yoghurts, and added to juice blends for colour and for strengthening antioxidant properties. Juice from the ripe berries is astringent, semi-sweet (moderate sugar content), sour (low pH), and contains a comparatively low level of vitamin C (Skupien & Oszmianski, 2007). Pressing chokeberry juice produces large amounts of pomace (also called press-cake or marc), which is frequently discarded as waste. This results in a loss of valuable constituents and causes environmental problems. Therefore, biorefining of chokeberry pomace into higher added value ingredients is an important task. Research for valorization of agricultural waste and by-products has increased during the last decade; however, it has been focussed mainly on the waste of the major horticultural products such as citrus fruits (Mamma & Christakopoulos, 2014), grapes (Arvanitoyannis, Ladas, & Mavromatis, 2006), raspberries (Kryževičiūtė, Kraujalis, & Venskutonis, 2016), pomegranates (Ayala-Zavala et al., 2011) and apples (Rabetafika, Bchir, Blecker, & Richel, 2014), while numerous other vegetables, fruits and berry species, including chokeberry, remain underexplored. It should also be noted that a number of innovative techniques and technologies have been developed for the recovery of added-value components from food waste (Galanakis, 2012). Such methods as ultrasound (Ghafoor, Hui, & Choi, 2011), microwave (Kaufmann & Christen, 2002), supercritical fluid (Ghafoor, AL-Juhaimi, & Choi, 2012; Ghafoor, Park, & Choi, 2010; Wang, Weller, Schlegel, Carr, & Cuppett, 2008), pressurized liquid (Kaufmann & Christen, 2002; Smith, 2002) extractions and others have been widely introduced for the isolation of plant bioactives. They have several common objectives: (i) extracting targeted bioactive compounds from a complex plant sample; (ii) increasing the selectivity of analytical methods; (iii) increasing the sensitivity of bioassays by increasing the concentration of targeted compounds; (iv) converting bioactive compounds into a more suitable form for detection and separation; (v) and providing a reproducible method that is independent of variations in the sample matrix (Smith, 2003).

The aim of this study was to investigate various extraction techniques and different solvents for the isolation of valuable fractions from *A. melanocarpa* berry pomace and to evaluate their antioxidant properties and composition. The findings of this study are expected to assist in valorization of chokeberry pomace by the development of their effective biorefining processes. Polyphenol rich preparations may find various applications, e.g. as natural food additives in fish and fish products (Maqsood, Benjakul, & Shahidi, 2013), fruit purees (Bobinaitė et al., 2016), and meat products (Šulniūtė, Jaime, Rovira, & Venskutonis, 2016). Such additives, being strong antioxidants, may impart to food a double effect, firstly, by protecting against autoxidation and, secondly, by enriching them with health beneficial phytochemicals.

2. Materials and methods

2.1. *Black chokeberry pomace and chemicals*

Fresh black chokeberry pomace was obtained from the company 'Obuolių namai' (Kaunas, Lithuania) and immediately freeze-dried. 2,2-Diphenyl-1-picrylhydrazyl radical (DPPH$^\bullet$, 95%), gallic acid (GA), tetramethylchromane-2-carboxylic acid (Trolox 97%), anhydrous sodium carbonate, 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH), and ammonium hydroxide were purchased from Sigma-Aldrich Chemie (Steinheim, Germany); fluorescein (FL) was purchased from Fluka Chemicals (Steinheim, Germany); 2.0 M Folin–Ciocalteu's reagent, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), NaCl, KCl, Na_2HPO_4, and $K_2S_2O_8$ were purchased from Merck (Darmstadt, Germany); KH_2PO_4 was purchased from Jansen Chimica (Beerse, Belgium); and chlorogenic acid, hyperoside and rutin were obtained from Chromadex (Irvine,