Food Chemistry 175 (2015) 516–522

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Antioxidant and antiproliferative activity of chokeberry juice phenolics during *in vitro* simulated digestion in the presence of food matrix

Nemanja Stanisavljević [a,*], Jelena Samardžić [a], Teodora Janković [b], Katarina Šavikin [b], Marija Mojsin [a], Vladanka Topalović [a], Milena Stevanović [a]

[a] University of Belgrade, Institute of Molecular Genetics and Genetic Engineering, Vojvode Stepe 444a, 11000 Belgrade, Serbia
[b] Institute for Medicinal Plants Research "Dr Josif Pančić", Tadeuša Košćuška 1, 11000 Belgrade, Serbia

ARTICLE INFO

Article history:
Received 16 September 2014
Received in revised form 1 December 2014
Accepted 3 December 2014
Available online 10 December 2014

Keywords:
Chokeberry
In vitro digestion model
Food matrix
Polyphenols
Antioxidant activity
Antiproliferative

ABSTRACT

Chokeberry juice was subjected to *in vitro* gastric digestion in the presence of food matrix in order to determine the changes in polyphenol content and antioxidant activity. Addition of food matrix immediately decreased the total phenolic content, anthocyanin content, DPPH scavenging activity as well as total reducing power by 36%, 90%, 45% and 44%, respectively. After *in vitro* digestion, total phenolic content, anthocyanin content and reducing power are slightly elevated, but they are still lower than in initial non-digested juice. The effect of digested juice on Caco-2 cells proliferation was also studied, and the reduction of proliferative rate by approximately 25% was determined. Our results suggested that although a large proportion of chokeberry phenolics undergo transformation during digestion they are still potent as antioxidant and antiproliferative agents.

1. Introduction

Berries of *Aronia melanocarpa* (Michx.) Elliott (black chokeberry, aronia) have a long history of usage in the Native North American Herbal medicine as a tea for treating cold and in diet as juice, syrup, wine and jam (Kulling & Rawel, 2008). The interest for chokeberry in the European countries has been recently increased mainly because of its health promoting effects. A large number of intervention studies have demonstrated chokeberry beneficial effects on various risk factors for cardiovascular diseases, such as high levels of cholesterol, triglycerides, glucose, as well as its vasoactive and vasoprotective properties (Bell & Gochenaur, 2006; Valcheva-Kuzmanova, Kuzmanov, Tancheva, & Belcheva, 2007). Biological activity of chokeberry is highly connected to its chemical composition. Chokeberries are rich source of polyphenolic compounds, such as phenolic acids, proanthocyanidins, anthocyanins, flavonols, and flavanones (Koponen, Happonen, Mattila, & Torronen, 2007; Wu, Gu, Prior, & McKay, 2004). Anthocyanins are dominant flavonoids in chokeberry, representing about 25% of total polyphenols (Benvenuti, Pellati, Melegari, & Bertelli, 2004). The major anthocyanins in chokeberry are glycosides of cyanidin such as cyanidin-3-*O*-galactoside, cyanidin-3-*O*-arabinoside, cyanidin-3-*O*-xyloside and cyanidin-3-*O*-glucoside (Oszmianski & Sepia, 1988). The beneficial effects of anthocyanins on health, such as vasoprotective, anti-inflammatory, anticarcinogenic, antiobesity and antidiabetic ones, have been documented (Kulling & Rawel, 2008).

One of the main limiting factors regarding the beneficial effects of polyphenols is their bioavailability which depends on digestive stability and their release from the food matrix as well as the efficiency of their transepithelial passage (Tagliazucchi, Verzelloni, Bertolini, & Conte, 2010). Although many studies reported the effects of an *in vitro* digestion on polyphenols from the fruit juices (Bermúdez-Soto, Tomás-Barberan, & García-Conesa, 2007; Cilla, Gonzalez-Sarrias, Tomas-Barberan, Espin, & Barbera, 2009; Gil-Izquierdo, Gil, Ferreres, & Tomas-Barberan, 2001), data about interaction of polyphenols and food matrix during digestion are lacking. The total amount of ingested polyphenolic compounds does not always reflect the amount that is available to the body. Certain studies indicate that the bioavailability of phenolics could be significantly different depending on the consumed food source (D'Archivio, Filesi, Vari, Scazzocchio, & Masella, 2010). Bioavailability of phenolic compounds is affected by food related factors such as interaction with proteins, carbohydrates, fibers and fat (D'Archivio et al., 2010). For instance, Roura et al. (2007) showed that milk significantly affected bioavailability of epicatechin metabolites. Higher dietary fat content could either enhance or delay the absorption of certain flavonoids (Lesser, Cermak, & Wolffram, 2004). In addition, Adam et al. (2002) showed that

* Corresponding author.

http://dx.doi.org/10.1016/j.foodchem.2014.12.009
0308-8146/

bioavailability of ferulic acid in rats digestive tract depends on type of dietary fibers to which it is linked. Phenolic compounds could also bind to proteins in aqueous media through different mechanisms such as hydrogen bonding, covalent bonding, hydrophobic interactions thus making them unavailable for absorption (Xu & Diosady, 2000). Another important factor regarding the bioavailability of polyphenols is the pH of the environment. It has been generally accepted that anthocyanins are stable at low pH values between 1 and 3, and according to the numerous studies low bioavailability of anthocyanins can be attributed to their low stability in the alkaline conditions of small intestine (Perez-Vicente, Gil-Izquierdo, & Garcia-Viguera, 2002; Tagliazucchi et al., 2010). However, studies of Cabrita, Fossen, and Andersen (2000) showed that certain anthocyanin 3-glucosides exhibited relatively high stability in the alkaline region. It has been previously observed that pH value in the upper part of the human gastrointestinal tract during feeding state varies from 1.7 in the gaster to 5.4 in the upper and medium part of duodenum (Dressman et al., 1990). The pH value in duodenum is returning to fasted state values of 6.7 in approximately 4 h. Other factors such as salt concentration in the medium should be also taken into consideration when stability of anthocyanins is examined (Cabrita et al., 2000).

Data concerning the fate of chokeberry polyphenols during the digestion process in the presence of food matrix are limited. We directed our research towards understanding the impact of digestion and food matrix on bio-accessibility and stability of certain phenolic compounds from chokeberry juice in stomach and upper duodenum acid environment. Since the release of phenolics from food matrix is prerequisite for their uptake, we simulated a simplified *in vitro* model of the human digestive tract using a complex food matrix of defined composition in controlled conditions (pH, temperature, bile salts, gastric and pancreatic enzymes), which allows measurement of selected phenolic compounds released from the ingested product as an indicator of their bioavailability. The main objectives of this research were to determine the changes in total phenolic content, total proanthocyanidin content and some highly abundant anthocyanins as well as changes in their antioxidant and antiproliferative activity during gastro-intestinal digestion of chokeberry juice under acid conditions in the presence of complex food matrix.

2. Materials and methods

2.1. Plant material

Chokeberry (*A. melanocarpa*) Nero cultivar, donated from Conimex trade d.o.o. was collected in the third year of cultivation from the experimental field certificated for organic production, Šabac locality, Serbia, in August 2013. The average diameter and weight of berries were 7.2 mm and 1.2 g, respectively. After harvesting, berries were stored at +5 °C for 10 days until the start of the experiment. Berries were crushed and squeezed in a wine horizontal screw press to obtain juice.

2.2. In vitro gastrointestinal digestion

We have applied previously reported and slightly modified *in vitro* digestion model consisting of a three step procedure which simulates the digestion in mouth, stomach and small intestine (Oomen et al., 2003). The existing model was improved according to Versantvoort, Van de Kamp, and Rompelberg (2004) in order to apply physiologically based model of human digestive tract which simulates feeding conditions. Infant formula (Juvitana, Swisslion Product d.o.o. Indjija, Serbia) of defined composition was used to simulate standard meal (food matrix). According to original manufacture specification, formula consisted of 20% turkey meat, 25% boiled corn paste, 10% boiled potato paste, 5% rice flour, 0.1% NaCl and 39.9% water. The mixture contained 3% protein, 10% carbohydrate and 1% fat. Food matrix was free of preservatives, antioxidants, artificial flavors and aromas. Synthetic juices used for *in vitro* digestion were prepared according to method of Oomen et al. (2003), and their composition was presented in detail in Table 1. All the enzymes used for *in vitro* digestion were purchased from Sigma–Aldrich Co. (St. Louis, USA). The general set-up of the digestion model is as follows: the digestion starts by introducing 6 mL of artificial saliva to a mixture prepared from 4.5 g of food matrix (infant formula) and 4.5 mL of chokeberry juice. Mixture was gently stirred for 5 min at 37 °C, transferred to 50 mL conical flask and supplemented with 3 mL of gastric juice. Flasks were placed to rotary shaker at 37 °C (1 h, 55 rpm). In the next step, 9 mL of gastric juice was added, pH was adjusted to 2.0 and

Table 1
Composition of artificial juices applied during *in vitro* digestion.

Artificial saliva	Gastric juice	Intestinal juice	Bile
Inorganic compounds[a]			
10 mL KCl 89.6 g/L	15.7 NaCl 175.3 g/L	40 mL NaCl 175.3 g/L	30 mL NaCl 175.3 g/L
10 mL KSCN 20 g/L	3 mL NaH_2PO_4 88.8 g/L	40 mL $NaHCO_3$ 84.7 g/L	68.3 mL $NaHCO_3$ 84.7 g/L
10 mL NaH_2PO_4 88.8 g/L	9.2 mL KCl 89.6 g/L	10 mL KH_2PO_4 8 g/L	4.2 mL KCl 89.6 g/L
10 mL Na_2PO_4 57 g/L	18 mL $CaCl_2 \times 2H_2O$ 22.2 g/L	6.3 mL KCl 89.6 g/L	200 μL HCl 37% g/g
1.7 mL NaCl 175.3 g/L	10 mL NH_4Cl 30.6 g/L	10 mL $MgCl_2$ 5 g/L	
1.8 mL NaOH 40 g/L	8.3 mL HCl 37% g/g	180 μL HCl 37% g/g	
Organic compounds[a]			
8 mL urea 25 g/L	10 mL glucose 65 g/L 10 mL glucuronic acid 2 g/L 3.4 mL urea 25 g/L 10 mL glucosamine hydrochloride 33 g/L	4 mL urea 25 g/L	10 mL urea 25 g/L
Compounds added to mixture of organic and inorganic solutions[b]			
145 mg α-amylase	1 g BSA	9 mL $CaCl_2 \times 2H_2O$ 22.2 g/L	10 mL $CaCl_2 \times 2H_2O$ 22.2 g/L
15 mg uric acid	1 g pepsin	1 g BSA	1.8 g BSA
50 mg mucin	3 g mucin	3 g pancreatin 0.5 g lipase	6 g bile
pH 6.5 ± 0.1	pH 1.0 ± 0.1	pH 7.8 ± 0.2	pH 8.0 ± 0.1

[a] Both inorganic and organic solutions were augmented with distilled water to a total volume of 500 mL and mixed together.
[b] The enzymes and other compounds assigned in table were added before use, and pH was additionally adjusted by 1 M NaOH or concentrated HCl in cases where it was necessary.