Food & Function

PAPER

View Article Online
View Journal | View Issue

Cite this: *Food Funct.*, 2018, **9**, 2998

Fermentation alters the bioaccessible phenolic compounds and increases the alpha-glucosidase inhibitory effects of aronia juice in a dairy matrix following *in vitro* digestion

Xue Du and Angela D. Myracle *

The prevalence of diabetes reached 415 million worldwide in 2015. Polyphenol-rich food intake can benefit the glycemic control for individuals with diabetes. Fermentation may increase the bioavailability of polyphenols, which is generally low. Aronia (*Aronia melanocarpa*) is a polyphenol-rich berry that is native to North America. Proanthocyanins and anthocyanins are the major phenolic compounds in aronia. In this study, aronia kefir was made by fermenting cow's milk with added aronia juice. The changes in bioaccessible polyphenols of aronia kefir during digestion were assessed using an *in vitro* model. The impact of fermentation on the potential bioactivity of aronia polyphenols was evaluated. Results showed that the bioaccessible polyphenols in aronia kefir were elevated during digestion and the antioxidant capacity increased (IC_{50} of DPPH scavenging decreased from 24.07 mg kefir per mL to 8.97 mg kefir per mL). Digested aronia kefir had less bioaccessible anthocyanins (cyanidin-3-galactoside, cyanidin-3-arabinoside and cyanidin-3-xyloside) but similar antioxidant capacity and stronger inhibitory activity on α-glucosidase (IC_{50}: 152.53 mg kefir per mL) compared to the non-fermented control (IC_{50}: 484.93 mg kefir per mL). These results indicate that fermentation may produce metabolites with higher antioxidant capacity and better α-glucosidase inhibitory activity. Utilizing aronia kefir in the diet is a good strategy to help control blood glucose levels without abdominal side effects. Fermentation may be an effective method to increase the bioavailability of dietary polyphenols in food. More studies about the effects of fermentation on polyphenol-rich food are needed to optimize the potential health-promoting properties.

Received 5th February 2018,
Accepted 9th May 2018

DOI: 10.1039/c8fo00250a

rsc.li/food-function

1. Introduction

Diabetes has been a global issue for the past few decades. In 2015, 30.3 million people (9.4%) in the United States and 415 million people in the world had diabetes.[1] Diabetes is associated with a decline in life expectancy and a reduction in life quality.[2] Tight blood glucose control is crucial for diabetic patients to delay the progression of complications, such as diabetic retinopathy, neuropathy and nephropathy.[3] The modulation of postprandial glucose absorption is one important method for management of hyperglycemia. Synthetic pharmaceutical agents are used to inhibit carbohydrate-hydrolyzing enzymes to delay and reduce the absorption of glucose. Despite the effectiveness of the drugs, most of them have side effects that impact the gastrointestinal tract and hepatic system.[3,4] Studies suggest that dietary polyphenols derived from plant-based food exhibit similar activity in inhibiting carbohydrate-hydrolyzing enzymes *in vitro* and have the potential to aid in blood glucose control without side effects.[3]

Dietary polyphenols are commonly ingested as a part of daily diet. They are found in high levels in plant-based food, especially in berries. Generally, polyphenols have low bioavailability due to their instability in the small intestine and their large molecular size.[5] The food matrix may limit or improve the absorption of polyphenols and influence the bioavailability.[6] Several methods have been suggested to increase the bioavailability of polyphenols, such as using encapsulation to increase the stability in the gastrointestinal tract.[7,8] Additionally, some microorganisms are capable of breaking down the complex phenolic compounds and the metabolites may be more bioactive.[9]

Aronia (*Aronia melanocarpa*) is a berry native to eastern North America.[10] Aronia contains more total polyphenols (7–12 mg gallic acid equivalent per g fresh weight) than many other plant-based food, such as blueberry (1 to 4 mg gallic acid equivalent per g fresh weight).[11–13] The astringent mouthfeel of aronia is caused by the high procyanidin content. Aronia is rarely consumed raw due to the astringency and the

5735 Hitchner Hall, University of Maine, Orono, Maine, 04469-5735 USA.
E-mail: xue.du@maine.edu, angela.myracle@maine.edu; Tel: +1 207 581 1617

lack of sweetness. Aronia was traditionally used by Native Americans as medicine to treat the common cold.[14] The phenolic compounds are thought to be the major bioactives that are responsible for the therapeutic effects of aronia. Park *et al.* observed the antiviral activity of aronia against different subtypes of influenza viruses.[15] In Russia, aronia has been used as a natural remedy to treat hypertension and atherosclerosis.[14] A recent study conducted by Loo *et al.* showed that consumption of aronia juice decreased low-grade inflammation in hypertensive patients;[16] Broncel *et al.* observed that consuming aronia extract reduced the oxidative stress in patients with metabolic syndrome.[17]

Kefir is a fermented dairy product consisting of up to 30 species of microorganisms including lactic bacteria, yeast and sometimes acetic acid bacteria.[18] Functional properties of kefir are well documented, including anti-bacterial, anti-carcinogenic and anti-inflammatory effects.[19] Kefir is naturally lactose-free, making it a good calcium and protein source for lactose-intolerance individuals. In this study, kefir was selected as the matrix to incorporate aronia polyphenols for the following reasons: (1) kefir is rich in protein, which can minimize the astringent mouth-feel of aronia[20] and may protect the polyphenols from degradation in the small intestine;[21] (2) the diverse microorganism community in kefir starter has the potential to metabolize phenolic compounds and increase the bioavailability.[22] Incorporating aronia into kefir may be a good way to optimize the potential health-promoting properties of aronia; (3) the acidic pH of kefir helps to protect the anthocyanins from degradation.[23]

Digestion is the key process influencing the bioavailability of a dietary component[24] because factors such as pH and enzymes in the digestive tract can modify the components and alter their liberation and absorption behavior.[8] Understanding the changes of the aronia polyphenols during digestion is important to assess their potential bioactivity. The functional properties of aronia before ingestion is well studied, but knowledge about the changes in a fermented matrix and the potential anti-diabetic properties after digestion remain unknown. To our knowledge, this is the first study to examine the bioaccessibility of aronia polyphenols in a fermented dairy matrix. The objectives of this study were: (1) to investigate the effects of kefir-fermentation on the potential bioactivity of aronia polyphenols in a dairy matrix; (2) to evaluate the changes in the bioaccessibility and antioxidant capacity of aronia kefir in the digestive tract using an *in vitro* model.

2. Materials and methods

2.1. Chemicals

Acetonitrile (HPLC grade), ammonium chloride, sodium phosphate dibasic, hydrochloric acid, methanol, potassium chloride, potassium thiocyanate, sodium hydroxide, sodium bicarbonate, soluble starch and urea were purchased from Fisher Scientific (Waltham, MA, USA). 2,2-Diphenyl-1-picrylhydrazyl (DPPH), dinitrosalicylic acid, formic acid, ox-bile, *p*-nitrophenyl β-D-glucopyranoside (pnp-G), potassium sodium tartrate tetrahydrate, porcine α-amylase, rat intestinal powder and HPLC standards (quercetin, chlorogenic acid, neo-chlorogenic acid and cyanidin-3-galactoside) were obtained from Sigma-Aldrich (St Louis, MO, USA). Bovine serum albumin, glucose, glucosamine hydrochloride, glucuronic acid, lipase, magnesium chloride, mucin, pepsin, sodium phosphate monobasic and uric acid were purchased from MP Biomedicals (Santa Ana, CA, USA). Calcium chloride was purchased from Ward's Science (Rochester, NY, USA), potassium dihydrogen phosphate was purchased from Alfa Aesar (Haverhill, MA, USA). All water used was obtained from a Millipore water system (EMD Millipore, Billerica, MA, USA).

2.2. Food material

Aronia (*Aronia melanocarpa*, variety 'Viking') were harvest based on the apparent ripeness of uniform deep purple color from the University of Connecticut (Storrs, CT, USA) from the 2014 growing season. Berries were de-stemmed, washed and stored at −20 °C. Frozen berries were heat treated (100 °C, 5 minutes) using a saucepan on a gas range stove and juiced with a domestic juicer (Hamilton Beach, Southern Pines, NC, USA). Juice was used immediately. Commercial kefir starter (Yogourmet®, Lachute, QC, Canada) and 2% milk (Oakhurst®, Portland, ME, USA) were purchased from a local supermarket.

2.3. Sample preparation

Aronia kefir was prepared by the following method: 2% milk was heated to 82 °C in an aluminum saucepan, cooled to 26 °C in an ice bath. The cooled milk and the commercial starter (5 g per quart of milk) were combined in a glass bowl. The mixture was stirred for 5 minutes to ensure that the starter was fully dissolved. Freshly made aronia juice was added to the milk-starter matrix (15%, w/w) and mixed well. The mixture was covered with a breathable cloth and kept at room temperature (23 °C) to ferment overnight. After 24 hours of fermentation, the aronia kefir was homogenized with an immersion blender (Hamilton Beach®, Southern Pines, NC, USA). The homogenized aronia kefir was transferred into a sealed glass jar and stored at 4 °C for 24 hours before carrying out the *in vitro* digestion. Three batches of aronia kefir were made and *in vitro* digestion was performed individually.

Milk and aronia juice without the addition of kefir starter was used as a non-fermented control. Non-fermented control was made by mixing 2% milk with 15% (w/w) freshly made aronia juice in a glass jar and sealed with a lid. The mixture was kept at 4 °C for 24 hours (the fermentation time of making kefir) and then acidified to pH 4.5 (the pH of kefir). The acidified non-fermented control was stored in the refrigerator (4 °C) for another 24 hours before the *in vitro* digestion process was carried out. Batches were made in triplicate.

2.4. *in vitro* digestion procedure

The digestion process was simulated using a modified method from Oomen *et al.* to assess the changes of polyphenols in the digestive tract after ingestion.[25] For each digestion, three com-

partments in the digestive tract were simulated: mouth, stomach and small intestine. Artificial digestive juices (saliva, gastric juice, intestinal juice and bile) were prepared fresh before the *in vitro* digestion was performed. The compositions of digestive juices are listed in Table 1. pH values of the digestive juices were adjusted with concentrated HCl or 2 M NaOH to the appropriate range. The digestion process was carried out as follows: the process was initiated by adding saliva to 27 mL aronia kefir (2 : 3, v/v). The mixture was stirred gently for 5 minutes at 37 °C in an Isotemp™ water bath (Fisher's Scientific, Waltham, MA, USA). One-third of the oral-digested sample was removed and collected as the oral-digested fraction. Gastric digestion was initiated by adding in gastric juice to the remaining oral-digested sample (4 : 5, v/v) and incubating the mixture at 37 °C in a shaking water bath (Edvotek®, Washington D.C., USA) for 2 hours. This process consisted of two parts because the pH environment in the stomach is not stable at the beginning of gastric digestion due to food influx: for the first hour, one portion of gastric juice was added to the remaining oral digesta and the pH of the mixture was not adjusted; for the second hour, three portions of gastric juice were added to the mixture, the pH was adjusted to 2.0 with concentrated hydrochloric acid. At the end of gastric digestion, half of the gastric digesta was removed and collected as the gastric-digested fraction. Digestion in the small intestine was initiated by adding $NaHCO_3$ (1 M) to the remaining gastric-digested sample, resulting in a pH of 5.7. Intestinal juice and bile were added to the mixture (4 : 2 : 9, v/v). pH of the mixture was adjusted to 7.5 with 2 M sodium hydroxide and the mixture was incubated at 37 °C in the shaking water bath for two hours. All of the intestinal digesta was collected as the small intestine-digested fraction. Though the collected volume of the individual fractions was different, each fraction contained an equal amount of aronia kefir (9 mL). All incubations were conducted in the dark and the mixtures were sealed with parafilm to reduce oxygen exposure. The aronia kefir controls for each stage of digestion were processed by the same procedure in the absence of enzymes and bile. Non-fermented control was treated with the same *in vitro* digestion procedure.

Table 1 The composition of digestive juices

Artificial saliva	Gastric juice	Intestinal juice	Bile
Inorganic compounds			
10 mL KCl	15.7 ml NaCl	40 ml NaCl	30 mL NaCl
89.6 g L^{-1}	175.3 g L^{-1}	175.3 g L^{-1}	175.3 g L^{-1}
10 mL KSCN	18 mL $CaCl_2{\cdot}2H_2O$	40 mL $NaHCO_3$	68.3 mL $NaHCO_3$
20 g L^{-1}	22.2 g L^{-1}	84.7 g L^{-1}	84.7 g L^{-1}
10 mL NaH_2PO_4	9.2 mL KCl	10 mL KH_2PO_4	4.2 mL KCl
88.8 g L^{-1}	89.6 g L^{-1}	8.0 g L^{-1}	89.6 g L^{-1}
10 mL Na_2PO_4	3 mL NaH_2PO_4	6.3 mL KCl	200 μl HCL
57.0 g L^{-1}	88.8 g L^{-1}	89.6 g L^{-1}	37% g g^{-1}
1.7 mL NaCl	10 mL NH_4Cl	10 mL $MgCl_2$	
175.3 g L^{-1}	30.6 g L^{-1}	5 g L^{-1}	
1.8 mL NaOH	8.3 mL HCl	180 μl HCl	
40.0 g L^{-1}	37% g g^{-1}	37% g g^{-1}	
Organic compounds			
8 mL urea	10 mL glucose	4 mL urea	10 mL urea
25.0 g L^{-1}	65.0 g L^{-1}	25.0 g L^{-1}	25.0 g L^{-1}
	10 mL glucuronic acid		
	2.0 g L^{-1}		
	3.4 mL urea		
	25.0 g L^{-1}		
	10 mL glucosamine Hydrochloride		
	33.0 g L^{-1}		
Others			
145 mg α-amylase	1.0 g BSA	9 mL $CaCl_2{\cdot}2H_2O$ 22.2 g L^{-1}	10 mL $CaCl_2{\cdot}2H_2O$ 22.2 g $^{-1}$L
15 mg uric acid	1.0 g pepsin	1.0 g BSA	1.8 g BSA
50 mg mucin	3.0 g musin	3.0 g pancreatin 0.5 g lipase	6.0 g bile
pH			
6.5 ± 0.2	1.07 ± 0.07	7.8 ± 0.2	8.0 ± 0.2

The organic and inorganic solutions were adjusted into 500 mL with distilled water separately. Other constituents were added to the mixture of organic and inorganic solutions and the pH was adjusted to the appropriate intervals with 2 M NaOH or concentrated HCl.

All collected samples were centrifuged at 16 639*g* (Eppendorf 5804R, Hamburg, Germany) for 10 minutes at 0 °C. The collected supernatant was acidified to pH 2.0 with concentrated hydrochloric acid to inactivate the digestive enzymes and to stabilize the phenolic compounds.[5] Methanol was added to the supernatant (2 : 1, v/v) and it was chilled at −20 °C to precipitate proteins. After 30 minutes, proteins in the mixture were removed by centrifugation at 0 °C for 30 minutes (16 639*g*, Eppendorf 5804R). Samples were filtered through a 0.20 μm syringe filter (Corning Inc., Corning, NY, USA) where an aliquot of the filtered supernatant was stored at −80 °C for phenolic compound quantification and antioxidant capacity evaluation. The remaining filtered supernatant was evaporated under a vacuum (Eppendorf Vacufuge plus, Hamburg, Germany) to remove methanol. The aqueous supernatant was purified with a C18 cartridge (Sigma-Aldrich, St Louis, MO, USA) and washed with water to remove reducing sugars. The phenolic compounds in the supernatant were eluted with methanol. The purified sample was dried under a vacuum and resuspended in ultrapure water. The re-suspended samples were stored at −80 °C for enzyme inhibition activity analyses. Digestion was carried out in triplicate.

2.5. UPLC analysis of phenolic compounds

The profile of polyphenols would be altered during digestion, monitoring these changes is important to understand the possible metabolism of polyphenols in the digestive tract and the impacts of digestion on the potential bioactivity of polyphenols. In this study, the quantification of the anthocyanins and phenolic acids (chlorogenic acid and neo-chlorogenic acid) in the collected digesta were performed on an Ultra

Performance Liquid Chromatography (UPLC) (Agilent Technologies1290 Infinity, Santa Clara, CA, USA) with a Photodiode Array (PDA) detector. The method used was modified from Teleszko *et al.*[26] Separation was carried out using a C18 column (3 μm, 150 × 4.6 mm, Thermo Scientific, Waltham, MA, USA) at 25 °C. Samples were injected at a flow rate of 1.3 mL min^{-1}. Phenolic compounds were eluted with a gradient mobile phase consisting 4.5% formic acid in water (phase A) and 4.5% formic acid in acetonitrile (phase B). The gradient was as follows: 0 min: 1% phase B; 4.5 min: 10% phase B; 7 min: 20% phase B; 10 min: 24% phase B; 14 min: 36% phase B; 15 min: 60% phase B; 16 min: 1% phase B. The post run time was 5 min. Samples were spiked with quercetin (25 μg mL^{-1}) as an internal standard. External calibration curve was drawn using cyanidin-3-galactoside (3.9, 7.8, 15.6, 31.2, 62.5, 125 and 250 μg mL^{-1}, r^2 = 0.9987), chlorogenic acid (3.9, 7.8, 15.6, 31.2, 62.5, 125 and 250 μg mL^{-1}, r^2 = 0.9999) and neo-chlorogenic acid (3.9, 7.8, 15.6, 31.2, 62.5, 125 and 250 μg mL^{-1}, r^2 = 0.9998) standards. Anthocyanins were detected at 520 nm and expressed as cyanidin-3-galactoside equivalents. The individual anthocyanins were identified by the elution order reported by Jakobek L., *et al.*[11] Chlorogenic acid and neo-chlorogenic acid were detected at 320 nm. Peak areas were used for quantification and the results were expressed as mg polyphenols per part (one part contains 9 mL aronia kefir). Measurements were conducted in triplicate.

2.6. DPPH free radical scavenging assay

Antioxidant capacity of polyphenols is a crucial parameter to evaluate their potential health benefits. In the presented study, the antioxidant capacity of each digested fraction was evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity. The method was slightly modified from Duymus *et al.*[27] Briefly, equal amounts of 0.3 mM DPPH solution (150 μL) and the diluted samples (150 μL) were loaded to a 96-well plate. The mixture was incubated in the dark at room temperature for 30 minutes and the absorbance was read at 515 nm with a Biotek plate reader (ELx800, Winooski, VT, USA). A mixture of DPPH solution and water was used as the negative control for this assay. Scavenging percentage was calculated with the following formula:

$$\%\ \text{Scavenging} = \frac{\text{Abs}_{\text{Control}} - \text{Abs}_{\text{Sample}}}{\text{Abs}_{\text{Control}}} \times 100$$

Scavenging activity of each fraction was measured at five different concentrations to calculate IC_{50} values, which is the concentration of the sample to scavenge 50% of the DPPH free radicals. Measurements were conducted in triplicate.

2.7. Rat intestinal α-glucosidase inhibitory activities

Alpha-glucosidase is a vital carbohydrate-hydrolyzing enzyme in charge of breaking down disaccharides and oligosaccharides to release glucose in the small intestine.[28] In this study, α-glucosidase inhibitory activity was evaluated using a method reported by Oki *et al.* with modification.[29] α-Glucosidase was extracted from rat intestine powder by using 0.1 M sodium phosphate buffer at pH 6.9 (1 : 30, w/v) in an ice bath with sonication. Sonication was performed 12 times (30 seconds for each round) and the mixture was vortexed after each sonication. The mixture was centrifuged at 0 °C for 10 minutes at 16 639*g*. The supernatant was filtered through a 0.45 μm syringe filter (Phenomenex, Torrance, CA, USA) and kept on ice until use in the assay. Samples (50 μL) and α-glucosidase extract (100 μL) were mixed and incubated at 37 °C for 10 minutes in the dark. Water was used to prepare controls. The reaction was initiated by the addition of 50 μL 4-nitrophenyl α-glucopyranoside (pnp-G, 5 mM). The mixture was incubated at 37 °C for 30 minutes in the dark and read at 405 nm. Phosphate buffer (0.1 M) was used to prepare sample blank to correct for the background color. The inhibitory activity of the sample on intestinal α-glucosidase was calculated as follows:

$$\%\ \text{inhibition} = \frac{\text{Abs}_{\text{control}} - (\text{Abs}_{\text{sample}} - \text{Abs}_{\text{sample blank}})}{\text{Abs}_{\text{control}}} \times 100$$

Five dilutions of each sample were measured to calculate IC_{50} values. Measurements were carried out in triplicate.

2.8. Porcine pancreatic α-amylase inhibitory activities

Pancreatic α-amylase is a key enzyme that starts the digestion of complex carbohydrates by hydrolyzing the glycosidic linkages in the small intestine. Inhibitory effects of samples on porcine pancreatic α-amylase were conducted with the method reported by Nampoothiri *et al.* with modification.[30] Briefly, sodium phosphate buffer (0.02 M, pH 6.9) with 0.006 M sodium chloride was used to dissolve α-amylase and the starch. 100 μL sample and 100 μL α-amylase solution (100 unit mL^{-1}) were mixed and incubated at 25 °C for 10 min. The reaction was initiated by adding 100 μL starch solution (1 g mL^{-1}). The mixture was incubated at 25 °C for an additional 10 min. The reaction was stopped by adding 200 μL dinitrosalicylic acid reagent and incubating the mixture in a water bath for 5 minutes at 100 °C. The dinitrosalicylic acid reagent was made of 1 g mL^{-1} dinitrosalicylic acid in water containing 2% NaOH (2 M, v/v) and 30% (w/v) potassium sodium tartrate tetrahydrate. When the mixture temperature reached the room temperature (23 °C), 50 μL of the mixture was loaded to a 96-well microplate, diluted with 200 μL water and read at 540 nm. Sample blank was prepared using sodium phosphate buffer to correct for the background color. The control was prepared with sodium phosphate buffer. The α-amylase inhibitory activity of the samples was calculated as follows:

$$\%\ \text{inhibition} = \frac{\text{Abs}_{\text{control}} - (\text{Abs}_{\text{sample}} - \text{Abs}_{\text{sample blank}})}{\text{Abs}_{\text{control}}} \times 100$$

The inhibition activities of individual samples were tested at five different dilutions. IC_{20} values, the concentration of the sample to inhibit 20% porcine pancreatic α-amylase, were calculated. The measurements were conducted in triplicate.

2.9. Statistical analysis

Data are shown as means ± standard deviations. Statistical analyses were conducted using SAS Studio (Cary, NC, USA). Analysis of Variance and Tukey's HSD *post hoc* were carried out to evaluate the differences. A significance level was set at $\alpha = 0.05$.

3. Results

3.1. Quantification of bioaccessible phenolic compounds

The individual contents of the bioaccessible phenolic compounds in aronia kefir and non-fermented control during gastrointestinal digestion are presented in Table 2 and the total anthocyanin contents are shown in Fig. 1. Four major monomeric anthocyanins (cyanidin-3-galactoside, cyanidin-3-glucoside, cyanidin-3-arabinoside and cyanidin-3-xyloside) and two dominant phenolic acids (chlorogenic acid and neo-chlorogenic acid) were identified and quantified *via* UPLC analyses. Caffeic acid, a metabolite of chlorogenic acid, was not detected.

Most phenolic compounds in aronia kefir increased after gastric digestion, with the exception of cyanidin-3-glucoside which showed no change compared to the oral-digested sample. During intestinal digestion, the chlorogenic acid content was increased (from 1.04 ± 0.02 mg per part to 1.29 ± 0.09 mg per part) and the other identified anthocyanins and phenolic acids remained the same. After the entire gastrointestinal digestion, total bioaccessible anthocyanins were increased by 96.9% compared to the undigested aronia kefir. Total anthocyanins in intestinal-digested kefir was 5.09 ± 0.40 mg per part (1 part = 9 mL aronia kefir). One serving of

Table 2 Quantification of individual phenolic compounds in aronia kefir and non-fermented control

Fractions			Cyanidin-3-galactoside	Cyanidin3-glucoside	Cyanidin-3-arabinoside	Cyanidin-3-xyloside	Chlorogenic acid	Neo-chlorogenic acid
Aronia kefir	Undigested[b]		1.87 ± 0.11d	0.09 ± 0.01cd	0.57 ± 0.03e	0.05 ± 0.01e	0.61 ± 0.03g	0.49 ± 0.02e
	Oral	C[a]	1.87 ± 0.15d	0.07 ± 0.02d	0.55 ± 0.07e	0.05 ± 0.01e	0.76 ± 0.02efg	0.59 ± 0.02cde
		D[a]	1.96 ± 0.13d	0.07 ± 0.02d	0.58 ± 0.06e	0.06 ± 0.01e	0.78 ± 0.02efg	0.60 ± 0.02cde
	Gastric	C[a]	2.60 ± 0.34cd	0.10 ± 0.03bcd	0.85 ± 0.14de	0.08 ± 0.01de	0.92 ± 0.04cde	0.69 ± 0.02abc
		D[a]	3.14 ± 0.42c	0.12 ± 0.04abcd	1.13 ± 0.19cd	0.11 ± 0.02cd	1.04 ± 0.02bc	0.75 ± 0.02ab
	Intestinal	C[a]	3.10 ± 0.33c	0.11 ± 0.02abcd	1.08 ± 0.12cd	0.10 ± 0.01cd	1.17 ± 0.11ab	0.74 ± 0.03ab
		D[a]	3.50 ± 0.29bc	0.13 ± 0.01abcd	1.33 ± 0.10bc	0.13 ± 0.01bc	1.29 ± 0.09a	0.70 ± 0.09abc
Non-fermented control	Undigested[b]		2.60 ± 0.13cd	0.13 ± 0.01abcd	0.87 ± 0.06de	0.08 ± 0.01de	0.70 ± 0.04fg	0.54 ± 0.03de
	Oral	C[a]	3.10 ± 0.45c	0.12 ± 0.03abcd	1.06 ± 0.21cd	0.11 ± 0.02cd	0.84 ± 0.06def	0.65 ± 0.04bcd
		D[a]	3.29 ± 0.16bc	0.13 ± 0.02abcd	1.15 ± 0.07cd	0.11 ± 0.01cd	0.85 ± 0.05def	0.64 ± 0.04bcd
	Gastric	C[a]	4.13 ± 0.32ab	0.16 ± 0.04abc	1.56 ± 0.17ab	0.16 ± 0.02ab	0.97 ± 0.02cd	0.72 ± 0.02ab
		D[a]	4.59 ± 0.41a	0.18 ± 0.04a	1.79 ± 0.21a	0.18 ± 0.02a	1.08 ± 0.05bc	0.77 ± 0.03a
	Intestinal	C[a]	4.67 ± 0.29a	0.17 ± 0.02ab	1.85 ± 0.11a	0.19 ± 0.01a	1.19 ± 0.07ab	0.78 ± 0.01a
		D[a]	4.69 ± 0.36a	0.17 ± 0.03ab	1.88 ± 0.19a	0.19 ± 0.02a	1.31 ± 0.06a	0.72 ± 0.07ab

Data are shown as means ± standard deviations ($n = 3$), values in the same column with the same letter are not significantly different at $p < 0.05$. [a] C stands for control (lack of digestive enzymes) and D stands for digesta (with digestive enzymes). [b] undigested samples are samples before digestion; anthocyanins were expressed as mg cyanidin-3-galactoside per part; chlorogenic acid was expressed as mg chlorogenic per part; neo-chlorogenic acid was expressed as mg neo-chlorogenic per part.

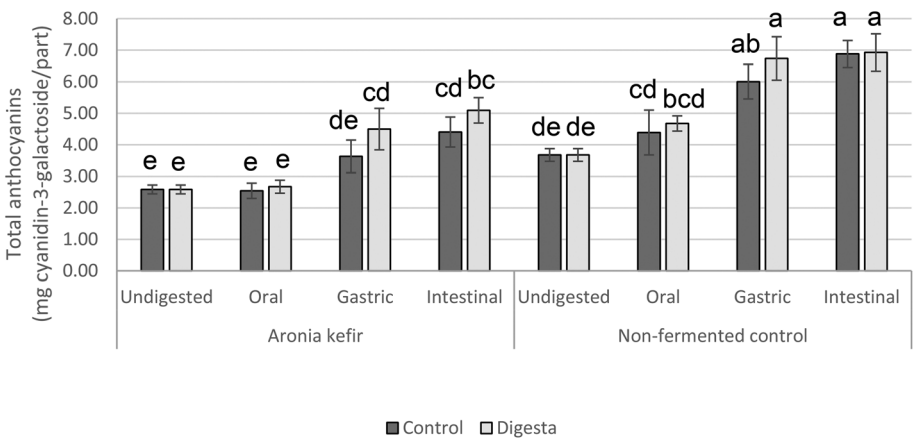

Fig. 1 Quantification of total anthocyanins in aronia kefir and non-fermented control, data are shown as means ± standard deviations ($n = 3$), bars with the same letter are not significantly different at $p < 0.05$.

commercial kefir is 240 mL thus one serving aronia kefir would provide 135.73 mg bioaccessible anthocyanins.

No difference was observed for phenolic compounds in the individual stages of digestion between aronia kefir and aronia kefir controls (Table 2 and Fig. 1). After intestinal digestion, the non-fermented control contained a larger amount of cyanidin-3-galactoside, cyanidin-3-arabinoside and cyanidin-3-xyloside (4.69 ± 0.36 mg per part, 1.88 ± 0.19 mg per part and 0.19 ± 0.02 mg per part) compared to aronia kefir (3.50 ± 0.29 mg per part, 1.33 ± 0.10 mg per part and 0.13 ± 0.01 mg per part respectively). There was no differences observed for chlorogenic acid and neo-chlorogenic acid between non-fermented control and aronia kefir. The total bioaccessible anthocyanins in intestinal-digested non-fermented control was 88.5% higher than in the undigested non-fermented control. After digestion, the increase of anthocyanins was lower in non-fermented control compared to the increase in aronia kefir.

3.2. Antioxidant capacity

Antioxidant activity of aronia kefir and non-fermented control was measured using the capacity to scavenge DPPH free radicals. IC_{50} values were calculated and the results are shown in Fig. 2. Aronia kefir exhibited antioxidant capacity during the entire gastrointestinal digestion. Antioxidant capacity of aronia kefir digesta was improved during gastric digestion (DPPH IC_{50} values from 24.07 ± 0.78 mg per part to 12.01 ± 0.57 mg per part) and held consistent after intestinal digestion (DPPH IC_{50}: 8.97 ± 0.93 mg per part). Aronia kefir digesta exhibited similar antioxidant capacity compared to the corresponding aronia kefir controls at each digestive stage. Aronia kefir and non-fermented control exhibited similar antioxidant capacity after gastric- and intestinal-digestion. A strong correlation between IC_{50} values of DPPH and total anthocyanins was observed (r = −0.89) as well as between IC_{50} values and the sum of chlorogenic and neo-chlorogenic acid contents (r = −0.90).

3.3. Inhibitory activity of carbohydrate-hydrolyzing enzymes

The inhibitory effects of intestinal digested aronia kefir and non-fermented control on α-glucosidase and pancreatic α-amylase were tested. The results are shown in Fig. 3 & 4.

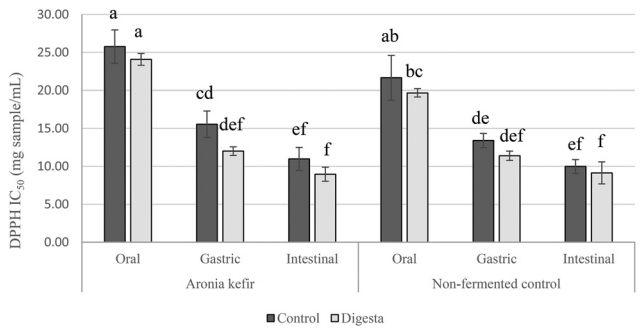

Fig. 2 Antioxidant capacity of aronia kefir and non-fermented control during digestion, data shown as means ± standard deviations (n = 3), bars with the same letter are not significantly different at p < 0.05.

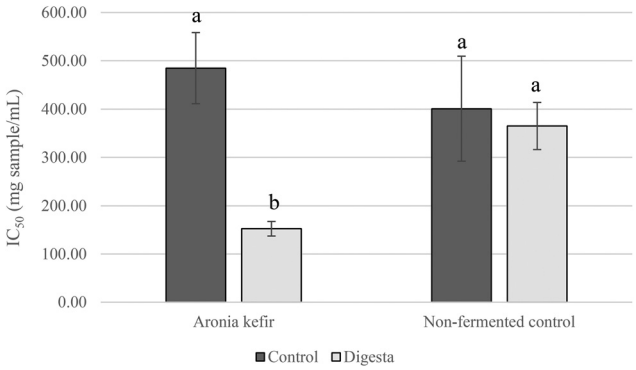

Fig. 3 α-Glucosidase inhibitory activity of intestinal-digested aronia kefir and non-fermented control, data shown as means ± standard deviations (n = 3), bars with the same letter are not significantly different at p < 0.05.

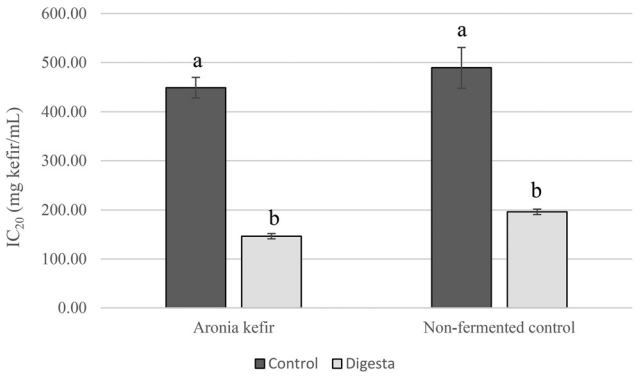

Fig. 4 Pancreatic α-amylase inhibitory activity of intestinal-digested aronia kefir and non-fermented control, data shown as means ± standard deviations (n = 3), bars with the same letter are not significantly different at p < 0.05.

Digested aronia kefir exhibited strong inhibitory activity toward α-glucosidase and weak inhibitory activity on α-amylase. Compared to the digested non-fermented control, digested aronia kefir had a stronger inhibitory effect on α-glucosidase. The IC_{50} values for α-glucosidase inhibition of aronia kefir and non-fermented controls were 152.53 ± 15.24 mg kefir per mL and 365.16 ± 48.84 mg non-fermented control per mL respectively. Digested aronia kefir as well as the digested non-fermented control exhibited similar inhibitory activity against pancreatic α-amylase. IC_{20} values of α-amylase for the aronia kefir and the non-fermented control were 146.52 ± 5.37 mg kefir per mL and 196.21 ± 5.50 mg non-fermented control per mL. Plain kefir was processed using the *in vitro* digestion system with the same method as the samples. Inhibitory activity of plain kefir on enzymes was not observed.

4. Discussion

This study examined the bioaccessibility and the antioxidant capacity of phenolic compounds in aronia kefir during a simu-

lated gastrointestinal digestion. The impacts of fermentation on aronia polyphenols and on their carbohydrate-hydrolyzing enzyme inhibitory activities were evaluated.

The *in vitro* digestion model used in this study simulated three compartments of the digestive tract: mouth, stomach and small intestine. Digestive juices (saliva, gastric juice, duodenal juice and bile) used in this model contained not only corresponding enzymes but also other compounds that exist in human digestive juices, such as calcium chloride which may chelate phenolic compounds in the digestive tract and alter their bioaccessibility.[31]

In this study, salivary α-amylase, which is the main digestive enzyme in the mouth, had negligible effects on the release of bioaccessible phenolic compounds in aronia kefir as expected, because the aronia kefir is a protein-rich beverage and the duration for the simulated oral digestion is short.

During gastric digestion, the acidic environment helps to stabilize the free anthocyanins and phenolic acids in aronia kefir. The low pH environment in the stomach contributes to the liberation of the phenolic compounds from the phenolic-protein complex and lead to the increase in bioaccessible anthocyanins and phenolic acids.[8,32,33] In addition, proanthocyanins, the oligomeric and/or polymeric flavan-3-ols, are the most abundant bioactive constituents in aronia. The depolymerization of proanthocyanins due to the acidic environment may contribute to the enhancement of the monomeric anthocyanin levels and potentially increase the bioavailability of aronia polyphenols.[34,35] Bermudezsoto *et al.* reported that digestive enzymes did not affect the aronia polyphenol content in the absence of food matrix.[5] In this study, a similar trend was observed where the digestive enzymes did not alter the amount of bioaccessible phenolic compounds in gastric-digested aronia kefir.

The small intestine is the major absorption site for most phenolic compounds so the quantity of bioaccessible polyphenols is important.[36] Many studies demonstrated that phenolic compounds are labile in the small intestine due to the mild alkaline environment. Bermudezsoto *et al.* conducted a study demonstrating that more than 35% of anthocyanins and 20% phenolic acids were lost after *in vitro* intestinal digestion of aronia juice.[5] Similar results were reported by Correa-Betanzo *et al.* where anthocyanins in blueberry decreased to 10%–15% during *in vitro* intestinal digestion.[37] Bouayed *et al.* reported a complete loss of anthocyanins but an increase in phenolic acids after *in vitro* intestinal digestion of apples.[38] However, depending on the type of polyphenols and the food matrix, the changes of bioaccessible polyphenols in the small intestine may be different. In the present study, the bioaccessible chlorogenic acid in aronia kefir increased and the anthocyanins content remained the same during intestinal digestion. The increases in chlorogenic acid may be attributed to degradation of anthocyanins in addition to liberation from the kefir matrix. Similar results were observed in other studies that utilized a protein-rich food matrix to protect the polyphenols from degradation in the small intestine. A study conducted by Lamothe *et al.* showed that the stability of tea polyphenol in the small intestine was improved by dairy matrices (milk, yogurt and cheese).[21] The protective effects of food matrices (dairy and egg) on the stability of grape anthocyanins during the intestinal digestion were observed by Pineda-Vadillo *et al.*[39] Stanisavljevic *et al.* reported that after *in vitro* digestion of aronia juice in a food matrix, bioaccessible anthocyanins and total phenolic compounds increased.[40] It is important to note that the referenced study only tested the anthocyanin and the total phenolic contents before and after the entire gastrointestinal digestion process (not at the individual digestive stage), the changes of the soluble anthocyanins in the small intestine remains unknown. There was no significant difference in anthocyanins, chlorogenic acid and neo-chlorogenic acid between the digested aronia kefir and the controls. Therefore, digestive enzymes and bile did not contribute to the liberation of phenolic compounds.

The antioxidant capacity of polyphenols is associated with their health-promoting properties. Consuming polyphenols helps to decrease oxidative stress, attenuate the production of pro-inflammatory biomarkers and may lower the risk of chronic diseases, such as type 2 diabetes.[41] Foods that have strong antioxidant capacity before consumption may lose their antioxidant activity during the digestion process. This is caused by the structural alterations that occur due to the harsh conditions in the digestive tract and/or the interaction with other food ingredients. A loss of antioxidant capacity of polyphenol-rich food after *in vitro* gastrointestinal digestion was documented in many studies and this loss was associated with the degradation of phenolic compounds.[37,42] In this study, the antioxidant capacity of intestinal-digested aronia kefir was higher than the oral-digested aronia kefir, the progressive release of phenolic compounds during digestion may contribute to the increase.[39] It is important for food to exhibit antioxidant capacity in the gut lumen, where dietary polyphenols could inhibit the proliferation of abnormal cells and the progression of cancer.[5] In addition, dietary polyphenols in the lumen may have protective effects on other food components during digestion, such as protecting unsaturated fatty acids from oxidation.[39,43] The protective activity of polyphenols on unsaturated fatty acids may contribute to a healthier cardiovascular status.

Alpha-glucosidase and pancreatic α-amylase are carbohydrate-hydrolyzing enzymes that play a vital role in breaking down complex carbohydrates. Inhibition of these enzymes can delay the absorption of carbohydrates and aid in the management of hyperglycemia and the progression of diabetic complications. In the present study, only intestinal-digested samples were tested for enzyme inhibitory activity because pancreatic α-amylase and α-glucosidase exist in the small intestine. It is important to note that yeast α-glucosidase was frequently used in other research, but this study used α-glucosidase extracted from rat small intestinal powder because mammalian α-glucosidase is more relevant to human α-glucosidase.[44] This study demonstrated that polyphenols in aronia were the major compounds affecting the enzyme inhibitory activity, because plain kefir treated in the same method did not show any

inhibitory activity (data not shown). The inhibitory effects of dietary polyphenols on pancreatic α-amylase and α-glucosidase are well documented.[45] In this study, intestinal-digested aronia kefir exhibited strong inhibitory activity on α-glucosidase and minor inhibitory effect on pancreatic α-amylase. Strong inhibition of pancreatic α-amylase may lead to undigested complex carbohydrates in the large intestine and cause abdominal pain, flatulence, and/or diarrhea.[46] Therapeutic agents, such as acarbose, can cause gastrointestinal side effects because of their non-specific inhibitory effects on both pancreatic α-amylase and α-glucosidase. Due to this effect, the specific inhibitory activity of aronia kefir on α-glucosidase over pancreatic α-amylase might be desirable for hyperglycemia management.[47,48] Incorporating aronia kefir into a normal diet may be a good strategy to control postprandial plasma glucose level without causing side effects.

Fermentation altered the composition of bioaccessible polyphenols in aronia kefir. Before digestion, a lower level of total anthocyanins was observed in aronia kefir than for the non-fermented control. The difference was not significant but a trend was observed. After oral-, gastric- and intestinal-digestion, aronia kefir contained significantly less anthocyanins than the non-fermented control. These results show that fermentation decreased the content of anthocyanins in aronia kefir. There was no difference in the levels of chlorogenic acid and neochlorogenic acid between aronia kefir and the non-fermented control. This demonstrates that fermentation did not alter the amount of these two phenolic acids. In addition, new peaks were observed in the chromatogram (at 320 nm and 280 nm) of aronia kefir compared to non-fermented control before and at individual stages of digestion (data not shown). This observation indicates that fermentation produced metabolites. The breakdown of anthocyanins may be a source of the phenolic metabolites.[49] Metabolites may be easier to absorb and more bioavailable compared to the parent compounds due to their smaller size.[9,50] The identification of the metabolites is needed to better understand the impacts of fermentation.

Fermentation did not affect the antioxidant capacity of aronia polyphenols even though it decreased the levels of total anthocyanins. These results indicate that fermentation may produce metabolites that exhibit higher antioxidant capacity than the parent compounds. The ability of fermentation to increase antioxidant capacity of phenolic-rich food was observed in other studies. A study conducted by Curiel *et al.* observed that fermentation by lactic acid bacteria increased the antioxidant capacity of Myrtle berry homogenate.[51] In addition, Hunaefi *et al.* stated that 24 hours lactic acid fermentation decreased the total phenolic compounds in red cabbage sprouts but increased the antioxidant activity.[52] Zhao *et al.* also reported that fermentation by lactic acid bacteria decreased the flavan-3-ols content and increased phenolic acid derivatives in tea extract.[22] There was also evidence that the antioxidant activity was elevated.[22] These results demonstrate that fermentation may be a feasible method to enhance the antioxidant capacity of dietary polyphenols in different food matrices.

Fermentation increased the α-glucosidase inhibitory activity of aronia polyphenols. This is concluded based on the lower IC_{50} value of aronia kefir (152.53 mg kefir per mL) than for the non-fermented control (365.16 mg non-fermented control per mL). The stronger carbohydrase inhibitory effects of digested aronia kefir may due to the metabolites of polyphenols generated by the fermentation. Frediansyah *et al.* observed similar results where fermentation by lactic acid bacteria increased the inhibitory activity of black grape juice for α-amylase and α-glucosidase.[53] Fermentation may be an effective strategy to increase the carbohydrase inhibitory activity of dietary polyphenols.

The carbohydrase inhibitory activity and antioxidant capacity are more relevant criteria to utilize for understanding the potential benefits of aronia polyphenols. In this study, reduced levels of anthocyanins (cyanidin-3-galactoside, cyanidin-3-arabinoside and cyanidin-3-xyloside) were observed in aronia kefir compared to the non-fermented control, but fermentation increased the α-glucosidase inhibitory activity of aronia polyphenols without altering the antioxidant capacity. Fermentation is a good strategy to increase the potential bioactivity of aronia polyphenols to facilitate postprandial blood glucose control. Thus, fermentation may be an effective method to enhance the health-beneficial properties of aronia kefir as a functional food. More research is needed to better understand the potential activity of fermentation on improving the bioavailability of dietary polyphenols.

5. Conclusion

In this study, the stability and bioaccessibility of the polyphenols in aronia kefir were evaluated using an *in vitro* gastrointestinal digestion model, where the impacts of fermentation on aronia polyphenols were evaluated. After digestion, the bioaccessible polyphenols in aronia kefir and its antioxidant capacity increased. The digested aronia kefir exhibited strong inhibitory activity toward α-glucosidase but weak inhibition of pancreatic α-amylase. Intestinal-digested aronia kefir contained less cyanidin-3-galactoside, cyanidin-3-arabinoside and cyanidin-3-xyloside compared to intestinal-digested non-fermented control but exhibited similar antioxidant capacity. Fermentation enhanced the inhibitory activity of aronia polyphenols on α-glucosidase. In conclusion, consuming aronia kefir may aid in controlling blood glucose level without side effects. Fermentation may be a good strategy to enhance the bioavailability of dietary polyphenols. In order to better understand the positive impacts of fermentation on the bioavailability of dietary polyphenols, the identification of the metabolites in aronia kefir is necessary.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

1 N. H. Cho, Q&A: Five questions on the 2015 IDF Diabetes Atlas, *Diabetes Res. Clin. Pract.*, 2016, **115**, 157–159.
2 A. Trikkalinou, A. K. Papazafiropoulou and A. Melidonis, Type 2 diabetes and quality of life, *World J. Diabetes*, 2017, **8**, 120–129.
3 A. Tanveer, K. Akram, U. Farooq, Z. Hayat and A. Shafi, Management of diabetic complications through fruit flavonoids as a natural remedy, *Crit. Rev. Food Sci. Nutr.*, 2017, **57**, 1411–1422.
4 Q. Liu, L. Chen, L. Hu, Y. Guo and X. Shen, Small molecules from natural sources, targeting signaling pathways in diabetes, *Biochim. Biophys. Acta*, 2010, **1799**, 854–865.
5 M. Bermudezsoto, F. Tomasbarberan and M. Garciaconesa, Stability of polyphenols in chokeberry (Aronia melanocarpa) subjected to in vitro gastric and pancreatic digestion, *Food Chem.*, 2007, **102**, 865–874.
6 C. Manach, A. Scalbert, C. Morand, C. Remesy and L. Jimenez, Polyphenols: food sources and bioavailability, *Am. J. Clin. Nutr.*, 2004, **79**, 727–747.
7 K. Kulandaivelu and A. K. A. Mandal, Improved bioavailability and pharmacokinetics of tea polyphenols by encapsulation into gelatin nanoparticles, *IET Nanobiotechnol.*, 2017, **11**, 469–476.
8 J. A. Dominguez-Avila, A. Wall-Medrano, G. R. Velderrain-Rodriguez, C. O. Chen, N. J. Salazar-Lopez, M. Robles-Sanchez and G. A. Gonzalez-Aguilar, Gastrointestinal interactions, absorption, splanchnic metabolism and pharmacokinetics of orally ingested phenolic compounds, *Food Funct.*, 2017, **8**, 15–38.
9 J. Z. Wang, G. G. Yousef, R. B. Rogers, E. G. de Mejia, I. Raskin and M. A. Lila, in *Emerging Trends in Dietary Components for Preventing and Combating Disease*, ed. B. S. Patil, G. K. Jayaprakasha, K. N. C. Murthy and N. P. Seeram, 2012, vol. 1093, pp. 95–116.
10 S. Borowska and M. M. Brzóska, Chokeberries (Aronia melanocarpa) and their products as a possible means for the prevention and treatment of noncommunicable diseases and unfavorable health effects due to exposure to xenobiotics, *Compr. Rev. Food Sci. Food Saf.*, 2016, **15**, 982–1017.
11 L. Jakobek, M. Drenjancevic, V. Jukic and M. Seruga, Phenolic acids, flavonols, anthocyanins and antiradical activity of "Nero", "Viking", "Galicianka" and wild chokeberries, *Sci. Hortic.*, 2012, **147**, 56–63.
12 L. Jakobek, M. Seruga, I. Novak and M. Medvidovic-Kosanovic, Flavonols, phenolic acids and antioxidant activity of some red fruits, *Dtsch. Lebensm.-Rundsch.*, 2007, **103**, 369–378.
13 D. Li, B. Li, Y. Ma, X. Sun, Y. Lin and X. Meng, Polyphenols, anthocyanins, and flavonoids contents and the antioxidant capacity of various cultivars of highbush and half-high blueberries, *J. Food Compos. Anal.*, 2017, **62**, 84–93.
14 A. Kokotkiewicz, Z. Jaremicz and M. Luczkiewicz, Aronia plants: A review of traditional use, biological activities, and perspectives for modern medicine, *J. Med. Food*, 2010, **13**, 255–269.
15 S. Park, J. I. Kim, I. Lee, S. Lee, M. W. Hwang, J. Y. Bae, J. Heo, D. Kim, S. Z. Han and M. S. Park, Aronia melanocarpa and its components demonstrate antiviral activity against influenza viruses, *Biochem. Biophys. Res. Commun.*, 2013, **440**, 14–19.
16 B. M. Loo, I. Erlund, R. Koli, P. Puukka, J. Hellstrom, K. Wahala, P. Mattila and A. Jula, Consumption of chokeberry (Aronia mitschurinii) products modestly lowered blood pressure and reduced low-grade inflammation in patients with mildly elevated blood pressure, *Nutr. Res.*, 2016, **36**, 1222–1230.
17 M. Broncel, M. Kozirog, P. Duchnowicz, M. Koter-Michalak, J. Sikora and J. Chojnowska-Jezierska, Aronia melanocarpa extract reduces blood pressure, serum endothelin, lipid, and oxidative stress marker levels in patients with metabolic syndrome, *Med. Sci. Monit.*, 2010, **16**, CR28–CR34.
18 Z. B. Guzel-Seydim, T. Kok-Tas, A. K. Greene and A. C. Seydim, Review: functional properties of kefir, *Crit. Rev. Food Sci. Nutr.*, 2011, **51**, 261–268.
19 Z. Ahmed, Y. Wang, A. Ahmad, S. T. Khan, M. Nisa, H. Ahmad and A. Afreen, Kefir and health: a contemporary perspective, *Crit. Rev. Food Sci. Nutr.*, 2013, **53**, 422–434.
20 P. Jauregi, J. B. Olatujoye, I. Cabezudo, R. A. Frazier and M. H. Gordon, Astringency reduction in red wine by whey proteins, *Food Chem.*, 2016, **199**, 547–555.
21 S. Lamothe, N. Azimy, L. Bazinet, C. Couillard and M. Britten, Interaction of green tea polyphenols with dairy matrices in a simulated gastrointestinal environment, *Food Funct.*, 2014, **5**, 2621–2631.
22 D. Zhao and N. P. Shah, Lactic acid bacterial fermentation modified phenolic composition in tea extracts and enhanced their antioxidant activity and cellular uptake of phenolic compounds following in vitro digestion, *J. Funct. Foods*, 2016, **20**, 182–194.
23 X. Du and A. D. Myracle, Development and evaluation of kefir products made with aronia or elderberry juice: Sensory and Phytochemical characteristics, *Int. Food Res. J.*, in press.
24 M. J. Cires, X. Wong, C. Carrasco-Pozo and M. Gotteland, The Gastrointestinal Tract as a Key Target Organ for the Health-Promoting Effects of Dietary Proanthocyanidins, *Front. Nutr.*, 2016, **3**, 57.
25 A. G. Oomen, C. J. Rompelberg, M. A. Bruil, C. J. Dobbe, D. P. Pereboom and A. J. Sips, Development of an in vitro digestion model for estimating the bioaccessibility of soil contaminants, *Arch. Environ. Contam. Toxicol.*, 2003, **44**, 281–287.

26 M. Teleszko and A. Wojdyło, Comparison of phenolic compounds and antioxidant potential between selected edible fruits and their leaves, *J. Funct. Foods*, 2015, **14**, 736–746.
27 H. G. Duymus, F. Goger and K. H. Baser, In vitro antioxidant properties and anthocyanin compositions of elderberry extracts, *Food Chem.*, 2014, **155**, 112–119.
28 V. Vadivel, C. N. Kunyanga and H. K. Biesalski, Antioxidant Potential and Type II Diabetes-Related Enzyme Inhibition of Cassia obtusifolia L.: Effect of Indigenous Processing Methods, *Food Bioprocess Technol.*, 2011, **5**, 2687–2696.
29 T. Oki, T. Matsui and Y. Osajima, Inhibitory effect of alpha-glucosidase inhibitors varies according to its origin, *J. Agric. Food Chem.*, 1999, **47**, 550–553.
30 S. V. Nampoothiri, A. Prathapan, O. L. Cherian, K. G. Raghu, V. V. Venugopalan and A. Sundaresan, In vitro antioxidant and inhibitory potential of Terminalia bellerica and Emblica officinalis fruits against LDL oxidation and key enzymes linked to type 2 diabetes, *Food Chem. Toxicol.*, 2011, **49**, 125–131.
31 S. C. Zivanovic, R. S. Nikolic and G. M. Nikolic, The influence of Mg(II) and Ca(II) ions on rutin autoxidation in weakly alkaline aqueous solutions, *Acta Fac. Med. Naissensis*, 2016, **33**, 163–171.
32 M. Friedman and H. S. Jurgens, Effect of pH on the stability of plant phenolic compounds, *J. Agric. Food Chem.*, 2000, **48**, 2101–2110.
33 J. Fang, Bioavailability of anthocyanins, *Drug Metab. Rev.*, 2014, **46**, 508–520.
34 M. J. Kruger, N. Davies, K. H. Myburgh and S. Lecour, Proanthocyanidins, anthocyanins and cardiovascular diseases, *Food Res. Int.*, 2014, **59**, 41–52.
35 K. Ou and L. Gu, Absorption and metabolism of proanthocyanidins, *J. Funct. Foods*, 2014, **7**, 43–53.
36 M. A. Lila, B. Burton-Freeman, M. Grace and W. Kalt, Unraveling Anthocyanin Bioavailability for Human Health, *Annu. Rev. Food Sci. Technol.*, 2016, **7**, 375–393.
37 J. Correa-Betanzo, E. Allen-Vercoe, J. McDonald, K. Schroeter, M. Corredig and G. Paliyath, Stability and biological activity of wild blueberry (Vaccinium angustifolium) polyphenols during simulated in vitro gastrointestinal digestion, *Food Chem.*, 2014, **165**, 522–531.
38 J. Bouayed, L. Hoffmann and T. Bohn, Total phenolics, flavonoids, anthocyanins and antioxidant activity following simulated gastro-intestinal digestion and dialysis of apple varieties: Bioaccessibility and potential uptake, *Food Chem.*, 2011, **128**, 14–21.
39 C. Pineda-Vadillo, F. Nau, C. G. Dubiard, V. Cheynier, E. Meudec, M. Sanz-Buenhombre, A. Guadarrama, T. Tóth, É. Csavajda, H. Hingyi, S. Karakaya, J. Sibakov, F. Capozzi, A. Bordoni and D. Dupont, In vitro digestion of dairy and egg products enriched with grape extracts: Effect of the food matrix on polyphenol bioaccessibility and antioxidant activity, *Food Res. Int.*, 2016, **88**, 284–292.
40 N. Stanisavljevic, J. Samardzic, T. Jankovic, K. Savikin, M. Mojsin, V. Topalovic and M. Stevanovic, Antioxidant and antiproliferative activity of chokeberry juice phenolics during in vitro simulated digestion in the presence of food matrix, *Food Chem.*, 2015, **175**, 516–522.
41 S. V. Joseph, I. Edirisinghe and B. M. Burton-Freeman, Berries: anti-inflammatory effects in humans, *J. Agric. Food Chem.*, 2014, **62**, 3886–3903.
42 L. Gayoso, A.-S. Claerbout, M. I. Calvo, R. Y. Cavero, I. Astiasarán and D. Ansorena, Bioaccessibility of rutin, caffeic acid and rosmarinic acid: Influence of the in vitro gastrointestinal digestion models, *J. Funct. Foods*, 2016, **26**, 428–438.
43 J. Kanner and T. Lapidot, The stomach as a bioreactor: Dietary lipid peroxidation in the gastric fluid and the effects of plant-derived antioxidants, *Free Radicals Biol. Med.*, 2001, **31**, 1388–1395.
44 J. Oh, S. H. Jo, J. S. Kim, K. S. Ha, J. Y. Lee, H. Y. Choi, S. Y. Yu, Y. I. Kwon and Y. C. Kim, Selected tea and tea pomace extracts inhibit intestinal alpha-glucosidase activity in vitro and postprandial hyperglycemia in vivo, *Int. J. Mol. Sci.*, 2015, **16**, 8811–8825.
45 A. Gökbulut, N. Orhan and D. Deliorman Orhan, Phenolic compounds characterization, carbohydrate digestive enzyme inhibitory and antioxidant activities of Hieracium pannosum Boiss, *S. Afr. J. Bot.*, 2017, **108**, 387–392.
46 S. Adisakwattana, T. Ruengsamran, P. Kampa and W. Sompong, In vitro inhibitory effects of plant-based foods and their combinations on intestinal alpha-glucosidase and pancreatic alpha-amylase, *BMC Complementary Altern. Med.*, 2012, **12**, 110.
47 Y. Tan, S. K. Chang and Y. Zhang, Comparison of alpha-amylase, alpha-glucosidase and lipase inhibitory activity of the phenolic substances in two black legumes of different genera, *Food Chem.*, 2017, **214**, 259–268.
48 S. Hogan, L. Zhang, J. R. Li, S. Sun, C. Canning and K. Q. Zhou, Antioxidant rich grape pomace extract suppresses postprandial hyperglycemia in diabetic mice by specifically inhibiting alpha-glucosidase, *Nutr. Metab.*, 2010, **7**, 71.
49 D. Sun-Waterhouse, J. Zhou and S. S. Wadhwa, Drinking yoghurts with berry polyphenols added before and after fermentation, *Food Control*, 2013, **32**, 450–460.
50 P. Filannino, Y. Bai, R. Di Cagno, M. Gobbetti and M. G. Ganzle, Metabolism of phenolic compounds by Lactobacillus spp. during fermentation of cherry juice and broccoli puree, *Food Microbiol.*, 2015, **46**, 272–279.
51 J. A. Curiel, D. Pinto, B. Marzani, P. Filannino, G. A. Farris, M. Gobbetti and C. G. Rizzello, Lactic acid fermentation as a tool to enhance the antioxidant properties of Myrtus communis berries, *Microb. Cell Fact.*, 2015, **14**, 67.
52 D. Hunaefi, N. Gruda, H. Riedel, D. N. Akumo, N. M. M. T. Saw and I. Smetanska, Improvement of antioxidant activities in red cabbage sprouts by lactic acid bacterial fermentation, *Food Biotechnol.*, 2013, **27**, 279–302.
53 A. Frediansyah, R. Nurhayati and F. Romadhoni, Enhancement of antioxidant activity, α-glucosidase and α-amylase inhibitory activities by spontaneous and bacterial monoculture fermentation of Indonesian black grape juices, *AIP Conf. Proc.*, 2017, **1803**, 020022.