Original Communication

Inhibitory effects of bioaccessible anthocyanins and procyanidins from apple, red grape, cinnamon on α-amylase, α-glucosidase and lipase

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Abstract: The goals of this study were to determine and evaluate the bioaccessibility of total anthocyanin and procyanidin in apple (Amasya, *Malus communis*), red grape (Papazkarası, *Vitis vinifera*) and cinnamon (Cassia, *Cinnamomum*) using an *in vitro* static digestion system based on human gastrointestinal physiologically relevant conditions. Also, *in vitro* inhibitory effects of these foods on lipid (lipase) and carbohydrate digestive enzymes (α-amylase and α-glucosidase) were performed with before and after digested samples using acarbose and methylumbelliferyl oleate (4MUO) as the positive control. While the highest total anthocyanin content was found in red grape (164 ± 2.51 mg/100 g), the highest procyanidin content was found in cinnamon (6432 ± 177.31 mg/100 g) ($p < 0.05$). The anthocyanin bioaccessibilities were found as 10.2 ± 1%, 8.23 ± 0.64%, and 8.73 ± 0.70% in apple, red grape, and cinnamon, respectively. The procyanidin bioaccessibilities of apple, red grape, and cinnamon were found as 17.57 ± 0.71%, 14.08 ± 0.74% and 18.75 ± 1.49%, respectively. The analyzed apple, red grape and cinnamon showed the inhibitory activity against α-glucosidase (IC_{50} 544 ± 21.94, 445 ± 15.67, 1592 ± 17.58 μg/mL, respectively), α-amylase (IC_{50} 38.4 ± 7.26, 56.1 ± 3.60, 3.54 ± 0.86 μg/mL, respectively), and lipase (IC_{50} 52.7 ± 2.05, 581 ± 54.14, 49.6 ± 2.72 μg/mL), respectively. According to our results apple, red grape and cinnamon have potential to inhibit of lipase, α-amylase and α-glucosidase digestive enzymes.

Keywords: Anthocyanin, procyanidin, bioaccessibility, α-amylase, α-glucosidase, lipase

Introduction

During the last years various *in vitro* and clinical studies have focused on the characterization and utilization of bioactive compounds in foods and their beneficial properties for human health. Also, the World Health Organization (WHO) emphasizes the importance of the bioactive compounds, especially from small colorful fruits for the prevention of the most important health problems namely cardiovascular diseases, diabetes, cancer, and obesity. Properties supporting the health benefits of bioactive compounds are preventing oxidation of cellular lipids, impacting cell cycle, inhibiting oxidant enzymes, inducing endogenous antioxidant enzymes, modulation of signal transduction, scavenging free radicals and intercellular communication. The last decade, consumer interest in the field of food production have changed thus, foods are no more intended to only satisfy hunger and to provide the necessary nutrients, but also to decrease of the risk of diet-related diseases. To day, the food industry has been facing new responsibilities to pay attention in the manufacturing and processing of food products. In recent years the potential of bioactive compounds to contribute to better nutrition through ingestion with functional foods has been widely discussed in the scientific community.

Obesity in recent years is one of the main underlying causes of many chronic, degenerative diseases and some cancers. Antiobesity drugs have disadvantageous due to their side effects. Therefore, there is a need for the exploration of the potential of natural products for the treatment of obesity. Inhibiting pancreatic lipase, α-glucosidase and amylase activities and/or delaying lipid and carbohydrate absorptions during digestion can be used as a strategy to prevent obesity. In addition to obesity, inhibition of *α*-glucosidase activity and carbohydrate absorption play an important role in the prevention and treatment of diabetes [1, 2]. There are varieties of natural sources, which consist of numerous bioactive components and can induce weight loss and prevent diet-induced obesity [3–5]. Polyphenols, terpens, saponins, and conjugated linoleic acids are some of the examples of bioactive compounds found in nature and have anti-obesity activity [4]. Especially, anthocyanins are the largest group of water-soluble pigments in the plant kingdom and belong to the family of

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compounds known as flavonoids [6]. These natural colorants are plant-derived flavonoids and found that in fruits and vegetables in a wide range of red, blue, purple and orange colors. They have an aromatic ring (A) bound to heterocyclic oxygen containing ring (C) and a third aromatic ring (B). The main differences among found in the fruits and vegetables anthocyanins (cyaniding, delphinidin, malvidin, pelargonidin, peonidin, petunidin) are the number and combination of hydroxyl and methoxyl groups on anthocyanins, the nature and number of sugar moieties, and the organic acids acylation on the sugar moieties [7]. Another bioactive compound, proanthocyanidins are condensed tannins; consist of monomeric units of flavans linked through carbon-carbon and ether linkages. Proanthocyanidins are the second most abundant group of natural phenolics after lignins and can be subdivided into at least fifteen subgroups based on their hydroxylation patterns on the A and the B-ring of the monomeric flavan-3-ol. Procyanidin is the most abundant subgroup present in foods and is a homogeneous group exclusively consisting of (epi)catechin units [8].

These bioactives have been associated with several health benefits including protect against neurological diseases, retard age-related decline in brain and cognitive functions, reduced risks of cardiovascular disease, diabetes, cancer and inflammation, modulated the gut microbial composition [9]. The inhibition of digestive enzymes including lipase and α-amylase by naturally occurring polyphenols in *in vitro* studies has been studied [10, 11]. Moreover, it has been reported that decrease in the blood levels of glucose, triglycerides, and LDL cholesterol, increase in energy expenditure and fat oxidation, and reduction in the body weight and adiposity were achieved by the action of polyphenol extracts [12].

The main target of this study was to determine the total anthocyanin and procyanidin contents in apple, red grape and cinnamon and their *in vitro* bioaccessibilities, and inhibitory effects on lipid and carbohydrate digestive enzymes (lipase, α-amylase and α-glucosidase) during *in vitro* digestion. We used standardized static *in vitro* digestion method, which is recently published by the COST FA1005 Action INFOGEST, an international network joined by more than 200 scientists from 32 countries working in the field of digestion, also we were participants of this action.

Materials and methods

Chemicals

4-nitrophenyl α-D-glucopyranoside (PNPG) (N0877), 4-methylumbelliferyl oleate (4MUO) (75164), potassium chloride (746436), α-amylase (A1031), pepsin (P7012), pancreatin (P1750), lipase (L3126), Pefabloc® SC (76307) and 4-(Dimethylamino)cinnamaldehyde (DMAC) (D4506) were purchased from Sigma (St. Louis, MO, US). All reagents were of analytical grade.

Samples

Cinnamon bark (Cassia, *Cinnamomum*) was purchased from local seller and ground. Apple (Amasya, *Malus communis*) and red grape (Papazkarası, *Vitis vinifera*) at commercial maturity stage, cultivated in Aegean District (Turkey) were purchased at different times from three different local markets in Izmir between September and November in 2016. The fruits were washed in cold water (4 °C) and after the apple kernels were separated from hulls, all samples were kept at 4 °C until use.

Total anthocyanin content

Total anthocyanin content assay was measured by the pH differential method [13, 14]. The assay was performed using the characteristic of the anthocyanins to change intensity of hue depending on pH shifting. Two buffer systems, potassium chloride buffer (pH 1.0, 0.025 M) and sodium acetate buffer (pH 4.5, 0.4 M) were used. Briefly, 0.4 ml sample was mixed with 3.6 ml of corresponding buffers and the corresponding maximum absorbance for both solutions was measured (at λ = 510 nm and λ = 700 nm). The results were expressed as mg of cyanidin-3-glucoside/100 g or mg of cyanidin-3-glucoside/100 ml.

Total monomeric anthocyanin absorbance (A) was calculated as

$$A = (A_{510} - A_{700})_{pH1.0} - (A_{510} - A_{700})_{pH4.5}$$

Total anthocyanin absorbance (A) was calculated as

$$A = (A_{510} - A_{700})_{pH1.0}$$

Total anthocyanin content of samples was calculated by following equation:

$$AC\,(mg/L) = (A)\,(MW)\,(DF)\,1000/(\varepsilon)\,\iota$$

where A: absorbance; MW: molecular weight (449.2 Da); DF: dilution factor; ε: molar absorptivity of cyanidin-3-glucoside (26,900); ι = thickness of spectrophotometric cuvette.

Total procyanidin content

Total procyanidins were measured using the 4-dimethylaminocinnamaldehyde (DMAC) assay [15]. One gram of the samples was placed in a 15 mL disposable

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centrifuge tube. 7 mL of 70:29.5:0.5 Acetone/Water/Acetic Acid was added to the centrifuge tube, then vortexed for 2 min to facilitate dispersion before homogenization in an Ultra Sonic Homogenizer for 5 min. The centrifuge tubes were centrifuged at 3250 g for 5 min. After the extraction, cuvettes were prepared with 20 μL of sample/standard, 2380 mL of methanol, and 100 μL of the prepared DMAC reagent (1:1 (v/v) 6 N H_2SO_4 and 2% DMAC (w/v) in methanol). Cuvettes were allowed to equilibrate for 15 min when the absorbance of the colored complex was measured at 640 nm. Total procyanidins were calculated as catechin equivalents based on a catechin standard curve ($y = 0.0752x + 0.0465$, $R^2 = 0.9923$).

In vitro digestion

Samples were subjected to *in vitro* static digestion according to the procedure recently described by Minekus et al. [16]. In this consensus protocol, within the COST FA1005 INFOGEST Network, the practical static digestion method is based on human gastrointestinal physiologically relevant conditions. They concluded that this protocol should be tested by different research groups for a variety of application on different food samples [17]. Pepsin (2000 U/mL) enzyme used for gastric phase. Trypsin (100 U/mL), pancreatic lipase (2000 U/mL) and pancreatic amylase (200 U/mL) enzymes were used for intestinal phase. Simulated digestion fluids were prepared according to the Minekus et al. [16]. The electrolyte stock solutions were 1.25 × concentrated i.e. 4 parts of electrolyte stock solution + 1 part of water gives the correct ionic composition in the simulated digestion fluids. The volumes of Simulated Salivary Fluid (SSF), Simulated Gastric Fluid (SGF) and Simulated Intestinal Fluid (SIF) were calculated for a final volume of 500 mL for each simulated fluid. Digestion procedure was applied as oral phase (final ratio of food to SSF of 50:50, w/v), gastric phase (final ratio of food to SGF of 50:50, v/v) and intestinal phase (final ratio of gastric chyme to SIF of 50:50, v/v). Anthocyanin or procyanidin bioaccesibilities were calculated as percentage of anthocyanin or procyanidin content of *in vitro* digested sample (S) and anthocyanin (AC) or procyanidin (PC) content of sample (C).

$$\%\,\text{Bioaccesibility} = (S/C) \times 100$$

Enzyme inhibition assays

Enzyme inhibition assays were performed before and after *in vitro* digestion of samples. Percentage of enzyme inhibition was calculated with the following equation.

$$\text{Inhibition}\,(\%) = 100 \times [(A_{\text{control}} - A_{\text{controlblank}}) - (A_{\text{sample}} - A_{\text{sampleblank}})/(A_{\text{control}} - A_{\text{controlblank}})]$$

where A_{control}, $A_{\text{controlblank}}$, A_{sample} and $A_{\text{sampleblank}}$ refer to the absorbance value of reaction vial containing active enzyme and buffer, inactive enzyme and buffer, active enzyme and inhibitor and inactive enzyme and inhibitor respectively. Substrate was present in all these vials. The curve of percentage inhibition against inhibitor concentration was plotted. IC_{50} (concentration of inhibitor required to produce a 50% inhibition of the initial rate of reaction) of each inhibitor was determined by interpolation from the curve. Also, the enzyme inhibition assays were performed for the samples at the concentration of 1 mg/mL after *in vitro* stomach digestion to observe the effect of samples on α-glycosidase, α-amylase and lipase enzymes during intestinal phase. The inhibition percentages of catechin as a standard were determined and compared with food samples. The enzyme inhibitions of standard catechin were determined at the concentration of 0.86, 1.47 and 64.32 μg/mL, and a curve of percentage inhibition against catechin concentration (μM) was plotted. The obtained curves for samples (Inhibition against concentration) and the curves for catechin (Inhibition against concentration in μM) were fitted to exponential equations. Then, the slope of the equations, which is equal to derivative of the equation at 50% inhibition of each inhibitor were used to find catechin equivalent inhibition capacity ($CEIC_{50}$, mM/g) as shown in the below equation.

$$\text{CEIC} = \text{Slope sample}/(\text{Slope catechin} \times \text{volume of sample})$$

α-Glucosidase inhibition assay

α-Glucosidase inhibition was measured using a spectrophotometric method from Koh et al. [18] with some modifications. Food samples and their digested fractions were mixed with 10 mL of phosphate buffer and centrifuged at 5000 g for 5 min. Reaction substrate 4-nitrophenyl α-D-glucopyranoside (PNPG) (30 mM) and α-glucosidase (AGH) (25 mg/mL) solutions were prepared in phosphate buffer saline (PBS), after vortexing for 10 min, the AGH mixture was centrifuged at 4 °C 10000 g for 30 min. Briefly, 340 μL of inhibitors of different concentrations were pipetted into separate reaction vials and 20 μL of AGH solution was added to each vial, followed by incubation at 37 °C for 10 min followed. Then, 40 μL of PNPG solution was added to initiate the reaction. After 15 min, 10 μL of 0.1 M EDTA and 190 μL of 1 M Na_2CO_3 were added for reaction termination. An aliquot of 200 μL was withdrawn from each vial and added into separate wells of a microplate reader (Thermo Scientific Varioskan Flash, Finland). Absorbance at 400 nm was measured. A control vial was prepared by replacing the inhibitor solution with phosphate

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buffer. The entire experiment was repeated by substituting the active AGH with inactive AGH treated at 100 °C for 10 min.

α-Amylase inhibition assay

α-Amylase inhibition was measured by a spectrophotometric method according to Koh et al. [18] and Yang et al. [19] with some modifications. First, 820 μL samples of different concentrations were pipetted into separate reaction vials and 100 μL of α-amylase enzyme solution was added into each vial, and incubated at 37 °C for 10 min. Next, 80 μL of potato starch solution (1%) was pipetted into each reaction vial. After 12 min incubation in a 37 °C water bath, 500 μL of HCl (10%) was added to each vial for reaction termination. Next, 150 μL of iodine solution (0.0025 M I_2/0.0065 M KI) and 500 μL of distilled water were added. Upon the addition of 500 μL of deionized water, an aliquot of 200 μL from each reaction vial was pipetted into separate wells of a microplate reader (Thermo Scientific Varioskan Flash, Finland). Absorbance at 620 nm was measured. A control vial was prepared by replacing the inhibitor solution with phosphate buffer. The entire experiment was repeated by substituting the active α-amylase enzyme with denatured α-amylase enzyme solution treated at 100 °C for 10 min.

Pancreatic lipase inhibition assay

Pancreatic lipase activity was measured according to Sugiyama et al. [20] using 4-methylumbelliferyl oleate (4MUO) as the substrate. Twenty-five microliters of the sample solution dissolved in water and 25 μL of the pancreatic lipase solution (1 mg/mL) were mixed in the well of a microplate reader. Fifty μL of 4MUO solution (0.1 mM) dissolved in Dulbecco's phosphate buffered saline (9.6 g/L) was then added to initiate the enzyme reaction. After incubation at 23 °C for 20 min, 100 μL of 0.1 M sodium citrate (pH 4.2) was added to stop the reaction. The amount of 4-methylumbelliferone released by lipase was measured using a fluorescence microplate reader at an excitation wavelength of 320 nm and an emission wavelength of 450 nm.

Statistical analysis

All experiments were performed in triplicate and parallel. Six values for each sample were averaged (n = 6). IC_{50} values were calculated with GraphPad Prism Version 5.01 (GraphPad Software, Inc., San Diego, CA). The MINITAB Statistical Software package (Version 17; State College, PA) was used for all statistical analyses. The two samples t-test was performed for investigating statistically significant differences within two samples. After the data was converted to log, the test for equal variances was conducted. It was found that the variances were not significantly different. Then one-way ANOVA was conducted with assuming equal variances, residuals were normally distributed. A p value of < 0.05 was considered to be significant.

Results and discussion

Anthocyanin content and bioaccessibility

Anthocyanins are the largest group of water-soluble pigments in the plant kingdom, and responsible for the blue, purple, and red color of many plant tissues. Anthocyanins also have important functions in plant physiology as well as possible human health effects [6, 21]. The total anthocyanin contents of apple (Amasya, *Malus communis*), red grape (Papazkarası, *Vitis vinifera*) and cinnamon (Cassia, *Cinnamomum*) are shown in Table 1. The content of total anthocyanin in red grape (164 ± 2.51 mg/100 g) was found higher than apple (24.2 ± 0.89 mg/100 g) and cinnamon (19.2 ± 0.4 mg/100 g) ($p < 0.05$). Wu et al. [21] stated the total anthocyanin content of apple (Fuji, Gala, Red Delicious) as 1.3 ± 0.7, 2.3 ± 0.8 and 12.3 ± 1.9 mg/100 g, respectively. Galet [22] stated that the amount of anthocyanin in 23 different grape varieties was between 42–4893 mg/kg but changed according to variety and year. Also, the anthocyanin content was reported as 233.9 mg/100 g in Cabernet sauvignon, 149.3 mg/100 g in Tempranillo and 54.3 mg/100 g in Pinot noir grapes. In the study of Wu et al. [21] the total anthocyanin content was found as 26.7 ± 10.9, 120.1 mg/100 g in red grape and Concord grape, respectively. Mazza [23] (1995) observed that the total anthocyanin content varied between approximately 30 – 750 mg/100 g in some varieties of grape. As we can see from these results, the anthocyanin content can be dependent on the variety of samples.

The simulated gastrointestinal digestion models are being extensively used at present because they are rapid, safe and do not have the ethical restrictions of *in vivo* methods. *In vitro* methods either simulate the digestion and absorption processes (for bioavailability) or only the digestion process (for bioaccessibility), and the response measured is the concentration of a nutrient and other dietary bioactive compound in some kind of final extract [16, 24]. In our study, anthocyanin bioaccessibilities were found as 10.2 ± 1.00, 8.23 ± 0.64 and 8.73 ± 0.70% in apple, red grape and cinnamon, respectively (see Table 1). There wasn't found any statistically significant difference ($p > 0.05$) between red grape and cinnamon.

Anthocyanins are unstable under *in vitro* conditions that mimic those of the upper gastrointestinal tract. The exposure to differences in pH, oxygen, and heating combines to greatly reduce anthocyanin availability to the serum

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Table 1. Total anthocyanin and procyanidin contents and bioaccessibilities*

Samples	Anthocyanin (AC)			Procyanidin (PC)		
	Total (AC) (mg/100 g)	After in vitro digestion (mg/100 g)	Bioaccessibility (%)	Total (PC) (mg/100 g)	After in vitro digestion (mg/100 g)	Bioaccessibility** (%)
Apple	$24.2 \pm 0.89^{B,a}$	$2.48 \pm 0.28^{B,b}$	10.2 ± 1.00^{A}	$147 \pm 3.88^{B,a}$	$25.9 \pm 0.71^{B,b}$	17.57 ± 0.71^{A}
Red grape	$164 \pm 2.51^{A,a}$	$13.6 \pm 0.98^{A,b}$	8.23 ± 0.64^{B}	$86 \pm 2.53^{B,a}$	$12.1 \pm 0.58^{B,b}$	14.08 ± 0.74^{B}
Cinnamon	$19.2 \pm 0.40^{C,a}$	$1.67 \pm 0.12^{B,b}$	8.73 ± 0.70^{B}	$6432 \pm 177.31^{A,a}$	$1206 \pm 113.14^{A,b}$	18.75 ± 1.49^{A}

*Different capital letters within the same column shows statistically significant difference. Values are means ± standard deviation (SD) (n = 6), different lower case letters within the same rows shows statistically significant difference ($p < 0.05$).
**Bioaccessibility is the fraction of an ingested nutrient that is released from the food matrix and is available for absorption in the gut after digestion (typically based on in vitro techniques).

fraction [25]. The absorbed anthocyanins are expected to appear rapidly in the blood stream. Most of the anthocyanins in the body are largely absorbed in the intestine, particularly the small intestine. While small intestine has a pH 7.0, the stability of anthocyanins is at maximum level at pH ~1.0–3.0. In the uptake process of anthocyanins, endogenic β-glucosidases are involved [15]. They cleave the sugar moiety from the aglycon, and the molecule becomes smaller and more hydrophobic. This makes passive diffusion of anthocyanidin possible. It has been reported that the amount of up-taken of anthocyanins in the intestinal tissue is about 75 to 78% of absorbed anthocyanins [15, 26]. After small intestine, the non-absorbed anthocyanins pass through the intestine. The gut microflora cleaves the glycosidic linkages to break down anthocyanins into phenolic acids [15, 26]. In summary, the absorption of anthocyanins may occur as in tact (glycosilated and/or acylated) or as free aglycons and they are often involved into glucuronidation, sulfation, and methylation reactions in the intestine enterocyte, liver, and kidney [15, 27, 28]. It has been reported that the absorption of anthocyanins varies between 0.02 to 0.2% in *in vivo* studies. The type of sugar moieties, acylation, and the food matrix affects the absorption of anthocyanins [15]. Moreover, Lapidot et al. [29] studied the bioavailabilities of several anthocyanins in red wine and found that 1.5–5.1% of ingested anthocyanins were detected in the urine, within 12 h of the wine drinking.

Procyanidin content and its bioaccessibility

Proanthocyanidins can be subdivided into at least fifteen subgroups based on their hydroxylation patterns on the A and the B-ring of the monomeric flavan-3-ol. The subgroups procyanidins, prodelphinidins and propelargonidins are of prime importance in terms of human intake. The other 12 subgroups have been detected mainly in non-food sources. Procyanidin is the most abundant subgroup present in foods and is a homogeneous group exclusively consisting of (epi)catechin units [8]. The content of procyanidin in cinnamon (6432 ± 177.31 mg/100 g) was found higher than apple (147 ± 3.88 mg/100 g) and red grape (86 ± 2.53 mg/100 g) ($p < 0.05$) (see Table 1). Gu et al. [30] reported that total procyanidin contents of apples (red delicious, golden delicious, granny smith, gala and fuji) were found as 125.8 ± 6.8, 91.1 ± 4.7, 141.0 ± 26.1, 92.4 ± 8.4, 69.6 ± 15.8 mg/100 g, respectively. Nemzer et al. [31] stated that total procyanidin contents of apples (Granny Smith, Red Delicious, Gala, Golden Delicious, Fuji, Reinette) were 131.01, 127.79, 92.42, 83.01, 65.59, 42.88 mg/100 g, respectively. In the study of Gu et al. [30], the total procyanidin content was found as 81.5 ± 15, 61.0 ± 12.3 mg/100 g in green grape and red grape, respectively. Gu et al. [30] also studied the total procyanidin content of cinnamon and found as 8108.2 ± 424.2-mg/100 g. Nemzer at al. [31] stated that the total procyanidin content of cinnamon was 7908.14 mg/100 g. Our results were close to the results reported in the literature. We determined the bioaccesibilities of procyanidin in apple, red grape and cinnamon as 17.57 ± 0.71, 14.08 ± 0.74 and 18.75 ± 1.49%, respectively (see Table 1). There wasn't found any statistically significant difference ($p > 0.05$) in procyanidin bioaccessibility between apple and cinnamon. The bioaccesibilities of these group bioactive compounds depend on the molecular weight. The absorption of proanthocyanidin polymers in the small intestine is limited due to their large molecular weight. There is not enough study to prove the absorption of proanthocyanidins through the gut barrier [32, 33]. In addition to its molecular weight, their high degree of polymerization makes proanthocyanidins the least bioavailable intact among all of the classes of flavonoids. It has been reported that the polymerized compounds are 10 to 100 times less bioavailable than their monomeric constituents [15, 34]. Ou and Gu [35] stated that proanthocyanidin dimers, trimers, and tetramers were absorbed in their intact form and their absorption rates were less than 10% of (−)-epicatechin. In the plasma, proanthocyanidins were found mainly in their conjugated form. Moreover, they also exist in the plasma as sulfated, glucuronidated, and

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methylated metabolites [15]. Ward et al. [36] reported the existence forms of proanthocyanidins in the plasma after performing a study including 69 human subjects given 1000 mg/day grape seed extracts. It has been also reported that the urine of rats had these metabolites [15, 34]. The gut microbiota may be responsible for the metabolism of proanthocyanidins in the intestine because they may help formation of smaller more bioactive compounds [15]. Proanthocyanidins in foods are of interest in nutrition and medicine because of their potential antioxidant capacity and possible protective effects on human health in reducing the risk of chronic diseases such as cardiovascular diseases and cancers [32, 37].

Inhibitory activities of apple, red grape, and cinnamon against digestive enzymes

Digestive enzyme inhibition in the samples was determined by calculating the IC_{50} with the lower numbers indicating the higher quality of enzymatic inhibition (Table 2). These results showed that apple, red grape and cinnamon were potent inhibitors on lipase, α-amylase and α-glucosidase activity. Enzyme inhibition curves, α-amylase, α-glucosidase and lipase inhibition of the samples were plotted as a function of concentration. Percent of enzyme inhibition of the sample was positively correlated with the concentration of sample. α-glucosidase inhibition (%) increased as concentration of samples increased (r^2 = 0.9). Percent α-amylase inhibition and the concentrations of samples were significantly correlated as well (r^2 = 0.9). Similarly, the correlation between lipase inhibition and concentration of samples was high (r^2 = 0.9). The samples (apple, red grape and cinnamon) showed the inhibitory activity against α-glucosidase (IC_{50} 544 ± 21.94, 445 ± 15.67 and 1592 ± 17.58 μg/mL), respectively. There wasn't found any statistically significant difference ($p > 0.05$) in the inhibitory activity against α-glucosidase between apple and red grape. Cinnamon sample showed the lowest inhibition against α-glucosidase among the samples. Red grape sample showed greater inhibition against α-glucosidase compared to apple and cinnamon samples. The reason of this could be having higher anthocyanin content than others. In the study of Hogan et al. [38], the red wine grape pomace (Cabernet Franc) inhibited over 64% of the rat α-glucosidase activity at a concentration of 2.5 mg/mL and the IC_{50} value were determined as 1.63 mg/mL. Hogan [39] stated that the Norton grape skin extract (*Vitis aestivalis*) had an IC_{50} value of 0.384 mg/mL on rat α-glucosidases. In the yeast α-glucosidase inhibitory activity of Norton grape skin extract (*Vitis aestivalis*) was 32-times stronger (IC_{50} = 10.5 μg/mL) than acarbose (IC_{50} = 341.8 μg/mL), a commercial oral hypoglycemic agent. Acarbose exhibited an IC_{50} value of 91 ± 10.8 μg/mL on α-glucosidases and red grape seed extracts showed IC_{50} values of 1.15 ± 0.16 μg/mL [40]. *In vitro* studies of Shihabudeen et al. [41] had indicated dose-dependent inhibitory activity of cinnamon extract against mammalian α-glucosidase with IC_{50} value of 670 μg/mL.

Table 2. IC_{50} values of samples for glucosidase, amylase, lipase

Samples	IC_{50} Values (μg/mL)		
	α-Glucosidase	α-Amylase	Lipase
Apple	$544 \pm 21.94^{D,a}$	$38.4 \pm 7.26^{D,b}$	$52.7 \pm 2.05^{B,b}$
Red grape	$445 \pm 15.67^{DE,b}$	$56.1 \pm 3.60^{D,c}$	$581 \pm 54.14^{A,a}$
Cinnamon	$1592 \pm 17.58^{C,a}$	$3.54 \pm 0.86^{E,c}$	$49.6 \pm 2.72^{B,b}$

Different capital letters within the same column shows statistically significant difference. Values are means ± standard deviation (SD) (n = 6), different lower case letters within the same rows shows statistically significant difference ($p < 0.05$).

The apple, red grape and cinnamon samples exhibited inhibitory activity against α-amylase with 38.4 ± 7.26, 56.1 ± 3.60 and 3.54 ± 0.86 μg/mL, respectively. There wasn't found any statistically significant difference ($p > 0.05$) in the inhibitory activity against α-amylase between apple and red grape. Cinnamon showed greater inhibition against α-amylase compared to apple and red grape samples. The reason of this could be having higher procyanidin content than others. In the study of Griffith [40], acarbose exhibited an IC_{50} value of 6.90 ± 0.81 μg/mL and grape seed extract showed an IC_{50} of 8.74 ± 0.81 μg/ml against α-amylase. Ponnusamy et al. [42] found IC_{50} of 1.0 μg/mL inhibition against pancreatic α-amylase by the isopropanol extracts of *Cinnamomum verum* and they also stated the concentration dependence of this inhibition. Pancreatic α-amylase inhibitors can be used to lower the levels of postprandial hyperglycemia since they can provide the control of starch breakdown. They also state found that the probable inhibitory compounds in this extract could be alkaloids, proteins, tannins, cardiac glycosides, flavonoids, saponins and steroids by phytochemical analysis.

The samples (apple, red grape and cinnamon) showed the inhibitory activity against lipase (IC_{50} 52.7 ± 2.05, 581 ± 54.14 and 49.6 ± 2.72 μg/mL, respectively) (see Table 2). There wasn't found any statistically significant difference ($p > 0.05$) in the inhibitory activity against lipase between apple and cinnamon. Similarly, Moreno et al. [43] reported the inhibition activity of red grape seed extract against lipase. The application of the red grape seed extract at a concentration of 1 mg/mL resulted in the inhibition of 80% of lipase enzyme activity during 5 min of incubation. Pancreatic lipase is the most important enzyme responsible for digestion of dietary fat, so its inhibition can lead to beneficial effects on overweight and obesity [44].

In addition to evaluation of enzyme inhibition of samples before consumption, the enzyme inhibition assays were

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Table 3. Enzyme inhibitions of samples before and after *in vitro* digestion

Samples (1 mg/mL concentration)	α-Glucosidase inhibition (%)	α-Amylase inhibition (%)	Lipase inhibition (%)
Apple			
Before *in vitro*	$63.7 \pm 1.30^{E,c}$	$90.9 \pm 0.44^{B,a}$	$68.2 \pm 1.26^{F,b}$
After *in vitro*	$44.3 \pm 0.84^{G,c}$	$79 \pm 0.32^{D,a}$	$60 \pm 0.60^{H,b}$
Red grape			
Before *in vitro*	$67.3 \pm 1.37^{D,b}$	$81.2 \pm 0.81^{D,a}$	$64.5 \pm 1.76^{G,b}$
After *in vitro*	$48.2 \pm 0.49^{F,c}$	$68.7 \pm 0.43^{GH,a}$	$57.3 \pm 1.01^{I,b}$
Cinnamon			
Before *in vitro*	$44.1 \pm 1.00^{G,c}$	$94 \pm 0.43^{A,a}$	$85.6 \pm 0.77^{C,b}$
After *in vitro*	$26.2 \pm 0.41^{H,c}$	$81 \pm 0.54^{D,a}$	$75.4 \pm 0.52^{E,b}$

Different capital letters within the same column shows statistically significant difference. Values are means ± standard deviation (SD) (n = 6), different lower case letters within the same rows shows statistically significant difference ($p < 0.05$).

Table 4. Enzyme inhibitions of the standard catechin

	Concentration of catechin		
Inhibition (%)	0.86 μg/mL	1.47 μg/mL	64.32 μg/mL
α-Glucosidase	$8 \pm 0.18^{c,C}$	$10.3 \pm 0.41^{b,C}$	$30.3 \pm 0.46^{a,C}$
α-Amylase	$33 \pm 0.62^{c,A}$	$35.8 \pm 0.53^{b,A}$	$71.2 \pm 0.12^{a,A}$
Lipase	$16.9 \pm 0.50^{c,B}$	$22.3 \pm 0.49^{b,B}$	$45.7 \pm 0.88^{a,B}$

Different capital letters within the same column shows statistically significant difference. Values are means ± standard deviation (SD) (n = 6), different lower case letters within the same rows shows statistically significant difference ($p < 0.05$).

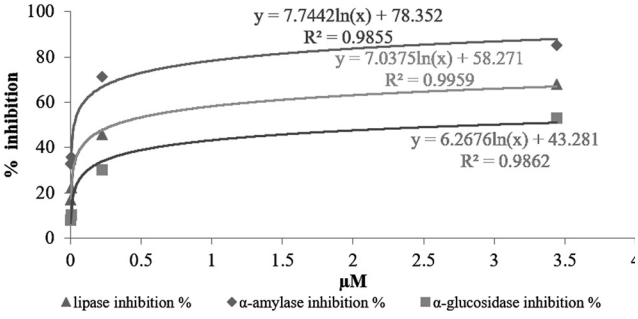

Figure 1. Enzyme inhibition-concentration graph of catechin standard.

Table 5. Catechin Equivalent IC_{50} values of samples for α-glucosidase, α-amylase, lipase

	$CEIC_{50}$ Values (mM catechin/g sample)		
Samples	α-Glucosidase	α-Amylase	Lipase
Apple	57	2.63	241
Red grape	64.6	0.69	91.7
Cinnamon	22	12	420

done after *in vitro* digestion of samples at 1 mg/mL concentration. The percentages of enzyme inhibition are shown in Table 3. The percentages of enzyme inhibition after in *vitro* digestion were found lower than before in *vitro* digestion for each enzyme and each sample. The reason of this could be the effects of the digestion process, temperature and pH change (acidic or basic environment). Cinnamon at 1 mg/mL concentration showed the lowest enzyme inhibition of 26.2 ± 0.41% for α-glucosidase enzyme after *in vitro* digestion. The highest percentage of enzyme inhibition was found as 81 ± 0.54% for α-amylase inhibition of cinnamon after *in vitro* digestion.

Procyanidin contents of apple, red grape and cinnamon at 1 mg/ml concentration, were found as 1.47, 0.86 and 64.32 μg/mL catechin equivalents, respectively. Therefore, the enzyme inhibitions of standard catechin were evaluated with 0.86, 1.47 and 64.32 μg/mL concentrations (Table 4). Enzyme inhibition curves, percent α-amylase, α-glucosidase and lipase inhibition of the catechin were plotted as a function of concentration as shown in the Figure 1. Enzyme inhibition (%) increased as concentration of catechin increased ($r^2 = 0.9$). The standard catechin exhibited highest inhibitory activity against α-amylase enzyme compared to its inhibitory activity against lipase and α-glucosidase enzymes ($p < 0.05$). The percentages of α-amylase enzyme of standard catechin at the concentrations of 0.86, 1.47 and 64.32 μg/mL were 33 ± 0.62, 35.8 ± 0.53 and 71.2 ± 0.12%, respectively. While the standard catechin at 0.86, 1.47 and 64.32 μg/mL concentrations provided 8 ± 0.18, 10.3 ± 0.41 and 30.3 ± 0.46% α-glucosidase enzyme inhibition, respectively; the lipase enzyme inhibition with 0.86, 1.47 and 64.32 μg/mL of standard catechin was 16.9 ± 0.50, 22.3 ± 0.49 and 45.7 ± 0.88%, respectively. However, apple, red grape and cinnamon at 1 mg/ml concentration, which included 0.86, 1.47 and 64.32 μg/mL catechin equivalents procyanidin, showed higher enzyme inhibitory activities against α-amylase, α-glucosidase, lipase than the standard catechin at the same concentration (Table 5). The higher inhibitor activity can be due to other biologically active substances and anti-nutritional factors except catechin. Catechin equivalent inhibition capacity ($CEIC_{50}$) was determined

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for each enzyme and sample (Table 5). The highest $CEIC_{50}$ value for α-glucosidase was found for red grape. The highest $CEIC_{50}$ value for α-amylase and lipase were found for cinnamon.

Conclusion

In conclusion, while the highest total anthocyanin content was found in red grape, the highest procyanidin content was found in cinnamon among the food samples ($p < 0.05$). The anthocyanin bioaccessibility of apple was found higher than grape and cinnamon ($p < 0.05$). The procyanidin bioaccessibilities of apple and cinnamon were found higher than grape. Also, present study demonstrated that apple, red grape and cinnamon samples exerted α-glucosidase, α-amylase and lipase inhibitory activity. Red grape sample showed the highest inhibitory activity against α-glucosidase, cinnamon showed the highest inhibitory activity against α-amylase and lipase according to IC_{50} and $CEIC_{50}$ values ($p < 0.05$). In summary, this study reported that apple, grape and cinnamon samples can inhibit activity of digestive enzymes *in vitro*. The consumption of these samples would be used in conjunction with a low-calorie diet for body weight management. These foods may provide safe, natural, and cost-effective alternatives to synthetic drugs for obesity and diabetes. As a future study, further isolation and identification of active inhibitory compounds in these samples are needed for developing antiobesity functional foods.

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