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DETERMINATION OF POLYPHENOLS BIOACCESSIBILITY BY IN VITRO GASTROINTESTINAL DIGESTION OF APPLE PEEL

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Summary

Bioaccessible polyphenols represent polyphenols that are released from the food matrix during digestion and become available for absorption. This work aimed to determine the bioaccessible polyphenols from the peel of commercial apple variety 'Idared' throughout oral, gastric, and intestinal simulated digestion. Polyphenols were extracted by the means of chemical and enzymatic extraction. *In vitro* gastrointestinal digestion of the peel of apples was conducted. Polyphenols present in the extracts and oral, gastric, and intestinal digest were analyzed with the use of high-performance liquid chromatography. The amount of polyphenols released during the simulated digestion was lower than the one present in the extracts. Polyphenols bioaccessibility, expressed as a percentage of initial polyphenol concentrations, was 26%, 37%, and 22% for oral, gastric, and intestinal phases, respectively. Flavonols showed to be the most stable group with the intestinal recovery of 34%, followed by phenolic acids (11%) and dihydrochalcones (8%). Flavan-3-ols and anthocyanins were not found in the intestinal phase. These results suggest that polyphenols are released from the peel of apples during digestion and that the amount decreases in the intestines.

Keywords: apples, simulated digestion, polyphenols, bioaccessibility

Introduction

Polyphenols are a group of natural compounds that contain phenolic structural features, i.e. a phenyl group ($-C_6H_5$) to which a hydroxyl group (-OH) is attached. They are widespread in the plants and can generally be divided into phenolic acids, flavonoids, stilbene and lignans (Belščak-Cvitanović et al., 2018; Mancha et al., 2004). Their role in plants has not been fully elucidated, but they are thought to protect plants from pathogens and herbivores. Furthermore, they contribute to plant color and flavor thereby indirectly increasing the chances of seed dispersal (Juadjur and Winterhalter, 2012). A number of scientific studies have been published suggesting a possible negative correlation between a polyphenol-rich diet and the risk of diseases such as cardiovascular disease, specific cancers, and diabetes (Anhê et al., 2103; Mendonça et al., 2018; Giacco et al., 2019 ; Yi et al., 2019; Sajadimajd et al., 2020). In order to show a positive effect on the human body, polyphenols must firstly be released from the food matrix and then absorbed in a certain amount (Jakobek, 2015).

Here we come to the problem of bioaccessibility, which can be defined as the amount of ingested compound that is available for absorption in the digestive tract (Palafox-Carlos et al., 2011). Several authors determined polyphenol bioaccessibility from different sources such as red chicory, white grape, jaboticaba fruit, plum and cabbage varieties (Bergantin et al., 2017; Kaulmann et al., 2016; Lingua et al., 2019; Quatrin et al., 2020). The common conclusion between these papers is that polyphenols are available for absorption throughout the human gastrointestinal system but in an amount that is lower than in the original source. However, Lingua et al. (2019) reported that the bioactivity of digested polyphenols, compared to the nondigested ones, did not change (Lingua et al., 2019). The extent to which polyphenols could be released from certain food depends on several factors among which are the characteristics of the food matrix, processing, and preservation, as well as the pH of the intestine environment (Stübler et al., 2020; Thakur et al., 2020). Out of total released polyphenols, only a small amount is actually absorbed, while the majority of polyphenols reaches the large intestine. Here, polyphenols can interact with gut microbiota and exert beneficial effect, either in terms of promoting bacterial health (prebiotic effect) or by direct antimicrobial effect. Inversely, beneficial gut microbes could utilize and transform certain polyphenols into more easily absorbable phenolic catabolites, thus affecting polyphenol bioaccessibility (Rodríguez-Daza et al., 2021).

Apples, considering that they are one of the most popular fruits worldwide and relatively rich in polyphenols, represent a good day-to-day source of polyphenols in the human diet (Fernández-Jalao et al., 2020). Although traditional apple varieties gained a lot of attention in recent years, due to higher polyphenol content (Jakobek and Barron, 2016), they are not as readily available as commercial varieties. Recovery of both commercial and traditional apple polyphenols

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has been investigated before (Bouayed et al., 2012; Jakobek et al., 2021). However, in the study that assessed commercial apple polyphenol recovery, authors used whole fruit, which could hinder the contribution of peel polyphenols due to significantly lower mass (Bouayed et al., 2012). Furthermore, despite the fact that peel only represent around 10 % of the total apple mass, it contains high amounts of quercetin derivatives, which are not commonly found in the apple flesh (Jakobek and Barron, 2016). Unfortunately, apple peel is often discarded, both by consumers and food industries, which could affect the total intake and the recovery of polyphenols, especially quercetin derivatives.

Hence, we decided to investigate the recovery of polyphenols from the peel of the commercial apple variety 'Idared' by studying in vitro simulated digestion processes in the mouth, the stomach, and the small intestine.

Materials and methods

Chemicals

Calcium chloride ($CaCl_2$), magnesium chloride ($MgCl_2$ x $6H_2O$), potassium chloride (KCl), potassium dihydrogen phosphate (KH_2PO_4) and sodium hydrogen carbonate ($NaHCO_3$) were obtainted from Gram mol (Zagreb, Croatia). Ammonium carbonate ($(NH_4)_2CO_3$) and sodium chloride (NaCl) were purchased from Kemika (Zagreb, Croatia) and Carlo Erba Reagents (Val de Reuil, France), respectively. Orto-phosphoric acid (85% HPLC-grade) was from Fluka (Buchs, Switzerland) while methanol (HPLC grade) was obtained from J.T. Baker (Gliwice, Poland). Polyphenol standards were obtained from Extrasynthese (Genay, France) and Sigma-Aldrich (St. Louis, MO, USA). α-amylase (A3176, 13 U/mg), pepsin (P7000, 632 U/mg), pancreatin (P7545, 8 USP), bile salt (B 8756, microbiology grade) and barley β-D-glucan were from Sigma-Aldrich (St. Louis, MO, USA).

Reagents preparation

Concentrated stock solutions of electrolytes (KCl (0.5 M), KH_2PO_4 (0.5 M), $NaHCO_3$ (1 M), $MgCl_2$ (0.15 M), $(NH4)_2CO_3$ (0.5 M), NaCl (2 M) and $CaCl_2$ (0.3 M)) were used to prepare simulated salivary, gastric and intestinal fluids. According to Menikus et al. (2014), the simulated fluids were prepared with 1.25 times concentration described in their study in order to reach final proper concentrations when used in digestion experiment (Table 1).

Enzyme solution were prepared daily in following concentrations: α-amylase 1,000 mg/L in simulated salivary fluid (SSF), pepsin 31,660.61 mg/L in simulated gastric fluid (SGF), pancreatin 8,000 mg/L in simulated intestinal fluid (SIF). Bile salt was prepared in simulated intestinal fluid in 25,000 mg/L, while β-glucan was prepared in millipore water at 550 mg/L.

Table 1. Preparations of 1.25 times concentrated simulated digestion fluids

	Simulated salivary fluid			**Simulated gastric fluid**			**Simulated intestinal fluid**	
	pH 7			pH 3			pH 7	
Constituent	**Volume (mL)**	**Concentration in SSF (mmol /L)**		**Volume (mL)**	**Concentration in SGF (mmol/L)**		**Volume (mL)**	**Concentration in SIF (mmol/L)**
KCL (0.5 M)	3.775	18.875		4.3125	8.625		8.5	8.5
KH_2PO_4 (0.5 M)	0.925	4.625		0.5625	1.125		1	1
$NaHCO_3$ (1 M)	1.7	17		7.8125	31.25		53.125	106.25
NaCl (2 M)	-	-		7.375	59		12	48
$MgCl_2$ (0.15 M)	0.125	0.05625		0.25	0.15		1.375	0.4125
$(NH_4)_2CO_3$ (0.5 M)	0.015	0.06		0.3125	0.625		-	-
H_20 (Millipore)	93.46			229.375			424	
Total volume	100			250			500	
For pH adjustment								
	mL	**mmol/L**		**mL**	**mmol/L**		**mL**	**mmol/L**
HCL (6 M)	-	-		1	24		1.6	1.92

Apple samples

About 1 kg of commercial apple variety 'Idared' was purchased for local supermarket in Croatia. After peeling, apple peel was pooled and homogenized with a coffee grinder. The samples were stored in plastic bags in a refrigerator at – 18 °C and used for chemical and enzyme assisted extraction of polyphenols and for simulated digestion within one week of storage.

Polyphenol extraction

For the chemical extraction of polyphenols, apple peel (around 3 g) and 22.5 mL of 80% methanol in water were added into a plastic tube (Jakobek & Barron, 2016; Jakobek et al., 2020). After 15 min extraction in the ultrasonic bath (Bandelin Sonorex RK 100, Berlin, Germany), the solution was centrifuged (10 min at 9,500 rpm; SL 8R, Thermo Fisher Scientific, Waltham, MA, USA). The extract was separated from the residue. The process of extraction of the residue was repeated one more time with 10 mL of 80% methanol. After combining two extracts, a total volume of approximately 32.5 mL was obtained. Finally, 1 mL of that extract was taken from a plastic tube, filtered (0.45 syringe filter) and analyzed using HPLC system.
Enzyme assisted extraction followed after chemical extraction (Bergantin et al., 2017). Into the tube with the residue after the chemical extraction, 0.3 mL of pepsin, 0.6 mL of pancreatin, 1.2 mL of bile salts and 21 mL of millipore water were added. The solution was incubated (2 h at 37 °C, water bath with shaking, SW 22, Julabo, Seelbach, Germany), and centrifuged (5 °C, 9,500 rpm, 5 min). 1 mL of the obtained extract was filtered (0.22 μm PTFE syringe filter), placed in an ice bath and analyzed using HPLC system. The residue was extracted one more time. Both extracts were analyzed with HPLC.

Simulated digestion

Around 3 grams of the homogenized peel was weighed into a plastic tube. For the simulation of oral digestion (Bergantin et al., 2017; Minekus et al., 2014), 3.5 mL of SSF, 0.975 mL of H_2O, 25 μL of $CaCl_2$ (0.3 M), 0.5 mL of α-amylase were added into a plastic tube containing the sample of peel. After vortexing (30 seconds), 0.5 mL was taken from the solution, filtered, placed in an ice bath, and analyzed using HPLC system. Then, in order to simulate gastric digestion, 7.5 mL of SGF, 0.295 mL of H_2O, 5 μL of $CaCl_2$ (0.3 M), 0.2 mL of HCl (1 M) and 2 mL of pepsin were added into the solution after simulated oral digestion. The solution was incubated (2 hours, 37 °C, water bath with a shaking device), centrifuged (5 minutes, 5 °C, 9500 rpm), and 0.5 mL was taken from the solution, filtered, put in an ice bathand analyzed using HPLC system. Finally, for the intestinal digestion, 11 mL of SIF, 3.61 mL of H_2O, 40 μL of $CaCl_2$ (0.3 M), 0.15 mL of NaOH (1 M), 5 mL of pancreatin and 0.2 mL of bile salt were added to the solution after simulated oral and gastric digestion. The solution was incubated (2 hours, 37 °C, water bath with a shaking device), centrifuged (5 minutes, 5 °C, 9500 rpm), and 1 mL was taken from the solution, filtered, placed in an ice bath and analyzed using HPLC system.

The recovery was calculated as:

$$recovery\ (\%) = \frac{\gamma_{digestion\ phase}\ (mg/kg)}{\gamma_{before\ digestion}\ (mg/kg)} * 100 \quad (1)$$

where
$\gamma_{digestion\ phase}$ is the concentration of polyphenol after a particular digestion phase (mg/kg fresh weight (FW)), $\gamma_{before\ digestion}$ is a polyphenol concentration in fruit before digestion determined with chemical and enzyme assisted extraction (mg/kg FW).

Reversed phase high performance liquid chromatography

HPLC system (1260 Infinity II, a quaternary pump, a PDA detector, a vialsampler) (Agilent technology, Santa Clara, CA, USA) with Poroshell 120 EC C-18 column (4.6 × 100 mm, 2.7 μm) and a Poroshell 120 EC-C18 4.6 mm guard-column was used to analyze all the samples. 10 μL of each sample was injected into the system and polyphenols were separated using 0.1% H_3PO_4 (mobile phase A) and 100% methanol (mobile phase B). The gradient was: 0 min 5% B, 5 min 25% B, 14 min 34% B, 25 min 37% B, 30 min 40% B, 34 min 49% B, 35 min 50% B, 58 min 51% B, 60 min 55% B, 62 min 80% B, 65 min 80% B, 67 min 5% B, 72 min 5% B, with a flow of 0.8 mL min^{-1} (Jakobek et al., 2020). Spiking samples with authentic standards and by comparing UV/Vis spectrum (200 to 600 nm) of standards and samples was used to identify polyphenols.

Statistical analysis

All experiments were repeated three times. The results were reported as mean ± standard deviation. The differences between results were analyzed using post-hoc Tukey test (Minitab LLC., State College, PA, USA).

Results and discussion

Polyphenols in the peel

Peel polyphenols, are well documented in the literature, and are composed of four or five subclasses depending on the color of the apple peel. Red apples, besides flavan-3-ols, dihydrochalcones, phenolic acids and flavonols, contain anthocyanins (Jakobek and Barron, 2016; Kschonsek et al., 2018). Total of

fourteen polyphenols were found in the chemical extracts of the peel 'Idared' (Fig. 1). Table 2 shows the amount of polyphenols after chemical and enzymatic extractions. The most abundant polyphenol subclass was flavonol subclass (720.9 mg/kg), followed by flavan-3-ols (302.7 mg/kg), phenolic acids (93.8 mg/kg), dihydrochalcones (66.8 mg/kg) and anthocyanins (24.9 mg/kg). Similar amounts were reported earlier (Jakobek et al., 2020; Kschonsek et al., 2018; Lo Piccolo et al., 2019). Enzymatic extractions were used to solubilize polyphenols bound to dietary fiber (Bergantin et al., 2017). The amount of polyphenols after enzymatic extraction was significantly lower than the amount after chemical extraction, and it contributed to approximately 12 % of the total amount of extracted polyphenols.

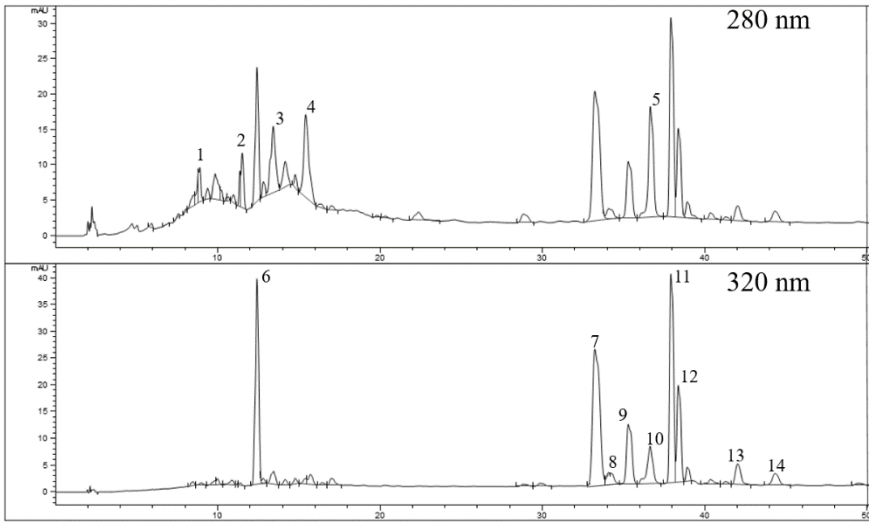

Fig. 1. RP-HPLC chromatographs of the polyphenols extracted from the peel of apple 'Idared. Peaks: 1-Procyanidin B1; 2- (+)-catechin; 3- (-)-epicatechin; 4- Cyanidin-3-galactoside; 5- Phloretin-2-glucoside; 6- Chlorogenic acid; 7- Quercetin-3-galactoside; 8- Quercetin-3-glucoside; 11- Quercein-3-xyloside; 12- Quercetin-3-rhamoside; 9, 10, 13, 14- Querectin derivatives

Table 2. The amounts of polyphenols from the peel of apple 'Idared' obtained after chemical extraction and first and second enzymatic extraction (mg/kg fresh weight (FW))

	Chemical extraction	Enzymatic extraction 1	Enzymatic extraction 2
Anthocyanins			
Cyanidin-3-galactoside	24.9 ± 1.1		
Total	24.9 ± 1.1		
Flavan-3-ols			
Procyanidin B1	45.2 ± 1.0		
(+)-catechin	45.5 ± 7.3	45 ± 14.0	
(-)-epicatechin	149.5 ± 4.4	7.1 ± 2.2	10.4 ± 0.1
Total	240.2 ± 12.7^{a}	52.1 ± 16.2^{b}	10.4 ± 0.1^{c}
Dihydrochalcones			
Phloretin-2-glucoside	60.0 ± 3.4	4.1 ± 1.6	2.7 ± 0.2
Total	60.0 ± 3.4^{a}	4.1 ± 1.6^{b}	2.7 ± 0.2^{b}
Phenolic acids			
Chlorogenic acid	88.9 ± 1.0	4.9 ± 1.9	
Total	88.9 ± 1.0^{a}	4.9 ± 1.9^{b}	
Flavonols			
Quercetin-3-galactoside	175.9 ± 3.5	8.8 ± 0.5	1.6 ± 0.1
Quercetin-3-glucoside	17.2 ± 4.4	1.5 ± 0.3	
Quercetin derivative 1	99.7 ± 4.5	9.9 ± 0.4	4.6 ± 0.1
Quercetin derivative 2	17.4 ± 4.0	4.5 ± 0.1	
Quercetin-3-xyloside	234.3 ± 14.0	20.3 ± 1.3	6.8 ± 0.3
Quercetin-3-rhamnoside	61.0 ± 2.5	9.1 ± 0.1	5.7 ± 0.1
Quercetin derivative 3	19.5 ± 0.7		
Quercetin-derivative 4	23.1 ± 0.9		
Total	648.1 ± 34.5^{a}	54.1 ± 2.7^{b}	18.7 ± 0.6^{c}
Total polyphenols	1062.1 ± 52.7^{a}	115.2 ± 22.4^{b}	31.8 ± 0.9^{c}

The means in a same row that do not share a letter are statistically different according to post-hoc Tukey pairwise comparison test at significance level 0.05. The results are reported as means ± standard deviations.

In vitro gastrointestinal digestion

The peel of apple 'Idared' underwent digestion simulation process and earlier identified polyphenols were tracked throughout the oral, gastric and intestinal phase of digestion. Table 3 shows the amounts of polyphenols before digestion and after each phase of digestion. For majority polyphenols, significantly lower amounts were detected after digestion in comparison to the amount present in undigested apple peel. These findings are similar to the earlier reported studies (Bouayed et al., 2012; Lingua et al., 2019; Quatrin et al., 2020). As can be seen from Fig. 2, the highest amount of total polyphenols was released in the gastric phase of digestion (37 % of the native amount), followed by oral phase (26 %) and intestinal phase (22 %). The same trend (gastric recovery > oral recovery > intestinal recovery) was established in our previous work, which investigated the recovery of traditional apple polyphenols (Jakobek et al., 2021). Similar results were reported in the studies that evaluated the effect of simulated digestion on polyphenols of white and red grapes. 34 %, 37 %, and 13 % of white grape polyphenols were recovered after oral, gastric, and intestinal digestion, respectively (Lingua et al., 2019), while red grapes had a slightly lower recovery (24 %, 29%, and 16 % for oral, gastric and intestinal phase, respectively) (Lingua et al., 2018). Fernández-Jalao et al. (2020) investigated the impact of gastrointestinal digestion on apple phenolic compounds. They reported gastric and intestinal recoveries similar to ours (32 and 28%, respectively) while oral recovery was somewhat higher (43 %).

Table 3. The amounts of polyphenols from peel of apple 'Idared' before digestion and recovered polyphenols after oral, gastric and intestinal digestion (mg/kg fresh weight (FW))

	Before digestion	Oral phase	Gastric phase	Intestinal phase
Anthocyanins				
Cyanidin-3-galactoside	24.9 ± 1.1^{a}	3.6 ± 0.9^{b}	4.4 ± 0.4^{b}	
Total	24.9 ± 1.1^{a}	3.6 ± 0.9^{b}	4.4 ± 0.4^{b}	
Flavan-3-ols				
Procyanidin B1	45.2 ± 1.0^{a}	11.8 ± 0.1^{c}	24.7 ± 0.4^{b}	
(+)-catechin	90.5 ± 21.3^{a}	11.5 ± 1.0^{b}	16.7 ± 1.5^{b}	
(-)-epicatechin	167.0 ± 6.7^{a}	15.2 ± 4.1^{b}	16.1 ± 0.9^{b}	
Total	302.7 ± 39.0^{a}	38.5 ± 5.2^{b}	57.5 ± 2.8^{b}	
Dihydrochalcones				
Phloretin-2-glucoside	66.8 ± 5.0^{a}	15.6 ± 2.6^{b}	17.4 ± 1.0^{b}	5.2 ± 1.2^{c}
Total	66.8 ± 5.0^{a}	15.6 ± 2.6^{b}	17.4 ± 1.0^{b}	5.2 ± 1.2^{c}
Phenolic acids				
Chlorogenic acid	93.8 ± 2.9^{a}	47.2 ± 0.1^{c}	55.2 ± 0.7^{b}	10.7 ± 0.6^{d}
Total	93.8 ± 2.9^{a}	47.2 ± 0.1^{c}	55.2 ± 0.7^{b}	10.7 ± 0.6^{d}
Flavonols				
Quercetin-3-galactoside	186.3 ± 4.1^{a}	47.5 ± 7.9^{c}	67.3 ± 8.2^{b}	48.4 ± 1.6^{c}
Quercetin-3-glucoside	18.7 ± 4.7^{c}	47.0 ± 9.9^{a}	75.2 ± 8.4^{b}	$54.7 \pm 8.2^{a,b}$
Quercetin derivative 1	114.2 ± 5.0^{a}	24.2 ± 4.0^{c}	37.0 ± 3.6^{b}	$29.3 \pm 0.1^{b,c}$
Quercetin derivative 2	21.9 ± 4.1^{a}	12.9 ± 2.0^{b}	24.3 ± 1.9^{a}	20.4 ± 0.2^{a}
Quercetin-3-xyloside	261.5 ± 15.6^{a}	15.3 ± 3.5^{c}	24.8 ± 3.3^{c}	46.7 ± 0.1^{b}
Quercetin-3-rhamnoside	75.8 ± 2.7^{a}	35.0 ± 2.0^{c}	53.9 ± 4.7^{b}	24.9 ± 0.1^{d}
Quercetin derivative 3	$19.5 \pm 0.7^{b,c}$	16.5 ± 2.4^{c}	23.2 ± 4.1^{a}	$21.1 \pm 0.3^{a,b}$
Quercetin-derivative 4	23.1 ± 0.9^{a}	13.5 ± 2.0^{b}	4.4 ± 0.4^{c}	
Total	721.0 ± 37.8^{a}	211.9 ± 33.7^{c}	310.1 ± 34.6^{b}	$245.5 \pm 10.6^{b,c}$
Total polyphenols	1209.2 ± 85.1^{a}	316.8 ± 42.5^{c}	444.6 ± 39.5^{b}	261.4 ± 12.4^{c}

The means in a same row that do not share a letter are statistically different according to post-hoc Tukey pairwise comparison test at significance level 0.05. The results are reported as means ± standard deviations.

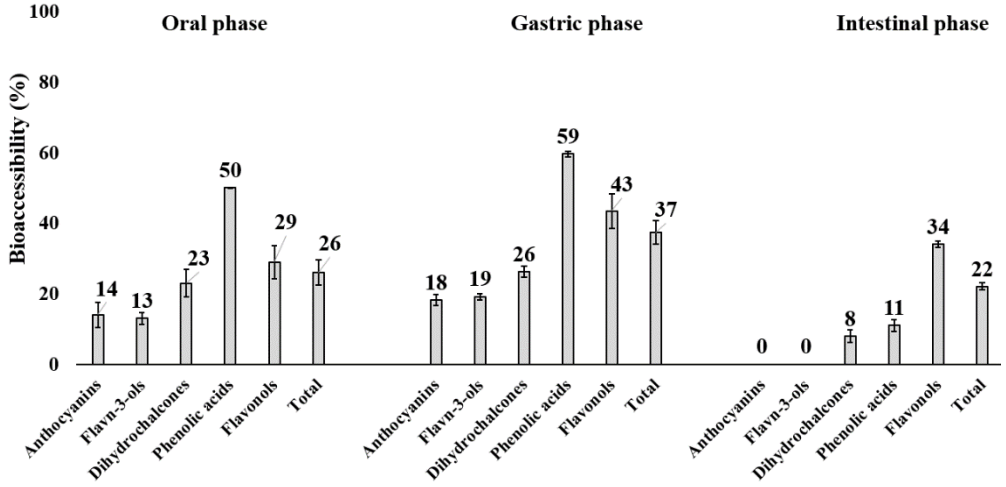

Fig. 2. The percentage recovery of polyphenols from the peel of apple 'Idared' after oral, gastric and intestinal digestion

Anthocyanins, namely cyanidin-3-galactoside, were found in the peel of 'Idared' in small amounts (Table 2). They were released in oral and gastric phases in similar amounts (3.6 and 4.4 mg/kg, respectively) (Table 3) which accounts for 14 and 18 %, respectively (Fig. 2). They were not found in the intestinal phase (Fig. 2). This might be due to their instability at higher pH such as pH 7 of the intestinal phase. At this pH anthocyanins undergo structural transformations from flavylium cation to colorless chalcone which could hinder their detectability (Pérez-Vicente et al., 2002). The disappearance of apple anthocyanins during intestinal digestion was reported in earlier studies (Bouayed et al., 2012; Jakobek et al., 2021). However, anthocyanins were found after intestinal digestion of jaboticaba fruit and strawberries, although in a small amount. This could be due to much higher initial amounts of anthocyanins compared to apples (Fernández-Jalao et al., 2020; Hilary et al., 2020; Stübler et al., 2020; Quatrin et al., 2020).

Flavan-3-ols showed the same trend as anthocyanins, where their amount increased (although not significantly) from oral to gastric phase (from 38.5 to 57.5 mg/kg) which accounts for 13 and 19 % of total flavan-3-ols, respectively (Fig 2). They were not present in the intestinal phase (Table 3). A much longer duration of the gastric phase compared to the oral phase might explain the observed increase of flavan-3-ols. Degradation of flavan-3-ols due to the transfer from the acidic gastric environment to neutral pH of the intestines might explain their disappearance. These findings are in accordance with earlier studies, in which degradation of flavan-3-ols to unknown compounds due to autooxidation, polymerization, transformation or complexation was suggested (Fernández-Jalao et al., 2020; Hilary et al., 2020).

Dihydrochalcones, namely phloretin-2-glucoside, were detected in the peel extracts, as well as in oral, gastric and intestinal phase of digestion. Similar amounts were recovered after oral and gastric phase (15.6 and 17.4 mg/kg, respectively), while the amount recovered after intestinal phase was significantly lower (5.2 mg/kg) (Table 3). Their oral, gastric, and intestinal recovery was 23, 26 and 8 %, respectively (Fig 2). Suggested gastric stability of phloretin-2-glucoside might be the reason for similar amounts in oral and gastric phase (Fernández-Jalao et al., 2020). However, other authors reported an increase in the amount of dihydrochalcones after intestinal phase ((Bouayed et al., 2012; Fernández-Jalao et al., 2020; Jakobek et al., 2021), which was not the case in this study.

Chlorogenic acid was the only phenolic acid identified in the peel of apple 'Idared'. As can be seen from Fig. 2, out of all polyphenol subclasses phenolic acids had the highest recovery throughout oral and gastric phases (50 and 59 %, respectively) (Fig. 2). However, their intestinal recovery decreased to 11 %. These results are in accordance with our previous work (Jakobek et al., 2021). A significant increase of phenolic acids from oral to gastric phase was detected (from 47.2 to 55.2 mg/kg), followed by a significant decrease after intestinal phase (from 55.2 to 10.7 mg/kg) (Table 3). The decrease of phenolic acids in the intestinal phase was reported in other studies of apple polyphenols (Bouayed et al., 2012) or other sources (Hilary et al., 2020; Lingua et al., 2018).

Flavonols were the most abundant polyphenols both in extracts and after gastrointestinal digestion (Table 3). Unlike other polyphenol subclasses, flavonols had a higher recovery in the intestinal phase than in the oral phase. The same trend was observed in other studies as well (Fernánndez-Jalao et al., 2020; Jakobek et al.,

2021). Furthermore, flavonols were the only polyphenol group that did not show a significant decrease from gastric to intestinal phase (from 310.1 to 245.5 mg/kg) (Table 3). It was suggested that quercetin derivatives were resistant to the mild alkaline environment of the intestine since quercetin was not detected, meaning that quercetin derivatives hydrolyzed to a lesser extend than other compounds (Jakobek et al., 2021). Their recovery was 29, 43 and 34 % for oral, gastric and intestinal phase, respectively (Fig. 2). These results are similar to those reported in our previous study (Jakobek et al., 2021).

Conclusion

This study investigated polyphenols from the peel of 'Idared' after gastrointestinal digestion. Five subclasses of polyphenols were found in the peel – anthocyanins, flavan-3-ols, dihydrochalcones, phenolic acids and flavonols. The amount of all polyphenols significantly decreased after the simulated digestion. Gastric recovery of total polyphenols was the highest (37 %), followed by oral (26 %) and intestinal (22 %). All polyphenol subclasses followed this trend as well, except for flavonols. Flavonols showed the best stability in the intestinal environment, while flavan-3-ols and anthocyanins were not found.

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