Kinetics of Phenolic Compound Release from the Peel of the Traditional Apple Variety 'Kolačara' During in vitro Gastrointestinal Digestion

Kinetika oslobađanja fenolnih spojeva iz kožice tradicionalne sorte jabuke 'Kolačara' tijekom in vitro gastrointestinalne probave

Ištuk, J., Matić, P., Skendrović-Babojelić, M., Jakobek, L.

Poljoprivreda / Agriculture

ISSN: 1848-8080 (Online)
ISSN: 1330-7142 (Print)

https://doi.org/10.18047/poljo.31.2.5

Fakultet agrobiotehničkih znanosti Osijek, Poljoprivredni institut Osijek

Faculty of Agrobiotechnical Sciences Osijek, Agricultural Institute Osijek

ISSN 1330-7142
UDK=634.12:591.131.4
https://doi.org/10.18047/poljo.31.2.5

KINETICS OF PHENOLIC COMPOUND RELEASE FROM THE PEEL OF THE TRADITIONAL APPLE VARIETY 'KOLAČARA' DURING *IN VITRO* GASTROINTESTINAL DIGESTION

Ištuk, J.[(1)], Matić, P. [(2)], Skendrović-Babojelić, M. [(3)], Jakobek, L. [(2)]

Original scientific paper
Izvorni znanstveni članak

SUMMARY

The peel of traditional apple varieties represents a rich source of phenolic compounds, known for their potential health benefits. This study investigated the release kinetics of phenolic compounds from the peel of the traditional apple variety 'Kolačara' during simulated gastrointestinal digestion using a modified first-order kinetic equation. During digestion, anthocyanins and flavan-3-ols were undetectable after the intestinal phase, while dihydrochalcones, phenolic acids, and flavonols demonstrated greater stability. Kinetic analysis revealed that the release followed first-order kinetics. During the gastric phase, half-life values ranged from 2.3 to 7.9 minutes, indicating that most phenolic compounds were released within the first 10 minutes of digestion. In the intestinal phase, half-life values reached up to 2.4, 6.6, and 31.1 minutes for phenolic acids, flavonols, and dihydrochalcones, respectively. The study highlights the rapid release of phenolic compounds from the peel of a traditional apple variety peel, with dihydrochalcones showing the highest resistance to intestinal degradation. These findings provide valuable insight into the digestive behavior of bioactive compounds derived from valuable traditional fruit sources.

Keywords: apple, peel, phenolic compounds, digestion, kinetics

INTRODUCTION

Phenolic compounds are natural bioactive compounds in the human diet, produced by various plants as secondary metabolites (Abbas et al., 2017). Apple peels, a somewhat underutilized by-product of apple processing industries, are relatively rich in these compounds (Asma et al., 2023). These compounds have been extensively studied for their potential health-promoting effects, including anticancer, antidiabetic, cardioprotective, and neuroprotective activities (Rana et al., 2022). To exert these potential benefits, phenolic compounds must be released from the food matrix, and the application of chemical kinetics can provide a clearer picture of the rate and extent of their release during digestion (Jakobek et al. 2023). Although not fully understood, the general fate of phenolic compounds involves their release from the food matrix due to chewing and the lowered pH of the stomach. In the small intestine, phenolic compounds are released further and are partially absorbed through passive diffusion or active transport (Nagar et al., 2019). Beyond absorption in the small intestine, certain phenolic compounds can reach the large intestine in their native form. There, these compounds can interact with the gut microbiota, potentially displaying prebiotic or antimicrobial effects. Conversely, beneficial gut bacteria may metabolize certain phenolic compounds into forms that are more readily absorbed (Rodríguez-Daza et al., 2021).

Although commercial apple varieties dominate the market, indigenous and traditional varieties represent a valuable source of genetic diversity and often exhibit a unique phytochemical profile. These varieties often contain higher concentrations of bioactive compounds, including phenolics, compared with modern commercial cultivars (Jakobek et al., 2020). Characterizing the behavior of these compounds from traditional sources during digestion is important for understanding their full nutritional and health potential.

(1) Jozo Ištuk, PhD (jozo.istuk@aptf.sum.ba) – University of Mostar, Faculty of Agriculture and Food Technology, 88 000 Mostar, Bosnia and Herzegovina, (2) Petra Matić, PhD, Assoc. Prof. Lidija Jakobek – Josip Juraj Strossmayer University of Osijek, Faculty of Food Technology Osijek, Franje Kuhača 18, Osijek, Croatia, (3) Assoc. Prof. Martina Skendrović-Babojelić – University of Zagreb, Faculty of Agriculture, Department of Pomology, Svetošimunska 25, 10000 Zagreb, Croatia

The aim of this research was to study the release kinetics of phenolic compounds from the peel of the traditional apple variety 'Kolačara' during simulated *in vitro* gastrointestinal digestion. To determine the kinetic parameters of the release, first-order kinetic equations, modified for gastrointestinal digestion, were used. To the best of our knowledge, the release kinetics of phenolic compounds from this traditional apple variety under simulated digestive conditions have not been previously investigated.

MATERIALS AND METHODS

Chemicals

The chemicals were purchased from various suppliers. Calcium chloride, hydrochloric acid, magnesium chloride, potassium dihydrogen phosphate, sodium hydroxide, and sodium hydrogen carbonate were obtained from Gram mol (Zagreb, Croatia). Ammonium carbonate was obtained from Kemika (Zagreb, Croatia), and sodium chloride from Carlo Erba Reagents (Val de Reuil, France). Orto-phosphoric acid (85% HPLC-grade) was acquired from Fluka (Buchs, Switzerland), and methanol (HPLC grade) from J. T. Baker (Gliwice, Poland). Enzymes, including α-amylase (A3176, 13 U/mg, from porcine pancreas), pepsin (P7000, 632 U/mg), pancreatin (P7545, 8 USP), as well as bile salts (B 8756, microbiology grade) were obtained from Sigma–Aldrich (St. Louis, MO, USA). Phenolic compound standards were obtained from Sigma–Aldrich (St. Louis, MO, USA) and Extrasynthese (Genay, France).

Apple Samples

The traditional apple variety 'Kolačara', described in the Croatian Plant Genetic Resources Database (https://cpgrd.hapih.hr/), was harvested from a private orchard in Perinci, Croatia (45°23'45.0 N, 17°34'06.3 E). The peel of 'Kolačara' apples was carefully separated from the flesh using a peeled and minced in an electric coffee grinder. The minced samples of apple peel were placed in plastic bags and stored in the freezer at -18 °C for further analysis.

Phenolic Compound Extraction

To determine the initial content of phenolic compounds, sequential chemical and enzyme-assisted extractions were performed. Briefly, 3 g of minced apple peel was extracted twice with 80% methanol using ultrasonication for 15 minutes, followed by centrifugation at 9000 rpm. The remaining residue was then subjected to two sequential enzymatic extractions using pepsin, pancreatin, and bile salts, and incubated for 2 hours at 37 °C. All extracts were filtered (0.45 μm PTFE), analyzed by HPLC, and the amounts were summed to determine the total phenolic content before digestion.

Simulated Digestion

The digestion simulation procedure was conducted according to the previously established method (Minekus et al., 2014; Bergantin et al., 2017). All enzyme solutions and simulated fluids were prepared as described by Minekus et al. (2014) and incubated at 37 °C prior to analysis. For each digestion simulation, 3 g of minced apple peel from the traditional variety 'Kolačara' were weighed into Falcon tubes, and the following steps were performed:

For the oral phase digestion simulation, 0.975 mL of Milli-Q water, 3.5 mL of Simulated Salivary Fluid (SSF), 0.025 mL of $CaCl_2$, and 0.5 mL of α-amylase were added to the Falcon tubes containing the apple material. The mixtures were vortexed for 30 seconds. The procedure then continued to the gastric and intestinal phases. Gastric phase digestion simulation involved adding 0.295 mL of Milli-Q water, 7.5 mL of Simulated Gastric Fluid (SGF), 2 mL of pepsin, 0.005 mL of calcium chloride, and 0.2 mL of HCl (1 mol L^{-1}) to the solutions after the oral phase. The solutions were vortexed and incubated in the water bath with agitation for 2 hours at 37 °C. During the simulated gastric digestion, 500 μL aliquots were taken from reaction mixtures at 10, 20, 30, 60, and 120 minutes to study the kinetics of polyphenol release. The aliquots were placed on ice, filtered through 0.45 μm PTFE filters, and injected into the HPLC system. After the 2-hour incubation, solutions were centrifuged at 5 °C and 9,500 rpm for 5 minutes. The intestinal phase was simulated by adding 3.61 mL of Milli-Q water, 11 mL of Simulated Intestinal Fluid (SIF), 5 mL of pancreatin, 0.2 mL of bile salts, 0.15 mL of NaOH (1 mol L^{-1}), and 0.040 mL of calcium chloride to solutions after the oral and gastric phases. During the simulated intestinal digestion, 500 μL aliquots were again taken from reaction mixtures at 10, 20, 30, 60, and 120 minutes. These aliquots were placed on ice, filtered through 0.45 μm PTFE filters, and injected into the HPLC system.

Reversed-Phase High Performance Liquid Chromatography (RP-HPLC)

Phenolic compounds in all samples were analyzed using a Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC) on an Agilent 1260 Infinity II system equipped with a quaternary pump and a diode array detector (DAD). Separation of phenolic compounds was performed on a Poroshell 120 column (EC-C18, 4.6×100 mm, 2.7 μm) with a Poroshell 120 UHPLC Guard pre-column (EC-C 18, 4.6 mm) (Agilent Technology, Santa Clara, CA, USA), using H_3PO_4 (mobile phase A) and 100% methanol (mobile phase B). The analysis was conducted using gradient elution, where the proportion of mobile phase was as follows: 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. The flow rate was set to 0.8 mL min^{-1}. The injection volume was 10 μL, and chromatograms were recorded in the wavelength range of 200 to 600 nm.

Kinetics of Phenolic Compound Release

The release of phenolic compound subgroups during simulated digestion was modeled using modified first-order and second-order kinetic equations, as described in our previous study (Jakobek et al., 2023). These models allow for both increasing and decreasing

concentration trends and are suited for analyzing the release and degradation of phenolic compounds during gastric and intestinal phases.

The equations used for first and second order models, respectively, were as follows:

$$c_t = (c_0 - c_\infty)e^{-kt} + c_\infty \quad (1)$$

$$c_t = \frac{c_0 - c_\infty}{1 + |c_0 - c_\infty| kt} + c_\infty \quad (2)$$

For reaction half-time, the time required for the concentration to reach 50% of the difference between c_0 and c_∞, the modified equations provide the following expressions:

For first-order kinetics:

$$t_{1/2} = \frac{0{,}693}{k} \quad (3)$$

For second-order kinetics:

$$t_{1/2} = \frac{1}{k|C_0 - C_\infty|} \quad (4)$$

In the equations, c_t (mg kg^{-1}) is the concentration of phenolic compounds at a given time t, c_0 (mg kg^{-1}) represents the initial concentration at $t = 0$, while c_∞ (mg kg^{-1}) represents the final concentration of phenolic compounds at reaction endpoint. The constant k is the reaction rate constant (expressed as min^{-1} for first-order and kg mg^{-1} min^{-1} for second-order kinetics) t is time (min) and $t_{1/2}$ indicates the time required for the concentration to reach halfway between c_0 and $c\infty$.

Statistical Analysis

Chemical and enzymatic extraction of apple peel was conducted three times, and each extract was analyzed once with HPLC to obtain the phenolic compounds amount before simulated digestion (n=3). Simulated digestion of the fruit material was carried out in triplicate, and measurements during simulated digestion were performed once (n=3). Results are presented as mean values ± standard deviation. Statistical data analysis was performed using the Tukey post-hoc test with *Minitab* software (Minitab LLC., State College, PA, USA). Nonlinear regression of the kinetic equations was carried out using the Solver tool in MS Excel (Microsoft Corporation, Redmond, USA), minimizing the standard error (SE model) given by the equation:

$$SE = \sqrt{\frac{\sum(c_t - c_e)^2}{N - k}} \quad (5)$$

where (mg kg^{-1}) represents the measured concentration of phenolic compounds at a given time interval, (mg kg^{-1}) represents the concentration of phenolic compounds at a given time interval estimated by the kinetic model, N is the total number of measurements, and k is the number of parameters in the model.

RESULTS AND DISCUSSION

Individual Phenolic Compounds in the Apple Peel

Tables 1 and 2 present the amounts of phenolic compounds released from the peel of the traditional apple variety 'Kolačara', after the gastric and intestinal digestion phases, respectively, as well as the amounts determined before digestion. A total of 14 phenolic compounds were detected in the apple peel before digestion. Phenolic compounds found in the peel of 'Kolačara' belong to five subclasses of phenolic compounds –anthocyanins (13.2 mg kg^{-1}), flavan-3-ols (571.5 mg kg^{-1}), dihydrochalcones (406.6 mg kg^{-1}), phenolic acids (174.1 mg kg^{-1}), and flavonols (949.2 mg kg^{-1}). Phenolic compounds identified in the peel of apples are consistent with the literature, with similar amounts reported in previous studies (Giomaro et al., 2014; Kschonsek et al., 2019).

Table 1. Amounts of phenolic compounds (mg kg^{-1} FW) in the peel of the traditional apple variety 'Kolačara' before digestion and after different periods of gastric digestion

Tablica 1. Količine fenolnih spojeva (mg kg^{-1} s.t.) u pokožici jabuke tradicionalne sorte 'Kolačara' prije probave i nakon različitih perioda probave u želudcu

	Before Digestion */ Prije probave*	**10 min.**	**20 min.**	**30 min.**	**60 min.**	**120 min.**
Anthocyanins						
cyanidin-3-galactoside	13.2±3.6^{a}	4.6±1.2^{b}	4.2±0.8^{b}	5.3±0.4^{b}	4.0±0.8^{b}	4.8±0.3^{b}
total	13.2±3.6^{a}	4.6±1.2^{b}	4.2±0.8^{b}	5.3±0.4^{b}	4.0±0.8^{b}	4.8±0.3^{b}
Flavan-3-ols						
(+)-catechine	281.1±3.1^{a}	87.3±7.0^{e}	108.5 ±8.0^{d}	151.3±4.0^{b}	146.7±2.9$^{b.c}$	131.8±8.3^{c}
(-)epicatechine	166.8±9.6^{a}	67.0±1.9^{b}	80.0±6.0^{b}	76.2±2.2^{b}	70.7±4.1^{b}	70.0±0.4^{b}
procyanidine B1	123.6±5.1^{a}	44.3±2.5^{d}	46.1±0.3^{c}	56.0±1.3^{b}	54.6±0.8^{b}	52.0±1.0$^{b.c}$
total	571.5 11.4^{a}	200.6±11.4^{e}	234.6±9.7^{d}	283.5±7.0^{b}	271.9±4.7$^{b.c}$	253.9±8.9$^{c.d}$
Dihydrochalcones						
phloretine-2-glucoside	342.9±13.7^{a}	200.2±21.3^{c}	215.7±36.7$^{b.c}$	239.4±9.4^{b}	212.9±11.5$^{b.c}$	212.9±8.7$^{b.c}$
phloretin-2'-xyloglucoside	63.7±10.2^{a}	41.7±6.1$^{b.c}$	42.7±1.0^{b}	33.0±1.7$^{b.c}$	32.5±0.2^{c,d}	22.8±4.8^{d}
total	406.6±23.9^{a}	241.9±10.7$^{b.c}$	285.4±26.7$^{b.c}$	272.4±11.1$^{b.c}$	245.4±7.9$^{b.c}$	235.8±12.4^{c}
Phenolic acids						
chlorogenic acid	174.1±14.9^{a}	132.0±3.7^{b}	140.2±4.6^{b}	141.8±0.7^{b}	136.1±5.1^{b}	133.0±4.8^{b}
total	174.1±14.9^{a}	132.0±3.7$^{b.c}$	140.2 ±4.6$^{b.c}$	141.8±0.7$^{b.c}$	136.1±5.1$^{b.c}$	133.0±4.8$^{b.c}$
Flavonols						
quercetin-3-galactoside	169.6±10.6^{a}	117.4±34.8^{b}	115.8±27.3^{b}	135.4±2.5$^{a.b}$	120.6±13.4^{b}	131.8±15.8^{b}
quercetin-3-glucoside	309.7±17.8^{a}	115.4±28.4^{b}	128.2±0.7^{b}	126.7±1.8^{a}	114.5±13.3^{b}	129.2±11.9^{b}
quercetin derivative	165.6±8.7^{a}	64.6±1.4^{c}	72.5±18.0$^{b.c}$	79.9±4.8$^{b.c}$	79.1±3.5$^{b.c}$	76.8±7.5$^{b.c}$
quercetin-3-xyloside	218.7±8.9^{a}	112.9±44.9^{b}	111.5±51.4^{b}	150.3±1.7^{b}	133.3±9.4^{b}	135.5±10.7^{b}
quercetin-3-rhamnoside	85.6±3.6^{a}	38.2±7.3^{b}	41.8±11.4^{b}	46.8±1.1^{b}	41.2±3.1^{b}	41.2±2.2^{b}
total	949.2±40.7^{a}	448.6±82.6^{b}	469.9±76.1^{b}	539.1±2.4^{b}	488.8±30.2^{b}	514.5±47.8^{b}
TOTAL	2114.9±69.8^{a}	1027.7±88.8^{c}	1107.4±109.2$^{b.c}$	1242.1±16.9^{b}	1146.3±44.9$^{b.c}$	1142.1±65.8$^{b.c}$

Amounts are mean values of three parallel experiments measured once on HPLC (n = 3). The values in rows with different letters are significantly different according to the post-hoc Tukey test ($p < 0.05$).

Količine su srednje vrijednosti triju usporednih eksperimenata mjerenih jedanput na HPLC-u (n = 3). Vrijednosti u redu s različitim slovima značajno su drukčije prema Tukeyevu post-hoc *testu ($p < 0.05$)*

Table 2. Amounts of phenolic compounds (mg kg^{-1} FW) in the peel of the traditional apple variety 'Kolačara' before digestion and after different periods of intestinal digestion

Tablica 2. Količine fenolnih spojeva (mg kg^{-1} s.t.) u pokožici jabuke tradicionalne sorte 'Kolačara' prije probave i nakon različitih perioda probave u tankome crijevu

	Before digestion / *Prije probave*	**10 min**	**20 min**	**30 min**	**60 min**	**120 min**
Anthocyanins						
Cyanidin-3-galactoside	13.2±3.6^{a}	n.d.	n.d.	n.d.	n.d.	n.d.
total	13.2±3.6^{a}					
Flavan-3-ols						
(+)-catechine	281.1±3.1^{a}	n.d.	n.d.	n.d.	n.d.	n.d.
(-)epicatechine	166.8±9.6^{a}	n.d.	n.d.	n.d.	n.d.	n.d.
procyanidine B1	123.6±5.1^{a}	n.d.	n.d.	n.d.	n.d.	n.d.
total	571.5 11.4^{a}					
Dihydrochalcones						
phloretine-2-glucoside	342.9±13.7^{a}	193.7±16.1$^{b.c}$	192.2±5.4$^{b.c}$	168.5±5.1$^{d.e}$	182.2±2.0$^{b.c.d.e}$	155.0±5.5^{e}
phloretin-2'-xyloglucoside	63.7±10.2^{a}	6.1±3.8$^{c.d}$	7.2±0.9$^{c.d}$	17.9±5.8$^{b.c}$	n.d.	n.d
total	406.6±23.9^{a}	199.8±17.9^{b}	199.4±6.1^{b}	186.4±6.7$^{b.c}$	182.2±1.4$^{b.c}$	155.0±5.5^{c}
Phenolic acids						
chlorogenic acid	174.1±14.9^{a}	26.5±0.8$^{b.c}$	20.3±1.6$^{c.}$	15.9±0.4^{c}	37.4±0.8^{b}	24.0±0.1$^{b.c}$
total	174.1±14.9^{a}	26.5±0.8$^{b.c}$	20.3±1.6^{c}	15.9±0.4^{c}	37.4±0.8^{b}	24.0±0.1$^{b.c.}$
Flavonols						
quercetin-3-galactoside	169.6±10.6^{a}	90.6±5.8^{c}	89.1±6.1^{c}	74.7±0.5^{c}	114.0±5.0^{b}	78.6±2.8^{c}
quercetin-3-glucoside	309.7±17.8^{a}	82.4±1.0$^{b.c}$	85.8±5.2$^{b.c}$	76.8±8.8^{c}	102.4±5.2^{b}	73.7±3.6^{c}
quercetin derivative	165.6±8.7^{a}	67.1±4.3$^{b.c}$	62.0±3.1$^{c.d}$	54.8±2.2$^{d.e}$	76.9±1.9^{b}	47.4±1.9^{e}
quercetin-3-xyloside	218.7±8.9^{a}	112.0±5.7$^{b.c}$	106.1±4.9$^{b.c.d}$	93.7±1.8^{d}	123.9±0.7^{b}	98.0±3.8$^{c.d}$
quercetin-3-rhamnoside	85.6±3.6^{a}	34.0±2.6^{b}	34.4±0.9^{b}	32.8±4.9^{b}	36.9±0.4^{b}	36.8±1.7^{b}
total	949.2±40.7^{a}	386.1±13.5^{c}	377.4±18.4^{c}	332.9±14.0^{c}	454.1±9.6^{b}	334.4±11.0^{c}
TOTAL	2114.9±69.8^{a}	618.2±39.4$^{b.c}$	597.1±17.7$^{b.c.d}$	535.3±19.5$^{c.d}$	673.7±10.6^{b}	513.4±13.6^{d}

Amounts are mean values of three parallel experiments measured once on HPLC (n = 3). The values in rows with different letters are significantly different according to the post-hoc Tukey test ($p < 0.05$).
n. d. – not detected

Količine su srednje vrijednosti triju usporednih eksperimenata mjerenih jedanput na HPLC-u (n = 3). Vrijednosti u redu s različitim slovima značajno su drukčije prema Tukeyevu post-hoc *testu ($p < 0.05$)*
n. d. – nije detektirano

The Release of Phenolic Compounds in the Gastric Digestion

The amount of total and individual phenolic compounds released in the first 10 minutes of gastric digestion of apple peel was statistically significantly lower compared to the number of phenolic compounds present in the apple peel before digestion ($p < 0.05$; Table 1). Similarly, the amounts of total and individual phenolic compounds at the end point of gastric digestion (120 minutes) were lower than the amounts before digestion ($p < 0.05$). This is in accordance with our previous work (Jakobek et al., 2023), as well as the work of other researchers (Bouayed et al., 2012; Fernández-Jalao et al., 2020).

During the subsequent gastric digestion period (10 - 120 minutes), the amount of individual and total phenolic compounds did show some fluctuations but generally remained statistically similar to that released in the initial 10 minutes (Table 1). This stability in phenolic compound levels throughout the gastric digestion process suggests that anthocyanins, flavan-3-ols, dihydrochalcones, phenolic acids, and flavonols exhibited resilience to gastric conditions, which is consistent with prior research. For example, anthocyanins in blueberries showed good stability during gastric digestion, with 54 % recovery (Jiao et al., 2018). The stability of flavan-3-ols was noted during the gastric digestion simulation of the traditional Italian apple variety 'Annurca' and four commercial apple varieties (Tenore et al., 2013). Similarly, dihydrochalcones have exhibited good stability under acidic conditions (Bouayed et al., 2012; Tenore et al., 2013; Tarko et al., 2020), suggesting that they reach the small intestine without forming the corresponding aglycone (phloretin). Bouayed et al. (2012) simulated the digestion of four commercial apple varieties and observed good stability of chlorogenic acid in the stomach. Tenore et al. (2013) monitored the quantities of quercetin-3-rutinoside in five different apple varieties after digestion in the mouth, stomach, and small intestine. They proposed the resistance of quercetin glycosides to

hydrolysis in the stomach and their delivery to the small intestine in their initial form.

Release of Phenolic Compounds in Intestinal Digestion

Similar to the gastric digestion phase, the amounts of individual and total phenolic compounds quantified after 10 minutes of intestinal digestion were statistically significantly lower than the amounts present in apple peels before digestion ($p < 0.05$) (Table 2). Anthocyanins and flavan-3-ols were not detected in the intestinal phase. The amount of other individual and total phenolic compounds did show some fluctuations during 120 min of digestion, but generally remained statistically similar to that released in the initial 10 minutes.

The absence of detectable anthocyanins during and after intestinal digestion of apple peel has also been reported before (Jakobek et al., 2023). This may result from the pH increase in the simulated intestinal environment. Anthocyanins can exist in various forms depending on pH: as flavylium cations (pH 1-3) in the stomach, transitioning to carbinol pseudo-base (pH 4-5), quinoidal base (pH 7-8), or chalcones (pH 12). In the small intestine, anthocyanins mainly exist as quinoidal base, which may pose a challenge for their determination (Pérez-Vicente et al., 2002).

Flavan-3-ols were another subclass of phenolic compounds that were not detected after transition from gastric to intestinal digestion. Several studies observed a reduction or complete disappearance of flavan-3-ols after simulated small intestine digestion (Bouayed et al., 2012; Annunziata et al., 2018; Fernández-Jalao et al., 2020; Sousa et al., 2021). Bouayed et al. (2012) suggested that the disappearance of flavan-3-ols in the small intestine may be due to degradation into unknown products caused by the transition from the acidic environment of the stomach to the slightly alkaline environment of the small intestine, as well as the possible action of pancreatin and bile salts. Similarly, Kahle et al. (2011) reported complete degradation of procyanidin B2 during incubation with simulated small intestine fluid, suggesting that chemical conditions, rather than enzymatic activity, were responsible for these changes. Other authors have attributed the poor recovery of flavan-3-ols to neutral conditions in simulated intestinal digestion, favoring their epimerization and auto-oxidation (Annunziata et al., 2018).

Dihydrochalcones were detected throughout the entire intestinal digestion. Their amounts at the end of intestinal digestion (120 min) were lower than those measured at the beginning (10 min), and this decrease was statistically significant ($p < 0.05$) (Table 2). In our previous study, phloretin-2-glucoside, found in apple peel, was not detected at the end of intestinal digestion (Jakobek et al., 2023). Similar can be observed for phloretin-2-xyloglucoside in this study, but not for phloretin-2'-glucoside. This might be due to higher initial amounts of phloretin-2-glucoside in this study (343 mg kg^{-1} *vs* 42 mg kg^{-1} (Jakobek et al., 2023)). Other studies have also observed lower amounts of dihydrochalcones after small intestine digestion compared to gastric digestion (Tarko et al., 2020). The hydrolysis of phloretin glycosides into phloretin under mildly alkaline conditions has been proposed as the main reason for the decrease in dihydrochalcone content in the small intestine (Tarko et al., 2020). However, phloretin was not detected in our samples, which may indicate a lower degree of glycoside degradation under the applied digestion conditions. Some studies simulating apple digestion in the small intestine have shown an increase in dihydrochalcone amount (Bouayed et al., 2012; Tenore et al., 2013; Fernández-Jalao et al., 2020).

The amounts of phenolic acids (chlorogenic acid) remained somewhat stable (Table 2). A similar trend was observed in our previous study (Jakobek et al., 2023). In a recent study, chlorogenic acid from apples exhibited isomerization into neochlorogenic and cryptochlorogenic acid during digestion in the oral and intestinal phases, as well as in simulated salivary and intestinal fluid electrolyte solutions, suggesting a dynamic transformation process (Jakobek et al.,2024).

The amount of total and individual flavonols during further intestinal digestion was generally stable, with some fluctuations that were generally not statistically significant (Table 2). This is in accordance with our previous study (Jakobek et al., 2023), as well as other studies that showed good stability of quercetin glycosides upon intestinal digestion simulation (Bouayed et al., 2012; Tenore et al., 2013; Fernández-Jalao et al., 2020). Bouayed et al. (2012) and Jakobek et al. (2021) did not detect quercetin aglycone after intestinal digestion simulation, suggesting that quercetin glycosides might be resistant to hydrolysis under mildly alkaline conditions of the small intestine.

This study's results, along with the cited literature, indicate that dihydrochalcones and flavonols are more stable in a simulated intestinal environment and could potentially be available for absorption in relatively large amounts. Conversely, anthocyanins and flavan-3-ols might be more susceptible to degradation under these conditions, while chlorogenic acid might exhibit isomerization into other phenolic acids.

Kinetics of Phenolic Compounds Release from Apple Peel During Gastric and Intestinal Digestion

Total phenolic compounds released during simulated gastric and intestinal digestion (c_t) over time (t) were analyzed using nonlinear regression with modified first-order and second-order equations. Both models adequately fit the experimental data (Figure 1), but the standard errors were slightly lower for the first-order model (75.9 *vs.* 85.1 for gastric digestion and 63.0 *vs.* 70.0 for intestinal digestion). Similarly, our prior research concluded that the first-order model provided lower standard errors and better correlation with experimental data compared to the second-order model (Jakobek et al., 2023). Previous studies have also employed first-order kinetics to describe the release of phenolic compounds (Villanueva-Carvajal et al., 2013) and starch (Zhang et al., 2021) during *in vitro* digestion. Thus, due to its higher accuracy and better correlation with experimental data, the first-order model was selected for further analysis.

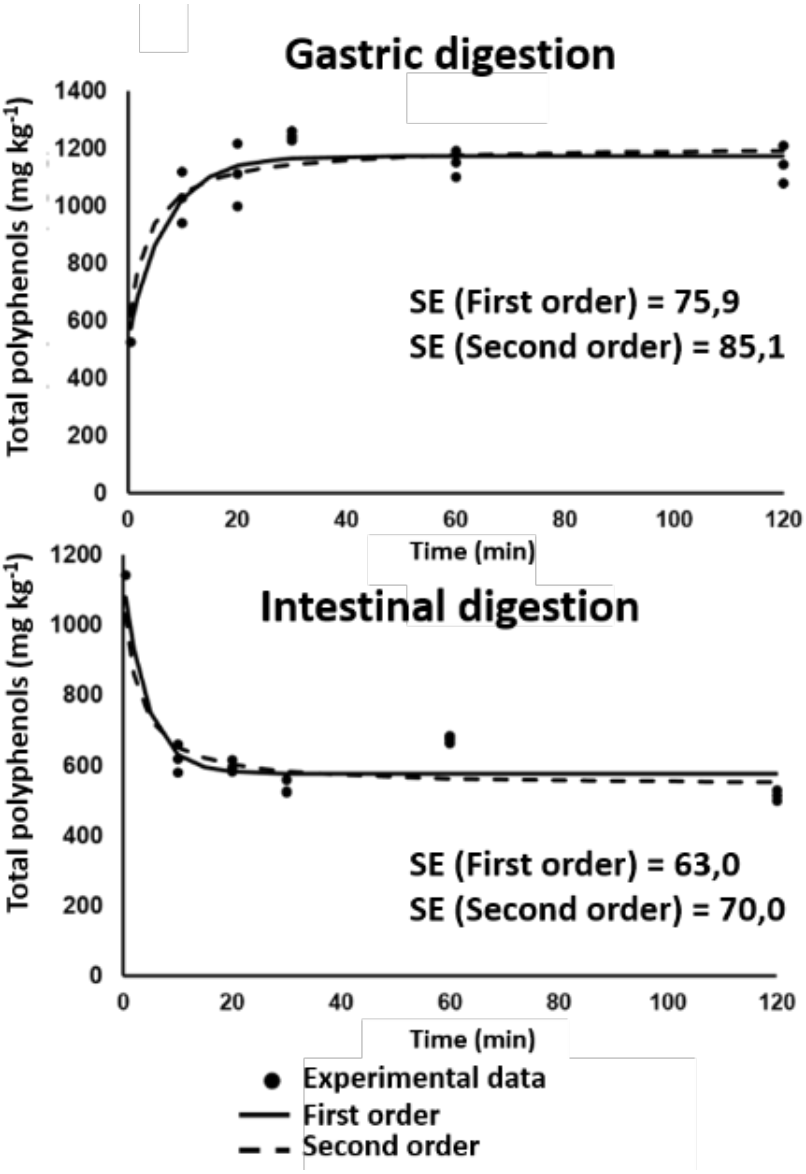

Figure 1. Modeling of experimental data with modified first-order and second-order equations.
Grafikon 1. *Modeliranje eksperimentalnih podataka s modificiranim jednadžbama prvoga i drugog reda.*

The parameters of the first-order reaction for phenolic compounds released during simulated digestion of apple peel are presented in Table 3. Based on predictions for c_∞, the amounts of phenolic subgroups released after gastric digestion can reach 4.6 to 509.0 mg kg^{-1}. The predicted amounts depend on the specific subgroup analyzed and the apple variety. For the total phenolic compounds, the model predicted c_∞ to reach 1170.5 mg kg^{-1}. In the intestinal digestion, the first-order model predicted c_∞ values for phenolic subgroups from 23.7 to 374.1 mg kg^{-1}. The model predicted the release of total phenolic compounds of 576.8 mg kg^{-1}. In both gastric and intestinal digestion, the amounts of total phenolic compounds and subgroups predicted by the model (c_∞) were similar to those determined experimentally (c_{exp}).

The half-life values ($t_{1/2}$) for total phenolic compounds and their subgroups are presented in Table 3. For gastric digestion, the model predicted $t_{1/2}$ values from 2.3 to 7.9 min. The predicted amounts depend on the specific subgroup or total phenolics. These results suggest that most phenolic compounds are released from apple peel within the first few minutes of gastric digestion, aligning with our previous findings (Jakobek et al., 2023). For intestinal digestion, the model predicted $t_{1/2}$ values from 2.4 to 31.1 min. Dihydrochalcones showed the highest half-life values (31.1 min), suggesting they may be more resistant to intestinal digestion compared to other phenolic compounds. This resistance could indicate slower degradation or release rates, which might influence their bioaccessibility.

Table 3. Parameters of first-order reaction for phenolic compound release during simulated digestion of apple peel

Tablica 3. Parametri reakcije prvoga reda za oslobađanje fenolnih spojeva tijekom simulirane probave pokožice jabuke

	Gastric digestion / *Želučana probava*					**Intestinal digestion** / *Crijevna probava*				
	c_{exp} (mg kg^{-1})	c_{∞} (mg kg^{-1})	k (min^{-1})	$t_{1/2}$ (min)	SE*	c_{exp} (mg kg^{-1})	c_{∞} (mg kg^{-1})	K (min^{-1})	$t_{1/2}$ (min)	SE*
Total polyphenols	1142.1	1170.5	0.148	4.7	75.9	513.4	576.8	0.228	3.0	62.3
Anthocyanins	4.8	4.6	0.305	2.3	0.7					
Flavan-3-ols	253.9	269.6	0.087	7.9	16.1					
Dihydrochalcones	235.8	253.3	0.234	2.9	18.6	155.0	154.4	0.022	31.1	10.4
Phenolic acids	132.9	137.9	0.222	3.1	4.6	24.0	23.7	0.288	2.4	8.6
Flavonols	514.5	509.0	0.155	4.5	52.6	334.4	374.1	0.105	6.6	51.5

*SE – Standard error

c_{exp} – experimentally measured concentration of phenolic compounds at the end of digestion; c_{∞} – concentration predicted by the kinetic model at the reaction's endpoint; k – reaction rate constant; $t_{1/2}$ – reaction half-time, representing the time required for the concentration to reach 50% of the difference between initial (c_0) and final (c_{∞}) concentration (min)

**SE – standardna pogreška*

C_{exp} – eksperimentalno izmjerena koncentracija fenolnih spojeva na kraju probave; C_{∞} – koncentracija na kraju reakcije predviđena kinetičkim modelom; k – konstanta brzine reakcije; $t_{1/2}$ – poluvrijeme reakcije, predstavlja vrijeme potrebno da koncentracija dosegne 50 % razlike između početne (C_0) i krajnje (C_{∞}) koncentracije (min)

CONCLUSION

This study investigated the release and stability of phenolic compounds from the peel of the traditional apple variety 'Kolačara' during in vitro gastrointestinal digestion. All major phenolic subgroups were identified, with dihydrochalcones, phenolic acids, and flavonols showing greater stability than anthocyanins and flavan-3-ols. Non-linear regression using modified first- and second-order kinetic models effectively described the release behavior, with the first-order model providing a slightly better fit to the experimental data. Most phenolic compounds were released or degraded within the first 5–10 minutes of each digestive phase, whereas dihydrochalcones demonstrated higher half-life values, indicating greater resistance to degradation. These findings contribute to a better understanding of phenolic compound behavior during digestion and highlight the value of traditional apple varieties as a source of bioactive compounds. However, the study used an in vitro model which has limitations, so future research should include colonic fermentation or in vivo studies to confirm these results.

AKNOWLEDGEMENT

This research was funded by the Croatian Science Foundation, grant number HRZZ-IP-2016-06-6777.

REFERENCES

1. Abbas, M., Saeed, F., Anjum, F. M., Afzaal, M., Tufail, T., Bashir, M. S., Ishtiaq, A., Hussain, S., & Suleria, H. A. R. (2017). Natural polyphenols: An overview. *International Journal of Food Properties*, *20*, 1689–1699. https://doi.org/10.1080/10942912.2016.1220393
2. Annunziata, G., Maisto, M., Schisano, C., Ciampaglia, R., Daliu, P., Narciso, V., Tenore, G., & Novellino, E. (2018). Colon bioaccessibility and antioxidant activity of white, green and black tea polyphenols extract after *in vitro* simulated gastrointestinal digestion. *Nutrients*, *10*(11), 1711. https://doi.org/10.3390/nu10111711
3. Asma, U., Morozova, K., Ferrentino, G., & Scampicchio, M. (2023). Apples and apple by-products: Antioxidant properties and food applications. *Antioxidants*, *12*(7), 1456. https://doi.org/10.3390/antiox12071456
4. Bergantin, C., Maietti, A., Cavazzini, A., Pasti, L., Tedeschi, P., Brandolini, V., & Marchetti, N. (2017). Bioaccessibility and HPLC-MS/MS chemical characterization of phenolic antioxidants in Red Chicory (*Cichorium intybus*). *Journal of Functional Foods*, *33*, 94–102. https://doi.org/10.1016/j.jff.2017.02.037
5. Bouayed, J., Deußer, H., Hoffmann, L., & Bohn, T. (2012). Bioaccessible and dialysable polyphenols in selected apple varieties following *in vitro* digestion vs. their native patterns. *Food Chemistry*, *131*(4), 1466–1472. https://doi.org/10.1016/j.foodchem.2011.10.030
6. Fernández-Jalao, I., Balderas, C., Sánchez-Moreno, C., & De Ancos, B. (2020). Impact of an *in vitro* dynamic gastrointestinal digestion on phenolic compounds and antioxidant capacity of apple treated by high-pressure processing. *Innovative Food Science & Emerging Technologies*, *66*, 102486. https://doi.org/10.1016/j.ifset.2020.102486
7. Giomaro, G., Karioti, A., Bilia, A. R., Bucchini, A., Giamperi, L., & Ricci, D. (2014). Polyphenols profile and antioxidant activity of skin and pulp of a rare apple from Marche region (Italy). *Chemistry Central Journal*, *8*, 45. https://doi.org/10.1186/1752-153x-8-45
8. Jakobek, L., Ištuk, J., Buljeta, I., Voća, S., Žlabur, J. Š., & Babojelić, M. S. (2020). Traditional, indigenous apple varieties, a fruit with potential for beneficial effects: Their quality qraits and bioactive polyphenol contents. *Foods*, *9*(1), 52. https://doi.org/10.3390/foods9010052
9. Jakobek, L., Ištuk, J., Barron, A. R., & Matić, P. (2023). Bioactive phenolic compounds from apples during simulated *in vitro* gastrointestinal digestion: Kinetics of their release. *Applied Sciences*, *13*(14), 8434. https://doi.org/10.3390/app13148434

10. Jakobek, L., Pöc, K., Valenteković, M., & Matić, P. (2024). The behavior of phenolic compounds from apples during simulated gastrointestinal digestion with focus on chlorogenic acid. *Foods*, *13*(5), 693. https://doi.org/10.3390/foods13050693

11. Jiao, X., Li, B., Zhang, Q., Gao, N., Zhang, X., & Meng, X. (2018). Effect of *in vitro* simulated gastrointestinal digestion on the stability and antioxidant activity of blueberry polyphenols and their cellular antioxidant activity towards HepG2 cells. *International Journal of Food Science & Technology*, *53*(1), 61–71. https://doi.org/10.1111/ijfs.13516

12. Kahle, K., Kempf, M., Schreier, P., Scheppach, W., Schrenk, D., Kautenburger, T., Hecker, D., Huemmer, W., Ackermann, M., & Richling, E. (2011). Intestinal transit and systemic metabolism of apple polyphenols. *European Journal of Nutrition*, *50*, 507–522. https://doi.org/10.1007/s00394-010-0157-0

13. Kschonsek, J., Wiegand, C., Hipler, U.-C., & Böhm, V. (2019). Influence of polyphenolic content on the *in vitro* allergenicity of old and new apple cultivars: A pilot study. *Nutrition*, *58*, 30–35. https://doi.org/10.1016/j.nut.2018.07.001

14. Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., Carrière, F., Boutrou, R., Corredig, M., Dupont, D., Dufour, C., Egger, L., Golding, M., Karakaya, S., Kirkhus, B., Le Feunteun, S., Lesmes, U., Macierzanka, A., Mackie, A., Marze, S., McClements, D. J., Ménard, O., Recio, I., Santos, C. N., Singh, R. P., Vegarud, G. E., Wickham, M. J., Weitschies, W., & Brodkorb, A. (2014). A standardised static *in vitro* digestion method suitable for food – an international consensus. *Food & Function*, *5*(6), 1113–1124. https://doi.org/10.1039/c3fo60702j

15. Nagar, E. E., Okun, Z., & Shpigelman, A. (2019). Digestive fate of polyphenols: Updated view of the influence of chemical structure and the presence of cell wall material. *Current Opinion in Food Science*, *31*, 38–46. https://doi.org/10.1016/j.cofs.2019.10.009

16. Pérez-Vicente, A., Gil-Izquierdo, A., & García-Viguera, C. (2002). *In vitro* gastrointestinal digestion study of pomegranate juice phenolic compounds, anthocyanins, and vitamin C. *Journal of Agricultural and Food Chemistry*, *50*(8), 2308–2312. https://doi.org/10.1021/jf0113833

17. Rana, A., Samtiya, M., Dhewa, T., Mishra, V., & Aluku, R. E. (2022). Health benefits of polyphenols: A concise review. *Journal of Food Biochemistry*, *46*(10), e14204. https://doi.org/10.1111/jfbc.14264

18. Sousa, M. M., Lima, R. M. T., Lima, A., Reis, A. C., Cavalcante, A. A. C. M., Sattler, J. A. G., Almeida-Muradian, L. B., Lima Neto, J. S., Moreira-Araujo, R. S. R., & Nogueira, N. N. (2021). Antioxidant action and enzyme activity modulation by bioaccessible polyphenols from jambolan (*Syzygium cumini* (L.) Skeels). *Food Chemistry*, *363*, 130353. https://doi.org/10.1016/j.foodchem.2021.130353

19. Tarko, T., Duda-Chodak, A., & Soszka, A. (2020). Changes in phenolic compounds and antioxidant activity of fruit musts and fruit wines during simulated digestion. *Molecules*, *25*(23), 5574. https://doi.org/10.3390/molecules25235574

20. Tenore, G. C., Campiglia, P., Ritieni, A., & Novellino, E. (2013). *In vitro* bioaccessibility, bioavailability and plasma protein interaction of polyphenols from Annurca apple (*M. pumila Miller cv Annurca*). *Food Chemistry*, *141*(4), 3519–3524. https://doi.org/10.1016/j.foodchem.2013.06.051

21. Villanueva-Carvajal, A., Bernal-Martínez, L. R., García-Gasca, M. T., & Dominguez-Lopez, A. (2013). *In vitro* gastrointestinal digestion of *Hibiscus sabdariffa* L.: The use of its natural matrix to improve the concentration of phenolic compounds in gut. *LWT - Food Science and Technology*, *51*(1), 260–265. https://doi.org/10.1016/j.lwt.2012.10.007

22. Zhang, H., Li, Z., Zhang, L., Lai, P. F. H., Tian, Y., Cui, S. W., & Ai, L. (2021). Effects of soluble dietary fibers on the viscosity property and digestion kinetics of corn starch digesta. *Food Chemistry*, *338*, 127825. https://doi.org/10.1016/j.foodchem.2020.127825

KINETIKA OSLOBAĐANJA FENOLNIH SPOJEVA IZ KOŽICE TRADICIONALNE SORTE JABUKE 'KOLAČARA' TIJEKOM *IN VITRO* GASTROINTESTINALNE PROBAVE

SAŽETAK

Pokožica autohtonih sorata jabuka predstavlja bogat izvor fenolnih spojeva s potencijalnim zdravstvenim koristima. U ovome radu istraživana je kinetika oslobađanja fenolnih spojeva iz pokožice jabuke tradicionalne sorte 'Kolačara' tijekom simulirane gastrointestinalne probave primjenom modificirane kinetičke jednadžbe prvoga reda. Tijekom probave, antocijani i flavan-3-oli nisu detektirani nakon intestinalne faze, dok su dihidrohalkoni, fenolne kiseline i flavonoli pokazali veću stabilnost. Kinetička analiza pokazala je da oslobađanje slijedi kinetiku prvoga reda. Tijekom želučane faze, poluvrijeme života kretalo se od 2,3 do 7,9 minuta, što ukazuje da se većina fenolnih spojeva oslobodi unutar prvih 10 minuta probave. U intestinalnoj fazi poluvrijeme života iznosilo je do 2,4, 6,6 i 31,1 minutu za fenolne kiseline, flavonole i dihidrohalkone. Ovo istraživanje sugerira brzo oslobađanje fenola iz pokožice tradicionalne sorte jabuke, pri čemu dihidrohalkoni pokazuju najveću otpornost na intestinalnu razgradnju. Ovi rezultati pružaju uvid u probavno ponašanje bioaktivnih spojeva iz vrijednih autohtonih izvora voća.

Ključne riječi: jabuka, pokožica, fenolni spojevi, probava, kinetika

(*Received on October 10, 2025; accepted on October 30, 2025* – Primljeno 10. listopada 2025.; prihvaćeno 30. listopada 2025.)