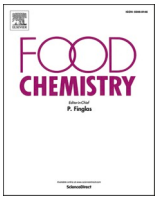
Food Chemistry 463 (2025) 141402

Contents lists available at ScienceDirect

Food Chemistry

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



The role of processing on phenolic bioaccessibility and antioxidant capacity of apple derivatives

Marilisa Alongi [a], Umberto Lanza [a], Andrea Gorassini [b], Giancarlo Verardo [a], Clara Comuzzi [a], Monica Anese [a], Lara Manzocco [a,*], Maria Cristina Nicoli [a]

[a] Department of Agriculture, Food, Environment and Animal Sciences, University of Udine, Italy
[b] Department of Humanities and Cultural Heritage, University of Udine, Italy

ARTICLE INFO

Keywords:
Apple pulp
High-speed homogenization
High-pressure homogenization
In vitro digestion
Polyphenols
Polyphenol oxidase
Radical scavenging capacity

ABSTRACT

Fruit derivatives are commonly obtained by applying processing operations deemed responsible for the loss of phenol compounds, but very little information is available on the fate of phenols upon digestion of these products. The present study evaluated the effect of thermal and mechanical treatments, commonly applied to turn apple pulp into puree and homogenate, on phenolic bioaccessibility and antioxidant activity.

Despite a 20 % decrease in polyphenols due to processing, their bioaccessibility was higher in apple derivatives (>20 %) compared to pulp (~2 %). Polyphenol oxidase (PPO), inactivated by thermal treatments in apple derivatives but not in the pulp, was hypothesized to be responsible for this difference. Results acquired on an unprocessed PPO-free apple model, only featuring quercetin-3-glucoside and pectin, actually exhibited similar bioaccessibility as processed derivatives.

The radical scavenging capacity was unaffected by the structural integrity of apples, indicating independence from the plant tissue's hierarchical arrangement. After digestion, radical scavenging capacity decreased in the real apple matrices, correlating with phenolic content, while it was retained in the apple model, further suggesting the pivotal food matrix role in modulating polyphenols bioaccessibility and subsequent biological activity.

Translating these results to an industrial scale, processing conditions can be optimized not only to guarantee that the quality requirements are met, but also to achieve desired nutritional benefits.

1. Introduction

Strong scientific evidence suggests that regular consumption of fruits and vegetables is negatively associated with the risk of developing chronic diseases. Such positive health effects are attributed to the biological activity of several naturally contained non-nutrient molecules, including dietary fibre, carotenoids and phenolic compounds (Zhu et al., 2018). The latter are the most important and large group of bioactive compounds, almost ubiquitous in plants. The common molecular trait of all phenolic compounds is the presence of one or more aromatic rings, with at least one hydroxyl group. They can be simple molecules with one phenol, such as phenolic acids, or complex structures with two or multiple phenol groups, such as stilbenes and flavonoids. In addition, their native form is often polymerized or conjugated with sugars or organic acids giving rise to a variety of glycosides (Rice-Evans et al., 1996). The peculiar structures of phenolic compounds are claimed responsible for a variety of biological activities including antioxidant, anti-inflammatory, and immunoregulatory activities (Arulselvan et al., 2016; Azab et al., 2016).

Despite phenol-rich fruit and vegetables are often consumed fresh, they are also subjected to several processing operations to obtain convenient derivatives (*e.g.,* purees, juices, snacks). These operations are expected to influence phenolic profile and bioactivity upon digestion. However, to the best of our knowledge, this matter has not been explored yet and there is no clear answer to the following research question: what is the effect of fruit and vegetable processing on the fate of their phenolic compounds upon digestion?

To exert their biological activity, phenolic compounds must be released from the vacuoles of plant cells into the gastrointestinal tract, absorbed by the brush border membrane, and transferred to the bloodstream (Al-Khayri et al., 2023). To this regard, bioaccessibility indicates the fraction of polyphenols that is released from the plant

* Corresponding author.
E-mail address: lara.manzocco@uniud.it (L. Manzocco).

https://doi.org/10.1016/j.foodchem.2024.141402
Received 27 June 2024; Received in revised form 17 September 2024; Accepted 21 September 2024
Available online 23 September 2024

matrix during digestion and becomes available for absorption in the small intestine (Ferruzzi, 2010). Conversely, bioavailability refers to the fraction that is effectively absorbed, distributed by the circulatory system and subjected to metabolism and elimination (Stahl et al., 2002). Scientific evidence regarding the bioaccessibility and bioavailability of dietary polyphenols, despite a general agreement on the fact that both are very low, is sometimes controversial (D'Archivio et al., 2010). The low bioaccessibility can be attributed to several factors such as polyphenol chemical structure, matrix interactions and applied technological operations (Arfaoui, 2021). The majority of polyphenols found in fruit and vegetables exist in glycosylated forms which need to be hydrolysed before absorption at the brush border membrane of the small intestine (D'Archivio et al., 2010). However, most polyphenols transit through the gastrointestinal tract without being metabolised, thereby entering the large intestine where they are metabolised by the gut microbiota into compounds that are effectively absorbed (Aura et al., 2005; Setchell et al., 2002). For example, this is the case of daidzein, a glycosylated polyphenol which is metabolised by gut microbiota into the active metabolite equol (Mayo et al., 2019). It must be pointed out that, being polyphenols strong antioxidants, even when not absorbed, they may contribute to the maintenance of the redox balance in the gastrointestinal tract (Garbetta et al., 2018).

The plant matrix may exert different and opposite effects on polyphenol bioaccessibility. For instance, by acting as a physical barrier, intact cells can hinder polyphenol release in the gut. In addition, the ability of polyphenols to interact with other components of the cellular matrix, such as polysaccharides, proteins and lipids, through covalent or hydrogen bonds, or hydrophobic forces, could hamper their release in the gastrointestinal tract (Jakobek, 2015). On the other hand, the plant matrix represents a useful barrier to protect polyphenols from endogenous oxidative enzymes, chemical oxidation and degradation induced by the harsh conditions experienced during the transit through the gastrointestinal tract (Quan et al., 2018). Based on these considerations, any technological intervention affecting the plant matrix is also expected to affect polyphenol bioaccessibility. For instance, processing might mechanically damage the cellular structure and the bonds between phenolic compounds and matrix macromolecules might be cleaved, favouring polyphenol release. Processing can also induce phenolic oxidation and/or hydrolysis of glycosylated forms (Arfaoui, 2021). Similarly, thermal treatments may be responsible for polyphenol degradation but at the same time they can enhance phenol release by impairing cellular integrity. Indeed, the polyphenol bioaccessibility in thermally treated fruit and vegetable derivates as compared to that of unprocessed ones results from the balance between the compounds that have been lost upon processing and those that have been released from the matrix in their intact form (Parada & Aguilera, 2007). The overall outcome mainly depends on the intensity and nature of the technological operations applied to the plant tissue. Moreover, the extent to which processing affects phenolic compounds depends on the protective effect that the matrix may exert but also on the chemical properties and thermal stability of the compounds themselves.

Although the bioaccessibility of polyphenols has gained popularity in the research community during the last decade, up to now, available data are insufficient to depict the effect of processing on plant tissue integrity and consequently on polyphenol bioaccessibility (D'Archivio et al., 2010).

In the case of apple derivatives, such as puree and homogenate, apple pulp is first subjected to blanching, a thermal treatment aimed at inactivating endogenous enzymes causing browning, such as polyphenol oxidase (PPO) (Beveridge & Weintraub, 1995). Following, the pulp is mechanically disintegrated. The derivative undergoes then another thermal treatment, *i.e.*, pasteurization, to inactivate the target alternative microorganism (Silva & Gibbs, 2001). Although these operations only slightly affect apple phenolic content (Alongi et al., 2023), they could play a role in determining the fate of polyphenols in the gastrointestinal tract. In unprocessed apple matrix instead, degradative events caused by enzymes may still proceed even during gastrointestinal events, possibly leading to substantial differences in the bioaccessibility of phenolic compounds.

In light of these considerations, the present study aims to evaluate the effect of some technological operations, commonly applied to obtain apple derivatives, on phenolic bioaccessibility. To this purpose, puree and homogenate obtained by mechanical disintegration of apple pulp through high-speed (HSH) or high-pressure (HPH) homogenization were considered. These processes were selected since they have been demonstrated to induce a different extent of cellular matrix disintegration (Alongi et al., 2023), leading to different polyphenol release. The polyphenol bioaccessibility of fresh apple pulp, apple puree and apple homogenate were then evaluated. The phenolic profile and concentration, as well as the overall antioxidant activity, were measured before and after *in vitro* digestion. Finally, with the purpose of better elucidating the mechanisms involved in determining polyphenol bioaccessibility, the same analyses were also conducted on highly simplified model systems containing quercetin-3-glucoside, selected as one of the most representative phenolic compounds in apple (Lee & Mitchell, 2012), and pectin, chosen as the main soluble dietary fibre fraction in apple (Du et al., 2022). Although these models were very simplistic in simulating apple derivatives, they allowed building an understanding about the impact of processing on phenolic bioaccessibility in a complex matrix, which currently represents a knowledge gap.

2. Material and methods

2.1. Chemicals

Methanol (MeOH), ethanol (EtOH), dimethyl sulfoxide (DMSO), 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$), formic acid (HCOOH), (+)-catechin, (−)-epicatechin, chlorogenic acid, phloridzin, quercetin-3-*O*-glucoside (Q-3-G), 3-hydroxycinnamic acid (internal standard; IS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), α-amylase from *Bacillus spp.*, porcine pepsin, porcine pancreatin, porcine bile extract, HCl, NaOH, $CaCl_2(H_2O)_2$, Na_2CO_3, $NaHCO_3$, NaCl, KCl, $MgCl_2(H_2O)_6$, $(NH_4)_2CO_3$, $MgSO_4$, resin Dowex® 50 W X8 were purchased from Sigma-Aldrich (Milan, Italy). Procyanidin B2 was obtained from ExtraSynthese (Lyon, France). Quercetin-3-O-arabinoside, quercetin-3-O-xyloside and quercetin-3-O-rhamnoside were purchased from Carbosynth (Berkshire, UK). Milli-Q grade water was produced by Elgastat UHQ-PS system (ELGA, High Wycombe Bucks, UK). Solid phase extraction (SPE) columns ISOLUTE C18, 1 g, 6 mL were from Biotage (Milan, Italy).

2.2. Preparation of apple derivatives

Freshly harvested apples (*Malus domestica* Borkh., cv. Golden Delicious) were provided by a local seller (Friulfruct, Spilimbergo, Italy).

Apple puree and homogenate were prepared as described by Alongi et al. (2023). After washing and peeling, 5-mm thick slices were obtained from the pulp, sealed in polyamide-polyethene pouches under vacuum (Combivac, Modena, Italy), blanched in boiling water for 3 min to inactivate PPO and cooled to 20 °C in an ice-water slurry (Beveridge & Weintraub, 1995).

Apple puree was obtained by subjecting the blanched apple pulp to high-speed homogenization (HSH) at 5000 rpm for 30 s (T 18 digital ULTRA-TURRAX®, IKA, Milan, Italy).

To produce the apple homogenate, the blanched apple pulp was first homogenized at high speed at 20,000 rpm for 15 min and then subjected to 3-pass high-pressure homogenization (HPH), at 20, 30, and 50 MPa respectively (GEA Lab Homogenizer PandaPLUS 2000, GEA Group, Düsseldorf, Germany).

The puree and the homogenate were than pasteurized at 100 °C for 6 min to obtain 2 Log reduction of *Alicyclobacillus acidoterrestris* (D_{90} =

7.4 min, z = 12 °C).

2.3. Pectin extraction from apple pulp

Pectin was extracted from commercial Golden delicious apples according to a slightly modified version of the method proposed by Westerlund et al. (1991). Apples were peeled, sliced, and a 100 g aliquot was transferred in 150 mL of an 80:20 (*v*/v) EtOH-H_2O solution preheated at 75 °C. Apple pulp was mashed with a high-speed mixer (MaxoMixx, BOSCH, Milano, Italy) for 4 min. A second homogenization step was performed with a high-speed homogenizer (Polytron PT-MR3000, Kinematica AG, Littau, Switzerland) at 20,000 rpm for 5 min. The sample was added with 450 mL 80:20 (v/v) EtOH-H_2O solution, refluxed for 2 h at 90 °C and then cooled to room temperature. The suspension was sieved (mesh size 0.8 mm), and the retentate was collected and dried in a thermostated oven (60 °C, 2 h).

The dried sample was then dissolved in Milli-Q water (250 mL) and refluxed for 1 h at 100 °C and cooled at room temperature. The suspension was filtered through cotton wool and passed through a resin pad (Dowex® 50 W X8). Absolute ethanol was added to the flowthrough until 80 % concentration was reached to allow pectin precipitation and the mixture was kept overnight at 4 °C. The precipitate was collected by filtration (Whatman no. 1), and finally freeze-dried (Epsilon 2–4 LSCplus, Martin Christ, Osterode am Harz, Germany).

2.4. Preparation of apple models

An apple model containing quercetin-3-glucoside (Q-3-G), chosen as a representative apple phenolic compound due to its concentration and bioaccessibility in apples (Alongi et al., 2019), was prepared by dissolving Q-3-G in DMSO (30 mg/mL) and by diluting this solution with Milli-Q water to a final concentration of 0.33 mg/mL, approximated to the total phenolic content in apple (Alongi et al., 2019). Model complexity was increased by preparing a second model which included pectin extracted from apple pulp, selected as the major soluble dietary fibre fraction in apples. To this purpose, Q-3-G DMSO solution was diluted at the final concentration of 0.33 mg/mL with a 2.8 mg/mL water-based pectin suspension, resembling pectin content in apples (Baker, 1997).

2.5. In vitro digestion

Apple pulp, puree and homogenate were *in vitro* digested based on the standardised Infogest protocol (Brodkorb et al., 2019).

Simulated salivary (SSF), gastric (SGF) and intestinal (SIF) fluids were prepared and stored at 4 °C until analyses and preheated to 37 °C just before use. Stock solutions of salivary α-amylase (2505 U/mL), pepsin (60,180 U/mL) in water, and pancreatin (800 U/mL) and bile (134 mM) in SIF were freshly prepared just before the experiments.

Fresh apple pulp was ground in a meat grinder (Trita express, R.G.V, Cermenate, Italy) fitted with a 7 mm-hole plate to simulate the oral processing (Gao et al., 2019).

Aliquots of 2.5 g of ground apple pulp, puree and homogenate were added with a volume of α-amylase solution providing 75 U/mL enzymatic activity in the final oral phase mixture. SSF and water were added to guarantee the osmolarity and the fluid-to-meal ratio. In particular, a 1:1 ratio between the dry weight of samples, *i.e.*, 13.5, 14.1, and 10.4 %, respectively for apple pulp, puree and homogenate (Alongi et al., 2023), and the volume of fluids was considered. For the models, a 1:1 ratio between the volume of samples and that of fluids was considered, since the dry weight was negligible (<0.5 %). The oral phase was simulated by maintaining the sample at 37 °C in a thermostat (Thermocenter TC- 40 T, SalvisLab, Rotkreuz, Switzerland) under stirring with a rotatory shaker (F205, Falc Instruments s.r.l., Treviglio, Italy) for 2 min at 15 rpm. The bolus was then mixed with SGF and a volume of pepsin solution providing 2000 U/mL, the pH was adjusted with HCl (6 M) to 3 and water was added to maintain a 1:1 (*v*/v) fluid to bolus ratio, and the gastric phase was carried out at 37 °C for 2 h under stirring at 15 rpm. The intestinal phase was started by mixing the chyme with SIF, bile salts (10 mM) and pancreatin providing 100 U/mL activity. The pH was adjusted with NaOH (1 M) to 7 and water was added to maintain a 1:1 (v/v) fluid to chyme ratio. The sample was stirred at 37 °C during 2 h at 15 rpm.

Blank digestion sample in which the apple pulp, puree or homogenate were replaced with the same weight of water was also digested.

At the end of the intestinal phase, samples were centrifuged at 30,000 ×*g* at 4 °C for 70 min (Beckman Avanti tm J-25, Beckman Instruments Inc., Palo Alto, CA, USA) to recover the bioaccessible fraction.

To clear the bioaccessible fraction from bile salts, formic acid was added under stirring until pH 2 was reached, the samples were centrifuged (7000*g* at 4 °C for 15 min) and the supernatant was filtered (0.20 μm), freeze-dried (Epsilon 2–4 LSCplus, Martin Christ GmbH, Osterode am Harz, Germany) and stored in desiccators in dark conditions until analysis.

2.6. Phenolic compound identification and quantification

The phenolic content was determined as previously described (Alongi et al., 2023). Briefly, freeze-dried samples obtained after *in vitro* digestion of 2.5 g of ground apple pulp, puree and homogenate were mixed with 30 μL of a methanolic solution of 3-hydroxycinnamic acid (100 μg/mL; IS), diluted with an aqueous solution of formic acid (0.4 %), loaded on a preconditioned C18 SPE column, washed with 10 mL aqueous solution of formic acid (0.4 %), and the phenolic fraction was eluted with 13 mL of MeOH/H_2O 1:1 (*v*/v) with 0.4 % of HCOOH. After removing the solvents by freeze drying, the residues were diluted with 1 mL of H_2O/MeOH 9:1 (v/v) with 0.4 % of formic acid and analysed by HPLC-DAD-ESI-MS^n. The HPLC was coupled with a diode array detector (Ultimate 3000 RS, Thermo Scientific, San Jose, CA, USA) and an electrospray ionization mass detector (Finnigan LXQ linear ion trap mass spectrometer, Thermo Scientific, San Jose, CA, USA). The HPLC was equipped with a column InfinityLab Poroshell 120 EC-C18 (4.6 × 150 mm, 2.7 μm; Agilent Technology, Milan, Italy) in a thermostatic oven. Elution was carried at 30 °C out using as mobile phases 0.2 % formic acid in methanol (A) and 0.2 % formic acid in water (B), with the following gradient: 0–6 min 10 % A, 20 min 35 % A, 46 min 40 % A, 48 min 100 % A, 60 min 100 % A, 62 min 10 % A, 70 min 10 % A. The flow rate was 0.6 mL/min and the injection volume was 20 μL. The mobile phase was split (1:1, v/v) and directed in parallel to the ESI for the identification of phenolic compounds and to the DAD for their quantitative analysis. ESI mass spectra were acquired in full scan (*m/z* 50–1500) and in full scan MS^2 (*m/z* 50–600) selecting the precursor ion $[M\text{-}H]^-$ at *m/z* 163 for 3-hydroxycinnamic acid (IS), *m/z* 289 for (+)-catechin and (−)-epicatechin, *m/z* 353 for chlorogenic acid, *m/z* 577 for procyanidin B2, *m/z* 435 for phloridzin, *m/z* 433 for quercetin 3-O-xyloside and quercetin 3-O-arabinoside, *m/z* 463 for quercetin 3-O-glucoside, *m/z* 447 for quercetin 3-O-rhamnoside, respectively. Phenolic compounds for which standards were not available, such as 4-O-*p*-Coumaroylquinic acid ($[M\text{-}H]^-$ at *m/z* 337), procyanidin B-type dimers ($[M\text{-}H]^-$ at *m/z* 577), trimers ($[M\text{-}H]^-$ at *m/z* 865), and tetramers ($[M\text{-}H]^-$ at m/z 1153), phloretin-xyloglucoside ($[M\text{-}H]^-$ at m/z 567), quercetin pentoside ($[M\text{-}H]^-$ at m/z 433) were tentatively characterized by comparison of their fragmentation patterns (Table S1) with those available in the literature.

Quantitative analysis of phenolic compounds listed in Table S1 was carried out in the range 200–400 nm and preparing calibration curves ($R^2 > 0.99$) of each standard with a constant concentration of the internal standard. When standards were unavailable, the quantification of the analytes was carried out using the calibration curve of available standards presenting similar chemical structures. The samples were analysed in duplicate. Data were acquired and elaborated by Chromeleon software (version 6.80) and results were reported on a dry basis ($\mu g/g_{dw}$).

2.7. Radical scavenging activity

An aliquot of 6 mg of freeze-dried digested apple pulp, puree, homogenate, or blank digestion sample was solubilized in 2 mL MeOH in an ultrasound bath for 10 min at 25 °C. A volume of 40 μL of sample was added with 110 μL of a freshly prepared 2,2-diphenyl-1-picrylhydrazyl DPPH$^{\bullet}$ methanolic solution (0.05 mg/mL).

Absorbance was measured in a microplate reader (Tecan, Argovia, Switzerland) at 517 nm for 20 min at 25 °C, upon gentle shaking for 5 s before measurement. Blank samples, in which DPPH$^{\bullet}$ solution was replaced by MeOH were also assayed. The radical scavenging activity was computed as reported in Eq. (2):

$$\textit{Radical scavenging activity}\ (\%) = \frac{(Abs_i - Abs_f)}{Abs_i} \times 100 \quad (2)$$

where Abs_i and Abs_f are the absorbances at the beginning and the end of the assay, respectively.

The radical scavenging activity measured on the blank digestion sample was subtracted to the values measured on digested apple pulp, puree, and homogenate.

Trolox® solutions at increasing concentrations (0, 5, 10, 20, 40, 60, 80 mg/L) were assayed under the same conditions described above to build a calibration curve, and the radical scavenging activity of samples was expressed as Trolox Equivalents on a dry basis (mg_{TE}/g_{dw}).

2.8. Statistical analysis

Results are averages of at least three technical replicates carried out on two biological replicates and are reported as means ± standard deviation. Statistical analysis was performed using R (version 3.2.3, The R Foundation for Statistical Computing, Vienna, Austria). Bartlett's test was used to check the homogeneity of variance, one-way ANOVA was carried out, and Tukey post-hoc test was used to determine statistically significant differences among means ($p < 0.05$).

3. Results and discussion

3.1. Bioaccessibility of phenolic compounds

Apple pulp, puree and homogenate are characterized by different levels of structural integrity. As previously reported by Alongi et al. (2023), while apple pulp is characterized by an organized cellular matrix consisting of intact cells tightly bound one to each other through cell walls, operations mimicking industrial production of apple derivatives caused different levels of matrix destructuring. Puree was a viscous system (0.73 Pa × s) in which the matrix was disaggregated and intact cells and large cell clusters were suspended in the juice, while homogenate was more fluid (0.27 Pa × s) due to the complete apple tissue disintegration and cell wall disruption.

Despite these differences, the concentration of total phenolic compounds only slightly decreased in apple derivatives compared to fresh apple pulp (Alongi et al., 2023). In this regard, Fig. 1 reports the concentration of phenolic compounds in apple puree and homogenate, expressed as a percentage of the total phenolic content in undigested apple pulp. This data representation allows accounting for the combined effect of processing and *in vitro* gastrointestinal digestion on phenolic compounds concentration. Apple puree and homogenate retained nearly 80 % of the phenolic content originally present in apple pulp (Fig. 1).

Looking more in detail to the depletion caused by processing for each phenolic class, in the case of the puree, flavonols, flavanols, dihydrochalcone derivatives and hydroxycinnamic acids decreased by 14, 16, 8 and 9 %, respectively. Overall similar data were also found in the homogenate (14, 15, 9 and 12 %, respectively) (data calculated from Table 1). This result shows that: (*i*) the same extent of depletion was found in apple puree and homogenate compared to apple pulp; (*ii*) in both cases, the most affected class was that of flavanols due to their thermolabile nature. Nevertheless, the relative abundance followed the order flavanols (55 %) > hydroxycinnamic acids (36 %) > dihydrochalcone derivatives (7 %) > flavonols (2 %) in all samples.

The moderate total phenolic depletion observed after the processing operations applied to obtain both the puree and the homogenate was mainly due to the decrease of the most abundant phenolic class, *i.e.*, flavanols. Since these compounds are quite thermolabile, their decrease could likely be attributable to both the applied mechanical operations triggering enzymatic polyphenol oxidation and the thermal treatments (*i.e.*, blanching and pasteurization). In this regard, it must be kept in mind that the same thermal treatments (*i.e.*, blanching and pasteurization) were applied during processing of both the puree and the homogenate, while apple pulp did not undergo any thermal nor mechanical treatment. Although thermal processing typically depletes phenolic compounds, the slow kinetics of phenol oxidation in plant-based matrices subjected to thermal enzymatic inactivation, combined with potential chemical interactions with released dietary fibre, may mitigate phenolic degradation (Oliveira & Pintado, 2015).

Since limited information is available on the influence of food processing on phenolic concentration potentially reaching the intestinal level after gastrointestinal events, the concentration of the major classes of phenolic compounds was assessed after *in vitro* digestion.

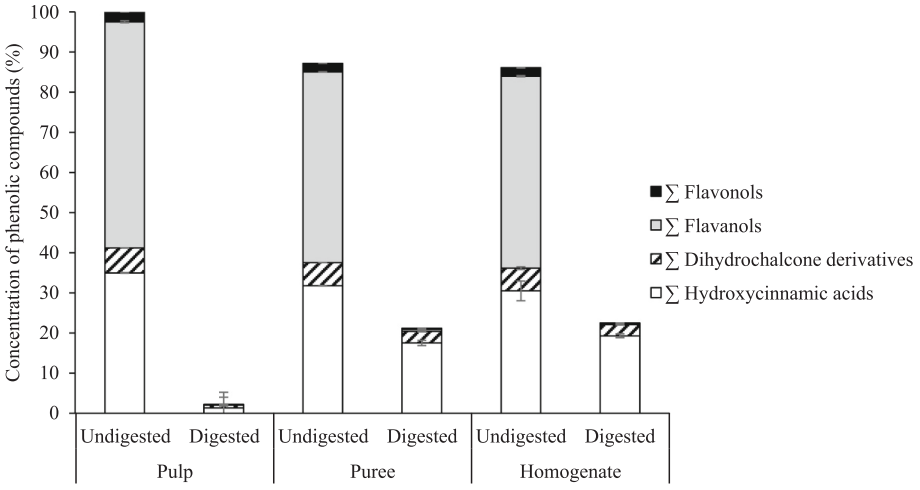


Fig. 1. Profile of the major classes of phenolic compounds in apple pulp, puree and homogenate before and after *in vitro* digestion. Results are expressed as the percentage compared to undigested apple pulp.

Table 1
Concentration (μg/g_{dw}) of phenolic compounds in apple pulp, puree and homogenate before and after *in vitro* digestion (data expressed as mean ± SD of at least two experimental replicates).

	Pulp				Puree				Homogenate			
Compound	Undigested*		Digested		Undigested*		Digested		Undigested*		Digested	
Chlorogenic acid	682.28	± 1.91	18.47	± 13.53	624.12	± 51.04	350.35	± 49.23	598.54	± 1.26	381.58	± 9.36
trans-4-p-Cumaroylquinic acid	60.55	± 0.20	10.38	± 0.92	51.07	± 5.36	22.10	± 2.80	49.33	± 0.06	28.53	± 0.70
∑ Hydroxycinnamic acids	742.83	± 1.71	28.85	± 13.90	675.19	± 56.40	372.45	± 51.97	647.87	± 1.32	410.11	± 10.06
Phloretin Xyloglucoside	53.09	± 0.28	6.68	± 1.13	52.20	± 0.45	34.43	± 3.67	52.50	± 0.02	36.85	± 1.16
Phloridzin	79.22	± 0.28	7.79	± 2.63	69.90	± 4.14	27.29	± 3.76	67.91	± 0.28	22.26	± 1.45
∑ Dihydrochalcone derivatives	132.31	± 0.56	14.48	± 3.73	122.11	± 4.59	61.72	± 7.18	120.42	± 0.31	59.11	± 2.61
Procyanidin B2	315.33	± 1.86	n.d.		264.23	± 15.79	5.05	± 1.46	274.12	± 0.88	0.26	± 0.02
Procyanidin B type dimers	91.19	± 0.46	n.d.		68.53	± 5.86	n.d.		69.86	± 0.13	n.d.	
Procyanidin B type trimers	235.00	± 1.28	n.d.		205.23	± 16.13	n.d.		205.72	± 1.20	n.d.	
Procyanidin B type tetramers	252.27	± 1.60	n.d.		206.92	± 17.77	n.d.		200.77	± 0.82	n.d.	
(−) Epicatechin	238.17	± 0.03	n.d.		210.23	± 8.30	4.58	± 1.58	213.38	± 0.42	2.04	± 0.03
(+) Catechin hydrate	64.89	± 0.04	n.d.		54.28	± 3.91	n.d.		51.90	± 0.14	n.d.	
∑ Flavanols	1196.86	± 5.27	n.d.		1009.43	± 67.73	9.63	± 3.01	1015.75	± 0.65	2.30	± 0.01
Quercetin-3-O-glucoside	3.40	± 0.01	n.d.		2.87	± 0.27	0.37	± 0.16	2.98	± 0.00	0.29	± 0.06
Quercetin-3-O-xyloside	6.95	± 0.04	0.33	± 0.10	5.44	± 0.14	0.71	± 0.14	5.51	± 0.02	0.58	± 0.22
Quercetin-3-O-rhamnoside	33.46	± 0.02	3.13	± 0.66	28.94	± 1.38	5.27	± 0.71	28.59	± 0.07	5.69	± 0.13
Quercetin-3-O-arabinoside	0.74	± 0.00	n.d.		0.63	± 0.05	n.d.		0.62	± 0.01	n.d.	
Other quercetin-pentosides	8.15	± 0.02	n.d.		7.25	± 0.08	n.d.		7.26	± 0.05	n.d.	
∑ Flavonols	52.70	± 0.10	3.47	± 0.75	45.13	± 1.91	6.34	± 1.00	44.96	± 0.15	6.56	± 0.14
Total phenolic content	2124.70	± 6.51	46.80	± 16.76	1851.85	± 130.60	450.14	± 61.90	1829.00	± 1.13	478.08	± 12.81

* Data elaborated from Alongi et al. (2023).

Upon *in vitro* digestion of apple pulp, puree and homogenate, the lowest total phenolic concentration was found in digested apple pulp, and accounted for ~2 % compared to the undigested apple pulp (Fig. 1). Such content represents the bioaccessible fraction, namely the one released from the food matrix during digestion and available for intestinal absorption (Ferruzzi, 2010).

Conversely, puree and homogenate showed a higher concentration of phenolic compounds after digestion, corresponding to ~20 % compared to the undigested apple pulp. These results indicate that the process-induced phenolic depletion previously observed (Alongi et al., 2023) was counteracted by digestion, with a 10-fold higher amount of phenolic compounds potentially available for absorption at the intestinal level after the ingestion of apple derivatives compared to intact apple pulp (Fig. 1).

Considering the phenolic classes in digested samples, the relative abundance trend changed compared to undigested samples, overall decreasing in the order hydroxycinnamic acids (60–85 %) > dihydrochalcone derivatives (12–30 %) > flavonols (1–7 %) ~ flavanols (0–2 %) (data elaborated from Table 1).

This change is in agreement with Feng et al. (2021) who reviewed the impact of processing on phenolic bioaccessibility, pointing out that the technological interventions applied to obtain apple derivatives resulted in different effects depending on phenolic compound classes. In this regard, Table 2 reports the bioaccessibility of phenolic classes in apple pulp, puree and homogenate. Flavanols were the less bioaccessible class (Table 2), even though these were the most abundant in undigested samples, *i.e.*, 1624 ± 18 μg/g_{dw} (Table 1). After digestion, flavanols could not be detected at all in the case of apple pulp, while only ~1.5 % was found for puree and homogenate (Fig. 1). Flavanols were reported to undergo glucuronidation, conjugation, or degradation into monomer or dimer units following digestion and absorption by the small intestine (Hackman et al., 2008; Zhang et al., 2007). The severe flavanol depletion occurring during digestion accounts for the overall low total phenolic concentration after digestion. The bioaccessibility of flavonols ranged between 10 and 13 % (Table 2), however, since this class was the less abundant in apple pulp and derivatives, *i.e.*, ~ 35 μg/g_{dw} (Alongi et al., 2023), its concentration was even more negligible in the digested samples. Hydroxycinnamic acids presented a low bioaccessibility in the apple pulp (<1 %) while this value increased considerably in apple puree and homogenate accounting for 25 and 29 %, respectively (Table 2). These data give reason for the higher bioaccessibility observed in the total phenolic content of apple puree and homogenate compared to the pulp. Finally, dihydrochalcone derivatives were the most bioaccessible phenolic compounds. However, given their low initial concentration ~ 120 μg/g_{dw} (Alongi et al., 2023), their resilience against digestion did not affect the overall phenolic bioaccessibility.

bioaccessibility values ranging from 5 to 50 % were already reported for apple derivatives depending on the phenolic compound class (Pollini et al., 2022).

Looking more in detail into single phenolic compounds, Table 1 and Table 2 also report the concentration of single phenolic compounds before and after digestion and their bioaccessibility. Before digestion, the phenolic profile was comparable among apple pulp, puree and homogenate, with the prevalence of chlorogenic acid, procyanidins and epicatechin in all cases.

After digestion, the profile considerably changed compared to undigested samples. In digested apple pulp, flavonols and hydroxycinnamic acids accounted together for more than half of phenolic compounds, while in puree and homogenate, chlorogenic acid prevailed, accounting alone for 60 % of total phenolic compounds. These data confirm that phenolic compounds differently suffered a depletion upon digestion depending on the technological intervention applied.

The role of processing in steering bioactive compounds bioaccessibility as compared to unprocessed vegetable matrices has been addressed by several authors. Even though it is generally believed that industrial processing may lead to detrimental changes in the phenolic

Table 2
Bioaccessibility (%) of phenolic compounds in apple pulp, puree and homogenate after *in vitro* digestion (data expressed as mean ± SD of at least two experimental replicates).

	Bioaccessibility %								
Compound	Pulp			Puree			Homogenate		
Chlorogenic acid	2.71	±	1.98	56.13	±	7.89	63.75	±	1.56
trans-4-*p*-Cumaroylquinic acid	17.14	±	1.52	43.28	±	5.49	57.84	±	1.42
∑ Hydroxycinnamic acids	3.88	±	1.87	55.16	±	7.70	63.30	±	1.55
Phloretin Xyloglucoside	12.58	±	2.12	65.95	±	7.03	70.19	±	2.21
Phloridzin	9.84	±	3.32	39.04	±	5.38	32.77	±	2.13
∑ Dihydrochalcone derivatives	10.94	±	2.82	50.54	±	5.88	49.09	±	2.17
Procyanidin B2	n.d.			1.91	±	0.55	0.09	±	0.01
Procyanidin B type dimmers	n.d.			n.d.			n.d.		
Procyanidin B type trimers	n.d.			n.d.			n.d.		
Procyanidin B type tetramers	n.d.			n.d.			n.d.		
(−) Epicatechin	n.d.			2.18	±	0.75	0.96	±	0.01
(+) Catechin hydrate	n.d.			n.d.			n.d.		
∑ Flavanols	n.d.			0.95	±	0.30	0.23	±	
Quercetin-3-O-glucoside	n.d.			12.77	±	5.69	9.79	±	1.86
Quercetin-3-O-xyloside	4.81	±	1.48	12.97	±	2.56	10.54	±	3.92
Quercetin-3-O-rhamnoside	9.37	±	1.99	18.19	±	2.46	19.89	±	0.45
Quercetin-3-O-arabinoside	n.d.			n.d.			n.d.		
Other quercetin-pentosides	n.d.			n.d.			n.d.		
∑ Flavonols	6.58	±	1.43	14.05	±	2.21	14.59	±	0.32
Total phenolic compounds	2.20	±	0.79	24.31	±	3.34	26.14	±	0.70

profile of plant-based food matrices, depending on the nature and intensity of the applied technological interventions, different and opposite outcomes can be observed (Arfaoui, 2021). Some authors reported that processing can be used to modify the native barriers in plant-based foods, potentially enhancing nutrient release (Verkempinck et al., 2020). In this regard, it must be kept in mind that in the intact apple matrix, most phenolic compounds are typically compartmentalized into the vacuoles and the remaining fraction is covalently bound to the polysaccharides building the cell wall, hindering their release into the bioaccessible fraction (Liu et al., 2019). In the apple matrix, pectin is known to non-covalently bind phenolic compounds (Chirug et al., 2018) and to interact with them even after processing, once the matrix is disrupted (Arfaoui, 2021), by hydrogen bonding, electrostatic interactions, and hydrophobic ones. These interactions depend on the features of fibre (*e.g.*, degree of polymerization, branching) and of phenolic compounds (*e.g.*, polarity), possibly affecting the bioavailability and subsequent bioactivity of polyphenols (Chirug et al., 2018).

The higher bioaccessibility observed in apple puree and homogenate compared to the intact apple pulp (Table 2) can thus be attributed to the disaggregation of the apple tissue occurring upon HSH and HPH, which likely favour the release of phenolic compounds from the cell matrix.

On the other hand, polyphenol release in the medium induced by processing could be, in principle, responsible for a higher exposure of these compounds to oxygen favouring their oxidation. Nonetheless, acquired results suggest that polyphenols present in apple derivatives seem to be less affected than those present in intact pulp. In the light of this, it can be inferred that the dramatic reduction of phenolic bioaccessibility in apple pulp can be attributed to enzymatic oxidation. Polyphenol oxidases (PPO) are naturally contained in apple cells in dedicated vacuoles (Murata et al., 1997). When the apple pulp undergoes *in vitro* digestion, during chewing simulation, some cellular structures, including walls and vacuoles are damaged and PPO come into contact with polyphenols and oxygen, thus triggering oxidation. It is reasonable to hypothesise that PPO could remain active at gastrointestinal temperature and pH (Chow et al., 2011) thus continuing polyphenol enzymatic oxidation during the subsequent digestion steps. Chow et al. (2011) observed that after 2 h exposure to acidic pH, *i.e.*, conditions close to those of the gastric phase, PPO retained its activity by over 50 %. This condition is not expected to happen in the case of apple puree and homogenate which both underwent enzymatic inactivation by blanching and further pasteurization (Beveridge & Weintraub, 1995; Yemenicioğlu et al., 1997). As a result, after digestion the phenolic depletion in apple pulp was much more pronounced than in apple puree and homogenate. In addition, the wide range of bioaccessibility values observed for different phenolic classes (Table 2) could also be attributed to the substrate specificity of PPO (Li et al., 2023).

This hypothesis is supported by the results acquired on apple model systems. To mimic the composition of apple, quercetin-3-glucoside (Q-3-G), selected as one of the most representative phenolic compounds in apple (Lee & Mitchell, 2012), was combined or not with pectin, which is the most abundant soluble fraction of dietary fibre playing a structural role in apple (Du et al., 2022). These compounds were combined considering concentrations that resembled those found in apple (Alongi et al., 2023). Although these models greatly approximate the real food matrix, they were used to understand the actual role of the food matrix on phenolic bioaccessibility starting from basic building blocks. To enable the comparison between the results obtained with real apple matrices and the results obtained with the model system, Q-3-G concentration in the models was expressed as $\mu g/g_{dw\ equivalent}$ given the fact that Q-3-G concentration was analogous to the total phenolic content of apple. Q-3-G concentration was assessed after *in vitro* digestion of the model and independently from the presence of pectin it accounted for ~100 $\mu g/g_{dw\ equivalent}$. The bioaccessibility of Q-3-G was 16.10 ± 1.07 % in the model constituted by Q-3-G alone, and 18.88 ± 1.36 % in the presence of pectin with no significant differences between the two. This value was comparable to that observed for total phenolic compounds in apple puree and homogenate. Since the models did not contain PPO and no cell-like structures able to compartmentalise Q-3-G, these results support the hypothesis that PPO activity and matrix entrapment plays a key role in determining phenolic bioaccessibility in apple pulp.

Such a reduction can be attributed to the chemical oxidation suffered by the phenolic compounds during gastrointestinal events. The presence of oxygen is critical along the gastrointestinal tract until the small intestine. It becomes negligible only in the colon where an anaerobic environment is present (Vieira et al., 2017). In addition, although the digestive enzymes and the bile extract used during *in vitro* digestion exert an antioxidant effect (0.69 $mg_{TE}/g_{dw\ equivalent}$), this is not expected to be able to counter phenolic chemical oxidation. It must also be mentioned that the simulated static digestive conditions do not account for other endogenous antioxidant systems that are present in the gastrointestinal tract (Fridovich, 2011; Rahman, 2007; Turunen et al., 2004). Nevertheless, other authors also reported on the oxidation of phenolic compounds occurring along the gastrointestinal tract (Sadeghi Ekbatan et al., 2016).

3.2. Interplay between phenolic bioaccessibility and antioxidant capacity

Most of the claimed health-promoting properties of apples are associated with the antioxidant activity of phenolic compounds. Therefore, we measured the radical scavenging capacity of apple pulp, puree and homogenate after *in vitro* digestion and compared the data to what previously observed in undigested samples.

After digestion, the radical scavenging capacity decreased by around 80 % in all samples. In particular, the radical scavenging capacity of apple pulp, puree and homogenate before digestion was 2.39 ± 0.25, 2.45 ± 0.36, and 2.06 ± 0.18 mg_{TE}/g_{dw} (Alongi et al., 2023) and after digestion, these values respectively decreased to 0.42 ± 0.05, 0.51 ± 0.04, 0.44 ± 0.05 mg_{TE}/g_{dw}. Overall, such a reduction is in line with the depletion in the phenolic content observed after digestion (Fig. 1).

To explore the causal-link between antioxidant activity and phenolic compounds, Fig. 2 shows the correlation between the radical scavenging capacity and the total phenolic content.

The correlation between the radical scavenging capacity and the total phenolic content was very strong ($r = 0.98$), in agreement with other authors (Chinnici et al., 2004).

To get more insights into the interplay among apple components and antioxidant activity, we measured the radical scavenging capacity in apple models containing quercetin, pectin and their combination (Fig. 3). To allow the comparison of model results with those acquired on apple matrices, the radical scavenging capacity was expressed on dry weight equivalents, given that the concentration of Q-3-G and pectin in the model was analogous to that of apple.

The radical scavenging capacity values of apple models were in the same range as that observed in real apple matrices (Fig. 3).

In the undigested models, Q-3-G presented a radical scavenging capacity of 3.58 $mg_{TE}/g_{dw\ equivalent}$. These results are in line with previous studies on the contribution of quercetin and its glucosides to the antioxidant capacity of different vegetable matrices (Zielinska et al., 2008).

Pectin also showed a radical scavenging capacity, corresponding to ~1 $mg_{TE}/g_{dw\ equivalent}$ and in line with literature reporting pectin to be 200 times less effective than Trolox (Wikiera et al., 2021).

When Q-3-G was combined with pectin, the radical scavenging capacity decreased to 2.5 $mg_{TE}/g_{dw\ equivalent}$ compared to Q-3-G alone, indicating that the concomitant presence of quercetin and pectin did not have an additive effect but rather an antagonistic one. This could be attributed to the binding of quercetin and pectin, possibly leading to a shielding of antioxidant active sites. Other authors (Liang et al., 2021) found that the antioxidant activity of phenolic compounds decreased while binding with pectin.

The radical scavenging capacity of Q-3-G + pectin was comparable to the values observed in apple pulp, puree and homogenate that ranged from 2 to 2.4 mg_{TE}/g_{dw} (Fig. 3). Given that the radical scavenging capacity was not affected by the different structural integrity of apple pulp, puree and homogenate (Alongi et al., 2023), overall these results suggest that interactions between polyphenols and pectin occur regardless the structural integrity and the hierarchical arrangement of the plant tissue.

After digestion, the radical scavenging capacity of pectin was negligible. Structural changes in pectin have been hypothesized to be induced under gastrointestinal conditions (Capuano, 2017) possibly modulating its capacity to interact with Q-3-G but further research is still required to shed light on this aspect.

After digestion, the radical scavenging capacity of the Q-3-G model decreased by only 30 %, although the concentration of Q-3-G decreased by more than 80 % (Table 2). Therefore, it seems that after digestion the Q-3-G system was much more effective in exerting antioxidant activity compared to the undigested model. It can be hypothesized that the formation of quercetin aglycone, having a higher antioxidant activity compared to the glycosylated form (Zymone et al., 2022), may occur during digestion (Walle et al., 2000). However, studies regarding the deglycosylation of quercetin during digestion are controversial (Day et al., 2003; Wolffram et al., 2002). Given that the quercetin aglycone could not be identified in any sample, our hypothesis is purely speculative, and further studies are required to better explore the fate of phenolic compounds in the gastrointestinal tract, including the non bioaccessible fraction.

Upon digestion, the radical scavenging capacity of Q-3-G + pectin digested system was not different from that of the undigested sample. A concomitant effect of Q-3-G deglycosylation, pectin structural changes and changes in the interactive behaviour between these molecules may have contributed to the observed radical scavenging capacity.

Overall, the major differences between radical scavenging capacity between real apple matrices and models was observed after digestion. These differences reasonably lie in the different composition of the systems considered, and in particular in the different susceptibility of phenolic compounds to digestion, leading to the formation of other

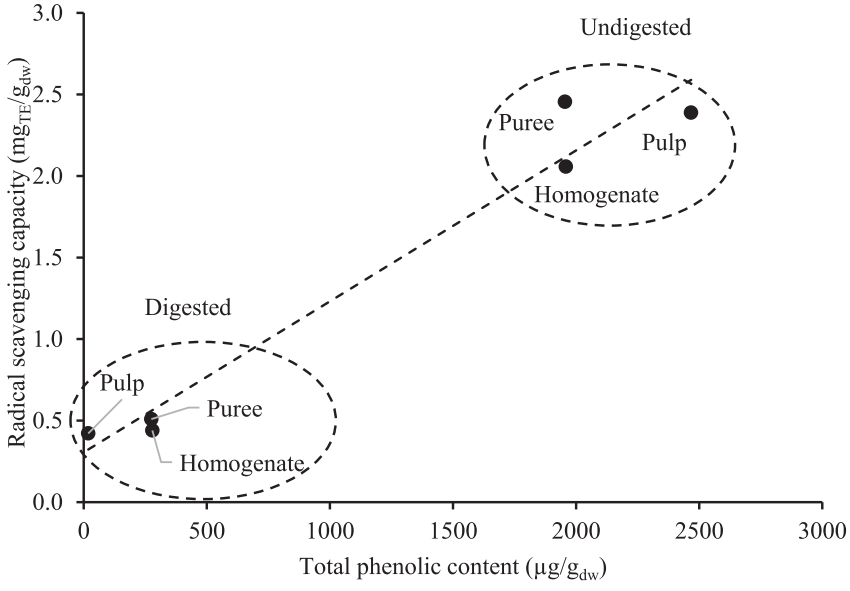


Fig. 2. Correlation between the total phenolic content and the radical scavenging capacity.

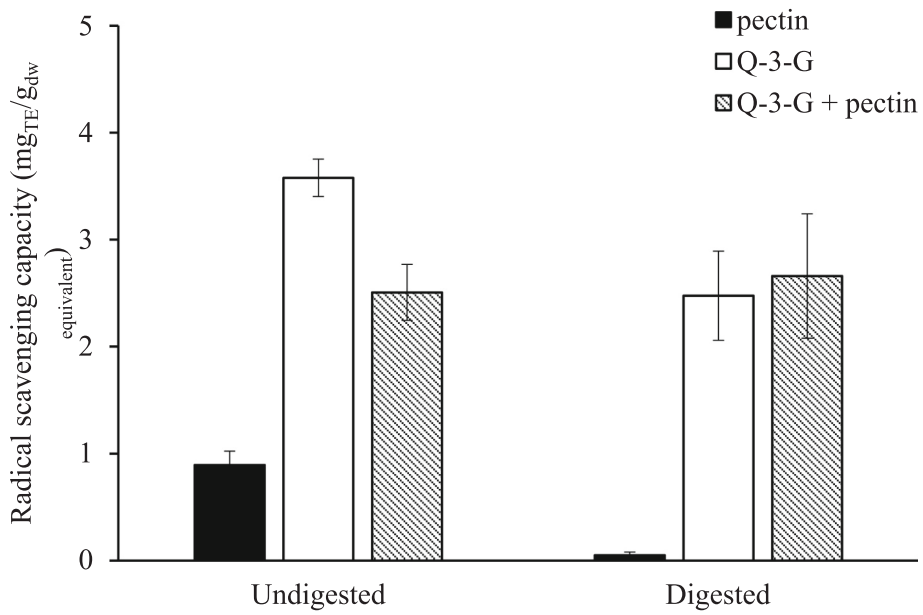


Fig. 3. Radical scavenging capacity in apple models containing pectin, quercetin-3-glucoside (Q-3-G), and Q-3-G+ pectin.

derivatives that may have a more or less pronounced radical scavenging activity.

4. Conclusions

This study highlights the significant impact of processing on the bioaccessibility of polyphenols in apples. Despite processing reduced phenolic content, after digestion their concentration remained higher in apple derivatives than in apple pulp. Substantial depletion of phenolic compounds was observed across all samples due to chemical oxidation in the gastrointestinal tract, but this was more pronounced in fresh apple pulp. This was hypothesized to be due to enzymatic oxidation, as polyphenol oxidase (PPO) was inactivated by thermal treatments in apple derivatives but remained active in the pulp, suggesting that simulated digestion conditions acted as an incubator for PPO activity.

A model containing quercetin-3-glucoside (Q-3-G) and pectin, devoid of PPO and not processed like apple pulp, showed bioaccessibility comparable to apple derivatives, confirming the hypothesis. The radical scavenging capacity, indicative of the biological activity of phenolic compounds, remained unaffected by the structural integrity of apples, suggesting independence from the plant tissue's arrangement. While radical scavenging capacity decreased after digestion, correlating with total phenolic content, apple models retained this capacity post-digestion, unlike real apple matrices.

Indeed, the model was oversimplified not only in structural terms but also in the phenolic profile, featuring only Q-3-G. The latter can undergo structural changes during digestion, resulting in higher antioxidant activity even at lower concentrations.

Future research should delve into the degradation pathway of phenolic compounds during digestion. In this regard, using models containing not only polyphenols but also PPO could be useful to verify the retention of PPO activity during digestion and understand if factors other than PPO are at play in affecting phenolic bioaccessibility.

The interaction of phenolic compounds with other components, such as dietary fibre, to form complexes and their impact on bioaccessibility also represent a topic to be explored in future studies.

Furthermore, investigating colonic fermentation of the non-bioaccessible fraction of polyphenols is an interesting perspective. Indeed, polyphenols characterized by low bioaccessibility might easily reach the colon to be metabolised by local microbiota, which allows them to exert their biological activity in that context.

Additionally, enhancing the complexity of *in vitro* simulations through cell culture experiments could account for endogenous antioxidant systems present in the gastrointestinal tract.

Finally, in view of translating the results acquired in the present study to an industrial level, it must be highlighted that processing conditions not only affect the technological and sensory properties of food, but they can be also modulated to steer food nutritional functionality.

Since consumers tend to associate technological interventions with detrimental nutritional effects, the results acquired in the present study could contribute to reverting this misconception and be used to communicate the potential benefits of processed food consumption.

CRediT authorship contribution statement

Marilisa Alongi: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Umberto Lanza:** Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **Andrea Gorassini:** Writing – review & editing, Methodology, Investigation. **Giancarlo Verardo:** Writing – review & editing, Resources, Methodology. **Clara Comuzzi:** Writing – review & editing, Resources, Methodology. **Monica Anese:** Writing – review & editing, Resources, Methodology. **Lara Manzocco:** Writing – review & editing, Project administration, Conceptualization. **Maria Cristina Nicoli:** Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

This research was carried out within the interdisciplinary research project of the University of Udine "Il tempo della mela – TEAM"

supported by Fondazione Friuli.

The University of Udine provides Open Access Funding within the CRUI-CARE Agreement.

The authors thank Dr. Anna Natali, Dr. Anna Naibo and Mr. Nicola Barbui for contributing to *in vitro* digestion and radical scavenging capacity experiments.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2024.141402.

References

Al-Khayri, J. M., Rashmi, R., Toppo, V., Chole, P. B., Banadka, A., Sudheer, W. N., … Rezk, A. A. S. (2023). Plant secondary metabolites: The weapons for biotic stress management. *Metabolites, 13*(6), 1–37. https://doi.org/10.3390/metabo13060716

Alongi, M., Manzocco, L., Comuzzi, C., Gorassini, A., Verardo, G., Rossi, A., … Nicoli, M. C. (2023). The impact of apple destructuring and thermal treatment on bioactive compounds: The fate of dietary fibre and polyphenols in puree and homogenate. *International Journal of Food Science and Technology, 58*(6), 3189–3200. https://doi.org/10.1111/ijfs.16446

Alongi, M., Verardo, G., Gorassini, A., Lemos, M. A., Hungerford, G., Cortella, G., & Anese, M. (2019). Phenolic content and potential bioactivity of apple juice as affected by thermal and ultrasound pasteurization. *Food & Function, 10*(11), 7366–7377. https://doi.org/10.1039/c9fo01762c

Arfaoui, L. (2021). Dietary plant polyphenols: Effects of food processing on their content and bioavailability. *Molecules, 26*(10), Article 2959. https://doi.org/10.3390/MOLECULES26102959

Arulselvan, P., Fard, M. T., Tan, W. S., Gothai, S., Fakurazi, S., Norhaizan, M. E., & Kumar, S. S. (2016). Role of antioxidants and natural products in inflammation. *Oxidative Medicine and Cellular Longevity, 2016*. https://doi.org/10.1155/2016/5276130

Aura, A. M., Martin-Lopez, P., O'Leary, K. A., Williamson, G., Oksman-Caldentey, K. M., Poutanen, K., & Santos-Buelga, C. (2005). In vitro metabolism of anthocyanins by human gut microflora. *European Journal of Nutrition, 44*(3), 133–142. https://doi.org/10.1007/S00394-004-0502-2

Azab, A., Nassar, A., & Azab, A. N. (2016). Anti-inflammatory activity of natural products. *Molecules, 21*(10), 1–19. https://doi.org/10.3390/molecules21101321

Baker, R. A. (1997). Reassessment of some fruit and vegetable pectin levels. *Journal of Food Science, 62*(2), 225–229. https://doi.org/10.1111/j.1365-2621.1997.tb03973.x

Beveridge, T., & Weintraub, S. E. (1995). Effect of blanching pretreatment on color and texture of apple slices at various water activities. *Food Research International, 28*, 83–86. https://doi.org/10.1016/0963-9969(95)93335-R

Brodkorb, A., Egger, L., Alminger, M., Alvito, P., Assunção, R., Ballance, S., Bohn, T., Bourlieu-Lacanal, C., Boutrou, R., Carrière, F., Clemente, A., Corredig, M., Dupont, D., Dufour, C., Edwards, C., Golding, M., Karakaya, S., Kirkhus, B., Le Feunteun, S., … Recio, I. (2019). INFOGEST static in vitro simulation of gastrointestinal food digestion. *Nature Protocols, 14*(4), 991–1014. https://doi.org/10.1038/s41596-018-0119-1

Capuano, E. (2017). The behavior of dietary fiber in the gastrointestinal tract determines its physiological effect. *Critical Reviews in Food Science and Nutrition, 57*, 3543–3564. https://doi.org/10.1080/10408398.2016.1180501

Chinnici, F., Bendini, A., Gaiani, A., & Riponi, C. (2004). Radical scavenging activities of peels and pulps from cv. Golden delicious apples as related to their phenolic composition. *Journal of Agricultural and Food Chemistry, 52*(15), 4684–4689. https://doi.org/10.1021/jf049770a

Chirug, L., Okun, Z., Ramon, O., & Shpigelman, A. (2018). Iron ions as mediators in pectin-flavonols interactions. *Food Hydrocolloids, 84*, 441–449. https://doi.org/10.1016/j.foodhyd.2018.06.039

Chow, Y. N., Louarme, L., Bonazzi, C., Nicolas, J., & Billaud, C. (2011). Apple polyphenoloxidase inactivation during heating in the presence of ascorbic acid and chlorogenic acid. *Food Chemistry, 129*, 761–767. https://doi.org/10.1016/j.foodchem.2011.05.017

D'Archivio, M., Filesi, C., Varì, R., Scazzocchio, B., & Masella, R. (2010). Bioavailability of the polyphenols: Status and controversies. *International Journal of Molecular Sciences, 11*(4), 1321–1342. https://doi.org/10.3390/ijms11041321

Day, A. J., Gee, J. M., DuPont, M. S., Johnson, I. T., & Williamson, G. (2003). Absorption of quercetin-3-glucoside and quercetin-4′-glucoside in the rat small intestine: The role of lactase phlorizin hydrolase and the sodium-dependent glucose transporter. *Biochemical Pharmacology, 65*(7), 1199–1206. https://doi.org/10.1016/S0006-2952(03)00039-X

Du, J., Anderson, C. T., & Xiao, C. (2022). Dynamics of pectic homogalacturonan in cellular morphogenesis and adhesion, wall integrity sensing and plant development. *Nature Plants, 8*(4), 332–340. https://doi.org/10.1038/s41477-022-01120-2

Feng, S., Yi, J., Li, X., Wu, X., Zhao, Y., Ma, Y., & Bi, J. (2021). Systematic review of phenolic compounds in apple fruits: Compositions, distribution, absorption, metabolism, and processing stability. *Journal of Agricultural and Food Chemistry, 69*, 7–27. https://doi.org/10.1021/acs.jafc.0c05481

Ferruzzi, M. G. (2010). The influence of beverage composition on delivery of phenolic compounds from coffee and tea. *Physiology & Behavior, 100*(1), 33–41.

Fridovich, I. (2011). Superoxide Radical. *Encyclopedia of Cancer*. https://doi.org/10.1007/978-3-642-16483-5_5580, 3563–3563.

Gao, J., Lin, S., Jin, X., Wang, Y., Ying, J., Dong, Z., & Zhou, W. (2019). In vitro digestion of bread: How is it influenced by the bolus characteristics? *Journal of Texture Studies, 50*, 257–268. https://doi.org/10.1111/jtxs.12391

Garbetta, A., Nicassio, L., D'Antuono, I., Cardinali, A., Linsalata, V., Attolico, G., & Minervini, F. (2018). Influence of in vitro digestion process on polyphenolic profile of skin grape (cv. Italia) and on antioxidant activity in basal or stressed conditions of human intestinal cell line (HT-29). *Food Research International, 106*, 878–884. https://doi.org/10.1016/j.foodres.2018.01.072

Hackman, R. M., Polagruto, J. A., Zhu, Q. Y., Sun, B., Fujii, H., & Keen, C. L. (2008). Flavanols: Digestion, absorption and bioactivity. *Phytochemistry Reviews, 7*(1), 195–208. https://doi.org/10.1007/s11101-007-9070-4

Jakobek, L. (2015). Interactions of polyphenols with carbohydrates, lipids and proteins. *Food Chemistry, 175*, 556–567. https://doi.org/10.1016/J.FOODCHEM.2014.12.013

Lee, J., & Mitchell, A. E. (2012). Pharmacokinetics of quercetin absorption from apples and onions in healthy humans. *Journal of Agricultural and Food Chemistry, 60*(15), 3874–3881. https://doi.org/10.1021/jf3001857

Li, J., Deng, Z.y., Dong, H., Tsao, R., & Liu, X. (2023). Substrate specificity of polyphenol oxidase and its selectivity towards polyphenols: Unlocking the browning mechanism of fresh lotus root (*Nelumbo nucifera* Gaertn.). *Food Chemistry, 424*, Article 136392. https://doi.org/10.1016/j.foodchem.2023.136392

Liang, T., Jiao, S., & Jing, P. (2021). Molecular interaction between pectin and catechin/procyanidin in simulative juice model: Insights from spectroscopic, morphology, and antioxidant activity. *Journal of Food Science, 86*, 2445–2456. https://doi.org/10.1111/1750-3841.15743

Liu, D., Lopez-Sanchez, P., & Gidley, M. J. (2019). Cellular barriers in apple tissue regulate polyphenol release under different food processing and: In vitro digestion conditions. *Food & Function, 10*, 3008–3017. https://doi.org/10.1039/c8fo02528b

Mayo, B., Vázquez, L., & Flórez, A. B. (2019). Equol: A bacterial metabolite from the Daidzein isoflavone and its presumed beneficial health effects. *Nutrients, 11*(9), 1–20. https://doi.org/10.3390/nu11092231

Murata, M., Ani, M. T., Ara, S. H., & Homma, S. (1997). Subcellular location of polyphenoloxidase in apples. *Bioscience, Biotechnology, and Biochemistry, 61*, 1495–1499. https://doi.org/10.1271/bbb.61.1495

Oliveira, A., & Pintado, M. (2015). In vitro evaluation of the effects of protein-polyphenol-polysaccharide interactions on (+)-catechin and cyanidin-3-glucoside bioaccessibility. *Food & Function, 6*(11), 3444–3453. https://doi.org/10.1039/c5fo00799b

Parada, J., & Aguilera, J. M. (2007). Food microstructure affects the bioavailability of several nutrients. *Journal of Food Science, 72*(2), R21–R32. https://doi.org/10.1111/J.1750-3841.2007.00274.X

Pollini, L., Juan-García, A., Blasi, F., Mañes, J., Cossignani, L., & Juan, C. (2022). Assessing bioaccessibility and bioavailability in vitro of phenolic compounds from freeze-dried apple pomace by LC-Q-TOF-MS. *Food Bioscience, 48*, Article 101799. https://doi.org/10.1016/J.FBIO.2022.101799

Quan, W., Tao, Y., Lu, M., Yuan, B., Chen, J., Zeng, M., Qin, F., Guo, F., & He, Z. (2018). Stability of the phenolic compounds and antioxidant capacity of five fruit (apple, orange, grape, pomelo and kiwi) juices during in vitro-simulated gastrointestinal digestion. *International Journal of Food Science and Technology, 53*(5), 1131–1139. https://doi.org/10.1111/ijfs.13682

Rahman, K. (2007). Studies on free radicals, antioxidants, and co-factors. *Clinical Interventions in Aging, 2*(2), 219–236.

Rice-Evans, C. A., Miller, N. J., & Paganga, G. (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology and Medicine, 20*(7), 933–956. https://doi.org/10.1016/0891-5849(95)02227-9

Sadeghi Ekbatan, S., Sleno, L., Sabally, K., Khairallah, J., Azadi, B., Rodes, L., … Kubow, S. (2016). Biotransformation of polyphenols in a dynamic multistage gastrointestinal model. *Food Chemistry, 204*, 453–462. https://doi.org/10.1016/j.foodchem.2016.02.140

Setchell, K. D. R., Brown, N. M., & Lydeking-Olsen, E. (2002). The clinical importance of the metabolite equol-a clue to the effectiveness of soy and its isoflavones. *The Journal of Nutrition, 132*(12), 3577–3584. https://doi.org/10.1093/JN/132.12.3577

Silva, F. V. M., & Gibbs, P. (2001). Alicyclobacillus acidoterrestris spores in fruit products and design of pasteurization processes. *Trends in Food Science and Technology, 12*(2), 68–74. https://doi.org/10.1016/S0924-2244(01)00070-X

Stahl, W., Van Den Berg, H., Arthur, J., Bast, A., Dainty, J., Faulks, R. M., … Astley, S. B. (2002). Bioavailability and metabolism. *Molecular Aspects of Medicine, 23*(1–3), 39–100. https://doi.org/10.1016/S0098-2997(02)00016-X

Turunen, M., Olsson, J., & Dallner, G. (2004). Metabolism and function of coenzyme Q. *Biochimica et Biophysica Acta - Biomembranes, 1660*(1–2), 171–199. https://doi.org/10.1016/j.bbamem.2003.11.012

Verkempinck, S., Pallares Pallares, A., Hendrickx, M., & Grauwet, T. (2020). Processing as a tool to manage digestive barriers in plant-based foods: Recent advances. *Current Opinion in Food Science, 35*, 1–9. https://doi.org/10.1016/j.cofs.2019.11.007

Vieira, S. A., Zhang, G., & Decker, E. A. (2017). Biological implications of lipid oxidation products. *JAOCS, Journal of the American Oil Chemists' Society, 94*(3), 339–351. https://doi.org/10.1007/s11746-017-2958-2

Walle, T., Otake, Y., Walle, U. K., & Wilson, F. A. (2000). Quercetin glucosides are completely hydrolyzed in ileostomy patients before absorption. *The Journal of Nutrition, 130*(11), 2658–2661. https://doi.org/10.1093/JN/130.11.2658

Westerlund, E., Åman, P., Andersson, R., Andersson, R. E., & Rahman, S. M. M. (1991). Chemical characterization of water-soluble pectin in papaya fruit. *Carbohydrate Polymers, 15*(1), 67–78. https://doi.org/10.1016/0144-8617(91)90020-D

Wikiera, A., Grabacka, M., Byczyński, Ł., Stodolak, B., & Mika, M. (2021). Enzymatically extracted apple pectin possesses antioxidant and antitumor activity. *Molecules, 26* (5). https://doi.org/10.3390/molecules26051434

Wolffram, S., Blo, M., & Ader, P. (2002). *Arts, Sesink, Hollman - 2002 - Quercetin-3-glucoside is transported by the glucose carrier SGLT1 across the brush border membrane of rat.pdf. September 2001* (pp. 630–635).

Yemenicioğlu, A., Özkan, M., & Cemeroğlu, B. (1997). Heat inactivation kinetics of apple polyphenoloxidase and activation of its latent form. *Journal of Food Science, 62*, 508–510. https://doi.org/10.1111/j.1365-2621.1997.tb04417.x

Zhang, L., Zuo, Z., & Lin, G. (2007). Intestinal and hepatic glucuronidation of flavonoids. *Molecular Pharmaceutics, 4*(6), 833–845. https://doi.org/10.1021/mp700077z

Zhu, F., Du, B., & Xu, B. (2018). Anti-inflammatory effects of phytochemicals from fruits, vegetables, and food legumes: A review. *Critical Reviews in Food Science and Nutrition, 58*(8), 1260–1270. https://doi.org/10.1080/10408398.2016.1251390

Zielinska, D., Wiczkowski, W., & Piskula, M. K. (2008). Determination of the relative contribution of quercetin and its glucosides to the antioxidant capacity of onion by cyclic voltammetry and spectrophotometric methods. *Journal of Agricultural and Food Chemistry, 56*(10), 3524–3531. https://doi.org/10.1021/JF073521F/ASSET/IMAGES/LARGE/JF-2007-03521F_0005.JPEG

Zymone, K., Benetis, R., Trumbeckas, D., Baseviciene, I., & Trumbeckaite, S. (2022). Different effects of quercetin glycosides and quercetin on kidney mitochondrial function—Uncoupling, cytochrome C reducing and antioxidant activity. *Molecules, 27*(19). https://doi.org/10.3390/molecules27196377