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APPARENT BIOACCESSIBILITY OF PHENOLIC COMPOUNDS AND LYCOPENE FROM TOMATO POMACE DURING STATIC *IN VITRO* DIGESTION

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ABSTRACT. Tomato pomace, a major by-product of industrial tomato processing composed mainly of peels and seeds, represents a valuable source of bioactive compounds with potential nutraceutical applications. This study comparatively evaluated the apparent bioaccessibility of phenolic compounds and lycopene from tomato pomace using two distinct *in vitro* gastrointestinal digestion approaches: sequential phase sampling (R1) and cumulative digestion sampling (R2). A static digestion model including oral, gastric, and intestinal phases was applied to assess the release of bioactive compounds into digestive fluids. Total phenolic content was determined using the Folin–Ciocalteu method and expressed as gallic acid equivalents (GAE), while lycopene was quantified by UHPLC-DAD. Phenolic compounds showed the highest concentration in the oral phase (332.65±29.67 mg GAE L^{-1}), followed by the intestinal phase ((234.73±21.45 mg GAE L^{-1}), with the lowest value observed in the gastric phase (171.78±14.73 mg GAE L^{-1}). Lycopene bioaccessibility increased progressively during digestion, reaching 8.61% in the oral phase, 16.47% in the gastric phase, and 29.07% in the intestinal phase. The cumulative digestion fraction was lower (22.1%), suggesting partial degradation of lycopene and retention of carotenoids within the insoluble fiber fraction of the tomato pomace matrix. These results indicate that digestion conditions and matrix interactions significantly influence the release and apparent bioaccessibility of tomato-derived bioactive compounds.

Keywords: *Tomato pomace; phenolic compounds; lycopene; in vitro gastrointestinal digestion; bioaccessibility; food by-products.*

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INTRODUCTION

The tomato processing industry generates millions of tons of waste each year. Tomato pomace, the peels and seeds left after processing, makes up about 3–7% of the raw tomato weight. Typically discarded or used as low-value animal feed, this waste is now attracting growing interest as a promising source of valuable bioactive compounds. [1,2].

Tomato pomace contains two main types of health-promoting substances: phenolic compounds and lycopene. Phenolics, such as quercetin and rutin, have antioxidant and anti-inflammatory effects [3]. Lycopene, the pigment that gives tomatoes their distinctive red colour, has been associated with reduced risks of prostate cancer and cardiovascular disease [4]. Tomato peels are especially rich in lycopene, up to five times more than the pulp [5]. However, a critical limitation exists: simply having these bioactive compounds in food matrices does not ensure their physiological efficacy. Phenolic compounds and lycopene present fundamentally distinct physicochemical properties, phenolics are hydrophilic and readily soluble in aqueous environments, while lycopene is highly lipophilic and remains locked in the cellular structures of plants [6]. The determinant factor for health benefits is bioaccessibility, defined as the fraction of compounds released from the food matrix during digestion and made available for intestinal absorption [7,8]. The structural complexity of the food matrix has a profound influence: dietary fiber and proteins can form insoluble complexes with phenolics, while lycopene absorption requires the presence of lipids and emulsification by bile salts to facilitate micelle formation and subsequent uptake [9].

To study these processes without conducting clinical trials on humans, researchers use static *in vitro* digestion models. These systems replicate the physiological conditions of the human gastrointestinal tract, including appropriate pH gradients, enzymatic activities, and temporal dynamics, within a controlled and reproducible experimental setting [10,11]. The standardized INFOGEST protocol, originally developed by Minekus et al. [12] and later refined by Brodkorb et al. [13], has established itself as the internationally recognized gold standard methodology for such studies. Static *in vitro* digestion models, such as the INFOGEST protocol used in the present study, simulate gastrointestinal conditions using fixed experimental parameters including pH, enzyme activity, digestion time, and fluid composition. In contrast, dynamic digestion models continuously adjust these parameters during digestion and more closely reproduce physiological processes such as gradual gastric emptying, variable secretion rates, and peristaltic mixing. In the present work, only a static digestion model was applied, while dynamic digestion studies are discussed solely for comparative interpretation of the obtained results. Previous studies have shown distinct kinetic patterns for these bioactive

compounds. Phenolic compounds typically show high concentrations in the oral phase, decrease significantly under conditions of gastric acidity, and increase again during intestinal digestion [14,15]. In contrast, lycopene follows an inverse trajectory: initial concentrations remain low, followed by a progressive accumulation that reaches peak levels in the intestinal phase, where bile-mediated solubilization facilitates micelle formation and the release of the compound [16,17]. Although several studies have investigated either phenolic compounds or carotenoids individually during *in vitro* digestion, relatively few studies have simultaneously monitored hydrophilic and lipophilic bioactive compounds within the same tomato pomace matrix under standardized gastrointestinal conditions. Moreover, the methodological impact of sequential versus cumulative sampling strategies on apparent bioaccessibility estimates remains insufficiently explored. Since digestion sampling design may significantly influence the recovery and interpretation of matrix-bound compounds, a systematic comparison of these approaches is necessary for improving the reliability and comparability of *in vitro* digestion studies.

Therefore, this study aimed not only to evaluate the release kinetics of phenolic compounds and lycopene from tomato pomace during simulated gastrointestinal digestion, but also to systematically compare sequential (R1) and cumulative (R2) digestion protocols as methodological approaches for estimating apparent bioaccessibility.Using both sequential and cumulative sampling protocols, we monitored the apparent bioaccessibility profiles of these bioactive compounds, with distinct structures, throughout each digestive phase. The results contribute to a mechanistic understanding of the effects of the food matrix on nutrient availability and provide a scientific basis for the strategic utilization of byproducts resulting from tomato processing in functional food applications.

RESULTS AND DISCUSSION

Distribution of phenolic compounds during simulated gastrointestinal digestion

The initial total phenolic content of the undigested tomato pomace was 950 mg GAE/100g DW, while the initial lycopene content was 6.58 µg/g DW. These values were used as reference concentrations for calculating apparent bioaccessibility.

The release of phenolic compounds from tomato pomace during simulated gastrointestinal digestion was evaluated using two experimental protocols. In protocol R1, the supernatant was collected after each digestive phase, allowing the evaluation of phenolic compound distribution in the oral,

gastric, and intestinal fluids. In protocol R2, the digestion process was performed continuously without intermediate separation, and phenolic compounds were determined only in the final supernatant after complete digestion. This two-protocol approach remedies an important methodological gap identified in recent studies [18], as the choice between serial and cumulative sampling significantly influences estimates of apparent bioaccessibility and their biological relevance [19]. For protocol R1, the concentration of total phenolic compounds measured in the digestive fluids showed significant variation between phases. The highest concentration was observed in the oral phase (332.65±29.67 mg GAE/L), followed by the intestinal phase (234.73±21.45 mg GAE/L), while the gastric phase showed the lowest value (171.78±14.73 mg GAE/L) (Figure 1).

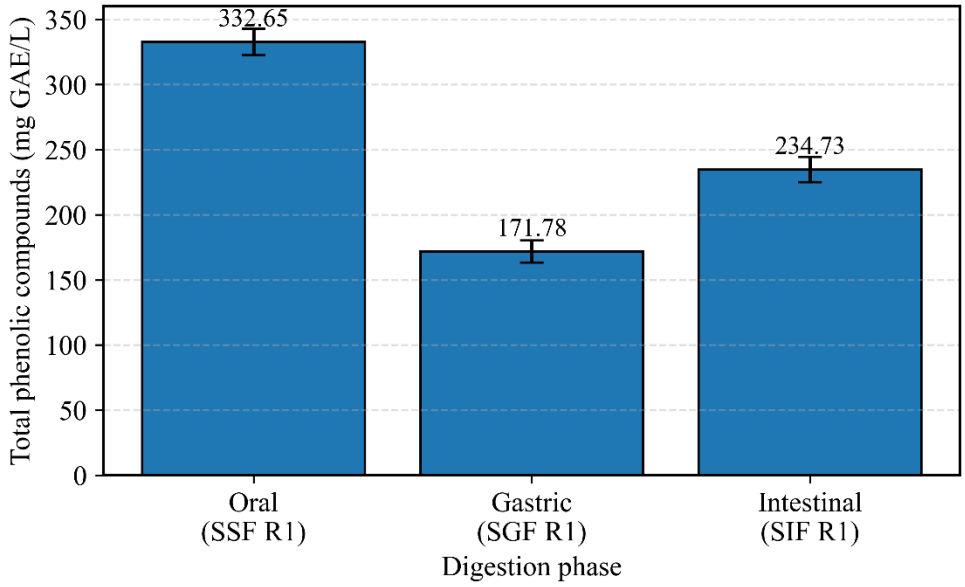

Figure 1. Concentration of total phenolic compounds released into the digestive supernatant during sequential *in vitro* digestion protocol R1. SSF, simulated salivary fluid; SGF, simulated gastric fluid; SIF, simulated intestinal fluid. Results are expressed as mg GAE/L. Error bars represent standard deviation of triplicate analyses.

One-way ANOVA indicated significant differences between digestion phases for total phenolic compounds ($p < 0.05$). The oral phase showed significantly higher phenolic release than the gastric phase, while the intestinal phase showed an intermediate recovery.

The peak observed during the oral phase is consistent with recent findings that surface phenolic compounds are rapidly released upon contact with saliva, before enzymes or acids break down the matrix [20, 21]. Similar patterns have been reported for anthocyanins in black carrots by Kamiloglu et al. [22] and for polyphenols in grapes by Tagliazucchi et al. [7], who also noted an initial rapid release followed by reassociation with the matrix. However, this early availability is short-lived, as phenolic compounds may be re-bound to fibers or degrade during longer incubation, a point that cumulative protocols often overlook [23], as demonstrated by Wang et al. [24] in studies on tomato waste.

The difference between our R1 and R2 results highlights the current debate regarding standards for digestion protocols. Recent studies conducted in multiple laboratories by Egger et al. [25] show that the timing of sample collection alone can alter apparent bioaccessibility estimates by 30–60% for matrix-bound polyphenols, confirming the concerns expressed by Carbonell-Capella et al. [26] regarding the choice of analytical methods. Our data suggest that the phenolic compounds in tomato pomace may behave as three functional fractions during digestion: a rapidly released fraction observed in the oral phase, a potentially reversibly bound fraction influenced by gastrointestinal conditions, and a fraction retained within the insoluble matrix residues. However, this interpretation should be considered a mechanistic hypothesis inferred from the digestion profiles and supported by literature describing polyphenol–fiber and polyphenol–protein interactions in plant-based matrices.

The distribution of phenolic compounds between the digestive phases was 45.0% in the oral phase, 23.2% in the gastric phase, and 31.8% in the intestinal phase. These results indicate that a considerable fraction of the phenolic compounds present in tomato pomace is rapidly released during the oral stage of digestion. This pattern mirrors findings by Kamiloglu et al. [27] for black carrot anthocyanins who similarly reported early-phase dominance due to rapid solubilization of surface-localized compounds. The high concentration observed in the oral phase suggests that a significant proportion of these compounds are present in a soluble form or are weakly bound to the plant cell structures consistent with the “labile fraction” concept described by Pérez-Jiménez and Saura-Calixto [28] for non-extractable polyphenols in fruits and vegetables.

Under neutral pH conditions and short incubation time, these compounds are readily transferred into the aqueous phase [29]. In contrast, the decrease in phenolic concentration observed during the gastric phase can be attributed to the acidic environment and potential interactions between phenolic compounds and structural components of the tomato pomace matrix. Similar gastric-phase drops were reported by Bouayed et al. [30] for apple

varieties. Tomato pomace contains considerable amounts of dietary fiber and residual proteins, which can bind phenolic compounds through hydrogen bonding or hydrophobic interactions. These interactions may limit the transfer of phenolic compounds into the liquid phase under gastric conditions, as demonstrated by Le Bourvellec and Renard [31] in their comprehensive review of polyphenol-macromolecule binding.

During the intestinal phase, the concentration of phenolic compounds increased again compared to the gastric phase. This increase may be explained by the higher pH and the presence of bile salts and pancreatic enzymes, which can destabilize interactions between phenolic compounds and the plant matrix. Consequently, phenolic compounds previously retained within the solid structure may be partially released into the digestive fluid, consistent with the “re-release” mechanism described by Jakobek [32] for polyphenol interactions with food matrices.

Phenolic Compounds after Complete Digestion

In protocol R2, phenolic compounds were determined only in the final supernatant after completion of the entire digestion process. The measured concentration was 63.92 mg GAE/L, considerably lower than the values obtained in individual digestive phases in protocol R1. This significant difference is similar to the findings of Pellegrini et al. [33], who reported similar differences between dynamic and static digestion of tomato waste, as well as to those of Fernández-Jalao et al. [34] in their meta-analysis of protocol variations. This difference can be explained primarily by the larger total volume of digestive fluids used in the cumulative digestion protocol, leading to dilution of phenolic compounds. Additionally, prolonged interaction between the liquid phase and the solid tomato pomace matrix may promote adsorption and retention of phenolic compounds within the fibrous structure as demonstrated by Elleuch et al. [35] for dietary fiber matrices. Tomato pomace is characterized by high contents of insoluble fiber and porous plant structures that can retain both digestive fluids and dissolved compounds. This retention effect, described by Saura-Calixto [36] as a key factor limiting polyphenol bioaccessibility from fiber-rich foods, can reduce the amount of phenolic compounds recovered in the supernatant after centrifugation, resulting in lower measured concentrations.

These results highlight the importance of the digestion protocol when evaluating the apparent bioaccessibility of phenolic compounds. However, R1 and R2 should not be interpreted as directly equivalent digestion outputs. The sequential protocol R1 describes the amount of phenolic compounds released into the supernatant after each individual digestive phase, while the cumulative protocol R2 represents the final soluble fraction recovered after

the complete digestion sequence without intermediate separation. Therefore, the comparison between R1 and R2 is used here to illustrate the methodological impact of sampling strategy on phenolic recovery, rather than to compare identical analytical endpoints. The lower value obtained for R2 may reflect dilution, prolonged contact with the insoluble matrix, re-adsorption of released phenolics, degradation, or incomplete recovery from the final supernatant. (Figure 2)

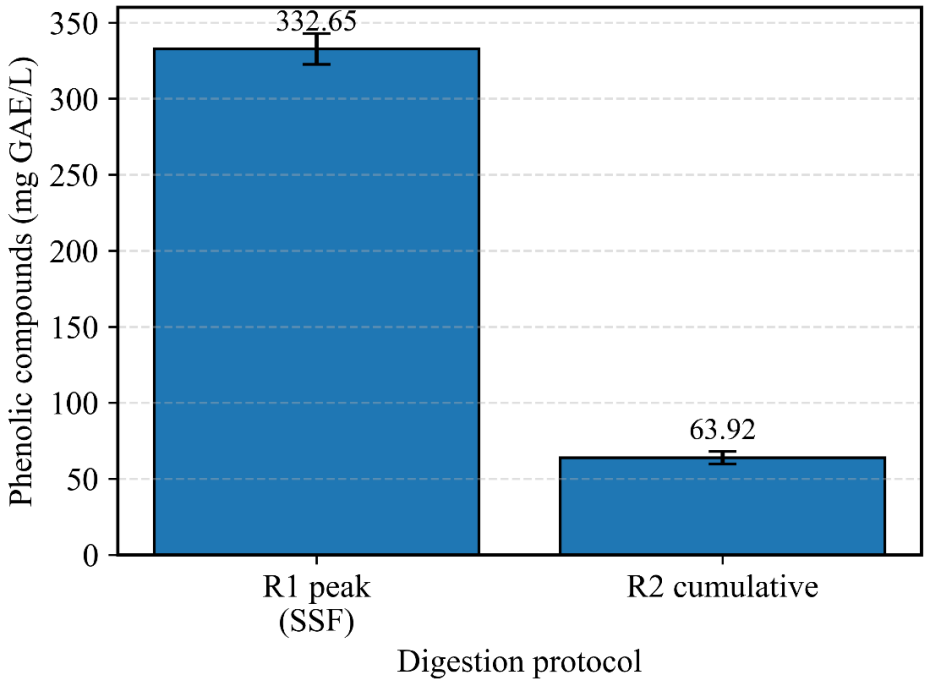

Figure 2. Comparison between sequential digestion protocol R1 and cumulative digestion protocol R2 for the release of total phenolic compounds from tomato pomace. Results are expressed as mg GAE/L in the recovered digestive supernatants. Error bars represent standard deviation of triplicate analyses.

The initial total phenolic content of the tomato pomace was 950 mg GAE/100 g DW. Based on this value, the apparent bioaccessibility of phenolic compounds released during simulated gastrointestinal digestion was calculated relative to the initial amount present in the undigested sample. (Tabel 1)

Table 1. Apparent bioaccessibility of phenolic compounds from tomato pomace during simulated gastrointestinal digestion

Digestion phase	Concentration in supernatant (mg GAE/L)	Digestion volume (mL)	Released phenolics (mg GAE)	Apparent bioaccessibility (%)
Oral phase (SSF R1)	332.65	2.5	0.832	17.5
Gastric phase (SGF R1)	171.78	2.5	0.429	9.0
Intestinal phase (SIF R1)	234.73	2.5	0.587	12.4
Cumulative digestion (R2)	63.92	17.5	1.119	23.6

The oral phase exhibited the highest apparent bioaccessibility (17.5%), followed by the intestinal phase (12.4%), while the gastric phase showed the lowest value (9.0%). In contrast, the cumulative digestion protocol (R2) resulted in a total apparent bioaccessibility of 23.6%, reflecting the overall soluble phenolic fraction remaining after prolonged interaction with the digestive matrix. These results indicate that the digestion protocol and sampling strategy significantly influence the estimation of phenolic compound release and apparent bioaccessibility from tomato pomace.

In vitro bioaccessibility of lycopene

The *in vitro* gastrointestinal digestion experiment revealed a gradual increase in lycopene release across the simulated digestion phases. The initial oral phase resulted in relatively low bioaccessibility of 8.61%, corresponding to approximately 0.57 μg g^{-1} released lycopene based on the average concentration determined in the samples (6.58 μg g^{-1} DW). This limited release aligns with findings by Reboul and Borel [37] who demonstrated that carotenoids remain largely sequestered within chromoplast structures and are poorly solubilized under neutral aqueous conditions of the oral environment. During the gastric phase, lycopene bioaccessibility increased to 16.47% (approximately 1.08 μg g^{-1}). The acidic conditions and mechanical agitation promote partial disruption of cellular structures and protein–carotenoid complexes, facilitating the release of lipophilic compounds from the tomato matrix (Figure 3), consistent with mechanisms described by Fernández-García et al. [38] for carotenoid apparent bioaccessibility from plant foods. Notably, this gastric-phase increment remains modest compared to intestinal values, reflecting the inherent limitation of aqueous acidic environments for solubilizing highly lipophilic compounds without lipid co-components or bile-mediated emulsification [39].

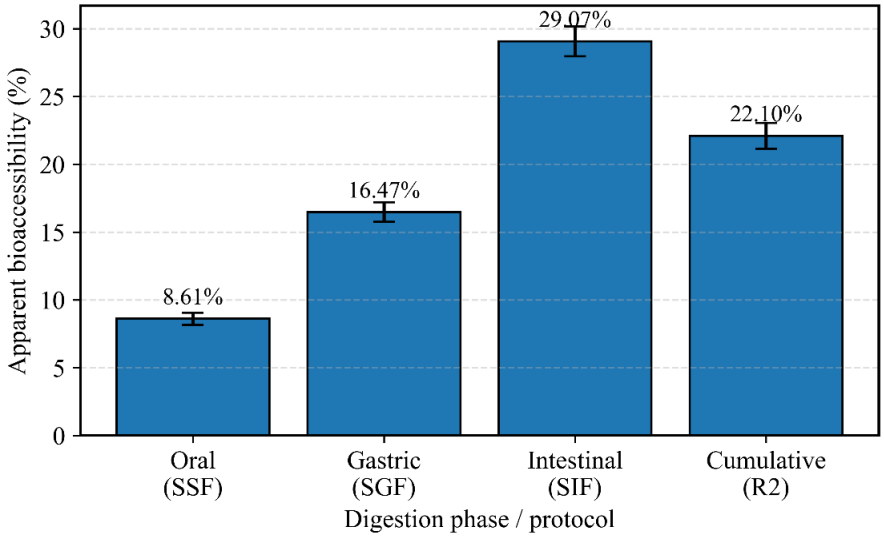

Figure 3. Apparent bioaccessibility of lycopene from tomato pomace during simulated *in vitro* gastrointestinal digestion. Values are expressed as percentage of the initial lycopene content in the undigested tomato pomace. Error bars represent standard deviation of triplicate analyses.

The highest bioaccessibility was observed during the intestinal phase, reaching 29.07%, equivalent to approximately 1.91 μg g^{-1} lycopene released from the tomato pomace matrix. In addition to micellar solubilization, digestive conditions may also induce structural changes in lycopene. Lycopene occurs predominantly as the all-trans isomer in raw tomato matrices, but thermal processing, acidic pH, bile salts, digestive enzymes, and prolonged incubation may promote partial trans-to-cis isomerization. This aspect is relevant because cis-isomers generally present higher solubility in lipid phases and may be more efficiently incorporated into mixed micelles than the all-trans form [6, 8]. Therefore, the increase observed during the intestinal phase may reflect not only the release of lycopene from the tomato pomace matrix, but also changes in isomeric profile that favor micellar incorporation. At the same time, lycopene is highly susceptible to oxidative degradation due to its conjugated double-bond structure. Exposure to oxygen, light, acidic conditions, and digestive incubation may contribute to partial degradation or loss of recoverable lycopene [38]. These processes may partly explain why the cumulative bioaccessibility value was lower than the maximum intestinal-phase value. One-way ANOVA also indicated significant differences in lycopene apparent bioaccessibility between digestion phases ($p < 0.05$), with the intestinal phase showing the highest bioaccessibility.

This increase is consistent with the presence of bile salts and digestive enzymes in the intestinal environment, which promote the formation of mixed micelles necessary for the solubilization and potential absorption of carotenoids. Interestingly, the overall cumulative bioaccessibility calculated across the digestion process was lower (22.1%) than the maximum value observed during the intestinal phase. This apparent decrease may be attributed to several factors, including oxidative degradation of lycopene during digestion, possible trans-to-cis isomerization followed by differential recovery of isomers, adsorption of carotenoids onto insoluble dietary fiber fractions, or losses during sample processing and phase separation [39,]. Similar discrepancies between phase-specific and cumulative recovery were reported by Corte-Real et al. [40]. Tomato pomace contains significant levels of dietary fiber and insoluble polysaccharides, which can retain lipophilic compounds within the solid fraction and limit their transfer to the micellar phase, as demonstrated by Saura-Calixto [36]. These findings are consistent with previously reported values for tomato matrices, where lycopene bioaccessibility typically ranges between 10 and 35%, depending on processing conditions, matrix structure, and lipid content. The presence of endogenous lipids from tomato seeds, particularly unsaturated fatty acids such as linoleic and oleic acids, may contribute to micelle formation and thus enhance carotenoid solubilization during the intestinal phase, consistent with the lipid effect described by Brown et al. [41] and Roodenburg et al. [42] for carotenoid absorption.

CONCLUSIONS

This study evaluated the apparent bioaccessibility of phenolic compounds and lycopene from tomato pomace during simulated *in vitro* gastrointestinal digestion. The results demonstrated that these bioactive substances follow significantly different release patterns during the digestive phases. Phenolic compounds exhibited the highest concentration in the oral phase, decreased in the gastric phase, and then partially recovered in the intestinal phase. This fluctuating profile may indicate the presence of phenolic compounds with different interaction strengths within the tomato pomace matrix, including readily soluble compounds and fractions more strongly associated with insoluble structural components. In contrast, lycopene showed a progressive release, confirming that bile-mediated emulsification is essential for the solubilization of carotenoids.

The cumulative digestion protocol (R2) yielded considerably lower values than sequential sampling (R1) for both classes of compounds. One of the most significant findings of this study is that the choice of digestion

protocol substantially affects apparent bioaccessibility estimations for both hydrophilic and lipophilic compounds. Sequential phase analysis (R1) provided a dynamic representation of compound release throughout digestion, whereas cumulative digestion (R2) reflected the final soluble fraction remaining after prolonged matrix interaction. The large discrepancies observed between the two approaches demonstrate that sampling strategy is not merely a procedural detail, but a critical methodological factor influencing the interpretation and comparability of *in vitro* digestion studies.

These findings demonstrate that the properties of the food matrix and digestive conditions influence the release of phytochemicals in a combined manner, but in ways specific to each compound. Phenolic compounds respond primarily to pH changes and the action of surfactants, while lycopene depends on lipid availability and micelle formation. Tomato pomace represents a promising dual source of hydrophilic and lipophilic bioactive compounds for nutraceutical applications. However, realizing this potential may require tailored processing strategies, like breaking down cell walls to boost the release of phenolic compounds or mixing in lipids to make lycopene more bioavailable. Therefore, the comparative evaluation of R1 and R2 protocols represents an important methodological contribution for future bioaccessibility studies involving complex plant-based matrices and food by-products.

EXPERIMENTAL SECTION

Chemicals and Reagents

All solvents used were of analytical or HPLC grade. Methanol, acetone, hexane, sodium carbonate, sodium chloride (NaCl), potassium chloride (KCl), potassium dihydrogen phosphate (KH_2PO_4), sodium hydrogen carbonate ($NaHCO_3$), calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$), magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$), and hydrochloric acid (HCl) were purchased from Merck (Darmstadt, Germany). Folin–Ciocalteu reagent and gallic acid standard were obtained from Sigma-Aldrich (Saint Louis, MO, USA). Digestive enzymes including α-amylase from porcine pancreas (≥5 U/mg), pepsin from porcine gastric mucosa (≥2500 U/mg protein), pancreatin from porcine pancreas (4× USP), and bile extract were also purchased from Sigma-Aldrich (Steinheim, Germany). Ultra-pure water was obtained using an ULTRACLEAR UV UF EVOQUA purification system (Pittsburgh, PA, USA).

Sample Preparation

Tomato pomace consisting of peels and seeds obtained as by-products from industrial tomato processing was used as raw material.The tomato pomace samples were dried in a laboratory oven (MEMMERT UN55, Schwabach, Germany) at 50 °C until constant weight, in order to reduce moisture while limiting thermal degradation of phenolic compounds and lycopene. The dried material was subsequently ground to obtain a homogeneous powder and stored in airtight containers at 4 °C until further analysis.

For digestion experiments, approximately 0.5 g of dried tomato pomace was used. For phenolic compound extraction, approximately 2.5 g of sample was extracted with 10 mL of methanol for 20 min in an ultrasonic bath (Sonorex RK 512H, BANDELIN electronic GmbH & Co. KG, Berlin, Germany) at room temperature. The extracts were subsequently centrifuged at 11,000 rpm for 2 min using a MicroCL 17 centrifuge (Thermo Fisher Scientific, Waltham, MA, USA), and the supernatant was filtered through a 0.45 µm cellulose membrane filter (Whatman, Sigma-Aldrich, St. Louis, MO, USA) prior to analysis.

Determination of Total Phenolic Compounds

Total phenolic compounds were determined using the Folin–Ciocalteu method. Briefly, 0.5 mL of sample extract was mixed with 5 mL of distilled water and 0.5 mL of commercial Folin–Ciocalteu reagent. After 3 min, 1.5 mL of sodium carbonate solution (10%, w/v) was added and the mixture was incubated for 45 min at room temperature in the dark. The absorbance was measured at 765 nm using a UV-Vis spectrophotometer (Lambda 25, Perkin Elmer, Waltham, MA, USA). Gallic acid was used for calibration, and the results were expressed as gallic acid equivalents (GAE).

Determination of lycopene by UHPLC–DAD

Lycopene was extracted from dried tomato pomace samples according to a modified solvent extraction procedure [43]. Approximately 0.5 g of homogenized dried sample was mixed with 10 mL of hexane: acetone: ethanol (2:1:1, v/v/v). The mixture was vortexed and allowed to stand for 10 min at room temperature to facilitate carotenoid solubilization. The samples were then centrifuged at 11,000 rpm for 2 min and the supernatant was filtered through a 0.45 µm cellulose membrane filter. Ultrasound-assisted extraction was not applied for lycopene in order to limit possible oxidative degradation and isomerization of this highly unsaturated carotenoid. All extraction steps were carried out under reduced light conditions to minimize carotenoid degradation.

Lycopene determination was performed using a UHPLC system (Vanquish, Thermo Fisher Scientific, Germany) equipped with a diode array detector. Chromatographic separation was achieved on an Acclaim™ C30 column (3 µm, 3.0 × 150 mm) maintained at 40 °C. The mobile phase consisted of methanol containing 3.2 g L^{-1} ammonium acetate (70%) and acetonitrile (30%) delivered at a flow rate of 1.7 mL min^{-1}. The injection volume was 8 µL. Detection was performed at 460 nm and quantification was achieved using external calibration curves prepared with certified lycopene standards in the concentration range 1–20 µg mL^{-1} with a coefficient of determination (R^2) of 0.9994. Results were expressed as µg g^{-1} dry weight. All analyses were carried out in triplicate. (Figure 4)

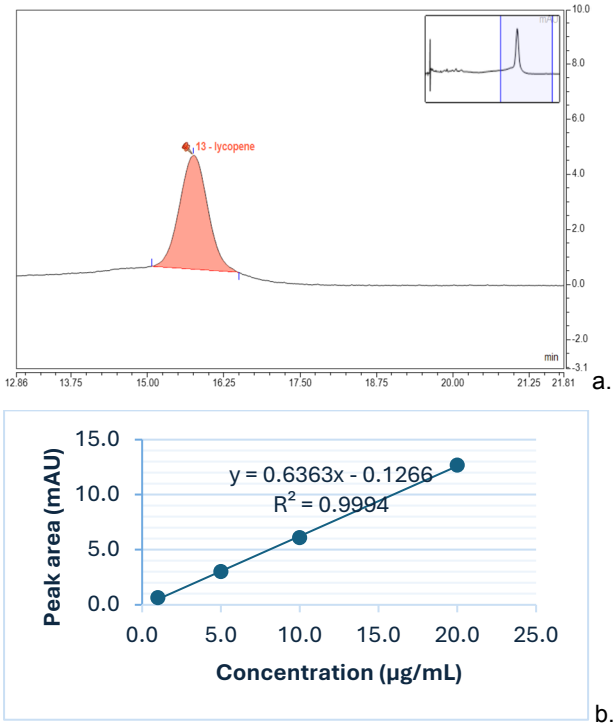

Figure 4. (a) Representative UHPLC-DAD chromatogram of lycopene standard; (b) External calibration curve used for lycopene quantification, showing excellent linearity within the investigated concentration range, y = 0.6363x - 0.1266, R^2 = 0.9994.

Apparent bioaccessibility (%) was calculated as follows: Bioaccessibility (%) = (amount of compound recovered in the digestive supernatant / initial amount of compound in the undigested tomato pomace) × 100.

In Vitro Gastrointestinal Digestion

A static *in vitro* gastrointestinal digestion model was applied to simulate the digestive conditions of the human gastrointestinal tract. The digestion protocol was adapted from the standardized method described by Minekus et al. and Brodkorb et al., which has been widely used for evaluating nutrient bioaccessibility in food matrices [12, 13]. The digestion process consisted of three sequential phases: oral, gastric, and intestinal.

The simulated digestive fluids were prepared using the electrolyte compositions recommended by the INFOGEST protocol, including sodium chloride (NaCl), potassium chloride (KCl), potassium dihydrogen phosphate (KH_2PO_4), sodium hydrogen carbonate ($NaHCO_3$), magnesium chloride hexahydrate ($MgCl_2 \cdot 6H2O$), calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$), ammonium carbonate ($(NH_4)2CO_3$), sodium hydroxide (NaOH), and hydrochloric acid (HCl).

For the digestion experiments, 0.5 g of dried tomato pomace sample was used. During the oral phase, the sample was mixed with 2.5 mL simulated salivary fluid (SSF) containing α-amylase (75 U/mL). The mixture was adjusted to pH 7.0 and incubated for 2 min at 37 °C under continuous agitation (100 rpm). For the gastric phase, the remaining solid fraction or oral bolus was mixed with 2.5 mL simulated gastric fluid (SGF) containing pepsin (2000 U/mL) and gastric lipase (60 U/mL). The pH was adjusted to 3.0 using 1 M HCl, under continuous monitoring with a calibrated pH meter, and the samples were incubated for 2 h at 37 °C under continuous agitation. or the intestinal phase, the gastric chyme was mixed with 2.5 mL simulated intestinal fluid (SIF) prepared using the electrolyte components recommended by the INFOGEST protocol, including NaCl, KCl, KH_2PO_4, $NaHCO_3$, $MgCl_2 \cdot 6H_2O$, and $CaCl_2 \cdot 2H_2O$. The SIF contained pancreatin corresponding to trypsin activity of 100 U/mL and bile salts at a final concentration of 10 mM. The pH was adjusted to 7.0 using NaOH solution under continuous monitoring with a calibrated pH meter, and the digestion continued for 2 h at 37 °C under continuous agitation at 100 rpm. Two digestion approaches were applied. In protocol R1, the supernatant was separated after each digestion phase by centrifugation and analyzed individually. In protocol R2, the digestion process was performed continuously without intermediate separation, with cumulative addition of digestive fluids throughout the digestion sequence.

At the end of digestion, enzyme activity was stopped by placing the samples in an ice bath for 10 min. The digested samples were centrifuged at 4500 rpm for 30 min using a Universal 320 centrifuge (Hettich, Tuttlingen, Germany). The supernatants were collected, filtered through 0.45 µm membrane filters, and stored at 4 °C until analysis.

Blank digestion samples containing the same digestive fluids and enzymes without tomato pomace were processed under identical conditions to correct possible interferences originating from enzymes and digestion buffers. All analyses were performed in triplicate.

Statistical Analysis

All analyses were performed in triplicate, and the results are expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way ANOVA to evaluate significant differences between digestion phases. Differences were considered statistically significant at $p < 0.05$.

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REFERENCES

1. M. Knoblich; B. Anderson; D. Latshaw; *J. Food Sci.*, **2005**, 70, C495–C500
2. N. Mirabella; V. Castellani; S. Sala; J. *Clean. Prod*., **2014**, 65, 28–41
3. A. Raiola; M. M. Rigano; R. Calafiore; L. Frusciante; A. Barone; *Mediators Inflamm*., **2014**, 139873.
4. E. Giovannucci; *Exp. Biol. Med*., **2002**, 227, 852–859
5. I. F. Strati; V. Oreopoulou; *Int. J. Food Eng*., **2011**, 7, 1–16
6. E. Reboul; *Nutrients*, **2013**, 5, 3563–3581
7. D. Tagliazucchi; E. Verzelloni; D. Bertolini; A. Conte; *Food Chem*., **2010**, *120*, 599–606
8. E. Fernández-García; I. Carvajal-Lérida; M. Jarén-Galán; J. Garrido-Fernández; A. Pérez-Gálvez; D. Hornero-Méndez; *Food Res. Int*., **2012**, *46*, 438–450
9. F. Saura-Calixto; J. Agric. *Food Chem*., **2011**, *59*, 43–49
10. C. H. M. Versantvoort; A. G. Oomen; E. Van de Kamp; C. J. M. Rompelberg; A. J. A. M. Sips; *Food Chem. Toxicol*., **2005**, *43*, 31–40
11. M. S. Brewer; Compr. Rev. *Food Sci. Food Saf*., **2011**, *10*, 221–247
12. M. Minekus; M. Alminger; P. Alvito; S. Ballance; T. Bohn; C. Bourlieu; F. Carrière; R. Boutrou; M. Corredig; D. Dupont; C. Dufour; L. Egger; M. Golding; S. Karakaya; B. Kirkhus; S. Le Feunteun; U. Lesmes; A. Maclerzanka; A. MacKie; et al.; *Food Funct*., **2014**, *5*, 1113–1124

13. A. Brodkorb; L. Egger; M. Alminger; P. Alvito; R. Assunção; S. Ballance; T. Bohn; C. Bourlieu-Lacanal; R. Boutrou; F. Carrière; A. Clemente; M. Corredig; D. Dupont; C. Dufour; C. Edwards; M. Golding; S. Karakaya; B. Kirkhus; S. Le Feunteun; U. Lesmes; A. Macierzanka; A. R. Mackie; C. Martins; S. Marze; D. J. McClements; O. Ménard; M. Minekus; R. Portmann; C. N. Santos; I. Souchon; R. P. Singh; G. E. Vegarud; M. S. J. Wickham; W. Weitschies; I. Recio; *Nat. Protoc.*, **2019**, *14*, 991–1014
14. J. Bouayed; L. Hoffmann; T. Bohn; *Food Chem.*, **2011**, *128*, 14–21
15. J. Pérez-Jiménez; F. Saura-Calixto; *Food Res. Int.*, **2015**, *74*, 315–323
16. G. C. Carvalho; B. A. F. de Camargo; J. T. C. de Araújo; M. Chorilli; *Trends Food Sci. Technol.*, **2021**, *118*, 447–458
17. G. Knockaert; S. K. Pulissery; I. Colle; S. Van Buggenhout; M. Hendrickx; A. V. Loey; *Food Chem.*, **2012**, *135*, 1290–1297
18. D. Dupont; M. Alric; S. Blanquet-Diot; et al.; Crit. Rev. *Food Sci. Nutr.*, **2019**, *59*, 1546–1562
19. L. Egger; O. Ménard; C. Delgado-Andrade; et al.; *Food Res. Int.*, **2022**, 154, 110962
20. V. Athanasiadis; T. Chatzimitakos; K. Kotsou; D. Kalompatsios; E. Bozinou; S. I. Lalas; *Int. J. Mol. Sci.*, **2023**, *24*, 15914
21. M. Morzel; F. Canon; S. Guyot; J. Agric. Food Chem., **2022**, *70*, 6317–6327
22. S. Kamiloglu; A. A. Pasli; B. Ozcelik; J. Van Camp; E. Capanoglu; *Food Chem.*, 2015, *186*, 74–82
23. F. Saura-Calixto; J. Pérez-Jiménez; Curr. Opin. Food Sci., **2021**, 42, 168–175
24. C. Wang; H. Wu; Z. Liu; C. J. Barrow; F. Dunshea; H. A. R. Suleria; *Food Funct.*, **2022**, *13*, 4954-4966
25. L. Egger; O. Ménard; C. Delgado-Andrade; P. Alvito; R. Assunção; et al.; *Food Res. Int.*, **2016**, *88*, 217–225
26. J. M. Carbonell-Capella; M. Buniowska; F. J. Barba; M. J. Esteve; A. Frígola; Compr. Rev. *Food Sci. Food Saf.*, **2014**, *13*, 155–171
27. S. Kamiloglu; A. A. Pasli; B. Ozcelik; et al.; *LWT-Food Sci. Technol.*, **2015**, *64*, 1305–1312
28. J. Pérez-Jiménez; F. Saura-Calixto; *Food Res. Int.*, **2015**, *74*, 315–323
29. A. Sridhar; M. Ponnuchamy; P. S. Kumar; A. Kapoor; D. N. Vo; S. Prabhakar; *Environ. Chem. Lett.*, **2021**, *19*, 3409–3443
30. J. Bouayed; L. Hoffmann; T. Bohn; *Food Chem.*, **2011**, *128*, 14–21
31. C. Le Bourvellec; C. M. G. C. Renard; Crit. Rev. *Food Sci. Nutr.*, **2012**, *52*, 213–248
32. L. Jakobek; M. Matošić; *Foods*, **2019**, *8*, 633
33. M. Pellegrini; R. Lucas-Gonzalez; E. Sayas-Barberá; et al.; *Food Res. Int.*, **2021**, *147*, 110546
34. I. Fernández-Jalao; C. Sánchez-Moreno; B. De Ancos; Crit. Rev. *Food Sci. Nutr.*, **2022**, *62*, 4892–4908
35. M. Elleuch; D. Bedigian; O. Roiseux; et al.; *Food Chem.*, **2011**, *124*, 411–421
36. F. Saura-Calixto; *J. Agric. Food Chem.*, **2011**, *59*, 43–49
37. E. Reboul; P. Borel; Prog. *Lipid Res.*, **2011**, *50*, 388–402

38. E. Fernández-García; I. Carvajal-Lérida; M. Jaren-Galan; et al.; *Food Res. Int*., **2012**, *46*, 438–450
39. P. Borel; C. Desmarchelier; U. Dumont; et al.; Br. J. Nutr., **2016**, *115*, 1701–1711
40. J. Corte-Real; E. Richling; L. Hoffmann; *Food Funct*., **2016**, *7*, 3235–3244
41. M. J. Brown; M. G. Ferruzzi; M. L. Nguyen; et al.; *Am. J. Clin. Nutr*., **2004**, *80*, 396–403
42. A. J. Roodenburg; R. Leenen; H. van Berg; et al.; *Am. J. Clin. Nutr.,* **2000**, *71*, 1187–1193
43. W. W. Fish; P. Perkins-Veazie; J. K. Collins; J*. Food Compos. Anal.*, **2002,** *15*, 309-317