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Tomato phenolic extract reduces starch digestibility: potential for developing low-glycaemic starchy meals

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Marina Corrado,^{*ab} Clara Abarca-Rivas,^{ab} Guillermo D. Manrique,^c Marta Titos-Zamora,^a Joan Romanyà,^{bd} Maria Pérez,^{ab} Anna Vallverdú-Queralt^{*ab} and Rosa M. Lamuela-Raventós^{ab}

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Interactions among food components can influence nutrient digestibility and absorption. This study investigates the relationship between starch and tomato polyphenols to predict potential effects on glycaemia. Phenolic extracts were prepared from raw tomato peels and processed tomato paste, characterised using LC-MS/MS targeted metabolomics, and applied to cooked starch to measure amylolysis using an *in vitro* single-enzyme digestion model. Two doses of tomato-derived phenolics were tested. Polyphenols are hypothesised to reduce starch hydrolysis by forming non-covalent interactions with starch or by inhibiting the enzyme, or both. At an equal starch-to-extract ratio (25 mg), starch digestibility was reduced by 10%. At a lower extract dose (12.5 mg, half the starch weight), only the tomato paste extract significantly reduced starch digestion, suggesting a synergistic effect of multiple phenolic compounds present in the tomato paste extract. These findings highlight the potential of tomatoes to moderate postprandial glycaemia when consumed with starch-rich foods. Furthermore, the use of tomato byproducts, such as the peel, could enhance the phenolic content of tomato products, possibly amplifying their health effects.

1. Introduction

The Mediterranean diet is defined by a predominant intake of plant-based foods, olive oil as the main fat source, moderate amounts of fermented dairy, moderate fish and poultry, low red meat, and typically a (moderate) wine consumption with meals.¹ Among the plant-based foods, it is common to find high-glycaemic foods such as bread, rice, potatoes and pasta, rich in starch content, frequently combined with vegetables or sauces. Tomatoes, a central element of this dietary pattern, are commonly consumed both raw and cooked, often as sauces accompanying starch-rich meals. They are a notable source of bioactive compounds, including phenolics and carotenoids, which may influence metabolic responses to meals. Understanding how food combinations influence postprandial glycaemia is particularly relevant in the context of this meal-based pattern. Reducing postprandial glycaemic excursions is a key dietary strategy to lower the risk of non-communicable diseases such as type 2 diabetes (T2D).

Several mechanisms have been proposed for polyphenols effects on glucose metabolism, including direct inhibition of pancreatic α -amylase,²⁻⁵ the primary starch-digesting enzyme, and formation of weak bonds with amylose that limit enzymatic access.⁶ In addition, systemic anti-inflammatory effects associated with (poly)phenols antioxidant activity may enhance glucose sensitivity and β -cell function. Studies on the antidiabetic effects of (poly)phenols show mixed results, indicating a need for further research on dosage, duration, and form of

intervention.⁷ While some findings report that (poly)phenol-rich foods or beverages reduce glucose response when consumed with carbohydrates, outcomes vary depending on the phenolic-carbohydrate combination and source. Most research has focused on grapes, tea, berries, and coffee,⁸ with limited work on tomatoes. This is likely due to the relatively low phenolic content in the tomato pulp, as most bioactive compounds are concentrated in the peel, which can be discarded during industrial processing of sauces and pastes.⁹ Therefore, by repurposing tomato by-products rich in bioactive compounds, it may be possible to develop novel functional foods with lower glycaemic impact and add value to a byproduct that is normally discarded.

Starch is the main carbohydrate in both Western and Mediterranean diets, present in most high-glycaemic foods. When digested, glucose is released by enzymatic hydrolysis and absorbed into the bloodstream, influencing the extent of the postprandial glycaemic response.¹⁰ However, meal ingredients may affect starch digestion and glucose release which directly influence glycaemic response. Understanding interactions between tomato phenolics and starch is therefore critical, as the meal composition and the presence of bioactive compounds can modulate glycaemic response. This is a way to move beyond the current strategies to reduce the glycaemic impact of starch-rich foods, primarily focused on ingredient modifications or processing adjustments.¹¹ Considering their dietary relevance, investigating how tomato-derived phenolics interact with starch is key to understanding their role in glycaemic regulation.

This study investigates the interaction between starch and tomato-derived phenolic extracts to assess the contribution of phenolics to starch digestibility, using a hydroalcoholic extraction that limits the presence of insoluble matrix components such as fibre. We hypothesised that differences in phenolic profile, rather than total content alone, drive their effects on starch digestion, and that these effects are dose-dependent. We compared extracts obtained from the peel of raw tomatoes and from processed tomato paste, focusing on a variety previously characterised as high in phenolic content (Cuban Pepper).¹²

^a Polyphenol Research Group, Department of Nutrition, Food Science and Gastronomy, Faculty of Pharmacy and Food Sciences, Institute of Nutrition and Food Safety (INSA-UB), University of Barcelona, 08028 Barcelona, Spain. Address here.

^b CIBER Physiopathology of Obesity and Nutrition (CIBEROBN), Institute of Health Carlos III, 28029 Madrid, Spain.

^c Faculty of Engineering, Universidad Nacional del Centro de la Provincia de Buenos Aires, Av. del Valle 5737, Olavarría 7400, Buenos Aires, Argentina.

^d Department of Biology, Health and the Environment, Faculty of Pharmacy and Food Sciences, Institute of Nutrition and Food Safety (INSA-UB), University of Barcelona, 08028 Barcelona, Spain

*Corresponding authors: marinacorrado@ub.edu and avallverdu@ub.edu



Two doses of tomato-derived polyphenols were tested to evaluate the dose-response effect on starch digestibility.

from the standard curve of gallic acid ranging from 1 mg/L to 24 mg/L.
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2. Materials and methods

2.1. Tomatoes

To evaluate the effect of variety and processing, we used the peel of two tomato varieties (*Solanum lycopersicum*) and one commercial tomato paste. The selected tomato varieties were the Cuban Pepper, also known as Peruvian Horn or Andine Cornue, characterised by an elongated and pointed shape, and the Mar Azul tomato, a Spanish heirloom non-transgenic tomato variety, known for its distinctive bluish-purple skin, shown in Supplementary Figure 1. Both tomatoes varieties were cultivated in Spain during 2023-2024. Tomatoes were peeled manually, separating the peel from the pulp and the seeds. The peels were then freeze-dried and stored for analysis.

The tomato paste was a commercial triple-concentrated tomato paste (Conesa) obtained from the concentration of washed, sound, ripe tomatoes. The final product did not have any additive except salt (28/30°BRX).

2.2. Production of phenolic extract

Phenolic compounds were extracted from 0.5 g of freeze-dried tomato peels or paste, using 5 mL of an ethanol and Milli-Q water solution (8:2, v/v). The samples were resuspended in the extraction solution and sonicated 5 min on ice, then vortexed for 30 sec. Subsequently, they were centrifuged at 2000×g for 20 min at 4°C. The resulting supernatant, containing the polyphenol extracted, was collected in a separate tube by filtering through a Buchner funnel with filtered paper using vacuum suction. The extraction process was repeated twice, and the combined filtered supernatants were then transferred into amber tubes. The extract solution was evaporated overnight using a MiVac sample concentrator (miVac DNA concentrator, Genevac™, Ipswich, UK). The extractions were carried out under UV-filtered light to avoid phenolic degradation.

2.3. Total Phenolic Content of extracts

The Total Phenolic Content (TPC) of the extracts were measured by Folin-Ciocalteu (F-C) Assay.¹³ Phenolic extracts were resuspended in 1 mL of methanol, and 20 µL of the extract solution were added to a 96-well plate (nunc, Roskilde, Denmark), in triplicate, followed by 188 µL of Milli-Q water, 12 µL of F-C reagent and 30 µL of sodium carbonate 20% (w/v), as described by Medina-Remón *et al.*(2)¹⁴ The blank, consisting of 20 µL of methanol, and the standard curve were prepared following the same procedure as the samples. They were incubated for 1 h at room temperature in the dark, after which, 50 µL of Milli-Q water were added, and the absorbance was measured at 765 nm in a UV/vis plate reader (Varioskan LUX 3020, Thermo Scientific, USA). Results were expressed as the mean ± SE (n = 3) in mg of gallic acid equivalents (GAE)/g of extract (DW) obtained

2.4. Targeted phenolic quantification by high-resolution UPLC-ESI-QTRAP-MS/MS

A targeted analysis was carried out using UHPLC-ESI-MS/MS according to Rinaldi de Alvarenga *et al.*¹⁵ Dry phenolic extracts were resuspended in acidified water (0.1% formic acid). They were filtered through 0.22 µm PTFE syringe filters, transferred into an amber glass vial and injected into an Acquity UHPLC (Waters, Milford, MA, USA) system equipped with a binary pump and autosampler coupled to an API 4000 triple-quadrupole mass spectrometer (PE SCIEX, Concord, ON, Canada) with electrospray-ionisation UHPLC-ESI-QTRAP-MS/MS operating in negative mode. Chromatographic separation was achieved using an Atlantis T3 3µm 2.1 x 100 mm column (Waters; Milford, MA, USA) at 40°C with a flow rate of 0.4 mL/min and an injection volume of 10 µL using as mobile phases water (A) and acetonitrile (B) both with 0.1% of formic acid, with a gradient of B of 40% in 16 min, 95% in 17 min, then maintained at 95% until 19 min to then return to initial condition. Turbo Ionspray source in negative mode was set as follows: capillarity voltage -4500 V, nebulizer gas (N₂) 10 arbitrary units (au); curtain gas (N₂) 25 au; collision gas (N₂) 4 au, focusing potential, -200 V; entrance potential, -10 V; drying gas (N₂), heated to 325°C and delivered at a flow rate of 8000 cm³/min. The declustering potential and collision energy were optimized for each compound in infusion experiments: individual standard solutions (10 µg/mL) dissolved in 50:50 (v/v) mobile phase were infused at a constant flow rate of 5 µL/min using a model syringe pump (Harvard Apparatus, Holliston, MA, USA). Full-scan data acquisition was performed scanning from m/z 100 to 700 in profile mode, with a cycle time of 2 sec, a step size of 0.1 u, and a pause of 2 millisecond between each scan.

Compounds quantification was carried out with MultiQuant software (Sciex, Foster City, CA, USA) and peaks with signal-to-noise (S/N) below 5 were excluded from the analysis. Compounds were quantified using external standards based on the retention time (RT) and exact mass (m/z) using standard calibration curves ranging from 0.1 to 2 mg/L. Ethyl gallate (0.5 mg/L) was used as internal standard for both calibration curves and samples. Identified compounds were quantified using reference standard curves of 4-caffeoylquinic acid, 5-caffeoylquinic acid, 3,4-dicaffeoylquinic acid, 4,5-dicaffeoylquinic acid, ferulic acid, caffeic acid, *p*-coumaric acid, 3,4-dihydroxycinnamic acid, protocatechuic acid, naringenin-7-*O*-glucoside, naringenin, rutin, quercetin, quercetin-3-β-D-glucoside. Caffeic-*O*-glucoside was quantified using a naringenin-7-*O*-glucoside standard curve. Concentrations were expressed as standard equivalents for each compound class and reported both as µg per mg of extract (Figure 1) and as µg per mg of tomato fresh weight (FW) in Table 1.

2.5. Starch amylolysis



Starch susceptibility to amylase digestion (amylolysis) was determined using an established amylolysis assay based on enzyme-kinetic principles.¹⁶ Although this simplified model does not fully replicate physiological digestion, it provides a robust and high-throughput system to directly assess starch–phenolic interactions under controlled conditions. A 5 mg/mL suspension of wheat starch (Merck, 9005-25-8) in Phosphate-Buffered Saline (PBS, Merk 524650) was gelatinised for 20 min at 90°C. Samples were cooled, and the phenol extract was added to achieve the same starch weight in suspension (25 mg) or half of the starch weight (12.5 mg). These concentrations are comparable to the upper range of polyphenol-to-starch ratios reported in co-digestion studies, where relatively high doses were applied to study interaction mechanisms and dose-dependent effects.¹⁷ The control sample was prepared as the other samples, but without the final addition of the extract, the final volume was the same in all samples. Samples were incubated at 37°C with end-over mixing, and ~2 U/mL of porcine pancreatic α -amylase per incubation mix were added to start the assay, after taking an aliquot to determine the baseline maltose concentration not originating from the enzymatic digestion (Y0). Amylase activity was determined as described elsewhere.¹⁸ Samples of incubation mix (100 μ L) were taken at 0 (before adding the enzyme), 3, 6, 9, 15, 30, 60 min of incubation. Each sample was added to a tube containing 100 μ L of 3,5-dinitrosalicylic acid (DNS), heated in a boiling water bath for 5 min to stop the reaction, and then immediately cooled on ice. Reducing sugars obtained from hydrolysis were measured at 560 nm in a plate reader (Varioskan LUX 3020, Thermos Scientific, USA) and the concentration of maltose equivalent in samples was calculated using the maltose standard curve.

2.6. Statistical analysis

Amylolysis curves were fitted to a first-order equation previously described using a non-linear regression model.¹⁹ The first order rate constant (k) and predicted starch digested at the end of the reaction (C_{∞}) were estimated from the equation, after subtracting the 'endogenous' maltose detected before the start of the reaction (Y0) from the subsequent timepoints. The experimental endpoint C_{60} and the incremental Area Under the Curve (iAUC) are reported as additional descriptors of the susceptibility to amylolysis.

Amylolysis parameters (C_{60} and iAUC) were compared across groups with mixed-effects models using the lmerTest R package (version 3.1.3),²⁰ with type of tomato and dose of the extract fixed effects and replicates as random effects to account for sample variability. As the k and C_{∞} parameters are covariant, 95% confidence ellipses were calculated based on the distribution of estimated values across replicates for each extract and tomato treatment. These ellipses show the correlation between parameters and help

to distinguish the joint behaviour of different digestion responses.

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Phenolic compounds concentrations were compared across tomato types using a linear model with tomato type as a categorical predictor, followed by pairwise comparisons with estimated marginal means differences and associated p -values. Statistical analysis and plots were generated in R (version 4.5.1, R Core Team, 2013, Vienna, Austria).²¹ Principal Component Analysis (PCA) was used to visualise differences in tomato phenolic profiles and their association with starch digestibility. This was applied to metabolite concentrations after reshaping to a sample (tomato, replicate and extract dose) \times compounds matrix. Missing values were set to zero (compound not detected). Prior to PCA, data were mean-centred and scaled to unit variance (*prcomp* function). The first two principal components explained 96.7% of the variance. Biplots were generated with samples coloured by tomato type and metabolite loadings shown as vectors. Starch digestion at 60 min was included as a supplementary quantitative variable; correlations of starch digestion with PC1 and PC2 were calculated separately for each extract concentration and represented as arrows on the biplot.

3. Results

3.1. Tomatoes characterisation

TPC was found to be higher in the Mar Azul peel extract (43.8 ± 1.5 mg GAE/g extract) compared to the Cuban Pepper (36.4 ± 0.4 mg GAE/g extract, $p = 0.002$) and the tomato paste (27.9 ± 0.9 mg GAE/g extract, $p < 0.001$). Targeted metabolomics analysis revealed different phenolic profiles across the extracts used. No differences in the concentration of ferulic acid (m/z 193) were found between extracts obtained from Mar Azul tomato and Cuban Pepper, as both extracts were obtained from the peel of the tomato (mean difference 0.0005 μ g/mg of extract, 95% CI (-0.001; 0.002), $p = 0.68$) while the extract from the tomato paste contained less ferulic acid than the other two extracts (mean difference -0.008 μ g/mg of extract, 95% CI (-0.010; -0.007), $p < 0.001$). The peel extracts were also higher in caffeoylquinic acids isomers (m/z 353) compared to the tomato paste extract. The Mar Azul extract had a higher concentration of 4-caffeoylquinic acid and 5-caffeoylquinic acid compared to Cuban Pepper (mean difference 0.35 μ g/mg of extract, 95% CI (0.31; 0.39), $p < 0.001$, mean difference 0.49 μ g/mg of extract, 95% CI (0.47; 0.52), $p < 0.001$, respectively) and to the tomato paste extracts (mean difference 0.56 μ g/mg of extract, 95% CI (0.52; 0.60), $p < 0.001$, mean difference 0.76 μ g/mg of extract, 95% CI (0.73; 0.79), $p < 0.001$, respectively). It was also richer in rutin (m/z 609) compared to the Cuban Pepper and tomato paste extracts (mean difference 0.05 μ g/mg of extract, 95% CI (0.04; 0.07), $p < 0.001$, mean difference 0.08 μ g/mg of extract, 95% CI (0.06; 0.09), $p < 0.001$, respectively). The



tomato paste extract was characterised by a small but higher naringenin-7-O-glucoside (m/z 433) concentration compared to the Mar Azul and

quantified because they were outside the quantification limits, shown in Figure 3B. For example, of the three chlorogenic acids isomers (m/z 353), the 3-caffeoylquinic

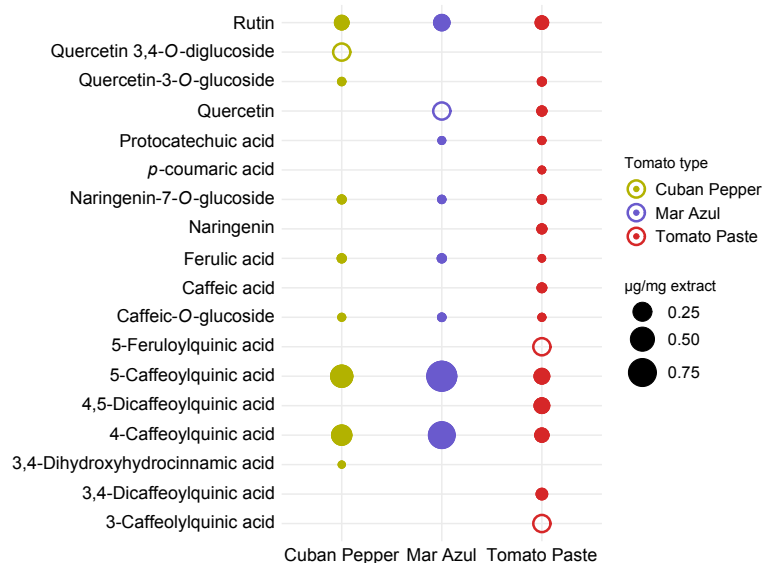


Figure 1 Phenolic compounds quantified in the tomato extracts, dot size is proportional to phenolic compound concentration per mg of extract. Hollow circles indicate compounds identified but not quantified (<LOQ), whereas absence of a circle indicates compounds not detected (ND).

Cuban Pepper extracts (mean difference 0.007 µg/mg of extract, 95% CI (0.006; 0.009), $p < 0.001$, mean difference 0.003 µg/mg of extract, 95% CI (0.002; 0.004), $p < 0.001$, respectively).

Interestingly, protocatechuic acid was not detected in the Cuban Pepper extract. As not all compounds were found in all samples, only the pairwise comparisons between detected compounds were reported in Supplementary Table 1. Some compounds were identified but not

acid was identified only in the tomato paste extract but was below the quantification limit.

Nonetheless, some compounds were not detected in the extracts obtained from tomato peels but were quantified in tomato paste such as the 3,4-dicaffeoylquinic acid (m/z 515), and the 4,5-dicaffeoylquinic acid (m/z 515). Compounds concentrations expressed as µg/g of fresh tomato are reported in Table 1.

Table 1. Phenolic compounds concentration expressed as µg/g of fresh tomato, mean ± SD of $n = 3$

Component Name	m/z	m/z ions	Cuban Pepper	Mar Azul	Tomato paste
Rutin	609	300, 271	0.85 ± 0.035	1.26 ± 0.069	1.92 ± 0.099
Quercetin 3-β-D-glucoside	463	301, 271	0.04 ± 0.0002	ND	0.24 ± 0.015
Quercetin	301	179, 151	ND	<LOQ	0.50 ± 0.003
Protocatechuic acid	153	109	ND	0.02 ± 0.001	0.09 ± 0.004
p-Coumaric acid	163	119, 117, 93	ND	ND	0.06 ± 0.009
Naringenin-7-O-glucoside	433	271, 151	0.07 ± 0.007	0.04 ± 0.002	0.31 ± 0.005
Naringenin	271	151, 119	ND	ND	0.48 ± 0.014
Ferulic acid	193	178, 149, 134	0.08 ± 0.008	0.09 ± 0.006	0.03 ± 0.001
Caffeic acid	179	135, 89	ND	ND	0.40 ± 0.01
Caffeic-O-glucoside	341	179	0.03 [§] ± 0.002	0.05 ± 0.002	0.09 ± 0.008
5-Caffeoylquinic acid	353	191, 93, 85	3.29 ± 0.045	7.33 ± 0.147	3.40 ± 0.054
4-Caffeoylquinic acid	353	173, 135, 93	2.49 ± 0.111	5.36 ± 0.215	2.36 ± 0.113
4,5-Dicaffeoylquinic acid	515	353, 173, 135	ND	ND	3.39 ± 0.095
3,4-Dicaffeoylquinic acid	515	135, 179, 173	ND	ND	0.98 ± 0.039
3,4-Dihydroxyhydrocinnamic acid	181	137, 109, 60	0.01 ± 0.002	ND	ND

[§] $n = 2$. ND= Not Detected, LOQ = Limit Of Quantification

3.2. Starch amylolysis



Starch amylolysis resulted in a lower proportion of starch digested after 60 min of incubation with α -amylase (C_{60}), in the presence of an equal quantity of phenolic extract and starch, compared to the starch control alone. This was observed for all tomato extracts. The extract (25 mg) from Mar Azul peel and the extract from tomato paste resulted in an 11% and 10% reduction in starch digested at 60 min of incubation, respectively, while Cuban Pepper peel extract resulted in a 7% reduction (C_{60} , $p < 0.001$ and 0.009 , respectively). Considering the intermediate extract dose of 12.5 mg, a modest effect of the tomato paste extract was observed on the C_{60} endpoint (-4.9% , $p = 0.05$) while no significant effect was observed for the other peel tomato extracts. These reductions, although observed in isolated starch, suggest phenolics can meaningfully modulate starch digestibility.

The overall starch digested at the end of the reaction (C_{∞}) was also lower in presence of tomato extract, especially with the highest dose (25 mg), compared to the control (no tomato extract), Table 2. The rate of digestion was similar for all tomato types and all extract doses, nonetheless this resulted in a lower iAUC for all tomato types and doses of extracts, with a greater effect when in presence of a higher extract dose.

The addition of polyphenol extracts had a small impact on the incubation pH, which was 6.5 at the end of the incubation with 25 mg of extract and 6.8 after incubating the starch with 12.5 mg of extract for 1h, compared to the control with only starch, where the pH remained close to the initial value of 7.4. Amylolysis curves and digestibility parameters are shown in Figure 2 and summarised in Table 2.

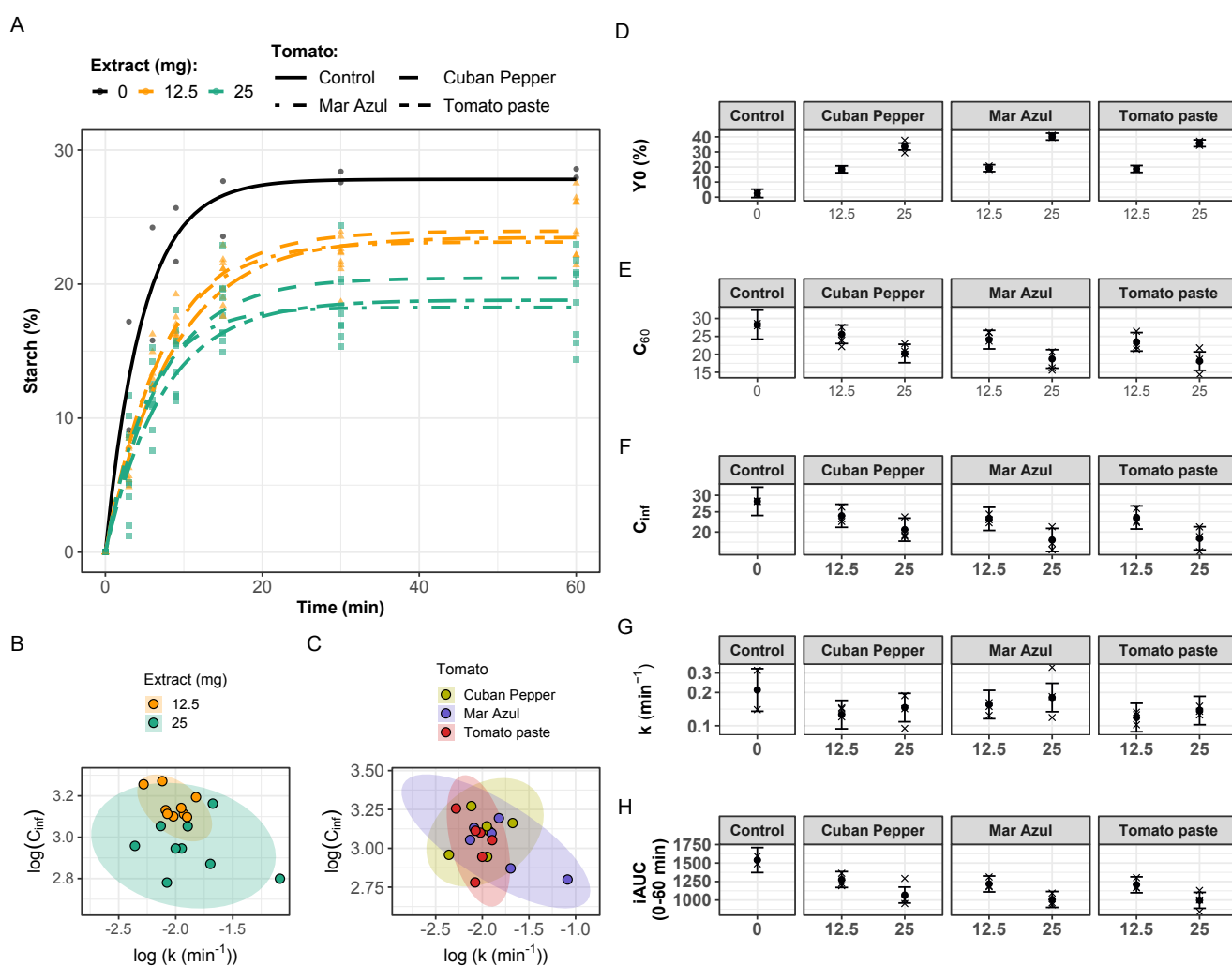


Figure 2. A. Starch digestibility curves of gelatinised wheat starch with increasing concentrations of extracts, $n =$ Different colours indicate the concentration of extract, the line type indicates the type of tomato. Experimental data points are shown fitting a first-order equation based on the estimates of k and C_{∞} values obtained from a non-linear regression model. B-C. Contour plots with 95% CI of rate (k) and predicted digestion endpoint (C_{∞}) showing a similar response between samples but a greater variability in amylolysis when the same amount of extract and starch is used (B), which may be due to the type of tomato extract used (C). D. Endogenous maltose Y_0 . E. C_{60} is the experimental endpoint. F. C_{∞} is the predicted endpoint of the reaction. G. k is the rate of the reaction. H. The incremental Area Under the Curve (iAUC).



Table 2. Starch amylolysis parameters.

Statistical significance is shown for $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***). Endogenous maltose Y_0 , C_{60} is the experimental endpoint, the incremental Area Under the Curve (iAUC). Amylolysis parameters k (rate) and C_{∞} (predicted endpoint of the reaction) are covariates so no statistical difference is shown here. 95% CI overlap can be seen in figure 3A showing no differences across samples.

Dose (mg)	Tomato	Y_0	k	C_{∞}	iAUC
0	Control	2.5 (-0.3; 5.3)	0.21 (0.13; 0.33)	28.1 (24; 32.8)	1540.3 (1357.8; 1722.7)
	Mar Azul	19.2 (16.9; 21.5)***	0.14 (0.09; 0.21)	23.1 (20.3; 26.3)	1227.5 (1086.2; 1368.8)**
12.5	Cuban Pepper	18.5 (16.2; 20.8)***	0.13 (0.09; 0.19)	23.9 (21.1; 27.2)	1260.4 (1119.1; 1401.7)**
	Tomato paste	18.7 (16.4; 20.9)***	0.12 (0.08; 0.17)	23.5 (20.6; 26.7)	1213.1 (1071.7; 1354.4)**
	Mar Azul	40.1 (37.8; 42.4)***	0.19 (0.13; 0.28)	18.3 (16.1; 20.8)	997.9 (856.6; 1139.2)***
25	Cuban Pepper	33.5 (31.3; 35.8)***	0.13 (0.09; 0.2)	20.5 (18.1; 23.3)	1084.2 (942.8; 1225.5)***
	Tomato paste	35.7 (33.5; 38.02)***	0.13 (0.09; 0.2)	18.6 (16.4; 21.2)	2.7 989.7 (848.3; 1130.9)***

The contour plots with 95% confidence intervals of the rate constant and the predicted digestion endpoint (Figure 2B) visually show similar responses between samples. They also reveal a greater variability in amylolysis parameters when the highest extract dose is used. This suggests a potential nested effect, where the type of tomato used to obtain the extract influences the effect of the dose used on starch digestion (Figure 2C). The PCA of metabolite concentrations in Tomato Paste, Mar Azul, and Cuban Pepper explained 96.7% of the variance in two dimensions (PC1 = 74.9%, PC2 = 21.8%), with samples clustering distinctly by tomato type

(Figure 3). A vector representing starch digested at 60 min showed weak alignment with phenolic composition at 12.5 mg extract dose, consistent with the small effect observed by the Tomato Paste. At 25 mg, the starch digestion vector shifted toward stronger suppression across all tomato types, with samples projecting closer to the origin along this vector, reflecting lower overall starch digestion at the higher extract concentration. These results support a dose-dependent inhibitory effect of tomato phenolics on starch digestion.

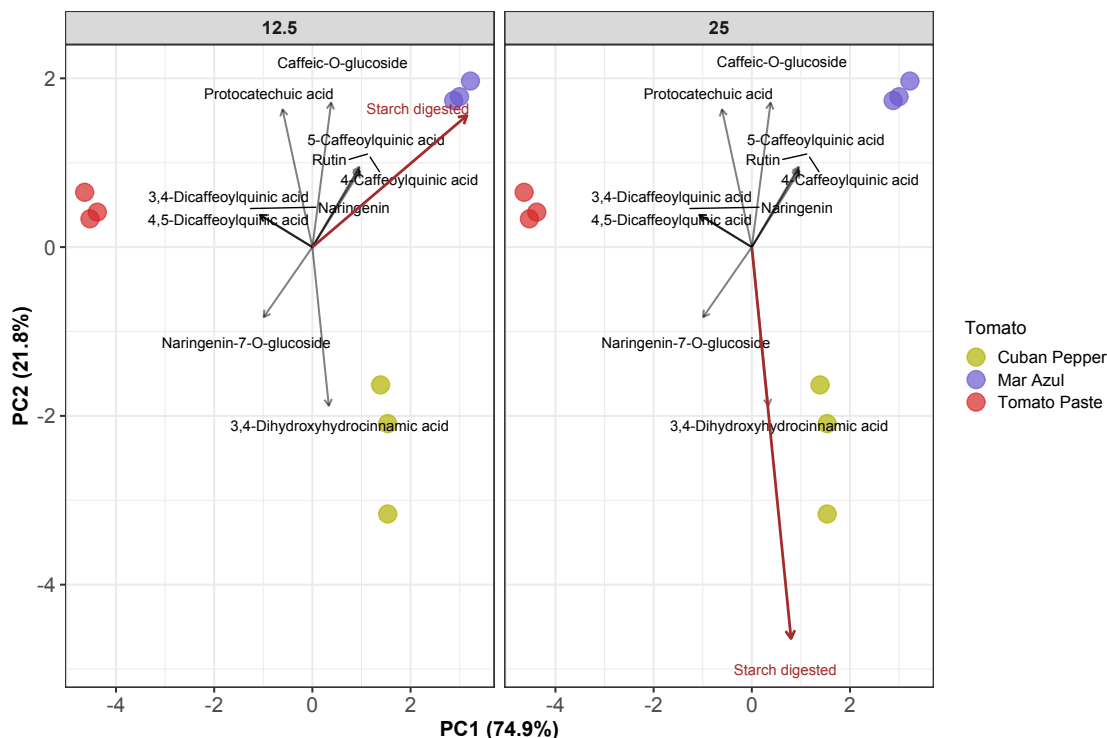


Figure 3. Principal Component Analysis (PCA) of tomato extracts at two doses (12.5 and 25 mg), showing that differences in phenolic composition contribute to variation in starch responses. Colours represent tomato types (dose-normalised phenolic composition). Black arrows indicate the top 10 compounds contributing most to separation along PC1 and PC2, while the red arrow represents starch digested. Arrow direction indicates alignment with the principal components, and length reflects the strength of this relationship.



4. Discussion

This study has shown that phenolic extracts from tomatoes can be used to lower starch digestibility *in vitro*. Starch digestibility was previously shown to correlate with *in vivo* glycaemic responses¹⁶ suggesting potential of the use of tomatoes combined with high-glycaemic starch-based foods as a way to lower their glycaemic index. Reductions in *in vitro* starch hydrolysis indices (C60, C90, HI) have been associated with decreases in glycaemic index of foods.¹⁶ This supports the physiological relevance of even modest (~10%) reductions in digestibility to guide phenolic dose selection for studies with full food matrices and bioaccessibility. From a mechanistic perspective, the observed effects can be interpreted in terms of enzyme inhibition and starch–phenolic interactions.

It should be noted that tomatoes have a relatively lower concentration of phenolic compounds compared with other fruits²² and, considering the outcome of this study, great quantities of tomato should be consumed to achieve the effects presented. Nonetheless, reusing by-products of tomato sauce production, peels and seeds, could be a suitable strategy to enrich tomato products of phenolic compounds.⁹ Additionally, tomato consumption in Europe should not be underestimated as it is one of the most produced horticultural crops (186 million tons in 2022).²³ The concentrations used in this study are higher than those typically achieved through consumption of whole tomatoes, reflecting the simplified nature of the model and the absence of matrix effects. These conditions were selected to enable clear detection of dose-dependent effects and to inform the levels required to observe measurable interactions. While crude extracts were used and minor co-extracted compounds may also contribute to the observed effects, phenolics are likely the primary drivers. Future studies should evaluate these effects in realistic meal scenarios and complex food matrices. Moreover, the highest extract-to-starch ratio used (1:1) was selected to explore maximal inhibitory potential under controlled *in vitro* conditions. Based on extraction yields, this corresponds approximately to 60 g of tomato paste combined with 60 g of starch. While consuming 60 g of tomato paste alone in a single serving would be unusual, such an amount in combination with a typical starchy portion is feasible. Furthermore, these results suggest that incorporating phenolic-rich byproducts, such as tomato peels or pomace, into traditional tomato-based foods could enhance their functional properties and modulate starch digestion.

The novelty of this study is that we focused on an existing starch-tomato combination that is highly consumed in the Mediterranean diet, which could have a great impact on glycaemia considering the frequency of consumption in Mediterranean culinary preparations.²⁴

Tomato fortification studies have primarily assessed bioactive content and product quality, rather than carbohydrate interactions or glycaemic impact. Only one study examined the effect of lycopene on starch digestibility in extruded snacks, finding reduced digestibility with increasing tomato powder concentrations.²⁵ However, because starch was partially replaced by tomato powder, differences in starch content across samples may have confounded glycaemic load comparisons. The role of

tomato phenolics on starch digestion and glycaemia remains largely unexplored.

Previous studies summarised elsewhere⁷ have shown that isolated phenolic compounds can reduce starch digestibility through competitive and non-competitive binding. In this study, phenolic extracts were added immediately after starch cooking, when gelatinised chains were still mobile and before substantial retrogradation had occurred; thus, the observed effects may be due to interactions with the starch chains, with the α -amylase, or both. Phenolics in foods, such as flavonoids, have been reported to exert hypoglycaemic effects,²⁶ although their mechanisms remain poorly understood. These studies focus mostly on tea or berries phenolics that are high in phenolic content. Dose-dependent inhibitory effects of phenolic-rich extracts on starch digestion have also been reported in co-digestion studies using more complex models (e.g., INFOGEST); however, differences in experimental design, including digestion conditions, food matrix, and phenolic-to-substrate ratios, make direct comparison with the present study challenging.^{17, 27} Among phenolic compounds, chlorogenic acid (also identified as caffeoylquinic acid) was found to have a greater inhibitory effect on amyolytic activity compared to caffeic and ferulic acids due to the number and position of hydroxyl groups.²⁸

In our study, peel extracts contained higher concentrations of chlorogenic acid isomers, yet overall starch digestibility was similar across extracts. The tomato paste extract, despite having lower concentrations of certain compounds such as caffeoylquinic acids, exhibited a more diverse phenolic composition compared to the peel of the other two the tomatoes variety (14 vs 8), and significantly reduced starch digestibility at 60 min when administered at an intermediate dose. This suggests that the phenolic profile, rather than the total phenolic content alone, may play a key role in reducing starch amyolysis.

Compounds such as quercetin and naringenin are known to inhibit salivary and pancreatic amylase activity, likely through interactions between their hydroxyl groups and enzyme catalytic sites.^{2, 29} These findings support the hypothesis that starch digestibility is reduced through the combined action of overall phenolic composition and individual bioactive compounds. While the contribution of individual compounds cannot be separated in this study, variation in the phenolic profile could help guide future work to identify specific compounds or compound classes that are most effective at reducing starch digestibility. For example, Future studies may further explore how cultivar selection and cultivation conditions influence tomato phenolic composition and its interaction with starch during digestion. The inhibitory activity of tomato paste extracts against α -amylase may depend not only on total phenolic content but also on the composition and accessibility of individual compounds. Aglycones generally inhibit α -amylase more effectively than their glycosylated derivatives,³⁰ while increased caffeoyl substitution does not enhance α -amylase inhibition and may even reduce activity.³¹ Therefore, the presence of dicaffeoylquinic acids in the tomato paste extract does not necessarily explain its stronger effect. It is more likely that the observed differences between extracts arise from a combination of phenolic composition, solubility, and interactions with co-extracted compounds, such as soluble carbohydrates or other low-molecular-weight constituents. These factors may also contribute



to the dose-specific effect at the intermediate level observed in this study. Given that crude extracts were used and individual compounds were not isolated, it is not possible to attribute the observed effects to specific molecules or to distinguish between additive and synergistic interactions.

A limitation of this study was that the targeted triple quadrupole LC-MS/MS approach, while highly sensitive for specific compounds, does not enable simultaneous quantification of all compounds of interest. Future studies could complement these results by analysing anthocyanins and other phenolics using additional LC-MS/MS approaches to provide a wider characterisation of Mar Azul tomatoes and their potential effects on glycaemic control.³² Another aspect to consider is that most phenolics are acidic, influencing the digestion conditions. In this experiment, phenolic extracts contributed to lowering the pH of the incubation however, the pH at the end of the incubation remained within the optimum limits of amylases, reported to be between 6.5 and 7.0.³³ However, higher extract doses could lower the pH further, leading to potential effects on digestibility that should be considered.

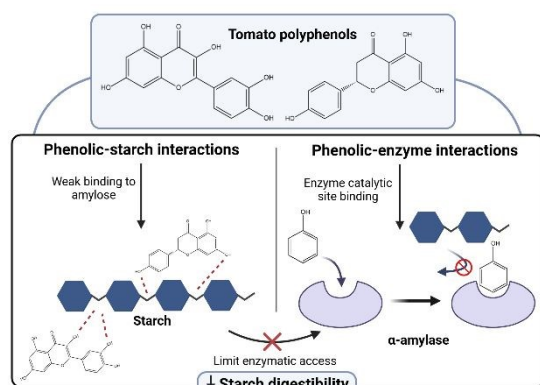


Figure 4. Summary of the proposed mechanism of action

This work highlights unique aspects of the phenolic composition of tomato extracts and their potential effects on starch digestion. The extracts displayed characteristics that raise questions about phenolic bioaccessibility and bioavailability. Mar Azul and Cuban Pepper extracts were obtained from tomato peels, whereas the tomato paste was made from all parts of the tomato, which may explain differences in composition. While all extracts lowered starch amylolysis at the highest dose, only the tomato paste extract significantly lowered the amount of starch digested at 60 min with the intermediate dose. This may be due to an effect of the processing that led to increased bioaccessibility of compounds from this matrix that were easily extracted compared to the tomato peels.³⁴ In this study, we focused on tomato peel as it is an abundant by-product of processing with potential nutritional value, and our aim was to specifically explore its phenolic contribution.

When considering dietary applications, the bioaccessibility of phenolic compounds within a food matrix must be considered. In whole tomatoes, bioactive compounds may be trapped within the cellular matrix and therefore may not be fully available to inhibit starch digestion. Future studies using more complex *in vitro* digestion models are needed to evaluate phenolic bioaccessibility within a starch-based meal and to study compound release during

digestion, as the single-enzyme system used here focused on starch kinetics rather than bioactive compound release.

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5. Conclusions

In summary, tomato-derived phenolics reduced starch digestibility *in vitro*, showing dose-dependent effects. These findings suggest that tomatoes, a staple food in the Mediterranean diet and one of the most consumed vegetables worldwide, could play a role in moderating postprandial glycaemic responses when combined with starch-rich foods. Although the phenolic content of tomatoes is modest compared with other crops, their ubiquity, versatility and cultural relevance make them a practical dietary vehicle for glycaemic control. Selecting cultivars with favourable phenolic profiles as well as the use of novel and sustainable agronomical techniques may further enhance their phenolic content. Moreover, the possibility of enriching tomato-based products with phenolic-rich by-products offers an opportunity to strengthen their beneficial effects on health as a functional component of a balanced diet.

Author contributions

MC Conceptualization, Methodology, Investigation, Formal analysis, Data Curation, Writing - Original Draft, Writing - Review & Editing, Funding acquisition. **CAR** Investigation, Data Curation, Writing - Original Draft, Writing - Review & Editing. **GDM** Investigation, Writing - Review & Editing. **MTZ** Investigation, Writing - Review & Editing. **JR** Investigation, Writing - Review & Editing. **MP** Writing - Review & Editing. **AVQ** Methodology, Formal analysis, Writing - Original Draft, Writing - Review & Editing. **RMLR** Conceptualization, Writing - Review & Editing, Funding acquisition.

Conflicts of interest

The authors have no relevant conflict of interests related to this work to declare.

Data availability

The Supplementary Material (SM) includes Figure 1. Tomatoes used without thermal processing. A. Cuban Pepper variety, B. Mar Azul variety. Table 1. Pairwise comparisons with Tukey-adjusted estimated marginal means. Raw data and annotated code used to generate the results reported in this manuscript have been deposited in the Zenodo repository 10.5281/zenodo.17879773.

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Data Availability Statement

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