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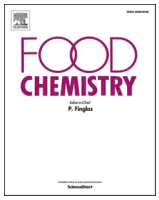

Neuroactive compounds in tomatoes: metabolic fate during in vitro digestion and colonic fermentation☆

Ana Kovačič [a,b,c,*], Mar Garcia-Aloy [c], Domenico Masuero [c], Cesare Lotti [c], Pietro Franceschi [d], Andrea Mancini [e], Josep Rubert [f,g], Urška Vrhovšek [c]

[a] Department of Environmental Sciences, Jožef Stefan Institute, Ljubljana, Slovenia
[b] International Postgraduate School Jožef Stefan, Ljubljana, Slovenia
[c] Metabolomics Unit, Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige, Italy
[d] Digital Agriculture Unit, Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige, Italy
[e] Natural Product Biotechnology Unit, Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige, Italy
[f] Food Quality and Design Group, Wageningen University & Research, Wageningen, the Netherlands
[g] Division of Human Nutrition and Health, Wageningen University & Research, the Netherlands

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ABSTRACT

Neurodegenerative diseases are hard to treat, and their progression is linked to diet and the gut microbiota. Tomatoes contain potentially neuroactive compounds, but their fate during digestion and colonic fermentation remains unclear. This study tracked neuroactive compounds and fecal fatty acids (<C12) during in vitro gastrointestinal digestion and fecal fermentation. Conditions included a fecal blank, digested inulin, digested tomato, tomato with neuro-disrupting compounds, and individual neuroprotective compounds. Targeted analyses and linear mixed-effects models showed samples were primarily differentiated by tomato presence and fermentation time. Several neuroprotective compounds (e.g., tomatine, serotonin) remained stable (>50%, 24 h) or increased, whereas others (e.g., caffeic acid, rutin) degraded rapidly (<LOQ, 3 h). Neuro-disrupting compounds, including bisphenol S and difenoconazole, persisted largely unchanged (>80%, 24 h). Digested tomato enhanced acetic and propionic acid production, though attenuated by neuro-disruptors. The study clarifies colonic bioaccessibility of neuroactive compounds and their potential influence on the gut–brain axis.

1. Introduction

Neurodegenerative diseases (NDs), such as Alzheimer's, Parkinson's, and Huntington's, are progressive conditions characterized by neuronal degeneration and cell death, leading to debilitating symptoms and reduced quality of life (Gao et al., 2025). Despite advancements in medical treatments, effective cures remain elusive, and the global prevalence of these disorders continues to rise, affecting millions of people (Ceppa et al., 2020). Recent research has highlighted the complex bidirectional communication between the gut microbiome and the central nervous system (CNS), known as the microbiota–gut–brain axis (GBA) (Lu et al., 2024). This axis plays a key role in regulating both health and disease, and is increasingly recognized as a promising target for therapeutic interventions (Loh et al., 2024). Among the various lifestyle factors that influence the GBA, diet has been shown to have a particularly significant impact. Certain dietary components, such as neuroactive compounds and their precursors (e.g., tryptophan), modulate the gut microbiota and, consequently, the gut–brain axis (Hou et al., 2022). Therefore, understanding the presence, bioaccessibility, and function of these compounds in foods is essential for evaluating their potential effects on human health, particularly in relation to NDs (Yılmaz & Gökmen, 2020).

By 2035, EU consumption of fresh fruits and vegetables is expected to remain stable or increase compared to the trimmed average of 2020–2024, driven by growing consumer awareness of the benefits of diets rich in fruits and vegetables, as well as public promotion initiatives (EU Commission, 2024). Tomatoes, a widely consumed vegetable, are rich in bioactive compounds, including polyphenols, carotenoids, vitamins, and glycoalkaloids, which are believed to have neuroprotective properties (Hwang & Kim, 2023; Wang et al., 2022). In Europe, the

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* Corresponding author at: Department of Environmental Sciences, Jožef Stefan Institute, Ljubljana, Slovenia.
E-mail address: ana.kovacic@ijs.si (A. Kovačič).

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average per capita consumption of fresh tomatoes is about 15 kg per year, corresponding to roughly 41 g per person per day (EU Commission, 2024). Although the bioactive composition of tomatoes has been well-characterized (Ali et al., 2021; Rapa et al., 2021), there is limited understanding of how these compounds impact neurodegenerative diseases, particularly in terms of bioaccessibility after digestion (Yong et al., 2023). Studies have shown that these compounds, by neutralizing free radicals, may protect against oxidative stress, a contributing factor to neurodegeneration (Shah et al., 2020). However, the influence of processing methods, digestion, and interactions between compounds on the bioaccessibility of phytonutrients is not fully understood (Reboredo-Rodríguez et al., 2021; Yong et al., 2023). This is a critical gap in the literature, as bioaccessibility influences the availability of these bioactive compounds for absorption and interaction with the gut microbiota.

In vitro models simulating gastrointestinal digestion have been widely used to predict the bioaccessibility and bioavailability of compounds (Brodkorb et al., 2019). Some studies have shown that bioactive compounds in tomatoes undergo significant alterations during digestion, including the decomposition and conversion of polyphenols, vitamins, and carotenoids (Izzo et al., 2022). For example, low recovery rates (< 30%) and high losses of rutin, naringenin, chlorogenic acids, and lycopene have been observed post-digestion (Izzo et al., 2022). Additionally, certain phenolic compounds may be bioaccessible in the small intestine, while others reach the colon, where they are fermented and bioconverted by the gut microbiota (de Paulo Farias et al., 2025; Dou et al., 2022; Wang et al., 2022; Zhao et al., 2023). The bioaccessibility of polyphenols and other compounds is influenced by their chemical structure and interactions with other food components, such as dietary fiber, which can impact their absorption and subsequent effects on health (Cárdenas-Castro et al., 2021). Moreover, fermentation in the colon leads to the production of short-chain fatty acids (SCFAs), which may influence the gut–brain axis, (Silva et al., 2020). In turn, bioactive compounds may modulate SCFA production, either by affecting the microbial community in the gut or by directly influencing the fermentation pathways (Cárdenas-Castro et al., 2021).

However, the majority of studies have focused on individual compounds, such as polyphenols and carotenoids, while the broader interplay between multiple bioactive compounds and their fate during digestion and fermentation remains understudied (Yong et al., 2023). Moreover, it is important to consider that tomato-based products may contain neuro-disrupting compounds, such as mycotoxins, pesticides, and industrial chemical residues (Aloizou et al., 2020). These contaminants can enter food at various stages, raising concerns about their potential effects on gut microbiota and brain health (Defois et al., 2018). For instance, studies have demonstrated that pesticides and bisphenols can disrupt neural functions and contribute to the development of NDs (Flores-Gutierrez et al., 2023). Yet, the bioavailability of these chemicals after digestion and their interaction with the gut microbiota is rarely explored, despite indications that up to 30–80% of some neuro-disrupting compounds, such as difenoconazole and bisphenol S, may remain bioavailable after digestion (Craggs et al., 2020; Lestido-Cardama et al., 2022).

This study aims to address these gaps by combining in vitro gastrointestinal and colonic fermentation models using tomatoes as a food model. Specifically, it investigates how digestion and fermentation affect the bioactive compounds in tomatoes, including both neuroprotective and neuro-disrupting compounds. The central hypothesis is that tomato digestion may increase the levels of neuroactive compounds reaching the colon, while neuro-disrupting compounds may alter the fate of neuroprotective compounds during digestion and fermentation. The findings will provide new insights into NDs and the role of the gut microbiota in modulating simultaneously bioactivity and toxic compounds. We also hypothesized that the presence of tomato could influence the fermentation dynamics of neuroactive compounds, potentially affecting their stability and bioaccessibility. Overall, this research offers new perspectives on how tomato consumption may shape the colonic bioaccessibility of neuroactive compounds, their behavior within the human body, and their potential relevance to NDs pathways.

2. Materials and methods

2.1. Chemicals and reagents

General information about the investigated neuroactive compounds is presented in the Supplementary information (Table SI 1). A detailed description of the chemical standards, short-, branched- and medium-chain fatty acids (< C12), as well as the LC-MS and GC–MS reagents and solvents please refer to Lotti et al. (Lotti et al., 2017) and our previous study (Kovačič, Masuero, Garcia-Aloy, Mancini and Vrhovšek, 2026). Reagents for ROS analysis, 1,1-diphenyl-2-picryl hydrazyl (DPPH) and Trolox were purchased from Sigma Aldrich. All standards, reagents and solvents were of an analytical grade quality.

2.2. Study design

2.2.1. Sample collection

Plump tomato was obtained from local supermarkets of Trento, Italy in August 2023 and washed with tap water. Tomato fruit was cut into pieces of approximately 2 × 2 × 2 cm and then frozen with nitrogen, grinded and lyophilized. Freeze-dried tomato paste was pooled into one homogenized sample, packed in closed plastic bottles and stored in the presence of molecular sieves, at room temperature until further analysis.

2.2.2. In vitro gastrointestinal digestion

The in vitro-simulated gastrointestinal digestion (Fig. 1) of tomatoes was performed as described in our previous study (Kovačič et al., 2026), following a general standardized and practical static INFOGEST digestion method (Brodkorb et al., 2019). The protocol comprises three sequential steps: oral, gastric and small intestinal digestion. The experimental design included digestion of 1) inulin, obtaining 16 g of digested inulin from 18 g, 2) tomato without addition of compounds, obtaining 40 g of digested tomato from 90 g freeze dried tomato, and 3) tomato with addition of neurotoxic compounds (bisphenol S, difenoconazole, aspartame) at environmentally relevant concentrations (50 μg/kg fresh weight), selected based on the most restrictive regulatory benchmark among the investigated compounds, namely the specific migration limit established for bisphenol S in food contact materials under European Union regulations (FitzGerald et al., 2020), obtaining 20 g from 54 g. To simulate passive intestinal absorption of water and hydrolytic products from digestion in the small intestine, after digestion the centrifugation of the total digested material was performed using 5000 rpm, 30 min, 4 °C. The remaining (undigested) pallet was freeze-dried, grinded, homogenized and stored in desiccator until use in the fecal fermentation experiment.

2.2.3. Fecal batch culture fermentation

Six healthy volunteers, ($N = 6$, aged 40–60, 3 males, 3 females), were used as fecal donors. They had not received antibiotic treatment within the previous 3 months of stool collection, had no history of bowel disorders, had not consumed pre- or probiotic supplements, and had not consumed any tomato-based food products 7 days prior to experiment. All donors were fully informed about the aims and procedures of the study and provided written consent (December 2023) for the use of their fecal material in the experiments, in compliance with the ethical procedures required by Fondazione Edmund Mach and the APSS (Azienda Provinciale per i Servizi Sanitari), Trento (TN), Italy. No personal biological data were collected or analyzed. The privacy rights of the donor were fully protected, and no identifying personal information has been disclosed.

The selection of the targeted 36 neuroactive compounds for the fecal fermentation experiment was based on findings from our previous studies, where we analyzed the neuroactive profile in tomatoes (Kovačič

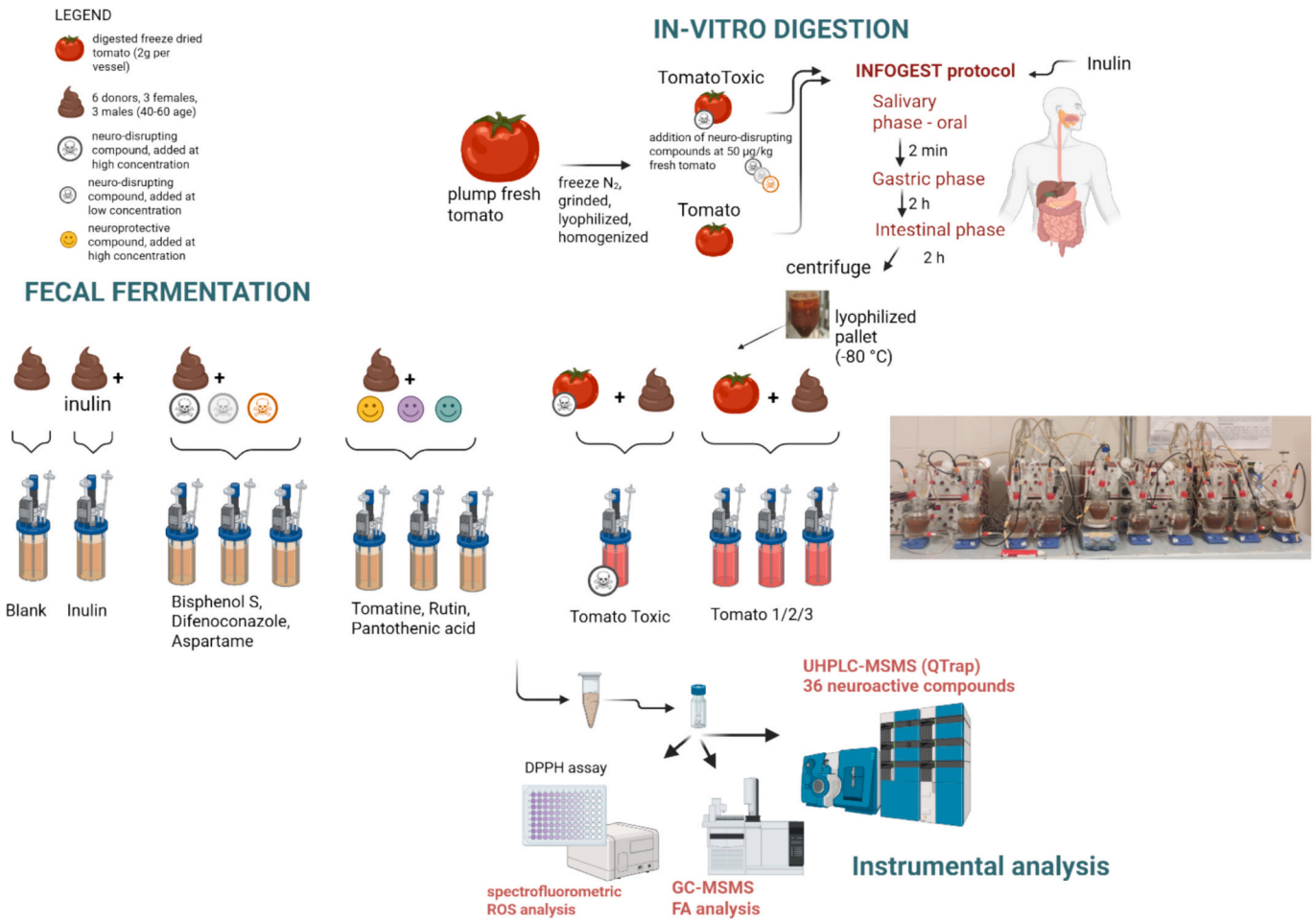

Fig. 1. Tomato extracts, with and without the addition of neuro-disrupting compounds and inulin, were subjected to in vitro digestion following the standardized INFOGEST protocol. Following digestion, the undigested pellet was collected and incorporated into a fecal fermentation model to simulate colonic microbial metabolism. The experimental design consisted of 10 different conditions: 1) Blank (fecal solution without any substrate), 2) Inulin (2 g of digested inulin as a control), 3) Digested tomato (2 g of digested tomato in triplicated, Tomato1/2/3), 4) Digested tomato with a mix of neuro-disrupting compounds (2 g of digested tomato adjusted with aspartame, difenoconazole, and bisphenol S at c = 50 µg/kg, TomatoToxic), and 5–10) Samples with individually added neuroprotective (rutin, pantothenic acid, tomatine) or neuro-disrupting compounds (aspartame, difenoconazole, bisphenol S) to a final concentration of 2 mg/L in the fecal vessel containing only the fecal solution media. After fermentation, the samples were processed for subsequent analyses, including LC-QTRAP-MS for neuroactive compounds, GC–MS/MS for fatty acids (< C12), and spectrofluorometric analysis for reactive oxygen species.

et al., 2025) and tracked the fate of these compounds during digestion using the INFOGEST protocol (Kovačič et al., 2025). For the present fermentation model, three neuro-disrupting compounds (bisphenol S, difenoconazole, and aspartame) and three neuroprotective compounds (rutin, tomatine, and pantothenic acid) were selected. These compounds were detected in tomatoes in our previous study (Kovačič et al., 2025), in which suspect screening was performed across different tomato types. The selection criteria included their confirmed occurrence in the matrix, limited available data regarding their behavior during gastrointestinal digestion and fecal fermentation, their classification as primarily tomato-derived compounds potentially reaching the colon intact or partially intact (>50% retention in the digested pellet), as observed in our previous study (Kovačič et al., 2025), and the need to ensure structural diversity among the selected representatives. They were also chosen for their potential to remain bioaccessible after digestion and stay in the digested tomato pellet This approach enabled the inclusion of compounds with distinct physicochemical properties and biological relevance, allowing for a comprehensive evaluation of their fate under colonic fermentation conditions. To investigate their fate during fermentation, we added higher concentrations of these compounds directly to the fecal solution. One sample also contained a digested tomato with environmentally relevant concentrations of a mix of the same neuro-disrupting compounds to simulate a real-life scenario. Although bisphenol S does not yet have an established Tolerable Daily Intake (TDI), it is expected to be set in the future, similar to bisphenol A (TDI = 0.2 ng per kilogram of body weight per day). For our experiment, we used an environmental concentration of 50 µg/kg of fresh weight, which aligns with the specific migration limit for bisphenol S (FitzGerald et al., 2020). This represents the maximum permitted amount that may migrate from food packaging into food and reflects a realistic yet conservative contamination level (Ighalo et al., 2024).

Fresh human fecal samples were collected in an anaerobic container within 2 h before the start of the fermentation experiment. The pH-controlled anaerobic batch culture fermentation was performed as described in our previous study (Kovačič et al., 2026). Briefly, sterilized glass vessels (200 mL) were filled with 180 mL of pre-sterilized basal nutrient medium, and anaerobic conditions were maintained overnight with a continuous N_2 flow. The temperature was set at 37 °C. Before inoculation, each vessel was dosed with 1 mL of a 10% L-cysteine hydrochloride solution (oxygen-reducing agent) and 20 mL of fecal slurry (10% *w/v* fresh human feces), homogenized in pre-reduced phosphate-buffered saline. The pH was adjusted to 5.5–6.1 and maintained within this range throughout the experiment using ammonium hydroxide or hydrochloric acid. The colonic model (Fig. 1) consisted of ten

experimental conditions: 1) Blank (fecal solution without any substrate), 2) Inulin (2 g of digested inulin as a control, a well-known prebiotic that is fermented by gut microbiota, resulting in a pH decrease that provides a measurable indication of active fermentation), 3) Digested tomato (2 g of digested tomato, Tomato), 4) Digested tomato with a mix of neuro-disrupting compounds (2 g of digested tomato adjusted with aspartame, difenoconazole, and bisphenol S at c = 50 μg/kg, TomatoToxic), and 5–10) Samples with individually added neuroprotective (rutin, pantothenic acid, tomatine) or neuro-disrupting compounds (aspartame, difenoconazole, bisphenol S) to a final concentration of 2 mg/L in the fecal vessel containing only the fecal solution media. Six replicates were performed using fecal donors, with sampling at 0, 3, 6, 9, 12, and 24 h. For three donors, samples with digested tomato were performed in triplicate to check for experimental variability (Tomato 1/2/3).

To cover all the analyses, target LC-MS/MS for the determination of 36 neuroactive compounds, GC–MS/MS for the analysis of 14 low-molecular-weight (< C12) fecal fatty acids (FAs), and ROS analysis using spectrophotometry, 2 mL aliquots were collected in triplicate at each sampling interval. Each aliquot was immediately centrifuged, and the resulting supernatant and pellet were separately stored at −80 °C until analysis. One day prior to extraction and analysis, one aliquot per condition was removed from the freezer and thawed overnight at +4 °C in the dark. The samples were then centrifuged at 1360 rad/s for 600 s at +4 °C and subsequently filtered using 0.45 μm regenerated cellulose membrane filters. The prepared supernatant from the same aliquot was used for all downstream analyses, including the determination of neuroactive compounds and FAs in the fecal solution, as well as ROS analysis.

2.3. Instrumental analysis

2.3.1. LC–MS/MS analysis of neuroactive compounds fecal samples

Samples were analyzed using an AB Sciex UHPLC QTRAP 6500+ (AB Sciex LLC, Marlborough, MA, U.S.A.). A detailed description of the sample preparation optimization and method development and validation is provided in our previous study (Kovačič et al., 2026). Briefly, the separation was achieved on a Waters Acquity HSS T3 column 1.8 μm, 100 mm × 2.1 mm (Waters Milford, MA, USA), kept at 40 °C. *mobile* phase A was ultrapure water containing 0.1% formic acid and 1 mM NH_4COOH and mobile phase B was methanol with 0.1% formic acid and 1 mM NH_4COOH at a flow rate of 0.3 mL min^{-1}. The injection volume of sample extract was 2 μL. The mass spectrometer was equipped with an electrospray ionisation (ESI) source and operated in positive and negative scheduled ion multiple reaction monitoring (MRM) mode using a Turbo V ion source. MultiQuant and Analyst from AB Sciex LLC (Framingham, MA, USA) were used for data acquisition and elaboration, respectively.

After centrifugation, as described in the "Study Design", 40 μL of the fecal solution (supernatant) was taken from the fecal samples and diluted five times with methanol containing an internal standard mixture (10_d -carbamazepine, $^{13}C_{12}$-bisphenol S) to achieve a final sample concentration of 0.05 μg/mL. During sample preparation, the samples were maintained at +4 °C. The prepared samples were analyzed in random order in different batches (one donor per batch).

A pooled quality control (QC) sample was prepared by combining aliquots from all collected samples (396 in total). QC samples and solvent blanks were injected at the beginning of each batch and after every 10 study samples. Solvent blanks were included to monitor for sample carryover. Instrumental stability was confirmed through consistent QC signal responses, with relative standard deviations (RSD) below 30% for most of quantified compounds above the limit of quantification (LOQ) and known to be stable, including citric acid, glutamic acid, glutamine, guanine, L-Dopa, linoleic acid, pantothenic acid, phenylalanine, rutin, tyramine, tyrosine, and tryptophan, over a total batch analysis time (as previously published; see Table SI 2). Some compounds showed signal reductions in QC samples between batches, which were attributed either to dilution effects (e.g., tomatine, serotonin, hesperetin) or to know instability beyond 48 h (e.g., 4-pyridoxic acid, quercetin), as reported in our earlier work. Importantly, however, all study samples were analyzed within 24 h, and the stability of all target compounds within this timeframe was previously confirmed and validated in our study. Additionally, the RSD of internal standard peak areas across all samples remained below 10% (data not shown), indicating high consistency in sample preparation and instrument performance.

Details on method validation parameters, including linearity, selectivity, accuracy, precision, and LOD and LOQ can be found in our previous study (Kovačič et al., 2026).

2.3.2. GC–MS/MS analysis of fecal fatty acid (FA) composition: short-, branched-, and medium-chain fatty acids (SCFAs, BCFAs and MCFAs)

The extraction and quantification of short-chain fatty acids (SCFAs: acetic, propionic, butyric), branched-chain fatty acids (BCFAs: valeric, isobutyric, isovaleric, 2-methylbutyric), and medium-chain fatty acids (MCFAs: hexanoic, heptanoic, octanoic, decanoic, dodecanoic, tetradecanoic, and hexadecanoic acid) were performed in fecal samples as previously described in Lotti et al. (2017) (Lotti et al., 2017). After centrifugation of fecal samples, a 100 μL aliquot from each sample was taken for analysis. A brief description of the procedure is provided in the Supplementary Material under "Materials and Methods". All fecal samples were freshly prepared and analyzed using GC–MS/MS system, consisting of Agilent 9000 Intuvo GC gas chromatograph (Agilent Technologies) coupled to an Agilent 7010B tandem mass spectrometer (Agilent Technologies); chromatographic separation was achieved on an Agilent DB-FATWAX UI 30mx0.25mmx0.25um GC column. Samples per batch (Donor) were randomly injected in a split ratio 10:1 or 150:1 when saturated (total run-time of 6.5 min). Method validation parameters included: linearity, specificity, selectivity, accuracy, precision, and LOD and LOQ. The LOQs ranged from 1 to 50 μM, with linearity observed up to 500–2500 μM depending on the compound. QC confirmed consistent instrument performance with <15% variation in QC samples, < 10% in calibration, and no carryover observed. Results are shown in Table SI 3.

2.3.3. ROS analysis - DPPH assay

The stock solution of DPPH (25 μg/mL) and Trolox, used as a reference standard (1.75 mg/mL), were prepared in methanol and filtered. The calibration curve (2, 10, 20, 50, 100, 200 and 500 μg/mL) was prepared with 190 μL of DPPH solution and addition of 10 μL appropriately diluted calibrant solution Trolox, control samples with addition of 10 μL of ultra-pure water, samples with the addition of 10 μL of filtered fecal samples (aliquot from each fecal sample) and blank samples containing 200 μL of ultra-pure water only. All samples, with a final sample volume 200 μL, were prepared on the 96-well Tissue culture plate with flat bottom with Lid and analyzed using 96 well plates and Vis -spectrophotometer - GEN5readed. Absorbance of samples, after being shaken for 4 s in the reader in the absence of light, was determined at 517 nm immediately and after 30 min and 60 min. The sample antioxidant activity was calculated using the following formula:

$$\text{antioxidant activity } [\%] = [(Ac - As) \div Ac] \times 100$$

where Ac—Control reaction absorbance; As—Testing specimen absorbance.

2.4. Data processing and statistical analysis

The datasets generated and analyzed during this study are deposited in the Zenodo repository (https://zenodo.org/uploads/15479190) and will be made publicly accessible upon publication. Statistical analyses and data visualizations were performed using R software (version 4.4.2). All custom R scripts for data processing and analysis, along with the corresponding input data, are available on GitHub (https://github.com/ana-kovacic/NeuroTOm).

Experimental variability was assessed by calculating the RSD (%) for three replicate samples taken at the same time point, under identical experimental conditions, and from the same fecal donor for each compound (Tables SI 4 and SI 5). For most compounds detected above the limit of quantification (LOQ), RSDs were below 40%, confirming repeatability.

Missing values for neuroactive compounds and fecal fatty acids (FAs) below the LOQ were imputed using random values between zero and the respective LOQ. Only compounds with ≥30% of values above the LOQ in at least one experimental condition were retained for further analysis. The imputed data were $\log_{10}$-transformed and scaled using Pareto scaling prior to Principal Component Analysis (PCA), which was used as an unsupervised approach to get an overview of the dataset.

To evaluate the behavior of individual compounds across experimental conditions, such as Tomato vs. Inulin (control), or Tomato vs. samples enriched with neuro-disrupting compounds (i.e., TomatoToxic group) we fitted linear mixed-effects models (LMMs). These models accounted for repeated measurements within donors by including a random effect for donor and treated experimental condition and time as fixed effects (i.e., value ~ experimental condition * time + (1 | donor)), with time modeled as a factor. Model performance was assessed using the marginal R^2, representing the variance explained by the fixed effects alone. Higher R^2 values indicate greater model explanatory power and reliability of the results. In this methodology, missing values were imputed to avoid biased results due to incomplete data, acknowledging that these missing entries mostly represent metabolite concentrations below the limit of detection rather than true missingness. While we acknowledge that imputation introduces estimates that may not represent true experimental values, it allows for not obtaining higher mean values, reflecting values that are theoretically between zero and the LOQs of the compounds. This approach was chosen to improve model robustness and ensure that analyses could be conducted even in the presence of missing data, though the imputed values should be interpreted with caution. To evaluate statistically significant differences between experimental conditions of interest and across time points within the same condition, we performed pairwise comparisons: 1) between experimental conditions at individual time points (e.g., Tomato vs. Inulin at 3 h), and 2) between consecutive time points within each condition (e.g., 0 h vs. 3 h within Tomato), using estimated marginal means and contrasts. Effect size (ES) was calculated as the ratio of the estimated difference to the standard deviation of the model residuals. The results of these analyses are presented in the form of plots, which are discussed in detail in the Results section. This approach allows for the statistical evaluation of compound behavior across experimental conditions and fermentation time. However, we acknowledge a limitation: gradual, linear changes over time may not be captured as statistically significant due to the focus on pairwise comparisons of consecutive time points. In such cases, although statistical confirmation may be lacking, consistent trends, such as a slow linear decline in compound levels, can still be visually observed in the plots. These trends are qualitatively interpreted and discussed where appropriate.

3. Results and discussion

3.1. The fate of neuroactive compounds during fecal fermentation

The gastrointestinal fate of the studied compounds was previously investigated in our published work (Kovačič et al., 2026). We found that most neuroprotective compounds, including amino acids, alkaloids, phenolics, vitamins, and fatty acids, showed high recovery (>75%) after digestion. Compounds detected in the liquid phase were considered bioaccessible for intestinal absorption, whereas those enriched in the pellet fraction were more likely to reach the colon. Some compounds (e.g., naringenin, resveratrol, quercetin) were more enriched in the pellet, whereas others (e.g., chlorogenic acid isomers, caffeic derivatives) were below LOQ in both fractions. Certain compounds (e.g., linoleic acid, pyridoxic acid, tyramine, guanine) even suggested increased release during digestion, indicating potential for both intestinal bioaccessibility and colonic delivery. In contrast, most neuro-disrupting compounds (e.g., bisphenols, acetaminophen, aspartame) were largely retained in the pellet, indicating likely colonic bioaccessibility, whereas difenoconazole showed minimal recovery (<10%), suggesting extensive transformation or degradation during digestion.

Based on the criterion of being detected in at least 30% of samples under at least one experimental condition, 10 out of 36 target compounds were excluded. Specifically, bisphenol A, caffeic aldehyde, dihydrokaempferol, naringin, resveratrol, and o-coumaric acid were not detected in any samples, while m-coumaric acid, neochlorogenic acid, crypto-chlorogenic acid, and chlorogenic acid were detected in only a few. Bisphenol S and difenoconazole, although included in further analyses, were detected only in samples where they were deliberately added. This observation aligns with previous findings, suggesting that certain compounds, though possibly present in tomatoes, they were not present in the digested tomato used in this study or, either exhibit high intestinal bioaccessibility (e.g., chlorogenic acid isomers) or undergo extensive transformation during digestion (e.g., difenoconazole).

To globally explore the dataset, a PCA was performed. The PCA score plot revealed a clear separation along PC1, distinguishing tomato-containing samples (digested tomato exposed to microbial community-fecal samples) from non-tomato-containing ones, with PC1 explaining 28.2% of the total variance in the dataset (Fig. 2, left). Most quantified compounds clustered on the same side as the tomato-containing samples (Fig. 2, right), further supporting the discriminative influence of tomato-derived metabolites.

3.1.1. Tomato vs. inulin fermentation

To better assess the specific effects of tomato on the monitored compounds within a multivariate framework, a PCA was performed on a subset of samples, including only those from the Tomato condition and its corresponding control (Inulin). This approach was chosen due to the high number of experimental conditions and the clear separation observed between digested tomato-containing and non-tomato fecal samples. We hypothesized that certain tomato-derived compounds are responsible for the separation which could also show distinct temporal changes during fecal fermentation, potentially due to microbial transformation or utilization (Wang et al., 2022). As expected, the score plot (Fig. 3A) shows a clear separation between the two groups along PC1, which explains nearly 40% of the total variance in the dataset. In the corresponding loading plot (Fig. 3C), most monitored compounds align with the Tomato samples, indicating that they strongly contribute to the separation along PC1 and are more abundant in Tomato samples. When the score plot is colored by fermentation time (Fig. 3B), Tomato samples show a distribution along PC2, indicating that tomato compounds undergo dynamic changes over fecal fermentation. In the loading plot, compounds were grouped into three clusters based on their location and distribution pattern. Compounds in the upper-left quadrant (Cluster 1, c1 – red), which were higher in Tomato samples at early time points, shifted over fecal fermentation, indicating that they are changing during the fermentation process, potentially due to microbial transformation or utilization. Compounds along the PC1 axis (Cluster 2, c2 – green), which had less weight on PC2, were more abundant in Tomato samples and suggesting minimal variation across time points. Cluster 3 (c3 – blue), with low absolute PC1 loadings, contained compounds that showed no clear association with either condition or fermentation time, likely representing molecules unaffected by the presence of digested tomato or microbial activity. Together, these patterns highlight distinct behaviors among the monitored compounds and support the hypothesis that specific tomato-derived molecules contribute to the observed separation and undergo characteristic transformations or remain stable throughout fermentation.

To further explore the observations from the multivariate analysis and to model the data in order to evaluate the behavior of individual

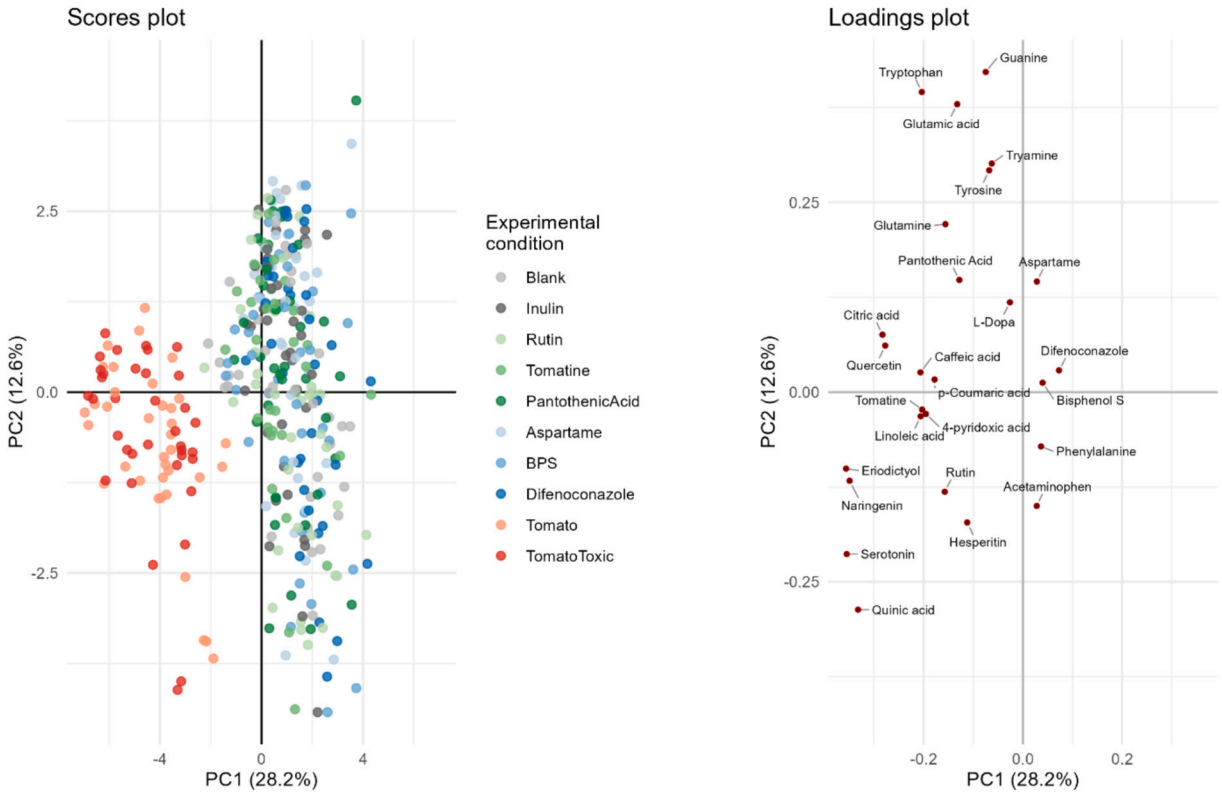

Fig. 2. PCA analysis of all fecal fermentation samples. Left: Score plot with samples colored according to experimental conditions, showing clear separation along PC1 between tomato-containing and non-tomato-containing samples. The experimental condition consist of 1) Blank (fecal solution without any substrate), 2) Inulin (2 g of digested inulin as a control), 3) Digested tomato (Tomato), 4) Digested tomato with a mix of neuro-disrupting compounds (aspartame, difenoconazole, bisphenol S, c = 50 μg/kg, TomatoToxic), and 5–10) Samples with individually added compounds exposed to microbial communities, namely neuroprotective (Rutin, Pantothenic acid, Tomatine) or neuro-disrupting compounds (Aspartame, Difenoconazole, bisphenol S (BPS)) to a final concentration of 2 mg/L in the fecal vessel. Right: Loading plot indicating the contribution of individual compounds to the observed variation.

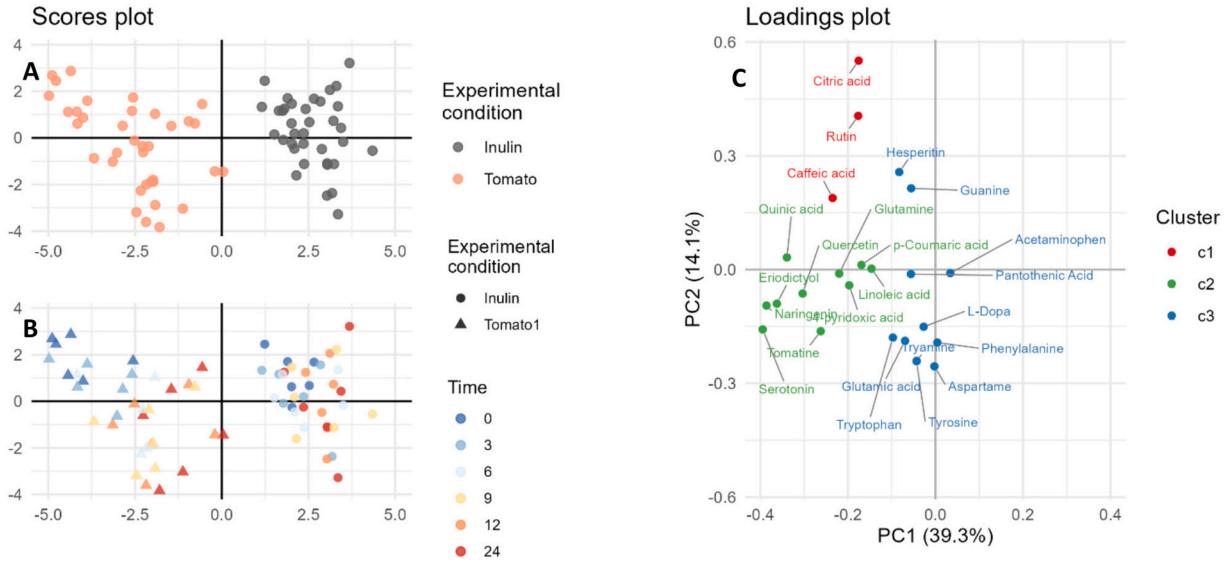

Fig. 3. PCA of fecal fermentation samples from Tomato and Inulin conditions. (A) Score plot showing clear separation between Tomato and Inulin samples along PC1. (B) Score plot colored by fermentation time, showing a time-dependent distribution of tomato samples along PC2. (C) Loading plot indicates that most compounds contribute to this separation, grouped into three clusters: c1 (red): Higher in tomato samples, changing over time; c2 (green): Higher in tomato samples, no clear time trend; c3 (blue): No distinct pattern across conditions or time points. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

compounds across experimental conditions (i.e., Tomato vs. Inulin (control)), we fitted linear mixed models (LMMs), as described in Material and methods (Subchapter 2.4, Data Processing and Statistical Analysis). The results of these analyses are presented in Fig. 4, grouped

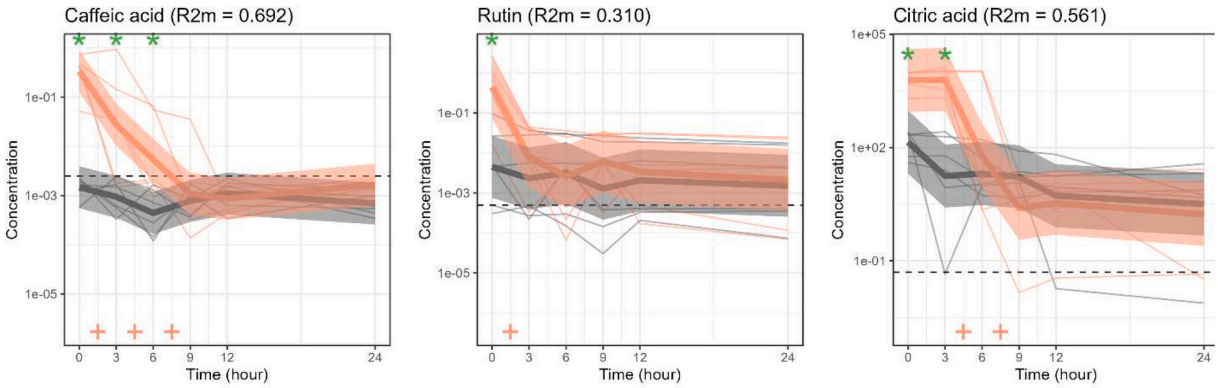

Fig. 4. (a) Predicted $\log_{10}$ concentrations and 95% confidence intervals from the mixed-effects model for each compound in the Tomato (red) and Control/Inulin (grey) samples. Cluster 1 includes compounds that were initially more abundant in tomato samples and showed progressive transformation or degradation during fecal fermentation. Thin lines represent individual sample measurements; thick lines indicate model estimates; shaded ribbons show 95% confidence intervals. The dashed horizontal line marks the limit of quantification (LOQ). Green asterisks at the top denote significant between-group differences ($p < 0.05$ and $|ES| > 1$); colored plus signs at the bottom indicate significant within-group changes over time. (b) Predicted $\log_{10}$ concentrations and 95% confidence intervals from the mixed-effects model for each compound in the Tomato (red) and Control/Inulin (grey) samples. Cluster 2 includes compounds that consistently showed higher concentrations in tomato samples across all time points, with limited temporal variation during fecal fermentation. Thin lines represent individual sample measurements; thick lines indicate model estimates; shaded ribbons show 95% confidence intervals. The dashed horizontal line marks the limit of quantification (LOQ). Green asterisks at the top denote significant between-group differences ($p < 0.05$ and $|ES| > 1$); colored plus signs at the bottom indicate significant within-group changes over time. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

according to the clusters defined in the PCA loading plots. Thin lines represent measured concentrations for individual samples, while thick lines indicate model estimates, with shaded ribbons showing the 95% confidence intervals. Dashed horizontal lines represent the LOQ. Green asterisks (at the top of each plot) denote time points with significant differences between-groups ($p < 0.05$ and $|ES| > 1$). Plus signs, colored according to the experimental condition (at the bottom of each plot), indicate significant differences between consecutive time points within a group ($p < 0.05$ and $|ES| > 1$).

The first cluster (Fig. 4a), C1, includes compounds that show initial differences between experimental groups (Tomato > Inulin), followed by a significant decrease over time in the Tomato condition, with no significant changes observed in the Inulin group. Specifically, caffeic acid shows significant between-group differences at 0, 3, and 6 h, and a time-dependent decrease in Tomato samples during this time. Rutin is significantly higher in Tomato at time zero but decreases rapidly, with a significant within-group drop occurring between 0 and 3 h. Citric acid also follows this pattern, showing significant between-group differences at time zero and after 3 h of fermentation and significant drop between 3 and 6 h. These compounds underwent rapid transformation during fermentation, with concentrations falling below the LOQ after 3–9 h. These patterns suggest that the initial differences were primarily driven by tomato-derived content, and the subsequent decline reflects compound degradation or biotransformation under fermentation conditions. In contrast, Inulin samples remained stable over time.

The second cluster (Fig. 4b, c2) comprises compounds that, based on the PCA, consistently distinguish the Tomato group from the Inulin group across all time points. This pattern likely reflects the presence of tomato-derived compounds that remain stable throughout fermentation, rather than changes in concentration over time. This observation was further supported by the LMMs, which fit the data well, with marginal R^2 values exceeding 0.5 for most compounds. The majority of compounds in this cluster showed no significant temporal changes in either group, indicating their stability during fecal fermentation. Glutamine was a notable exception, displaying a gradual decrease over time in both Tomato and Inulin conditions, while linolenic acid exhibited a modest, transient increase between 0 and 3 h in the Tomato group. Meanwhile, p-coumaric acid, although grouped in this cluster, exhibited an early decline in concentration, more characteristic of Cluster 1, suggesting it may represent a transitional kinetic profile between clusters. Despite differences in absolute compound levels between Tomato and Inulin conditions, their temporal evolution was generally similar. No consistent increasing or decreasing trends were observed over time for most compounds. These compounds retained more than 50% of their initial concentrations after 24 h of fecal fermentation, indicating potential for colon bioaccessibility.

The third cluster (C3) includes compounds that showed no significant differences between experimental conditions (Tomato vs. Inulin) and, in general, no consistent temporal trends throughout the fermentation period (Fig. SI 1). The few differences observed, such as phenylalanine, which showed higher concentrations in Tomato samples at the beginning and end of fermentation, and pantothenic acid, which increased between 12 and 24 h, appear to be sporadic and not robust. Similarly, the decrease in tyramine observed in the Inulin condition. An exception within this cluster is guanine, which exhibited a modest increase between 0 and 3 h, followed by a decrease between 3 and 9 h, although no significant differences between the conditions were observed. Overall, the compounds in Cluster 3 showed minimal variation and limited response to treatment or fermentation time, suggesting that they are unaffected by the tested conditions.

3.1.2. *Impact of neuro-disrupting compounds (Tomato vs. TomatoToxic)*

In the next step, we focused on the neuro-disrupting compounds effect, to examine whether the presence of neuro-disrupting compounds could influence the behavior of monitored compounds during fecal fermentation containing digested tomato. For this reason, we performed a PCA on the subset of samples from Tomato and TomatoToxic experimental conditions (Fig. 5). The score plot of the PCA (Fig. 5A) did not show a clear separation between samples with and without the addition of neuro-disrupting compounds at environmentally relevant concentrations, suggesting that these compounds, at the low concentrations tested, did not strongly perturb the system. This observation aligns with expectations, given that only a small number of neuro-disrupting compounds were added at realistic low concentrations, with the highest accepted values for the most critical compound used to adjust the concentrations of all three (aspartame, bisphenol S, difenoconazole). In the scores plot, colored by time, samples tend to separate among the PC1 by the time of fermentation (Fig. 5B). However, in the loadings plot

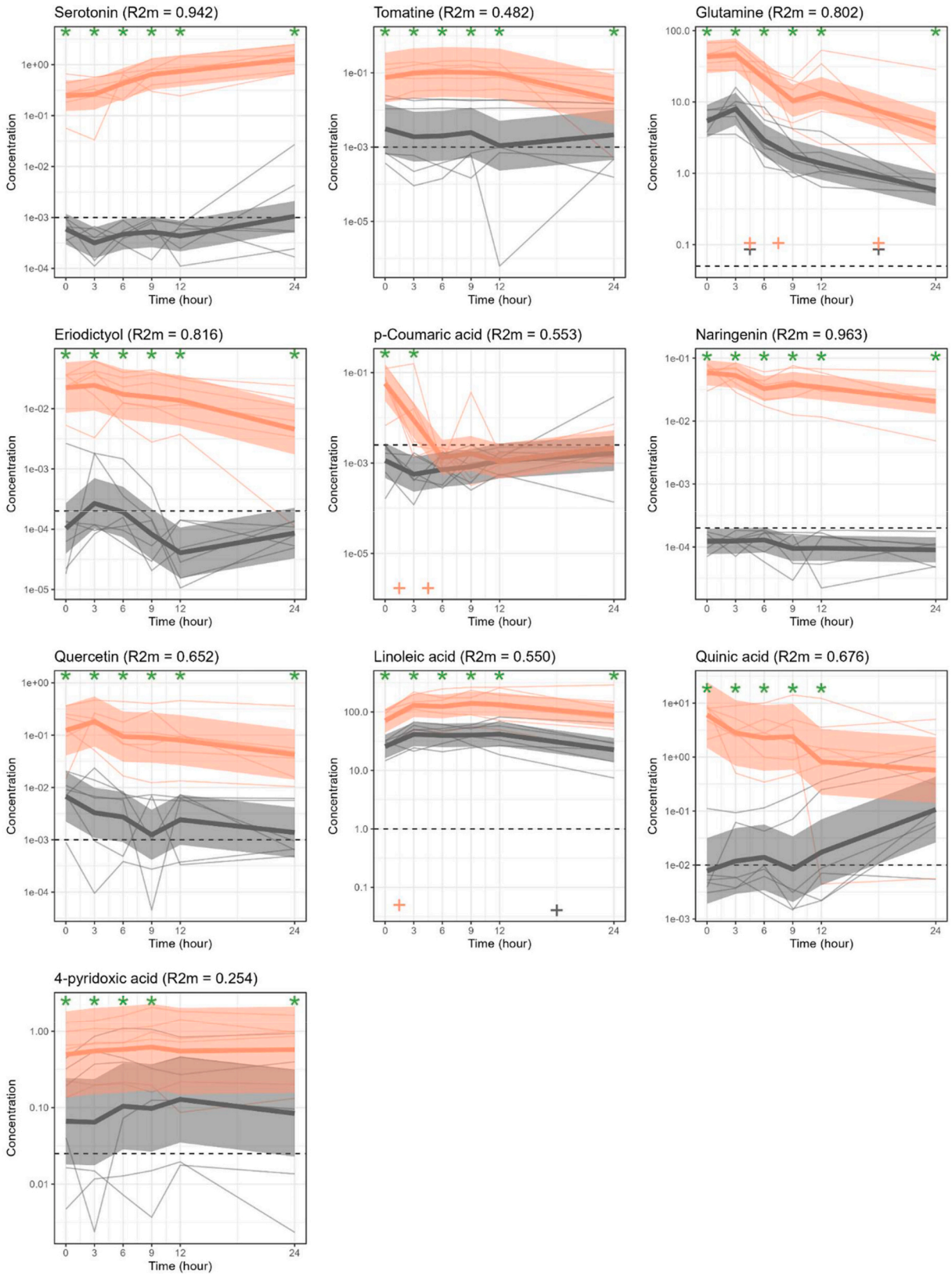

Fig. 4. (*continued*).

(Fig. 5C), no clear cluster formation was observed. Based on the direction and magnitude of PC1 loadings, compounds located on the right side of the plot (high positive PC1 loadings) appear to exhibit time-dependent trends. Following the previously described workflow, LMMs combined with pairwise comparisons were applied to this dataset to compare the Tomato experimental conditions with and without the addition of neuro-disrupting compounds (TomatoToxic). Compounds such as citric acid, caffeic acid, guanine, rutin, *o*-coumaric acid, and

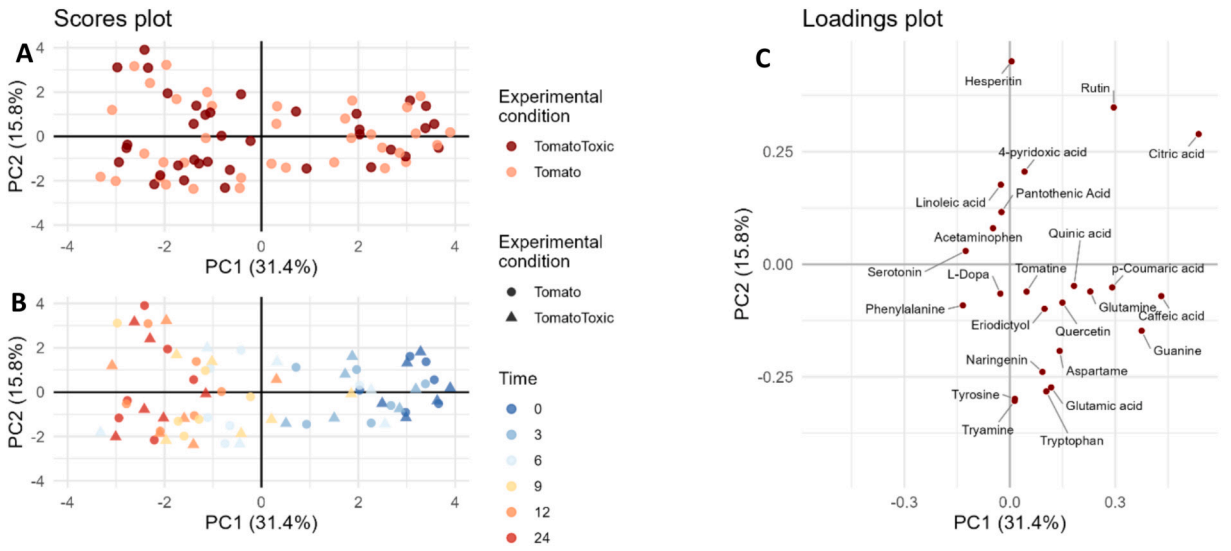

Fig. 5. Principal Component Analysis (PCA) of fecal fermentation samples from tomato with (TomatoToxic) and without (Tomato) the addition of neuro-disrupting compounds at environmentally relevant concentrations. (A) Score plot showing no clear separation between the two experimental conditions along PC1. (B) Score plot colored by fermentation time, showing a time-dependent distribution of tomato samples along PC1. (C) The accompanying loading plot.

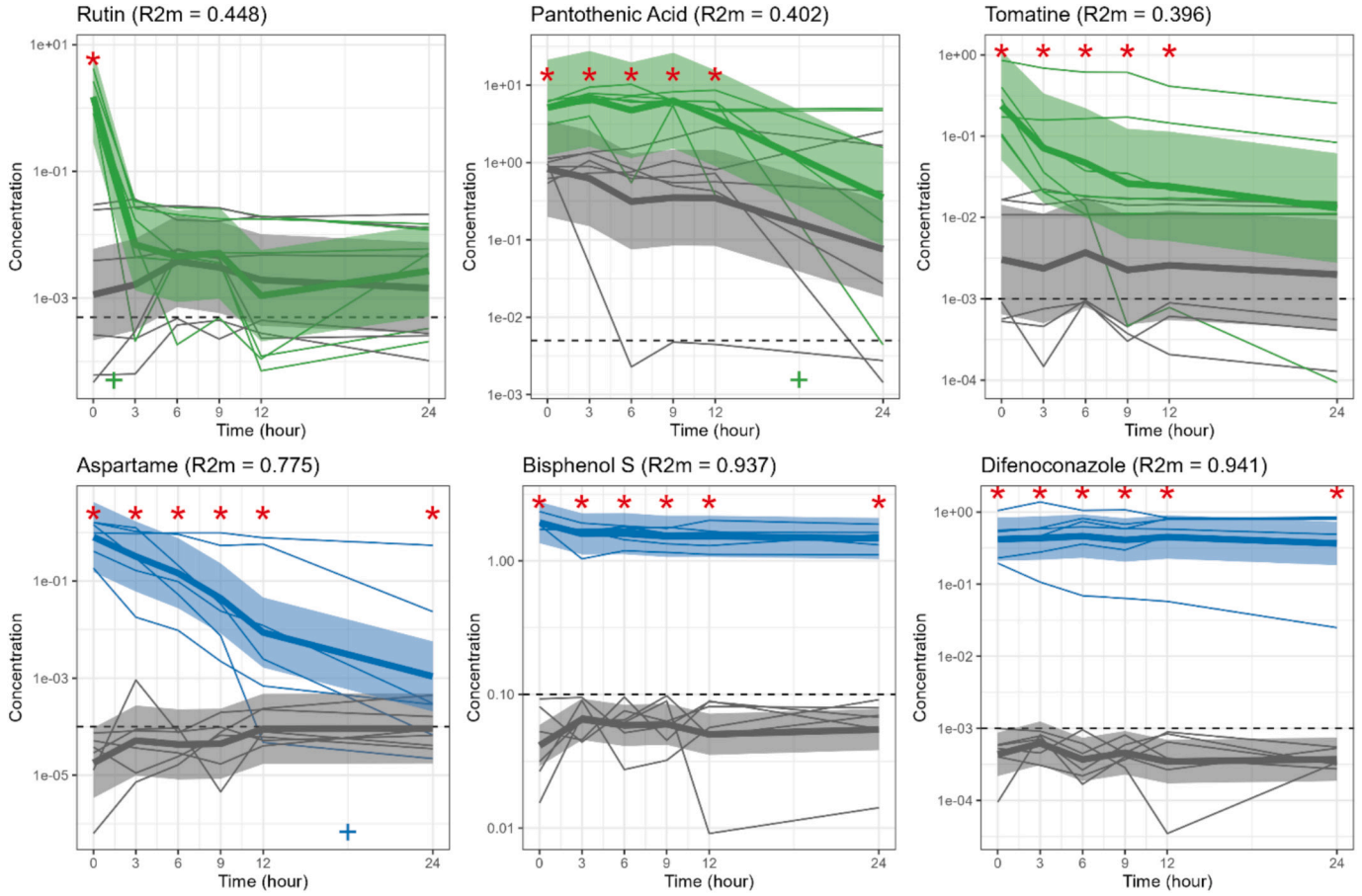

Fig. 6. Kinetics of individually added neuro-disrupting and neuroprotective compounds during fecal fermentation: Predicted $\log_{10}$ concentrations and 95% confidence intervals from the linear mixed-effects models (LMMs) are shown for each compound individually added to the fecal solution. Grey lines represent control samples (Blank, fecal solution only). Neuroprotective compounds (rutin, pantothenic acid, tomatine) are shown in green; neuro-disrupting compounds (aspartame, bisphenol S, difenoconazole) are shown in blue. Thin lines depict measured values for individual samples; thick lines represent model estimates; shaded ribbons indicate 95% confidence intervals. The dashed horizontal line denotes the limit of quantification (LOQ). Red asterisks indicate significant between-group differences ($p < 0.05$ and $|ES| > 1$); colored plus signs at the bottom denote significant within-group changes over time. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

glutamine exhibited the highest PC1 loadings and also showed statistically significant declines in concentration over fermentation time at specific time points. Among these, caffeic acid, rutin, and citric acid were also previously identified as exhibiting time-dependent differences when comparing Tomato with the Inulin (control) samples. In contrast, the majority of the remaining compounds, positioned closer to the center of the loadings plot, remained relatively stable throughout fermentation (Figure SI 2).

3.1.3. Fate of individually added neuroactive compounds

One of the aims of the study was also to individually evaluate the fate of selected compounds during fecal fermentation. To this end, three neuro-disrupting and three neuroprotective compounds were selected and separately added to the fecal solution prior to fermentation. Similar to the previous comparisons LMMs and pairwise comparisons were performed between each individually added compound and the control sample, which in this case was the Blank condition, containing only the fecal solution (Fig. 6). Grey lines represent the Blank samples; neuroprotective compounds (i.e., rutin, pantothenic acid, and tomatine) are shown in green, while neuro-disrupting compounds (i.e., aspartame, bisphenol S, and difenoconazole) are shown in blue. As expected, there was a significant difference between each individually added compound and the control. Kinetic data for rutin indicated that it began to undergo transformation within the first 3 h of fermentation, with its concentration eventually falling below the LOQ. This transformation pattern mirrored what was observed in the fecal fermentation samples containing digested tomato, where rutin also dropped below the LOQ after 3 h. In contrast, although the change in the kinetic profile of tomatine was not statistically significant, we observed a slow decrease over time. Pantothenic acid followed a distinct pattern. It underwent minimal transformation during the early stages of fermentation, with statistically significant changes observed only between 12 and 24 h. Interestingly, while pantothenic acid levels began to decrease in the individually spiked samples at this late time point, an increase was observed in the fecal fermentation samples containing tomato. This divergence could suggest that pantothenic acid is being formed from other tomato-derived compounds or reflects complex interactions within the tomato fermentation matrix. However, in this case, we must also consider the tenfold higher concentration compared to the samples with digested tomato, which could obscure the underlying tomato-related trends.

Among the neuro-disrupting compounds, aspartame was the only one that showed a significant decrease in concentration between 12 and 24 h of fermentation. Similar to tomatine, a linear decrease over time was observed, although it did not reach statistical significance, with less than 10% of the median concentration remaining at the end of the process. This decrease was not apparent in the Tomato condition, which may be explained by the high proportion of values below the LOQ in those samples. This suggests that aspartame was practically undetectable in the presence of digested tomato. Bisphenol S and difenoconazole remained relatively stable throughout the fermentation period, with more than 80% of their initial concentrations retained after 24 h and no statistically significant changes detected. Although both compounds were below the LOQ in the other fecal fermentation conditions, their presence cannot be completely excluded. Their low concentrations could be due to transformations occurring during the upper gastrointestinal digestion phase. In our previous study, we demonstrated that up to 50% of bisphenol S and less than 20% of difenoconazole can reach the colon, while the remaining portion becomes bioavailable in earlier digestive stages (Kovačič et al., 2026). The reason these compounds are not observed in the TomatoToxic condition could be that they are metabolized below the LOQ, present at concentrations lower than detectable, or already bioaccessible in the upper part of the digestive tract. Based on the results from the other experimental conditions, including TomatoToxic, we do not expect these compounds to reach the colon in significant amounts. However, if they do reach the colon, they appear to remain largely unaffected by the colonic microbiota.

Our findings suggest that fecal fermentation samples containing previously digested tomato were significantly richer in neuroprotective compounds, such as rutin, caffeic acid, citric acid, serotonin, tomatine, glutamine, p-coumaric acid, naringenin, eriodictyol, linoleic acid, quinic acid, 4-pyridoxic acid, and quercetin. Aside from rutin, citric acid, and caffeic acid—whose degradation, based on prior literature, may involve microbial glycosidases (e.g., α-L-rhamnosidase and β-glucosidase) in the case of rutin (Yang et al., 2012) and phenolic acid–modifying enzymes in the case of caffeic acid (Carmody & Turnbaugh, 2014), most of these neuroprotective compounds remained stable during fecal fermentation, indicating their potential colonic bioaccessibility and possible neuroactive effects if absorbed into the bloodstream. Moreover, as tomato appears to be their primary source, their presence underscores the nutritional relevance of tomatoes in contributing bioactive compounds with known neuroprotective potential (Silva and Pogačnik, 2020; Yılmaz & Gökmen, 2020). The concentrations of these compounds were elevated compared to the control, with elevated concentrations in line with literature data (Ali et al., 2021), showing the general concentrations of these compounds found in tomatoes, which additionally indicates that tomatoes are a key source of these neuroactive compounds. Specifically, the concentration of citric acid was found to be 50 times higher, and the remaining elevated concentrations of other compounds, such as rutin, caffeic acid, serotonin, tomatine, glutamine, p-coumaric acid, naringenin, eriodictyol, linoleic acid, quinic acid, and 4-pyridoxic acid, were observed to be approximately 5 to 20 times higher compared to the control samples. These results suggest that including tomatoes in the diet may enhance the bioaccessibility of a broad range of neuroactive compounds during digestion and to the colon. Increasing the concentrations of dietary phytochemicals, including phenolic compounds, neurotransmitters, amino acids, fatty acids, and alkaloids in the diet, could have beneficial effects on gut health due to their anti-inflammatory, antioxidant, and digestive-supportive properties (Rocchetti et al., 2022; Santhiravel et al., 2022). It is important and essential to balance the intake of positively active compounds to avoid potential adverse side effects, particularly if their concentrations exceed what is considered safe (Duda-Chodak & Tarko, 2023). Ideally, these compounds should be consumed within recommended limits for optimal gut health benefits. The gut microbiota plays a key role in brain health by producing metabolites and modifying host-derived molecules, including neuroactive substances such as serotonin, dopamine, and GABA (Loh et al., 2024). Previous studies have shown that gut microbes can produce or stimulate the synthesis of neurotransmitters, and food itself can serve as a source of bioactive compounds. For example, tomatoes contain bioavailable GABA (de Bie et al., 2022), and in our study, we observed increased and stable levels of serotonin in fecal samples containing digested tomato. Although higher serotonin levels in tomato, up to fivefold, as suggested by our data, may influence digestive function and gut health, their impact on the brain is limited, as dietary serotonin does not readily cross the blood–brain barrier (Jenkins et al., 2016). Nevertheless, indirect effects on mental health remain possible through gut-mediated pathways. In contrast, gut-derived serotonin also cannot cross the blood–brain barrier, but its precursor, 5-hydroxytryptophan, may influence brain function and contribute to neuroactive signaling (Loh et al., 2024). Amino acids such as tyrosine, tryptophan, and phenylalanine, which remained stable during fermentation, are key precursors for neurotransmitters and may have systemic effects (Chen et al., 2021). For example, tyramine, a bioactive amine and catecholamine-releasing agent derived from these amino acids, was also stable during fermentation (Yılmaz & Gökmen, 2020). L-DOPA, derived from tyrosine and further converted into dopamine (a key neuroactive compound synthesized in the gut), also remained stable under all tested fermentation conditions (Yılmaz & Gökmen, 2020). Our experiments confirmed an increased presence of polyphenols in fecal samples containing tomato at the onset of fermentation. Some compounds, like rutin and caffeic acid, underwent transformation, whereas others, such as naringenin, eriodyctiol, and quercetin, remained bioaccessible to the

colon. Additionally, linoleic acid levels were higher in tomato-containing fecal samples, which may influence gut microbiome composition (Huyan et al., 2022; Mercola, 2025). Tomatine, another key compound derived from tomatoes, showed a more pronounced decreasing trend in the absence of tomato and at a higher concentration when individually added and exposed to microbial communities, compared to the samples containing digested tomato. This finding is particularly relevant, as protective effects have been observed even at low, physiologically realistic concentrations, which may also explain the slower transformation observed (Bailly, 2021). Finally, pantothenic acid, an essential nutrient involved in various metabolic processes, showed a stable concentration and even a slight increase between 12 and 24 h of fermentation with tomato, further supporting its colonic bioaccessibility (Wan et al., 2022). In contrast, levels declined at this later stage in the individually spiked samples, in which neuroactive compounds were added separately to the fecal solution prior to fermentation, suggesting possible formation from tomato-derived compounds or matrix-related interactions. However, the tenfold higher concentration in the spiked samples may mask tomato-related effects.

Among the neuro-disrupting compounds examined, bisphenol S and difenoconazole stood out due to their marked stability during fecal fermentation. In samples where they were individually added, both compounds remained largely intact (> 80%) after 24 h of fecal fermentation, indicating limited degradation by gut microbiota. Although these compounds were undetectable in the other fecal fermentation samples (where they were not added or were added only at very low concentrations based on the strictest existing regulations, i.e., bisphenols) (FitzGerald et al., 2020), the selected levels reflect a realistic yet conservative contamination scenario for neuro-disrupting compounds in tomatoes (ranging from 0.3 to 400 μg/kg for difenoconazole and bisphenol S, and aspartame only if deliberately added or fortification occurred) (Gaouar et al., 2021; Ighalo et al., 2024). Their chemical persistence, even at considerably higher concentrations, suggests limited microbial metabolism and raises concerns about their potential to reach systemic circulation and affect the central nervous system (Cox et al., 2023). To ensure detectable levels for assessing colonic bioaccessibility, stability, transformation, and microbial interactions, the compounds were tested at elevated concentrations. The spiking scenario in the fecal fermentation experiment (simulating 300 mg/kg fresh tomato) represents an intentionally high exposure level used for mechanistic investigation. Such concentrations are not realistic under normal agricultural conditions, they are approximately 1000-fold higher than typical difenoconazole residues in tomatoes, and even more for bisphenol S in the case of possible contamination. Likewise, 0.3 mg/g of aspartame would only be plausible in sweetened or fortified products, not in raw or dried natural tomatoes. This use of elevated concentrations represents a limitation of the present study, and caution should be exercised when extrapolating these findings to realistic dietary exposure scenarios. Nevertheless, the observed stability implies high colonic bioaccessibility, consistent with previous reports showing that over 50% of ingested bisphenol S can reach the colon (Cox et al., 2023). Furthermore, bisphenol analogs like bisphenol S have been linked to alterations in gut microbiota composition and increased intestinal permeability, potentially enhancing their systemic effects (Calero-Medina et al., 2023). For difenoconazole also a similar pattern of persistence was reported, with its bioaccessibility during colonic fermentation reported to range from 59.4% to 27.1%, depending heavily on the digested food matrix (Ruiz-González et al., 2024). Our findings are especially relevant given a recent cross-sectional study in Andalusia (Spain), which correlated significantly higher rates of neurodegenerative diseases in districts with elevated pesticide use (Ruiz-González et al., 2024). Our results support these observations, suggesting that the resistance of bisphenol S and difenoconazole to microbial degradation could allow these compounds to persist in the gut environment and potentially exert neurotoxic effects if absorbed into the bloodstream (Cox et al., 2023). While artificial sweeteners offer certain benefits, such as reduced caloric intake and improved blood sugar regulation, their impact on gut microbiome health raises important concerns. Studies have shown that they can reduce beneficial bacterial populations while promoting the growth of potentially pathogenic strains, underscoring the need for caution in their consumption (Hetta et al., 2025). Based on prior studies, aspartame is likely hydrolyzed by microbial esterases and peptidases to aspartic acid, phenylalanine, and methanol (Plaza-Diaz et al., 2020). In our study, the observed decrease in aspartame during fecal fermentation highlights the importance of understanding the metabolic fate of food additives, as their breakdown products may still pose health risks; however, confirmation of these processes would require non-targeted meta-omics approaches.

The main limitations of this study include the use of a static in vitro model restricted to the colonic phase, which lacks absorption processes and host–microbiome interactions and therefore cannot fully replicate in vivo physiological conditions. For example, the absence of epithelial uptake and systemic distribution may result in an overestimation of colonic persistence for highly stable compounds, such as bisphenol S and difenoconazole, since no absorption or clearance mechanisms are simulated in this model. Moreover, although additional transformations at longer incubation times (e.g., 48–72 h) cannot be excluded, extending static batch fermentations beyond 24–48 h may reduce physiological relevance and increase the risk of artefactual effects due to nutrient depletion and metabolite accumulation (Isenring et al., 2023). In addition, the absence of microbial community characterization limits the identification of specific taxa contributing to the observed compound transformations. Nevertheless, the findings presented here provide a valuable foundation for selecting the most relevant samples for future metagenomic analyses. These analyses will be correlated with non-targeted compound profiling to evaluate microbial composition and its potential influence on compound fate, thereby addressing this gap in subsequent studies.

3.2. Fecal fatty acids production

Following the same criteria, 13 FAs were detected in at least 30% of samples under at least one experimental condition. Using the same data processing workflow as for neuroactive compounds, a PCA was performed on the full FAs dataset (Fig. 7). In contrast to the neuroactive compounds, the PCA revealed that fermentation time was the primary source of variance (Fig. 7B), with PC1 accounting for 40.8% of the total variance. Notably, no clear separation between tomato-containing and non-tomato-containing samples was observed (Fig. 7A). Most FAs clustered near the PC1 zero axis, while a few were positioned toward the right, suggesting that these compounds exhibit the most pronounced changes over the course of fermentation (Fig. 7C).

For the compounds positioned toward the right side of the loading plots, which account for variance related to the fermentation time, we applied LMMs and performed pairwise comparisons, similar to the analysis of neuroactive compounds. This included comparisons between Tomato vs. Inulin and TomatoToxic vs. Tomato (Fig. 8). The group of FAs in this category includes compounds that show significant temporal changes across all three experimental conditions (Inulin, Tomato, TomatoToxic). These primarily consist of SCFAs (acetic, propionic, butyric) and BCFAs (valeric, isovaleric, 2-methylbutyric acid). The kinetic data revealed a significant increase in the levels of these compounds over the course of fermentation, particularly between 12 and 24 h. Notably, acetic and propionic acids exhibited significant differences between groups at 6 and 9 h, and after 12 h, respectively (Tomato > Inulin). Additionally, LMM analysis identified significantly higher levels of acetic, propionic, and isovaleric acids in Tomato samples compared to TomatoToxic samples. This suggests that neuro-disrupting compounds, even at environmentally relevant concentrations, may inhibit SCFA production during fecal fermentation. Overall, the SCFAs and BCFAs in this group displayed marked temporal changes, in line with their known production and metabolic patterns during

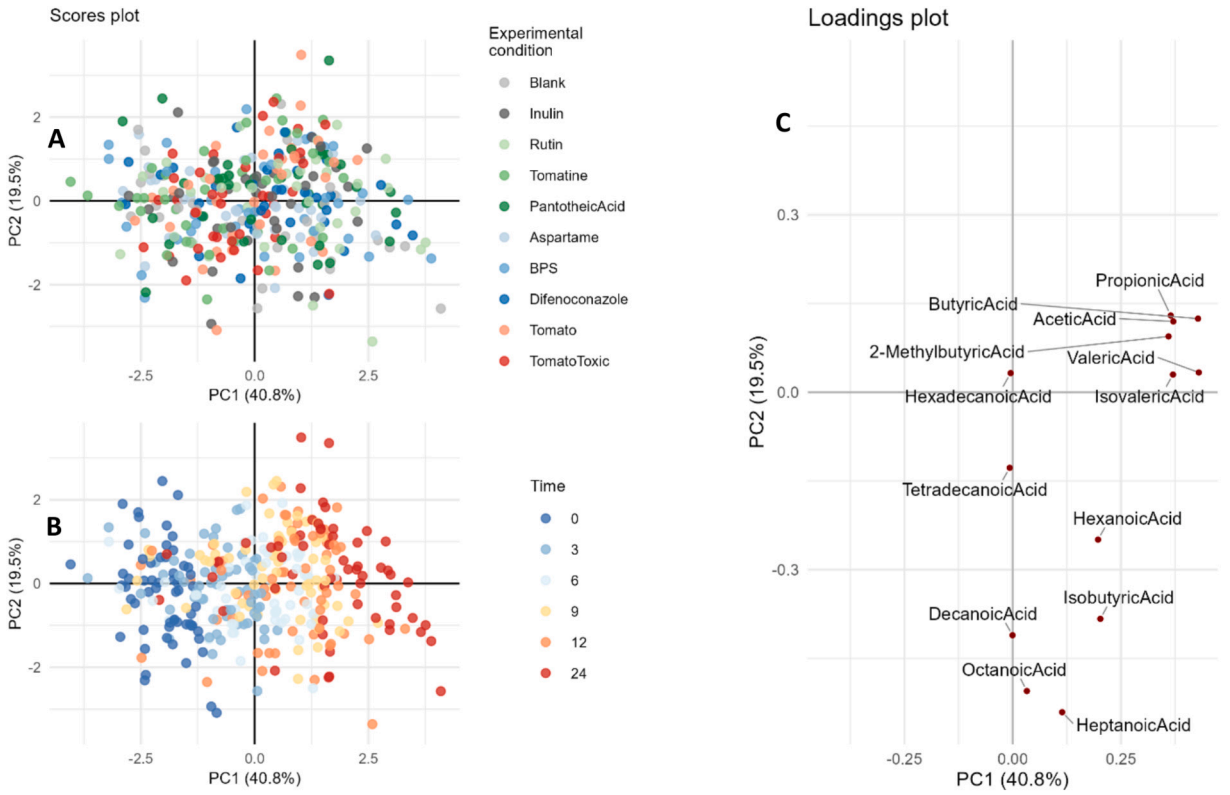

Fig. 7. PCA analysis of all fecal fermentation samples. Left: Score plot with samples colored according to experimental conditions (A), and colored according to time (B) showing clear separation along PC1 between different times of fermentation. (C): Loading plot indicating the contribution of individual compounds to the observed variation.

fermentation. In contrast, other FAs (Figure SI 3), positioned near the PC1 zero axis, including BCFAs such as isobutyric acid and MCFAs such as hexanoic, heptanoic, octanoic, decanoic, tetradecanoic, and hexadecanoic acids, did not exhibit consistent temporal changes throughout the fermentation process. Since these compounds are produced in smaller quantities and are primarily derived from dietary intake, their stability in this experiment suggests that their levels are independent of the fermentation conditions. Additionally, because tomato is not a significant source of MCFAs, the lack of temporal variation further supports the idea that these compounds are largely unaffected by the tested fermentation conditions.

In addition, the ratio between butyric and hexanoic acid has been proposed as an indicator of gut inflammation when it decreases under the examined condition. To assess the potential influence of neuro-disrupting compounds, we calculated the butyric-to-hexanoic acid ratio (BA/HA) and compared it between the Tomato and TomatoToxic conditions, as a decrease in this ratio has been suggested as a marker of gut inflammation (Dell'Olio et al., 2024). However, no statistically significant differences were observed in the comparison of the log-transformed BA/HA ratio during fecal fermentation between the TomatoToxic and Tomato conditions (Figure SI 4).

In summary, all treatments resulted in predominantly increased levels of acetic and propionic acids, consistent with previous findings (Aho et al., 2021). An increasing trend was also observed for butyric, isovaleric, methylbutyric, and valeric acids; however, these increases were mainly not statistically significant due to their slow and linear increase. Nevertheless, a general upward trend was evident for all of them. Acetic, propionic, and butyric acids, three key SCFAs, are principal end products of microbial fiber fermentation in the gut (Zhang et al., 2023). Diet is known to shape gut microbiome composition, which in turn drives SCFA production, as dietary fiber serves as the main substrate for microbial fermentation (Pirkola et al., 2023). Importantly, our results suggested that fecal samples with digested tomato promoted higher formation of certain SCFAs and BCFAs, notably acetic and propionic acids, which are recognized mediators of gut–brain communication through mechanisms such as activation of free FA receptors, modulation of immune signaling, and potential effects on blood–brain barrier integrity (Y. P. Silva et al., 2020). However, this effect was attenuated in the presence of neuro-disrupting compounds, suggesting that such environmental contaminants can interfere with the microbial fermentation of dietary substrates and reduce the production of key metabolites. This represents an important observation linking dietary components, environmental stressors, and microbial metabolism. In human physiology, SCFAs act not only as metabolic substrates but also as signaling molecules that influence energy homeostasis, immune function, and neuro-immunoendocrine regulation (Kim, 2023). Beyond their established roles in energy supply, trophic support, and immune modulation, increasing evidence indicates that SCFAs affect multiple organs, including the brain (Silva et al., 2020). However, the mechanisms by which SCFAs influence brain physiology and behavior remain to be fully clarified, and further explored.

3.3. ROS analysis

The same statistical methodology used for neuroactive compounds and SCFAs was also applied to the ROS data to investigate whether digested tomato or the presence of neuroactive compounds during fecal fermentation had any impact on ROS formation. The results revealed no statistically significant differences in ROS levels across treatment conditions or fermentation times within the investigated experimental setups (Figure SI 5). Additionally, neither the individually added neuroprotective nor the neuro-disrupting compounds caused significant changes in antioxidant activity compared to the control (Blank) samples. This suggests that the contribution of these compounds may be

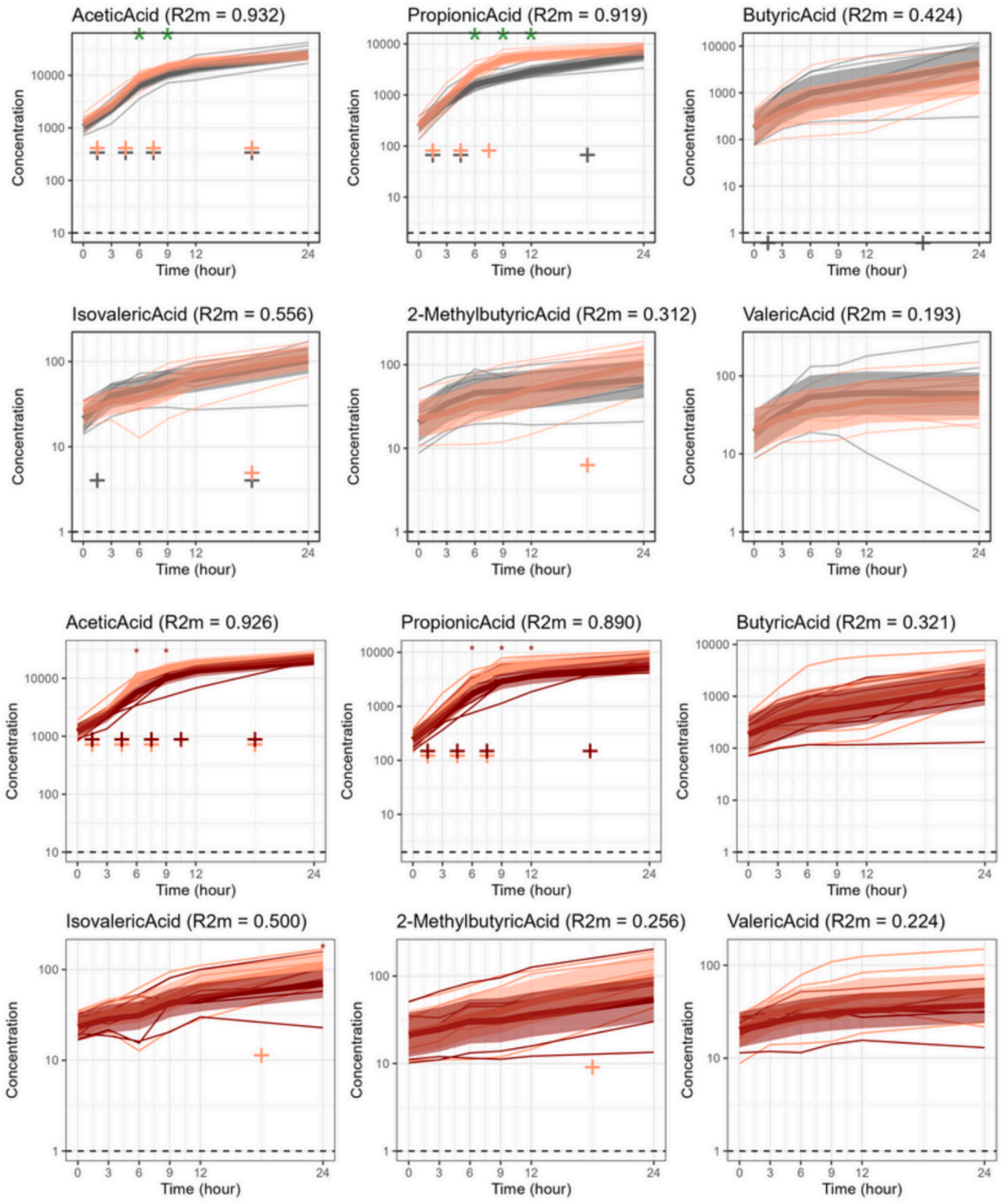

Fig. 8. Predicted $\log_{10}$ concentrations and 95% confidence intervals from the mixed-effects model for each compound in Tomato (light red) versus Control/Inulin (grey) samples, and Tomato (light red) versus TomatoToxic (dark red) samples. The figure highlights groups of FAs that showed temporal variation during fecal fermentation. Thin lines represent individual sample measurements; thick lines indicate model estimates; shaded ribbons show 95% confidence intervals. The dashed horizontal line marks the limit of quantification (LOQ). Green/dark red asterisks at the top denote significant between-group differences ($p < 0.05$ and $|ES| > 1$); colored plus signs at the bottom indicate significant within-group changes over time. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

negligible compared to the already high baseline antioxidant activity present during fecal fermentation, likely arising from the fermentation of nutrients in the medium itself.

4. Conclusion

This study provides new insights into how neuroprotective and neuro-disrupting compounds behave during digestion and colonic fermentation, illustrating the complex interplay between dietary components, environmental contaminants, and microbial metabolism. Our findings show that tomato digestion substantially enhances the colonic bioavailability of multiple neuroprotective compounds, including rutin, caffeic acid, quercetin, serotonin, and tomatine, many of which remained stable during fermentation, suggesting good bioaccessibility and potential systemic relevance. In contrast, neuro-disrupting contaminants such as bisphenol S and difenoconazole displayed high

stability and limited microbial degradation, indicating their capacity to persist in the gut and potentially interfere with host physiology. Tomato digestion also promoted production of beneficial SCFAs, including acetic and propionic acids, though this positive microbial response was weakened by neuro-disrupting compounds. Overall, tomato consumption appears to confer meaningful benefits by increasing exposure to neuroprotective phytochemicals and supporting beneficial microbial metabolism. However, these advantages may be compromised by the presence of persistent pollutants introduced through food or the environment. Although limited by the static in vitro model, this work contributes to a growing understanding of how foods, food components, additives, and environmental contaminants interact with the gut microbiota in ways that may influence neuroactive processes. This work lays a foundation for future research which should investigate these interactions to better assess both the health-promoting potential of tomato-rich diets and the risks posed by co-exposure to stable neuro-disrupting pollutants.

CRediT authorship contribution statement

Ana Kovačič: Writing – original draft, Visualization, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation. **Mar Garcia-Aloy:** Writing – review & editing, Supervision, Data curation, Conceptualization. **Domenico Masuero:** Writing – review & editing, Methodology, Formal analysis, Data curation, Conceptualization. **Cesare Lotti:** Methodology, Formal analysis, Data curation. **Pietro Franceschi:** Writing – review & editing, Data curation, Conceptualization. **Andrea Mancini:** Writing – review & editing, Methodology, Conceptualization. **Josep Rubert:** Writing – review & editing, Supervision, Conceptualization. **Urška Vrhovšek:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Ethical statement

The collection and use of fecal samples were conducted in accordance with relevant ethical procedures and institutional policies. According to the guidelines of Fondazione Edmund Mach and the APSS (Azienda Provinciale per i Servizi Sanitari), Trento (TN), Italy, formal review and approval by an institutional ethics committee were not required for this study. All donors were fully informed about the aims and procedures of the study and provided written informed consent (August 2023) for the use of their fecal material in the experiments. No personal biological data were collected or analyzed. Donor rights and privacy were fully protected, and no identifying personal information has been disclosed.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2026.148888.

Data availability

The data are deposited in the researcher's Zenodo repository and will be made publicly available upon manuscript acceptance. All in-house R scripts are available on GitHub.

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