



Impact of fermentation and *in vitro* gastrointestinal digestion on the bioaccessibility of phytochemicals and antioxidant capacity of tiger nut, carob and rice beverages

Matteo Vitali^{a,b}, Mónica Gandía^a, Antonio González-Sarrías^c, Fernando Vallejo^d, Antonio Cilla^{b,*}, Amparo Gamero^a

^a Bionutest Research Group, Food Technology area, Faculty of Pharmacy and Food Sciences, University of Valencia, Av. Vicente Andrés Estellés s/n, 46100 Burjassot, Valencia, Spain

^b Bionutest Research Group, Nutrition and Food Science area, Faculty of Pharmacy and Food Sciences, University of Valencia, Av. Vicente Andrés Estellés s/n, 46100 Burjassot, Valencia, Spain

^c Research Group on Quality, Safety and Bioactivity of Plant Foods, Centro de Edafología y Biología Aplicada del Segura, Consejo Superior de Investigaciones Científicas (CEBAS-CSIC), Campus de Espinardo, 30100 Murcia, Spain

^d Metabolomics Platform, Centro de Edafología y Biología Aplicada del Segura, Consejo Superior de Investigaciones Científicas (CEBAS-CSIC), Campus de Espinardo, 30100 Murcia, Spain

ARTICLE INFO

Keywords:

Antioxidants
Bioactive compounds
INFOGEST 2.0
Lactic acid fermentation
Phenolic compounds
Plant-based beverages

ABSTRACT

This study unveils the transformative effects of lactic acid fermentation and *in vitro* digestion on the phytochemical profile (UHPLC-QTOF) and antioxidant capacity (total polyphenols, TEAC and ORAC) of plant-based beverages made from local crops. Fermentation with the probiotic consortium VEGE061 and subsequent INFOGEST 2.0 digestion triggered antioxidant changes. Tiger nut beverages showed a dramatic increase in ORAC activity (1.8–4.0 mmol L⁻¹ Trolox) while preserving polyphenol levels, with the b-isomer of hydroxyoleic acid exhibiting high potential bioaccessibility (>90 %) and sinapoyl alcohol stability improving markedly. Carob beverages displayed elevated polyphenol content (348–413 mg GAE/L) and TEAC activity (1.9–2.3 mmol L⁻¹ Trolox), with 3-propyl malic acid bioaccessibility rising from 10 % to 64 % with fermentation. Rice beverages displayed modest improvements. Overall, digestion severely reduced phenolic stability (~8 % retention), highlighting the urgent need for strategies to improve the bioaccessibility and maximize health benefits. These findings open new avenues for innovative functional beverage design.

1. Introduction

Interest in human health has changed dietary habits, with consumers increasingly seeking healthier foods, especially those of plant origin (Alcorta et al., 2021). Fermented products represent a significant segment of the global market that has grown exponentially in both demand and supply. Plant-based beverages, often used as milk alternatives, are an increasingly common option, with a growing market presence (Grau-Fuentes et al., 2023). This trend towards healthier and more sustainable foods is part of a broader context of global challenges. The development of new foods is a growing necessity, driven by factors such as population growth, climate change, and scarcity of natural resources. These challenges require innovative solutions that contribute to

the Sustainable Development Goals (SDGs), particularly SDG 2 (Zero Hunger), SDG 3 (Good Health and Well-being), SDG 12 (Responsible Consumption and Production), and SDG 13 (Climate Action) (United Nations, 2015).

In this context, the use of local ingredients to make plant-based beverages such as tiger nut, carob, and rice in the Valencian Community represents a promising option, not only due to their regional economic importance and their contribution to reducing the carbon footprint, but also due to their valuable nutritional properties. These traditional raw materials are increasingly valued within the framework of sustainable diets, as they combine cultural heritage with health benefits. Specifically, carob stands out for its high fiber and mineral content, in addition to being one of the plant sources with the highest

* Corresponding author.

E-mail addresses: matteo.vitali@uv.es (M. Vitali), monica.gandia@uv.es (M. Gandía), agsarrias@cebas.csic.es (A. González-Sarrías), fvallejo@cebas.csic.es (F. Vallejo), antonio.cilla@uv.es (A. Cilla), amparo.gamero@uv.es (A. Gamero).

<https://doi.org/10.1016/j.foodchem.2025.146562>

Received 2 June 2025; Received in revised form 23 September 2025; Accepted 25 September 2025

Available online 27 September 2025

0308-8146/© 2025 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

levels of polyphenols (such as tannins, gallic acid derivatives, and flavonol glycosides). Tiger nut is especially rich in healthy fats (oleic acid) and provides moderate amounts of protein and carotenoids, while rice is primarily characterized by its high carbohydrate content, the presence of B vitamins, and a lower polyphenol content. These foods, besides being suitable for the preparation of plant-based beverages in various cultures, can also be valuable in predominantly plant-based diets (Vitali et al., 2023). Fermented versions of these beverages are particularly attractive to consumers due to their health properties, which may surpass those of their unfermented counterparts (Deziderio et al., 2023; Wang et al., 2021). The potential benefits derive from both direct microbial activity and interactions between microorganisms and the food matrix. These effects range from improved nutrient utilization through fermentative activity to enhanced gastrointestinal and immune function (Mukherjee et al., 2024). Fermentation can improve not only the organoleptic characteristics but also the physicochemical properties of these beverages, such as vitamin synthesis, structural changes, or the formation of bioactive compounds (Turkmen et al., 2019). Several studies have evaluated how fermentation acts specifically on a wide variety of different matrices, including tiger nut and rice (Satir, 2022; Da Silva et al., 2023; Yang et al., 2023). However, these beverages must undergo the digestive process before providing benefits to the organism, and given the complexity of digestion, it is important to study how this process modifies their bioactive properties (Yeo & Ewe, 2015; Ozturk et al., 2024; Llorens et al., 2024; Wang et al., 2024).

In this framework, it is necessary to evaluate how digestion and fermentation could work together to potentially enhance the bioactive properties of these beverages, an area that recent studies are beginning to explore (Cuvas-Limon et al., 2022; Méndez-Galarraga, Pirovani, García-Cayuela, & Van de Velde, 2025; Méndez-Galarraga, Pirovani, Vinderola, & Van de Velde, 2025; Nicolescu et al., 2023). Studies on similar matrices related to those of the present study have shown that although beverages made from ingredients such as carob and tiger nut present profiles rich in bioactive compounds (Custódio et al., 2011; Goulas et al., 2016), gastrointestinal digestion causes a drastic reduction in the concentration of total polyphenols and, in some cases, in the overall antioxidant capacity, as reported by other researchers in yerba mate infusions and grape juice (Correa et al., 2017; Dutra et al., 2023). In the case of tiger nut-based beverages, research has reported significant losses in antioxidant capacity after digestion (ABTS+ assay), while other works suggest that digestion can selectively modulate the degradation of certain compounds (Badejo et al., 2014; Carrasco-Chávez et al., 2024; Da Silva et al., 2023; Llorens et al., 2024). Moreover, recent studies have highlighted that factors such as the initial composition of the substrate and the conditions of the fermentation process determine the magnitude of these changes (Frühbauerová et al., 2022; Vilas-Boas et al., 2022). However, for these bioactive compounds to effectively benefit the body, they must survive the digestive process, which involves drastic changes in pH and the sequential action of enzymes. *In vitro* simulation of digestion, using standardized protocols such as INFOGEST 2.0 (Brodkorb et al., 2019), allows the stability of these compounds to be assessed under physiological conditions. In fact, studies in matrices similar to those in the present study have shown that, despite a significant reduction in total phenolic content, certain compounds can maintain their activity or even be protected by techniques such as fermentation (Jakobek, 2015; Llorens et al., 2024; Zhu, 2015). This phenomenon suggests that fermentation could modulate the degradation of bioactive agents during digestion, favoring their release and efficacy in the body. Previous results obtained by our research group showed that these matrices are suitable for fermentation and their antioxidant capacity is significantly and positively affected by fermentation. Several commercial starters were tested to optimize fermentation, and VEGE061 showed the best results in terms of antioxidant activity before digestion and better microbial count before and after digestion, so it was therefore selected for subsequent assays (Vitali et al., 2025). However, the impact of gastrointestinal digestion on their

antioxidant and phytochemical profile is unknown. Advanced analytical techniques allow for precise profiling of these changes, facilitating the identification of novel compounds or those arising as a result of enzymatic reactions.

The novelty of the present work lies in the comprehensive and comparative evaluation of the antioxidant capacity and phytochemical profile in fermented and unfermented plant-based beverages made from tiger nut, carob and rice, analyzed before and after *in vitro* gastrointestinal digestion. A multidimensional approach is employed that combines methods for quantifying antioxidant capacity (using Folin–Ciocalteu, TEAC, and ORAC assays) with advanced phytochemical analysis techniques (UPLC-ESI-QTOF-MS/MS), allowing for the elucidation of the changes induced by fermentation and the stability of key compounds during digestion. The objective, therefore, is to unravel the impact of lactic acid fermentation and gastrointestinal digestion on the phytochemical compounds and antioxidant capacity, which could support strategies for designing products with greater nutritional and functional value. Such advancements not only contribute to the scientific understanding of food processing but also support the development of innovative products aimed at improving overall health and preventing chronic diseases.

2. Materials and methods

2.1. Preparation of fermented beverages

Plant-based beverages were obtained as previously described (Vitali et al., 2025) and were subjected to fermentation using the commercial probiotic VEGE061 consortium (Danisco, Denmark). Fermentation of each beverage (tiger nut, carob or rice) was carried out in a volume of 800 mL using glass bottles of 1 L capacity. The starter culture VEGE061, which contains a mixture of probiotic strains (*Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus acidophilus* NCFM®, *Bifidobacterium animalis* subsp. *lactis* HN019® and *Lactobacillus paracasei*), was inoculated following manufacturer guidelines. Fermentation was conducted at 37 °C until reaching pH 4–4.5, which required different times depending on the food matrix (24, 72 or 48 h for the tiger nut, carob and rice beverages respectively).

2.2. Simulation of gastrointestinal digestion

Samples underwent a standardized three-phase *in vitro* digestion following the INFOGEST 2.0 protocol (Brodkorb et al., 2019). The enzyme activities and bile salts content were experimentally determined by comparing with the activity reported by the manufacturer to ensure reproducibility. The complete table characterizing the enzymatic activity can be found in the Supplementary Material (Table S1). To start the digestion process, first, 5 g of sample were combined with simulated saliva fluid containing α -amylase (75 U/mL final concentration), 25 μ L calcium chloride (0.3 mol L⁻¹), and water up to 10 mL. This oral phase mixture was incubated in a shaking bath (37 °C, 95 rpm, 2 min). For the gastric phase, 7.5 mL gastric fluid containing rabbit gastric extract (60 U lipase/mL) and pepsin (2000 U/mL) were added, adjusting pH to 3 and volume to 20 mL before 2 h incubation at 37 °C and 95 rpm. The intestinal phase involved adding 11 mL intestinal fluid, pancreatin (100 U/mL), bovine bile solution (10 mmol L⁻¹) and calcium chloride (0.3 mol L⁻¹), adjusting pH to 7 and volume to 40 mL, followed by 2 h incubation at 37 °C and 95 rpm. The bioaccessible fraction was obtained by centrifugation (3100g, 90 min, 4 °C) and quickly frozen at -21 °C to stop the digestion process.

2.3. Total antioxidant capacity

Due to the lack of a universal method for measuring total antioxidant capacity (TAC), three complementary methods were used, including both hydrogen atom transfer (HAT) and electron transfer (ET) based

reactions. Total soluble polyphenols (TSP) and Trolox equivalent antioxidant capacity (TEAC) assays represent ET-based mechanisms, while oxygen radical absorbance capacity (ORAC) reflects HAT reactions. These methods were selected due to their well-established use in evaluating the antioxidant capacity of foods and dietary supplements, as highlighted in a relevant review (Prior et al., 2005).

TSP was determined by mixing sample extracts (100 μ L) with sodium carbonate and Folin-Ciocalteu reagent. Absorbance was measured at 765 nm using a Perkin Elmer lambda 2 UV-VIS spectrophotometer. Quantification was performed using gallic acid (0–500 mg/L) as external standard, expressing results as gallic acid equivalents (GAE/L) according to Singleton and Rossi (1965).

For TEAC assay, the initial absorbance (A_0) was measured using 2 mL ABTS⁺ working solution. After adding diluted samples (1/1 to 1/20 v/v) or Trolox standard (100 μ L), final absorbance (A_f) was recorded at 3 min using a thermostated UV-Vis spectrophotometer (Re et al., 1999). The radical scavenging activity was obtained by means of the Eq. (1):

$$\% \text{Inhibition} = [1 - (A_f/A_0)] \times 100 \quad (1)$$

ORAC determination was performed using a Multilabel Plate Counter VICTOR³ 1420 (excitation 485 nm/emission 535 nm) at 37 °C. Reaction mixtures contained fluorescein (80 μ L), AAPH radical generator (40 μ L), and either diluted sample (1/50 v/v), Trolox standard, or phosphate buffer (blank) in 80 μ L. Fluorescence decay was monitored every 5 min for 70 min until reaching <5 % of initial values (Cilla et al., 2011). Results were calculated using the area under the curve difference between samples and blank, expressing both TEAC and ORAC results as mmol L⁻¹ Trolox equivalents.

2.4. UPLC-ESI-QTOF-MS/MS analysis of phytochemical profile

Phytochemical compounds were extracted using a sequential protocol (Ávila-Gálvez et al., 2024) with some modifications. Briefly, samples were extracted thrice with 2 mL methanol and centrifuged (14,000g, 5 min). Pellets underwent three additional extractions using 200 μ L DMSO plus 2 mL methanol (60 °C, 30 min). Combined supernatants (12 mL) were vacuum-concentrated overnight, resuspended in 100 μ L methanol and filtered (0.45 μ m PVDF).

Analysis was performed on an Agilent 1290 UPLC coupled to 6550 Q-TOF MS with Jet Stream ESI source. Separation utilized a Poroshell 120 EC-C18 column (3 $\times$ 100 mm, 2.7 μ m) at 30 °C. mobile phases consisted of (A) water:formic acid and (B) acetonitrile:formic acid (both 99.9:0.1). Mass spectra were acquired in negative mode (m/z 100–1100) at 1.5 spectra/s with internal mass calibration. Internal mass calibration by simultaneous acquisition of reference ions and mass drift compensation was used for obtaining low mass errors. The most interesting compounds were selected for targeted MS/MS analysis that provides high confidence to compound identification. MS/MS product ion spectra were collected at a m/z range of 50–800 using a retention time window of 1 min, a collision energy of 20 V and an acquisition rate of 4 spectra/s. Data were processed using the Mass Hunter Qualitative Analysis software (version B.10.00 Agilent Technologies). A target screening strategy was applied to all the samples for the qualitative screening of possible phytochemicals metabolites. This strategy consists of searching for a list of target compounds after MS full acquisition. Screening was based on mass filtering at the exact mass of the compound investigated using narrow mass extraction windows (0.01 m/z). The identification was possible with the valuable information given in QTOF-MS acquisition mode that provides possible molecular formulae for the compounds based on the accurate mass and isotopic pattern. Besides, the targeted MS/MS experiment offered fragmentation information providing more confidence in the compound identification process. When possible, direct comparison with authentic standards was performed. Data on the identification of phytochemicals by UHPLC-QTOF, as well as representative chromatograms for each plant-based beverage (tiger nut, carob,

and rice, respectively) unfermented non-digested and fermented non-digested, are provided as supplementary material (Tables S2-S4 and Figs. S1-S3 for tiger nut, carob and rice beverages, respectively).

2.5. Statistical analysis

All experimental measurements were carried out in triplicate and results are expressed as mean $\pm$ standard deviation from at least two different experiments. For comparisons between different conditions (unfermented vs fermented, before and after digestion), Welch's *t*-test was applied, which is a variant of Student's *t*-test that does not assume equal variances between groups. Statistical significance was established at 95 % ($p < 0.05$). Data analysis was performed using GraphPad Prism version 9.0 (GraphPad Software Inc., San Diego, CA, USA).

3. Results and discussion

3.1. Antioxidant capacity

The analysis of the three plant matrices reveals complex patterns in the interaction between fermentation, digestion and antioxidant capacity. According to current research on phytochemical potential bio-accessibility, scarce literature exists on fermented plant beverages. A fundamental aspect to consider is the influence of the initial composition of each matrix on its behavior during fermentation and digestion. Phenolic compounds must be in free form to have effects in the organism, although they can bind to various compounds, such as lipids, proteins and carbohydrates, and their release is seriously affected by various conditions, mainly the raw material (Jakobek, 2015; Zhu, 2015). Data on TSP, TEAC and ORAC antioxidant capacity methods of the three plant-based beverages (tiger nut, carob and rice) unfermented and fermented prior and after *in vitro* gastrointestinal digestion are presented in Figs. 1, 2 and 3.

3.1.1. Effect of fermentation on non-digested beverages (UF-WD vs. F-WD)

Initial values in unfermented samples (UF-WD) showed tiger nut beverage contained 302 $\pm$ 41 mg GAE/L of TSP, with TEAC and ORAC values of 313 $\pm$ 11 and 1.8 $\pm$ 0.4 mmol L⁻¹ Trolox, respectively. Carob beverage exhibited the highest antioxidant capacity with 348 $\pm$ 55 mg GAE/L of TSP, 1.9 $\pm$ 0.1 mmol L⁻¹ Trolox of TEAC and 8.5 $\pm$ 0.8 mmol L⁻¹ Trolox of ORAC. Rice beverage showed the lowest values among the three matrices, with 81.6 $\pm$ 4.8 mg GAE/L of TSP, 0.061 $\pm$ 0.004 mmol L⁻¹ Trolox of TEAC and 1.3 $\pm$ 0.2 mmol L⁻¹ Trolox of ORAC. Fermentation produced different effects depending on the matrix. In tiger nut beverage, while TSP values slightly decreased (from 302 $\pm$ 41 to 282 $\pm$

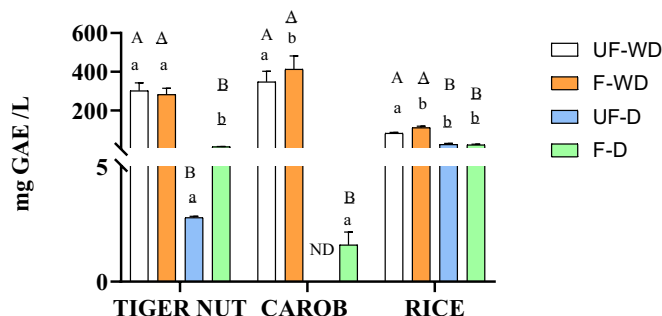


Fig. 1. Total soluble polyphenols of beverages fermented with the VEGE061 consortium and unfermented; digested and non-digested. UF-WD (unfermented, non-digested); F-WD (fermented, non-digested); UF-D (unfermented, digested); F-D (fermented, digested). Analyses were performed in triplicate. Different letters indicate statistically significant differences, Welch *t*-test. ($p < 0.05$). Lowercase letters compare UF-WD and F-WD. Underlined lowercase letters compare UF-D and F-D. Uppercase letters compare UF-WD and UF-D. Underlined uppercase letters compare F-WD and F-D. ND: (non-detected sample).

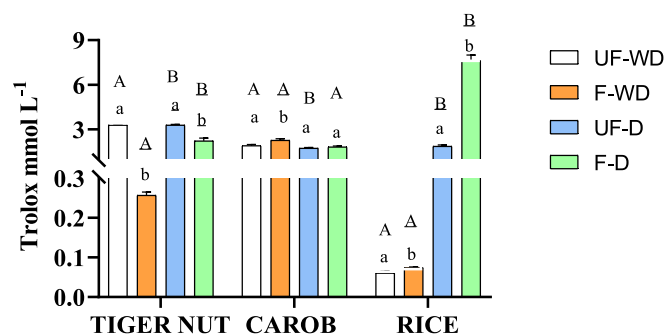


Fig. 2. Total antioxidant capacity (TEAC method) of beverages fermented with the VEGE061 consortium and unfermented; digested and non-digested. UF-WD (unfermented, non-digested); F-WD (fermented, non-digested); UF-D (unfermented, digested); F-D (fermented, digested). Analyses were performed in triplicate. Different letters indicate statistically significant differences, *Welch t-test*. ($p < 0.05$). Lowercase letters compare UF-WD and F-WD. Underlined lowercase letters compare UF-D and F-D. Uppercase letters compare UF-WD and UF-D. Underlined uppercase letters compare F-WD and F-D.

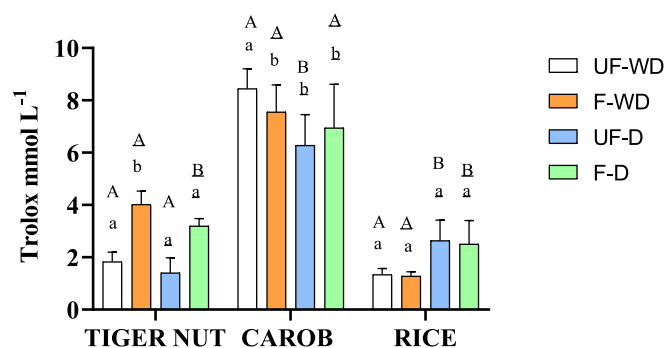


Fig. 3. Total antioxidant capacity (ORAC method) of beverages fermented with the VEGE061 consortium and unfermented; and digested and non-digested. UF-WD (unfermented, non-digested); F-WD (fermented, non-digested); UF-D (unfermented, digested); F-D (fermented, digested). Analyses were performed in triplicate. Different letters indicate statistically significant differences, *Welch t-test*. ($p < 0.05$). Lowercase letters compare UF-WD and F-WD. Underlined lowercase letters compare UF-D and F-D. Uppercase letters compare UF-WD and UF-D. Underlined uppercase letters compare F-WD and F-D.

33 mg GAE/L) and TEAC values decreased (from 0.31 ± 0.01 to 0.26 ± 0.01 mmol L⁻¹ Trolox), no significant differences were observed compared to unfermented samples. However, ORAC values showed a significant 2.2-fold increase (from 1.8 ± 0.4 to 4.0 ± 0.5 mmol L⁻¹ Trolox). As noted by Badojo et al. (2014), tiger nut is a potential source of bioactive compounds, showing that processing can significantly improve its antioxidant capacity. The content of TSP remained relatively stable during fermentation. The results are in line with both our previous work and others, although they are subject to the preparation methods and strains used (Naeem & Youssef, 2022; Vitali et al., 2025).

Carob beverage showed significantly higher values after fermentation in almost all assays, with TSP increasing 19 % (from 348 ± 55 to 413 ± 68 mg GAE/L), TEAC 19 % (from 1.9 ± 0.1 to 2.3 ± 0.1 mmol L⁻¹ Trolox), while ORAC displayed a slight decrease from 8.5 ± 0.8 to 7.6 ± 1.0 mmol L⁻¹ Trolox. Carob initially presented the highest values of TSP and ORAC, which is consistent with previous studies on its rich phytochemical profile such as organic acids, tannins, flavonol glucosides (Buzzanca et al., 2025; Goulas et al., 2016; Vitali et al., 2025). Fermentation evoked a significant increase in TSP and TEAC which correlates with the appearance of new compounds such as phloroglucinol, cynaroside A, gallotannins and gallic acid 4-O-(6-galloyl)glucoside, in addition to a 62 % increase in citric acid, suggesting active metabolic transformation by lactic acid bacteria. All this is possible

thanks to the wide range of enzymes that microorganisms possess to increase the release and availability of these compounds from the food matrix mainly through several types of glycosidases (Gaur & Gänzle, 2023; Liang et al., 2023). Rice beverage fermentation led to significantly higher TSP (35 % increase, from 81 ± 5 to 110 ± 8 mg GAE/L) and TEAC (23 % increase, from 0.061 ± 0.004 to 0.075 ± 0.002 mmol L⁻¹ Trolox) values, while ORAC remained unchanged from 1.3 ± 0.2 to 1.3 ± 0.2 mmol L⁻¹ Trolox. Unfermented rice beverage displayed the lowest content in terms of total antioxidant capacity. In the study by Da Silva et al. (2023), no significant differences were found after fermentation in rice beverage, measured through TEAC and DPPH assays, with modifications in antioxidant profiles being observed during lactic fermentation of these beverages.

3.1.2. Effect of digestion on unfermented beverages (UF-WD vs. UF-D)

The digestion of unfermented beverages induced significant variations in antioxidant capacity across different matrices. In tiger nut beverage, digestion dramatically increased TEAC values 10.5-fold (from 0.31 ± 0.01 to 3.3 ± 0.0 mmol L⁻¹ Trolox), while ORAC values showed a non-significant decrease from 1.8 ± 0.4 to 1.4 ± 0.6 mmol L⁻¹ Trolox. Carob beverage exhibited a non-significant reduction in TEAC values from 1.9 ± 0.1 to 1.7 ± 0.1 mmol L⁻¹ Trolox, and a significant 26 % decrease in ORAC values from 8.5 ± 0.8 to 6.3 ± 1.2 mmol L⁻¹ Trolox. Rice beverage showed a significant 30.7-fold increase in TEAC values (from 0.061 ± 0.004 to 1.9 ± 0.1 mmol L⁻¹ Trolox), and a 97 % increase in ORAC values from 1.3 ± 0.2 to 2.6 ± 0.8 mmol L⁻¹ Trolox. Total polyphenol content dramatically decreased in all matrices, with tiger nut reducing 99 % (from 302 ± 41 to 2.8 ± 0.1 mg GAE/L), carob 100 % (from 348 ± 55 to 0.010 ± 0.005 mg GAE/L), and rice 70 % (from 82 ± 5 to 24 ± 4 mg GAE/L). Nicolescu et al. (2023) showed that protein-polyphenol complexes can decrease total polyphenol content by 28 %, while Fröhbaurová et al. (2022) documented that processing methods significantly affect carob compound bioaccessibility. On the other hand, the digestion process revealed distinctive patterns between fermented and non-fermented samples, especially regarding rice. Our results confirm findings of Sęczyk et al. (2021), who found that rice flour showed the best polyphenol potential bioaccessibility among cereals studied, although it still experienced significant decreases like all other flours. In our non-fermented samples, a drastic increase was observed with TEAC showing a 30.7-fold increase and ORAC a 97 % increase, while TSP significantly decreased (70 % reduction). This dramatic antioxidant enhancement, as will be seen later, correlates with the exceptional 362 % increase in the β -isomer of dihydroxystearic acid and the high bioaccessibility of the α -isomer (79 %). As far as we are aware, no studies have been found specifically evaluating the antioxidant capacity of pure hydroxylated fatty acids using *in vitro* total antioxidant capacity assays. However, the presence of hydroxyl groups in the aliphatic chain of these lipophilic compounds suggests that these hydroxylated fatty acids could contribute to the total antioxidant capacity detected, which warrants systematic investigation in future studies. Several other hydroxylated acids showed moderate bioaccessibility (20–38 % range), suggesting that the post-digestion antioxidant capacity may derive from the transformation of these compounds rather than from native polyphenols.

3.1.3. Effect of digestion on fermented beverages (F-WD vs. F-D)

The digestion of fermented beverages revealed distinct changes in antioxidant capacity across different matrices. In tiger nut beverage, digestion of fermented samples significantly increased TEAC values 8.7-fold (from 0.26 ± 0.01 to 2.2 ± 0.2 mmol L⁻¹ Trolox), while ORAC values significantly decreased 21 % (from 4.0 ± 0.5 to 3.2 ± 0.3 mmol L⁻¹ Trolox). Carob beverage showed a significant 20 % reduction in TEAC values (from 2.3 ± 0.1 to 1.8 ± 0.1 mmol L⁻¹ Trolox), with non-significant changes in ORAC values from 7.6 ± 1.0 to 6.9 ± 1.7 mmol L⁻¹ Trolox. Rice beverage demonstrated the most remarkable response with a 101.9-fold increase in TEAC values (from 0.08 ± 0.002 to $7.6 \pm$

0.4 mmol L⁻¹ Trolox) and a significant 96 % increase in ORAC values from 1.3 ± 0.2 to 2.5 ± 0.9 mmol L⁻¹ Trolox.

TSP significantly decreased in all fermented matrices after digestion, with tiger nut reduction from 282 ± 33 to 11.6 ± 2.0 mg GAE/L, carob from 413 ± 68 to 1.6 ± 0.6 mg GAE/L, and rice from 110.3 ± 7.8 to 21.4 ± 3.8 mg GAE/L. As will be detailed in the phytochemical analysis, these antioxidant capacity changes correlate with selective compound retention patterns that vary across matrices, with complete loss of several compounds and significant degradation of others.

It is particularly notable that rice, despite having the lowest initial polyphenol content (81.6 mg GAE/L), showed the highest relative potential bioaccessibility and the most marked increases in TEAC after digestion. Yu et al. (2024) demonstrated that, while cooking caused significant initial decreases in TEAC values, the intestinal digestion phase resulted in substantial recovery of antioxidant capacity, with non-pigmented rice showing increases of 92–169 % in free phenolics compared to the gastric phase. These findings contrast with Ed Nignpense et al. (2024), who reported decreased ABTS•+ antioxidant activity (31 % retention) following simulated intestinal digestion of purple rice extracts, suggesting that beverage matrix composition and processing conditions significantly influence digestive stability of rice-derived bioactive compounds. Since starch is the main component of rice, the release of bound phenolic compounds from this matrix component could play a role in this antioxidant increase, but future research is warranted to clarify this observation.

3.1.4. Effect of fermentation on digested beverages (UF-D vs. F-D)

After digestion, the effect of fermentation revealed distinct changes in antioxidant capacity across different matrices. In tiger nut beverage, fermented and digested samples showed a significant 32 % decrease in TEAC values (from 3.3 ± 0.0 to 2.2 ± 0.2 mmol L⁻¹ Trolox), while ORAC values showed the opposite trend with a significant 127 % increase (from 1.4 ± 0.6 to 3.2 ± 0.3 mmol L⁻¹ Trolox). Carob beverage exhibited a non-significant increase in TEAC values (from 1.7 ± 0.1 to 1.8 ± 0.1 mmol L⁻¹ Trolox), as well as in ORAC values from 6.3 ± 1.2 to 6.9 ± 1.7 mmol L⁻¹ Trolox. Rice beverage demonstrated a remarkable 309 % increase in TEAC values (from 1.9 ± 0.1 to 7.6 ± 0.4 mmol L⁻¹ Trolox), while ORAC values remained relatively unchanged from 2.6 ± 0.8 to 2.5 ± 0.9 mmol L⁻¹ Trolox. TSP varied across matrices, with tiger nut beverage showing a significant 315 % increase (from 2.79 ± 0.05 to 11.6 ± 2.0 mg GAE/L), carob beverage increasing dramatically (from 0.010 ± 0.005 to 1.6 ± 0.6 mg GAE/L), whereas rice beverage showing a slight non-significant decrease (from 24.2 ± 4.4 to 21.4 ± 3.8 mg GAE/L). As detailed in the phytochemical analysis section, the role of fermentation in protecting specific compounds becomes evident, with fermented samples showing enhanced compound retention compared to unfermented counterparts. This apparent discrepancy between the drastic reduction of TSP in some matrices and the maintenance or increase of antioxidant capacity suggests that degradation products could maintain significant antioxidant activity even if they are not detectable by traditional analytical methods. Maybe some peptides released during protein digestion may contribute to antioxidant capacity, potentially explaining part of the enhanced activity observed despite phenolic compound losses (Chen et al., 2021; Zhao et al., 2024).

The rice beverage, despite having the lowest initial polyphenol content, showed the highest relative increases in TEAC after digestion. This dramatic increase post-digestion, especially in the fermented samples, suggests the formation of these new potential peptides. Such transformations have been noted in other studies, where researchers found that approximately 50 % absorption of sulfonate compounds and even higher values for glycosylated phenolic compounds could be achieved in fermented beverages (Salas-Millán & Aguayo, 2024). This phenomenon was less pronounced in carob and tiger nut, suggesting that the rice matrix provides unique conditions for these transformations.

3.2. Phytochemical profile modifications by lactic acid fermentation and *in vitro* digestion

After evaluating the antioxidant capacity of the non-digested and digested plant-based beverages, a comprehensive phytochemical profiling was conducted to provide deeper insights into the composition of these matrices, before and after fermentation and *in vitro* digestion. The results of this phytochemical analysis are presented in Tables 1–3, which display the concentrations of the major compounds identified, as well as the potential bioaccessibility values for each sample condition (fermented and unfermented).

3.2.1. Phytochemical changes due to fermentation on non-digested tiger nut beverages (UF-WD vs. F-WD)

Lactic acid fermentation induced distinctive changes in tiger nut beverage's phytochemical profile (Table 1). S-leucic acid showed a statistically significant decrease (−82 %), and ferulic acid emerged exclusively in fermented samples. Several compounds exhibited statistically significant changes: dihydroxystearic acid isomer a increased substantially (+184 %), followed by homovanillic acid (+79 %) and hydroxylinoic acid isomer a (+47 %). Among hydroxylated fatty acids, dihydroxystearic acid isomer b showed a statistically significant moderate increase (+44 %), while trihydroxy octadecenoic acids displayed varying responses, with isomer b increasing (+23 %) and isomer a decreasing (−11 %).

The increase or decrease in various fatty acid hydroxylates critically depends on both the matrix and the strain used. For example, Fiorino et al. (2023) evaluated several bacterial strains, including *Lactiplantibacillus plantarum*, in the production of these derivatives. The researchers found a decrease in hydroxy and dihydroxy acids in the fermented walnut sample compared to the control. This aligns with our result for tiger nut beverage but contrasts with the results obtained for rice beverage, where an increase in these acids was detected after fermentation. It is important to note that the researchers used single isolated strains, and we used a bacterial consortium, in addition to the critical matrix difference, which could indicate modulation by different conditions.

Tiger nut beverage ranked in second place in terms of antioxidant capacity and exhibited a complex behavior during fermentation. These changes were differently reflected in the antioxidant capacity: while TEAC decreased from 0.31 to 0.26 mmol L⁻¹ Trolox, ORAC increased significantly from 1.8 to 4.0 mmol L⁻¹ Trolox.

3.2.2. Phytochemical changes due to digestion on unfermented tiger nut beverages (UF-WD vs. UF-D)

The digestion process substantially affected the phytochemical profile of unfermented tiger nut beverages (Table 1). Seven compounds disappeared during digestion, including homovanillic acid, L-leucic acid, S-leucic acid, and all identified phenolic compounds (kaempferol 3',7-diglucoside, 4-vinylphenol, ethyl vanillin). Among hydroxylated fatty acids, bioaccessibility rates varied considerably: hydroxyoleic acid isomer b showed remarkable stability (93 %), while hydroxylinoic acid a and dihydroxystearic acid b maintained moderate levels (30 % and 16 %, respectively). Sinapoyl alcohol demonstrated notable resistance to digestion (41 % bioaccessibility), while citric acid showed moderate stability (16 %). This selective retention phenomenon aligns with findings from Zhu (2015) and Jakobek (2015), showing that matrix composition and processing conditions can significantly affect compound bioaccessibility. The non-covalent interactions between polyphenols and food macromolecules (starch, proteins, lipids, carbohydrates) form various complexes that substantially influence both the physicochemical properties of the matrix and the bioactivity of phenolic compounds. These interactions can either enhance or reduce polyphenol bioavailability, affect their delivery to the large intestine, and provide protection against oxidative degradation during digestion. Understanding these complex molecular interactions is crucial for

Table 1

Peak integration area values from the Extract ion Chromatograms (EICs) of the phytochemical compounds identified by UHPLC-QTOF in tiger nut beverages unfermented and fermented with consortium of lactic acid bacteria (VEGE061) for 24 h and prior and after digestion.*

Phytochemicals	UF-WD	UF-D	Bioaccessibility	F-WD	F-D	Bioaccessibility
Citric acid	$1.01 \times 10^8 \pm 4.00 \times 10^6$ ^A	$3.73 \times 10^5 \pm 2.83 \times 10^4$ ^B	16.44 %	$1.11 \times 10^8 \pm 1.93 \times 10^7$ ^A	$1.46 \times 10^7 \pm 4.88 \times 10^6$ ^B	13.23 %
Homovanillic acid *	$1.59 \times 10^6 \pm 9.67 \times 10^4$	ND	ND	$2.85 \times 10^6 \pm 5.46 \times 10^5$ ^A	$5.66 \times 10^5 \pm 5.18 \times 10^3$ ^B	19.87 %
L-leucic acid	$4.37 \times 10^6 \pm 2.01 \times 10^5$	ND	ND	$7.39 \times 10^6 \pm 5.47 \times 10^6$ ^A	$5.94 \times 10^5 \pm 8.66 \times 10^4$ ^B	8.03 %
S-leucic acid	$3.72 \times 10^7 \pm 1.60 \times 10^6$ ^A	ND	ND	$6.64 \times 10^6 \pm 5.58 \times 10^6$ ^{AB}	$4.10 \times 10^5 \pm 8.34 \times 10^4$ ^B	6.17 %
Kaempferol 3',7-diglucoside *	$1.46 \times 10^6 \pm 8.47 \times 10^4$	ND	ND	$1.05 \times 10^6 \pm 7.12 \times 10^5$	ND	0
4-vinylphenol *	$2.82 \times 10^6 \pm 1.39 \times 10^5$	ND	ND	$3.34 \times 10^6 \pm 3.80 \times 10^6$	ND	0
Ethyl vanillin *	$1.92 \times 10^7 \pm 3.41 \times 10^5$	ND	ND	$2.28 \times 10^7 \pm 2.66 \times 10^7$ ^A	$4.44 \times 10^5 \pm 4.61 \times 10^4$ ^B	1.94 %
Ferulic acid *	ND ^A	ND	ND	$1.05 \times 10^6 \pm 7.22 \times 10^5$ ^B	ND	0 %
Sinapoyl alcohol	$9.12 \times 10^5 \pm 6.20 \times 10^4$ ^A	$3.73 \times 10^5 \pm 2.83 \times 10^4$ ^B	40.85 %	$9.26 \times 10^5 \pm 1.25 \times 10^5$ ^A	$7.25 \times 10^5 \pm 1.08 \times 10^5$ ^B	78.28 %
Trihydroxy octadecenoic acid isomer a	$2.89 \times 10^7 \pm 4.56 \times 10^5$ ^A	$2.18 \times 10^6 \pm 1.47 \times 10^5$ ^B	7.56 %	$2.57 \times 10^7 \pm 2.30 \times 10^7$ ^A	$2.67 \times 10^6 \pm 5.47 \times 10^5$ ^B	10.37 %
Trihydroxy octadecenoic acid isomer b	$2.27 \times 10^7 \pm 1.17 \times 10^6$ ^A	$8.23 \times 10^5 \pm 2.92 \times 10^4$ ^B	3.63 %	$2.80 \times 10^7 \pm 2.04 \times 10^7$ ^A	$9.64 \times 10^5 \pm 4.31 \times 10^5$ ^B	3.44 %
Dihydroxyoleic acid isomer a	$4.59 \times 10^7 \pm 8.84 \times 10^5$ ^A	$5.29 \times 10^5 \pm 3.13 \times 10^4$ ^B	1.15 %	$3.94 \times 10^7 \pm 2.81 \times 10^7$ ^A	$1.23 \times 10^6 \pm 7.00 \times 10^5$ ^B	3.11 %
Dihydroxyoleic acid isomer b	$4.38 \times 10^7 \pm 9.00 \times 10^5$ ^{AA}	$1.10 \times 10^6 \pm 3.20 \times 10^4$ ^B	2.51 %	$4.45 \times 10^7 \pm 3.68 \times 10^7$ ^{AB}	$1.86 \times 10^6 \pm 8.68 \times 10^5$ ^B	4.18 %
Dihydroxystearic acid isomer a	$1.84 \times 10^7 \pm 4.15 \times 10^4$ ^{AA}	$1.92 \times 10^6 \pm 5.09 \times 10^4$ ^B	10.41 %	$5.23 \times 10^7 \pm 1.65 \times 10^7$ ^{AB}	$2.62 \times 10^6 \pm 1.90 \times 10^6$ ^B	5.01 %
Dihydroxystearic acid isomer b	$7.17 \times 10^7 \pm 1.55 \times 10^6$ ^A	$1.04 \times 10^7 \pm 4.19 \times 10^5$ ^B	15.55 %	$1.03 \times 10^8 \pm 4.18 \times 10^7$ ^A	$1.75 \times 10^7 \pm 1.88 \times 10^7$ ^B	17.02 %
Hydroxylinoleic acid isomer a	$4.69 \times 10^7 \pm 4.58 \times 10^5$ ^A	$1.40 \times 10^7 \pm 2.28 \times 10^5$ ^B	29.89 %	$6.90 \times 10^7 \pm 5.52 \times 10^7$ ^A	$2.15 \times 10^7 \pm 1.60 \times 10^7$ ^B	31.19 %
Hydroxyoleic acid isomer a	$3.65 \times 10^7 \pm 8.06 \times 10^5$ ^A	$4.28 \times 10^6 \pm 1.12 \times 10^5$ ^B	11.74 %	$2.75 \times 10^7 \pm 3.19 \times 10^7$ ^A	$2.89 \times 10^6 \pm 2.77 \times 10^6$ ^B	10.52 %
Hydroxyoleic acid isomer b	$3.91 \times 10^6 \pm 1.46 \times 10^5$ ^A	$3.62 \times 10^6 \pm 4.10 \times 10^4$ ^A	92.65 %	$4.19 \times 10^6 \pm 2.33 \times 10^6$ ^A	$3.79 \times 10^6 \pm 2.45 \times 10^6$ ^A	90.42 %

* Phenolic compounds. The symbol α/β indicates significant difference between UF-WD vs F-WD (indicates the effect of fermentation), A/B indicates significant difference between UF-WD vs UF-D (indicates the effect of digestion on unfermented beverages), and A'/B' indicates significant difference between F-WD vs F-D (indicates the effect of digestion on fermented beverages). Different letters indicate statistically significant differences ($p < 0.05$) according to Welch's *t*-test. ND: not detected.

developing functional foods and optimizing the nutritional benefits of polyphenol-rich products. Similarly, [Llorens et al. \(2024\)](#) found that the bioaccessibility of polyphenols in tiger nut beverage varied significantly, with approximately 27 % of the total compounds becoming bio-accessible. This stability of the b-isomer of hydroxyoleic acid is particularly remarkable.

3.2.3. Effect of *in vitro* gastrointestinal digestion on fermented tiger nut beverages (F-WD vs. F-D)

Three compounds were completely lost after digestion (kaempferol 3',7-diglucoside, 4-vinylphenol and ferulic acid). On the other hand, homovanillic acid maintained low but detectable levels (20 % bio-accessibility) while showing complete degradation in digested unfermented samples. Similarly, L-leucic acid showed limited resistance (8 % bioaccessibility), contrasting with its complete loss in digested unfermented samples ([Table 1](#)). Sinapoyl alcohol exhibited enhanced bio-accessibility after fermentation (78 % vs 41 % in unfermented). Among hydroxylated fatty acids, hydroxyoleic acid isomer b maintained remarkable stability (90 % bioaccessibility).

This apparent discrepancy between the drastic reduction of TSP and the maintenance or even increase in antioxidant capacity suggests that the degradation products could retain significant antioxidant activity even if they are not detectable by the analytical techniques employed; some of these could correspond to peptides with antioxidant activity ([Chen et al., 2021](#); [Zhao et al., 2024](#)).

3.2.4. Phytochemical changes due to fermentation in non-digested carob beverages (UF-WD vs. F-WD)

Carob initially presented the highest values of TSP and ORAC. Lactic acid fermentation profoundly modified carob beverage's phytochemical profile, with 19 compounds showing significant changes ($p < 0.05$) ([Table 2](#)). Fourteen compounds emerged exclusively after fermentation: phenolic compounds (phloroglucinol, cynaroside A, gallic acid 4-O-(6-galloyl)glucoside), gallotannin and its isomer, benzoic acid, kaempferide 7-glucoside), organic acids (ethylmalonic acid, 3'-methoxyfukiic acid), and other bioactive molecules (isorhamnetin, 3',5'-dihydroxy-flavanone, L-menthyl acetoacetate, 9,10-dihydroxy stearic acid, and laserpitin), in addition to a 62 % increase in citric acid, suggesting active metabolic transformation (degradation and/or biotransformation) by lactic acid bacteria or greater release of this compound from the matrix ([Gaur & Gänzle, 2023](#); [Liang et al., 2023](#); [Polat et al., 2023](#)). Among existing compounds, only citric acid showed a significant 62 % increase, while four compounds decreased significantly: isochinomin (−42 %), succinic acid (−16 %), gallic acid (−15 %), and 2-O-galloylglucose (−6 %).

3.2.5. Impact of *in vitro* gastrointestinal digestion on unfermented carob beverages (UF-WD vs. UF-D)

In vitro digestion substantially affected the phytochemical profile of unfermented carob beverage, with all detected compounds showing statistically significant decreases ($p < 0.05$) ([Table 2](#)). Several compounds were completely lost during digestion, including (r)-2-

Table 2

Peak integration area values from the Extract ion Chromatograms (EICs) of the phytochemical compounds identified by UHPLC-QTOF in carob beverages unfermented and fermented with consortium of lactic acid bacteria (VEGE061) for 72 h and prior and after digestion.

Phytochemicals	UF-WD	UF-D	Bioaccessibility	F-WD	F-D	Bioaccessibility
2-Dehydro-D-xylonate	$1.93 \times 10^7 \pm 3.48 \times 10^5$ ^A	$1.98 \times 10^6 \pm 3.94 \times 10^4$ ^B	10.30 %	$1.83 \times 10^7 \pm 4.09 \times 10^6$ ^A	$1.61 \times 10^6 \pm 1.66 \times 10^5$ ^B	8.75 %
Citric acid	$1.93 \times 10^7 \pm 1.01 \times 10^6$ ^A α	$1.64 \times 10^6 \pm 1.73 \times 10^5$ ^B	8.52 %	$3.13 \times 10^7 \pm 2.24 \times 10^6$ ^A β	$1.01 \times 10^7 \pm 1.13 \times 10^6$ ^B	32.39 %
2-Deoxy-D-Ribose	$4.36 \times 10^7 \pm 1.01 \times 10^6$ ^A	$4.85 \times 10^6 \pm 1.35 \times 10^5$ ^B	11.13 %	$4.24 \times 10^7 \pm 7.40 \times 10^6$ ^A	$3.61 \times 10^6 \pm 3.88 \times 10^5$ ^B	8.51 %
(R)-2-Methylmalate	$2.07 \times 10^7 \pm 7.71 \times 10^5$	ND	ND	$1.94 \times 10^7 \pm 5.30 \times 10^5$ ^A	ND	0.0 %
2-Galloylglucose *	$1.99 \times 10^7 \pm 6.42 \times 10^5$ ^A	$1.92 \times 10^6 \pm 7.13 \times 10^5$ ^B	9.62 %	$1.79 \times 10^7 \pm 5.93 \times 10^6$ ^A	$9.28 \times 10^5 \pm 1.31 \times 10^6$ ^B	5.20 %
Succinic acid	$1.61 \times 10^7 \pm 1.46 \times 10^5$ α	ND	ND	$1.35 \times 10^7 \pm 7.02 \times 10^5$ ^{AB}	ND	ND
2-Methylcitrate	$1.30 \times 10^7 \pm 2.85 \times 10^4$	ND	ND	$1.26 \times 10^7 \pm 1.61 \times 10^5$ ^A	ND	ND
Gallic acid *	$9.88 \times 10^7 \pm 1.98 \times 10^6$ ^A	$1.08 \times 10^7 \pm 3.21 \times 10^5$ ^B	10.92 %	$8.43 \times 10^7 \pm 1.91 \times 10^6$ ^{AB}	$1.79 \times 10^5 \pm 2.53 \times 10^5$ ^B	0.21 %
2-O-Galloylsucrose *	$7.07 \times 10^7 \pm 7.81 \times 10^5$ ^A α	ND	ND	$6.64 \times 10^7 \pm 7.09 \times 10^5$ ^{AB}	ND	ND
Phloroglucinol *	ND α	ND	ND	$5.76 \times 10^7 \pm 1.23 \times 10^7$ ^{AB}	ND	ND
Ethylmalonic acid	ND α	ND	ND	$1.33 \times 10^7 \pm 1.44 \times 10^6$ ^A β	ND	ND
b-D-Xylopyranosyl-(1-4)-a-L-rhamnopyranosyl-(1-2)-L-arabinose	$2.13 \times 10^7 \pm 1.64 \times 10^6$ ^A	$1.69 \times 10^6 \pm 4.42 \times 10^4$ ^B	7.92 %	$1.80 \times 10^7 \pm 7.27 \times 10^6$ ^A	$9.79 \times 10^5 \pm 1.38 \times 10^5$ ^B	5.43 %
a-L-Fucopyranosyl-(1-2)-b-D-galactopyranosyl-(1-2)-D-xylose	$2.87 \times 10^7 \pm 2.14 \times 10^6$ ^A	$4.48 \times 10^6 \pm 1.12 \times 10^5$ ^B	15.63 %	$3.65 \times 10^7 \pm 1.43 \times 10^7$ ^A	$1.51 \times 10^6 \pm 2.14 \times 10^6$ ^B	4.13 %
Cynaroside A *	ND α	ND	ND	$2.85 \times 10^7 \pm 8.50 \times 10^5$ ^{AB}	ND	ND
3'-Methoxyfukiic acid	ND α	ND	ND	$1.31 \times 10^7 \pm 1.33 \times 10^6$ ^A β	ND	ND
Gallic acid 4-O-(6-galloylglucoside) *	ND α	ND	ND	$2.99 \times 10^7 \pm 5.14 \times 10^5$ ^A β	ND	ND
3-propylmalic acid	$3.86 \times 10^7 \pm 6.39 \times 10^5$ ^A	$3.96 \times 10^6 \pm 8.23 \times 10^4$ ^B	10.26 %	$3.40 \times 10^7 \pm 4.47 \times 10^6$ ^A	$2.19 \times 10^7 \pm 2.51 \times 10^7$ ^B	64.38 %
3-O-Methylgallate *	$1.35 \times 10^7 \pm 7.49 \times 10^5$ ^A	$1.58 \times 10^6 \pm 2.57 \times 10^4$ ^B	11.74 %	$1.04 \times 10^7 \pm 4.25 \times 10^6$ ^A	$9.27 \times 10^5 \pm 1.31 \times 10^6$ ^B	8.88 %
Eriocitrin *	$2.26 \times 10^7 \pm 1.06 \times 10^6$ ^A	$1.91 \times 10^5 \pm 1.44 \times 10^4$ ^B	0.85 %	$1.58 \times 10^7 \pm 1.08 \times 10^7$ ^A	$1.10 \times 10^5 \pm 1.55 \times 10^5$ ^B	0.69 %
Gallotannin *	ND α	ND	ND	$3.76 \times 10^7 \pm 7.03 \times 10^5$ ^A β	ND	ND
Gallotannin (isomer) *	ND α	ND	ND	$3.09 \times 10^7 \pm 5.19 \times 10^5$ ^A β	ND	ND
Ellagic acid +*	$1.26 \times 10^8 \pm 1.56 \times 10^7$ ^A	$5.01 \times 10^5 \pm 9.53 \times 10^2$ ^B	0.40 %	$1.10 \times 10^8 \pm 3.69 \times 10^7$ ^A	$1.24 \times 10^5 \pm 1.76 \times 10^5$ ^B	0.11 %
Myricitrin +*	$2.58 \times 10^7 \pm 9.93 \times 10^5$ ^A	$1.75 \times 10^6 \pm 8.69 \times 10^3$ ^B	6.76 %	$2.00 \times 10^7 \pm 7.26 \times 10^6$ ^A	$6.83 \times 10^5 \pm 9.66 \times 10^5$ ^B	3.41 %
Quercetin 3-O-glucoside +*	$1.54 \times 10^7 \pm 1.62 \times 10^6$ ^A	$1.92 \times 10^6 \pm 9.24 \times 10^4$ ^B	12.49 %	$1.13 \times 10^7 \pm 3.82 \times 10^6$ ^A	$7.81 \times 10^5 \pm 1.10 \times 10^6$ ^B	6.92 %
Benzoic acid *	ND α	ND	ND	$1.38 \times 10^7 \pm 1.03 \times 10^6$ ^A β	ND	ND
Quercetin 3-arabinoside +*	$2.71 \times 10^7 \pm 6.69 \times 10^6$ ^A	$1.79 \times 10^6 \pm 1.03 \times 10^4$ ^B	6.62 %	$1.85 \times 10^7 \pm 4.20 \times 10^6$ ^A	$1.02 \times 10^6 \pm 1.44 \times 10^6$ ^B	5.50 %
Quercitrin +*	$1.51 \times 10^8 \pm 4.97 \times 10^6$ ^A	$2.25 \times 10^6 \pm 1.61 \times 10^5$ ^B	1.49 %	$1.22 \times 10^8 \pm 3.23 \times 10^7$ ^A	$1.04 \times 10^6 \pm 1.47 \times 10^6$ ^B	0.86 %
Isochinomin	$1.41 \times 10^7 \pm 8.65 \times 10^5$ ^A	$9.33 \times 10^5 \pm 1.76 \times 10^4$ ^B	6.60 %	$8.20 \times 10^6 \pm 8.35 \times 10^5$ ^{AB}	$3.93 \times 10^5 \pm 5.55 \times 10^5$ ^B	4.79 %
Kaempferide 7-glucoside +*	ND α	ND	ND	$1.10 \times 10^7 \pm 1.41 \times 10^6$ ^A β	ND	ND
Phloretin 2'-O-glucuronide +*	$6.80 \times 10^7 \pm 4.01 \times 10^6$ ^A	$2.00 \times 10^5 \pm 2.49 \times 10^3$ ^B	0.29 %	$5.45 \times 10^7 \pm 1.40 \times 10^7$ ^A	$1.32 \times 10^5 \pm 1.87 \times 10^5$ ^B	0.24 %
Apigenin 7-O-glucoside +*	$1.92 \times 10^7 \pm 7.31 \times 10^5$ ^A	$1.37 \times 10^5 \pm 2.12 \times 10^4$ ^B	0.71 %	$1.35 \times 10^7 \pm 5.49 \times 10^6$ ^A	$3.19 \times 10^5 \pm 1.74 \times 10^5$ ^B	2.37 %
6-Hydroxykaempferol +*	$2.29 \times 10^7 \pm 1.96 \times 10^6$ ^A	$1.81 \times 10^6 \pm 7.21 \times 10^4$ ^B	7.87 %	$2.49 \times 10^7 \pm 2.88 \times 10^6$ ^A	$9.78 \times 10^5 \pm 1.38 \times 10^6$ ^B	3.92 %
Isorhamnetin +*	ND α	ND	ND	$1.50 \times 10^7 \pm 8.50 \times 10^5$ ^{AB}	ND	ND
Quercetin +*	$2.89 \times 10^7 \pm 1.19 \times 10^6$ ^A	$1.99 \times 10^6 \pm 3.48 \times 10^4$ ^B	6.89 %	$2.93 \times 10^7 \pm 2.74 \times 10^6$ ^A	$1.16 \times 10^6 \pm 1.63 \times 10^6$ ^B	3.95 %
9S,12S,13S-trihydroxy-10E-octadecenoic acid	$5.91 \times 10^7 \pm 1.31 \times 10^6$ ^A	$1.82 \times 10^6 \pm 1.05 \times 10^5$ ^B	3.08 %	$1.05 \times 10^8 \pm 5.20 \times 10^7$ ^A	$6.88 \times 10^5 \pm 9.73 \times 10^5$ ^B	0.66 % ^{A/B}
3',5'-Dihydroxyflavanone *	ND α	ND	ND	$1.68 \times 10^7 \pm 2.86 \times 10^5$ ^A β	ND	ND

(continued on next page)

Table 2 (continued)

Phytochemicals	UF-WD	UF-D	Bioaccessibility	F-WD	F-D	Bioaccessibility
Octadecanedioic acid	$4.46 \times 10^7 \pm 8.52 \times 10^5$ ^A	$3.98 \times 10^6 \pm 3.98 \times 10^4$ ^B	8.93 %	$3.61 \times 10^7 \pm 1.71 \times 10^7$ ^A	$1.89 \times 10^6 \pm 2.68 \times 10^6$ ^B	5.24 %
9,10-DiHOME	$5.74 \times 10^6 \pm 1.83 \times 10^6$ ^A	$3.93 \times 10^6 \pm 1.18 \times 10^5$ ^B	6.85 %	$3.17 \times 10^6 \pm 2.77 \times 10^6$ ^A	$1.98 \times 10^6 \pm 2.80 \times 10^6$ ^B	6.25 %
L-Menthyl acetoacetate	ND ^α	ND	ND	$1.98 \times 10^6 \pm 4.66 \times 10^5$ ^{Aβ}	ND	ND
9,10-dihydroxy stearic acid	ND ^α	ND	ND	$3.28 \times 10^6 \pm 1.14 \times 10^6$ ^{Aβ}	$3.24 \times 10^6 \pm 4.58 \times 10^6$ ^B	9.86 %
Laserpitin	ND ^β	ND	ND	$2.17 \times 10^6 \pm 8.12 \times 10^5$ ^{Aβ}	$4.04 \times 10^5 \pm 5.71 \times 10^5$ ^B	1.86 %
alpha,alpha'-Trehalose 6-palmitate	$2.24 \times 10^6 \pm 7.96 \times 10^5$ ^A	$1.06 \times 10^6 \pm 5.56 \times 10^5$ ^B	4.74 %	$1.27 \times 10^6 \pm 6.74 \times 10^6$ ^A	$6.29 \times 10^5 \pm 8.90 \times 10^5$ ^B	4.97 %
12R-hydroxy-9Z-octadecenoic acid	$1.69 \times 10^6 \pm 6.89 \times 10^5$ ^A	$1.09 \times 10^6 \pm 1.04 \times 10^5$ ^B	6.41 %	$3.82 \times 10^7 \pm 3.25 \times 10^7$ ^A	$1.27 \times 10^6 \pm 2.22 \times 10^5$ ^B	3.32 %
16-hydroxy hexadecanoic acid	$3.22 \times 10^7 \pm 2.91 \times 10^6$ ^A	$1.07 \times 10^6 \pm 9.78 \times 10^4$ ^B	3.31 %	$1.57 \times 10^7 \pm 1.87 \times 10^7$ ^A	$6.91 \times 10^5 \pm 5.84 \times 10^5$ ^B	4.40 %

* Phenolic compounds. The symbol α/β indicates significant difference between UF-WD vs F-WD (indicates the effect of fermentation), A/B indicates significant difference between UF-WD vs UF-D (indicates the effect of digestion on unfermented beverages), and A'/B' indicates significant difference between F-WD vs F-D (indicates the effect of digestion on fermented beverages). Different letters indicate statistically significant differences ($p < 0.05$) according to Welch's *t*-test. ND: not detected.

Table 3

Peak integration area values from the Extract ion Chromatograms (EICs) of the phytochemical compounds identified by UHPLC-QTOF in rice beverages unfermented and fermented with consortium of lactic acid bacteria (VEGE061) for 48 h and prior and after digestion.

Phytochemicals	UF-WD	UF-D	Bioaccessibility	F-WD	F-D	Bioaccessibility
Citric acid	$7.36 \times 10^6 \pm 1.21 \times 10^6$ ^A	$1.25 \times 10^6 \pm 1.50 \times 10^6$	17.05 %	$7.28 \times 10^6 \pm 1.04 \times 10^6$ ^A	$2.04 \times 10^6 \pm 3.37 \times 10^5$ ^B	28.04 %
L-leucic acid	$3.02 \times 10^5 \pm 1.36 \times 10^5$ ^α	ND	ND	$9.39 \times 10^5 \pm 3.82 \times 10^4$	ND	ND
S-leucic acid	$3.22 \times 10^5 \pm 8.91 \times 10^4$ ^α	ND	ND	$5.87 \times 10^7 \pm 5.80 \times 10^5$	ND	ND
p-coumaric acid *	$1.91 \times 10^6 \pm 1.22 \times 10^5$ ^α	ND	ND	$1.16 \times 10^6 \pm 6.20 \times 10^3$	ND	ND
Ethyl vanillin *	ND ^α	ND	ND	$5.26 \times 10^7 \pm 2.56 \times 10^6$	ND	ND
Sinapoyl alcohol	$4.78 \times 10^6 \pm 3.66 \times 10^5$ ^{Aα}	$5.06 \times 10^5 \pm 3.00 \times 10^4$ ^B	10.59 %	$6.04 \times 10^6 \pm 1.65 \times 10^5$ ^{AB}	$7.94 \times 10^5 \pm 3.54 \times 10^5$ ^B	13.16 %
Trihydroxy octadecenoic acid isomer a	$7.99 \times 10^7 \pm 9.45 \times 10^7$ ^A	$6.99 \times 10^6 \pm 8.18 \times 10^6$ ^B	8.75 %	$1.58 \times 10^8 \pm 2.28 \times 10^7$ ^A	$3.37 \times 10^6 \pm 4.77 \times 10^6$ ^B	2.13 %
Trihydroxy octadecenoic acid isomer b	$1.09 \times 10^8 \pm 9.27 \times 10^6$ ^A	$1.29 \times 10^6 \pm 5.14 \times 10^4$ ^B	1.19 %	$9.87 \times 10^7 \pm 3.54 \times 10^7$ ^A	$5.46 \times 10^5 \pm 7.72 \times 10^5$ ^B	0.55 %
Dihydroxyoleic acid isomer a	$2.62 \times 10^7 \pm 2.76 \times 10^6$ ^{Aα}	$9.98 \times 10^6 \pm 4.87 \times 10^5$ ^B	38.12 %	$9.65 \times 10^7 \pm 1.87 \times 10^7$ ^{AB}	$7.31 \times 10^6 \pm 4.02 \times 10^6$ ^B	7.58 %
Dihydroxyoleic acid isomer b	$5.72 \times 10^7 \pm 4.37 \times 10^6$ ^{Aα}	$6.19 \times 10^6 \pm 6.12 \times 10^4$ ^B	18.82 %	$1.47 \times 10^8 \pm 3.23 \times 10^7$ ^{AB}	$4.58 \times 10^6 \pm 2.53 \times 10^6$ ^B	3.11 %
Dihydroxystearic acid isomer a	$6.58 \times 10^6 \pm 6.37 \times 10^5$ ^{Aα}	$5.18 \times 10^6 \pm 4.67 \times 10^5$ ^A	78.82 %	$7.97 \times 10^7 \pm 2.42 \times 10^7$ ^{AB}	$3.16 \times 10^6 \pm 1.64 \times 10^6$ ^B	3.96 %
Dihydroxystearic acid isomer b	$9.84 \times 10^6 \pm 1.15 \times 10^6$ ^{Aα}	$3.56 \times 10^7 \pm 1.51 \times 10^6$ ^B	362.31 %	$8.84 \times 10^7 \pm 4.09 \times 10^7$ ^A	$1.13 \times 10^7 \pm 4.18 \times 10^6$ ^B	12.84 %
Hydroxylinoleic acid isomer a	$8.90 \times 10^7 \pm 1.48 \times 10^7$ ^A	$2.31 \times 10^7 \pm 1.41 \times 10^6$ ^B	25.98 %	$1.02 \times 10^8 \pm 4.74 \times 10^7$ ^A	$1.65 \times 10^7 \pm 4.74 \times 10^6$ ^B	16.18 %
Hydroxyoleic acid isomer a	$1.81 \times 10^7 \pm 2.36 \times 10^6$ ^{Aα}	$6.04 \times 10^6 \pm 1.41 \times 10^6$ ^B	33.30 %	$9.09 \times 10^7 \pm 2.42 \times 10^7$ ^{AB}	$1.98 \times 10^6 \pm 1.01 \times 10^6$ ^B	2.17 %
Hydroxyoleic acid isomer b	$1.45 \times 10^7 \pm 3.10 \times 10^6$ ^A	$3.00 \times 10^6 \pm 1.98 \times 10^5$ ^B	20.68 %	$1.78 \times 10^7 \pm 9.88 \times 10^6$ ^A	$1.89 \times 10^6 \pm 6.99 \times 10^5$ ^B	10.58 %

* Phenolic compounds. The symbol α/β indicates significant difference between UF-WD vs F-WD (indicates the effect of fermentation), A/B indicates significant difference between UF-WD vs UF-D (indicates the effect of digestion on unfermented beverages), and A'/B' indicates significant difference between F-WD vs F-D (indicates the effect of digestion on fermented beverages). Different letters indicate statistically significant differences ($p < 0.05$) according to Welch's *t*-test. ND: not detected.

methylmalate, succinic acid, 2-methylcitrate, and 2-O-galloylsucrose. Among the compounds showing resistance to digestion, despite their significant reduction, α-l-fucopyranosyl-(1-2)-β-d-galactopyranosyl-(1-2)-d-xylose exhibited the highest bioaccessibility (16 %), followed by quercetin 3-O-glucoside (12 %), 3-O-methylgallate (12 %), and 2-deoxy-D-ribose (11 %). gallic acid and 3-propylmalic acid showed moderate bioaccessibility (11 % and 10 % respectively), while 2-O-galloylglucose and 2-dehydro-d-xylonate maintained around 10 %. Several phenolic compounds displayed low retention rates: 6-hydroxykaempferol (8 %), myricitrin (7 %), quercetin (7 %), quercetin 3-arabinoside (7 %), and

quercitrin (1 %). Particularly, low *in vitro* bioaccessibility was observed for eriocitrin (0.9 %), ellagic acid (0.4 %), and phloretin 2'-O-glucuronide (0.3 %). Among fatty acid derivatives, retention rates varied from moderate to low: octadecanedioic acid (9 %), 9,10-DiHOME (7 %), 12R-hydroxy-9Z-octadecenoic acid (6 %), 9 s,12 s,13 s-trihydroxy-10e-octadecenoic acid (3 %), and 16-hydroxy hexadecanoic acid (3 %). Digestive processes significantly altered the phytochemical profile of fermented carob beverages, revealing intricate interactions between fermentation, digestion, and compound stability with a sharp decrease on phenolic compounds.

3.2.6. Impact of *in vitro* gastrointestinal digestion on fermented carob beverages (F-WD vs. F-D)

In vitro digestion substantially affected the phytochemical profile of fermented carob beverages, with all detected compounds showing statistically significant decreases ($p < 0.05$) (Table 2). Numerous compounds were eliminated during digestion, including cynaroside A, 3'-methoxyfukiic acid, gallic acid 4-O-(6-galloylglucoside), gallotannin, isorhamnetin, benzoic acid, L-menthyl acetoacetate, and 3',5'-dihydroxyflavanone. Most phenolic compounds showed very low retention rates: quercetin 3-O-glucoside (7 %), quercetin 3-arabinoside (6 %), and 6-hydroxykaempferol (4 %). The low bioaccessibility may be due to the formation of phenolic complexes with digestive enzymes (Chait et al., 2021). Furthermore, it is notable that some organic acids showed higher bioaccessibility in fermented compared to unfermented samples: 3-propylmalic acid increased its retention from 10 % to 64 %, and citric acid from 9 % to 32 %. The fermentation process seems to play an important role in protecting these target compounds in carob. Chait et al. (2021) observed high bioaccessibility of numerous phenolic compounds, such as gallic and ellagic acids, myricetin, and quercetin. However, given that their beverage was a mixture of milk and carob, the role of milk in the gastrointestinal protection of these compounds cannot be ruled out in relation to our study. No additional studies on the bioaccessibility of polyphenols in carob plant-based beverages were found. This selective preservation of certain compounds after digestion has been previously observed in carob matrix, where processing methods link grinding o size particle can significantly affect the bioaccessibility of specific compounds (Frühbauerová et al., 2022).

3.2.7. Phytochemical changes due to fermentation in non-digested rice beverages (UF-WD vs. F-WD)

Lactic acid fermentation induced significant changes in rice beverage's phytochemical profile (Table 3). The most dramatic increase was observed in S-leucic acid (182.2-fold increase), followed by substantial increases in several hydroxylated fatty acids: dihydroxystearic acid isomer a (12.1-fold), hydroxyoleic acid isomer a (5.0-fold), dihydroxyoleic acid isomer a (3.7-fold), and dihydroxyoleic acid isomer b (2.6-fold). Other hydroxylated compounds also showed notable increases: dihydroxystearic acid isomer b (9.0-fold), trihydroxy octadecenoic acid isomer a (2.0-fold), and hydroxyoleic acid isomer b (1.2-fold). Ethyl vanillin emerged exclusively in fermented samples. UPLC-ESI-QTOF-MS/MS analysis revealed that these increases in antioxidant capacity were correlated with specific modifications in the phytochemical profile. Particularly notable was the appearance of ethyl vanillin and a sharp increase in S-leucic acid. However, fermentation also caused some significant decreases, such as a 0.6-fold reduction in p-coumaric acid and a 0.9-fold decrease in trihydroxy octadecenoic acid isomer b. Furthermore, fermentation seemed to specifically favor the formation of hydroxylated acids and the appearance of ethyl vanillin, indicating different metabolic pathways predominating in this matrix compared to the other plant-based beverages studied. This was also observed by Bocchi et al. (2021) who found a certain level of protection between fermented and unfermented variants of a commercial oat beverage, particularly for compounds like 4-hydroxybenzaldehyde, p-coumaric acid and avenanthramide derivatives.

3.2.8. Impact of *in vitro* gastrointestinal digestion on unfermented rice beverages (UF-WD vs. UF-D)

In vitro digestion revealed distinctive patterns in unfermented rice beverages (Table 3). Remarkably, dihydroxystearic acid isomer b showed an exceptional 362 % increase after digestion, while its isomer a demonstrated unique stability (79 % bioaccessibility). Several hydroxylated fatty acids displayed moderate *in vitro* bioaccessibility: dihydroxyoleic acid isomer a (38 %), hydroxyoleic acid isomer a (33 %), hydroxylinoleic acid isomer a (26 %), and hydroxyoleic acid isomer b (21 %). Citric acid and sinapoyl alcohol showed limited resistance to digestion (17 % and 11 % respectively). Several compounds were

completely lost during digestion, including L-leucic acid, S-leucic acid, and p-coumaric acid.

In unfermented samples, a dramatic increase was observed in both TEAC (from 0.06 to 1.9 mmol L⁻¹ Trolox) and ORAC (from 1.3 to 2.6 mmol L⁻¹ Trolox), while the TSP decreased significantly (from 81.6 to 24.2 mg GAE/L). Several other hydroxylated acids showed moderate bioaccessibility, suggesting that the post-digestion antioxidant capacity may derive from the transformation of these compounds rather than from native polyphenols.

3.2.9. Effect of *in vitro* gastrointestinal digestion on fermented rice beverages (F-WD vs. F-D)

All compounds in fermented rice beverage were significantly affected by *in vitro* digestion ($p < 0.05$) (Table 3). Several compounds were completely lost during digestion: L-leucic acid, S-leucic acid, p-coumaric acid, and ethyl vanillin. Among the remaining compounds, hydroxylinoleic acid isomer a (16 %), sinapoyl alcohol (13 %) and dihydroxystearic acid isomer b (13 %) showed selective retention. Citric acid demonstrated notable bioaccessibility (28 %). Other hydroxylated fatty acids showed low bioaccessibility: dihydroxyoleic acid a (8 %), dihydroxystearic acid isomer a (4 %), dihydroxyoleic acid isomer b (3 %), hydroxyoleic acid isomer a (2 %), trihydroxy octadecenoic acid isomer a (2 %), and trihydroxy octadecenoic acid isomer b (0.6 %). This pattern of selective compound retention and transformation during digestion has been observed by Inada et al. (2020), who found varying degrees of compound retention after digestive processes in jaboticaba (*Plinia cauliflora*) peel and seed.

The selective retention of compounds during digestion deserves special attention when comparing the three matrices. The compounds most resistant to digestion were generally those with more complex or fermentation-modified structures, as evidenced by the exceptional retention of hydroxyoleic acid isomer b in tiger nut beverage (>90 %) and the higher retention of 3-propylmalic acid in fermented (64 %) compared to unfermented (10 %) carob beverage. These differences in retention suggest that matrix-compound interactions play a crucial role in the potential bioaccessibility of bioactive compounds. It is important that bioaccessible bioactive compounds can remain bound to fiber as well as to the microbial biomass itself, which in turn can be influenced by the endogenous microbiota. This limitation has been observed in other studies; for example, Inada et al. (2020) found approximately 60 % decrease in bioaccessibility in jaboticaba peel and seed after digestive processing, which decreased further to 19 % after *in vitro* colonic fermentation. Similar magnitudes of significant losses were also reported by Grzelczyk et al. (2024) in coffee extract varieties. Importantly, the phenolic compounds remaining after digestive processing, even if not assimilated, can potentially act together with bacterial biomass in a postbiotic or symbiotic effect (Al-Habsi et al., 2024). Furthermore, to our knowledge, this work is currently the only one to have explicitly reported the use of the INFOGEST 2.0 method in these types of studies. Small variations in pH, time and temperature conditions during digestion can significantly alter phenolic compound bioaccessibility. The apparent contradiction between the decrease in TSP and the increase in antioxidant capacity observed under various conditions, particularly in rice beverage, suggests limitations in traditional methods of polyphenol quantification. Degradation or transformation products of polyphenols, although not detectable by the Folin-Ciocalteu method, could contribute significantly to the antioxidant capacity, especially after digestion. This result has been observed in other studies where a decrease in phenolic content after digestion was found, while it was also noted that milk could help improve bioaccessibility, particularly for compounds with lipophilic character (Cilla et al., 2012; Chait et al., 2021; Civas-Limon et al., 2022; Nicolescu et al., 2023; Jiménez-Monreal et al., 2025; Méndez-Galarra, Pirovani, García-Cayuela, & Van de Velde, 2025). They also detected decreases in ORAC levels. This complex relationship between compound degradation and antioxidant activity highlights the importance of using multiple complementary analytical techniques to

assess the bioactive potential of these beverages. Lactic acid bacteria enzymatic machinery described in the literature contributes to the formation of new metabolites, fact that can be attributed to specific biotransformation pathways involving β -glucosidase, tannase, and gallate decarboxylase activities that convert precursor compounds through established biochemical routes (e.g., gallic acid $\rightarrow$ pyrogallol $\rightarrow$ phloroglucinol) (Gaur & Gänzle, 2023; Liang et al., 2023; Polat et al., 2023). This could explain the appearance of novel bioactive compounds like phloroglucinol and cynaroside A in fermented samples, representing genuine enzymatic biotransformation rather than simple compound release from the food matrix (Fig. 4).

4. Conclusions

The synergistic role of fermentation and digestion in modulating the phytochemical profile and antioxidant capacity emerges as a central finding of this study. Fermentation not only modifies the initial profile of bioactive compounds in a matrix-specific manner but also alters their susceptibility to digestion and potential bioavailability. This effect was particularly evident in rice beverage, where fermented samples showed the highest increase in total antioxidant capacity (TEAC method) values after digestion suggesting that fermentation may enhance the bioactive potential through complex mechanisms beyond simple changes in polyphenol content. The study shows that lactic fermentation induces significant changes in antioxidant capacity and in the polyphenol content of plant-based beverages, before and after *in vitro* digestion. In case of tiger nut beverages, it is observed that, despite an average global bioaccessibility of phenolic compounds of around 8 % after digestion, there are exceptions such as the b-isomer of hydroxyoleic acid (a hydroxylated fatty acid) that shows a marked retention followed by sinapoyl alcohol which shows medium retentions. In carob beverages, besides the marked global degradation of antioxidant compounds, a significant increase in the retention of 3-propyl malic acid (an organic acid) is evident, which goes from approximately 10 % in unfermented

samples to 64 % in fermented samples after digestion. In contrast, the rice beverage, despite having initial lower antioxidant capacity, showed relatively greater stability after digestion, with bioaccessibility of approximately 30 % in unfermented samples and 19 % in fermented samples, a fact that translates into lower variations in TAC and TSP. These results first demonstrate that fermentation induces substantial changes in phenolic compounds and antioxidant activity, depending on the plant-based matrix. Altogether, these findings reinforce the relevance of this study for the design of functional plant-based beverages. Specifically, our quantitative results demonstrate matrix-specific optimization strategies: (i) rice fermented beverages show the highest antioxidant enhancement (101.9-fold TEAC increase) through synergistic fermentation-digestion effects, (ii) carob beverage fermentation optimally protects organic acids (6.3-fold increase in 3-propylmalic acid bioaccessibility), (iii) tiger nut matrices preserve hydroxylated fatty acids (>90 % bioaccessibility), and (iv) matrix-specific fermentation times (24 h tiger nut, 72 h carob, 48 h rice) are required for optimal results.

CRediT authorship contribution statement

Matteo Vitali: Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization. **Mónica Gandía:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition. **Antonio González-Sarriás:** Writing – review & editing, Methodology, Investigation. **Fernando Vallejo:** Writing – review & editing, Methodology, Investigation. **Antonio Cilla:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **Amparo Gamero:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

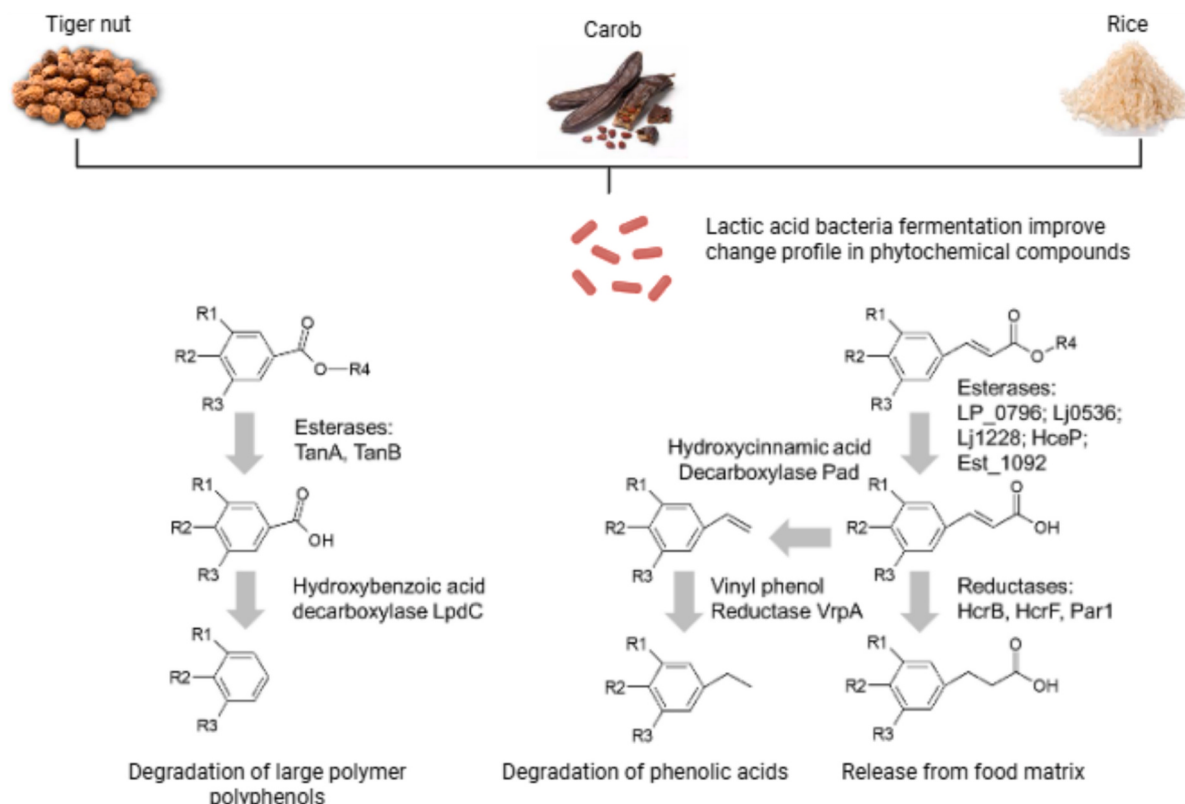


Fig. 4. Possible changes in phytochemicals following lactic acid fermentation. Adapted from Gaur and Gänzle (2023). (Created using BioRender.com, version 2023).

Funding

This study is part of the AGROALNEXT program (AGROALNEXT/2022/047) with the support of the MCIN and funding from the European Union Next Generation EU (PRTR-C17-I1) and the Generalitat Valenciana (Spain). Matteo Vitali has a research staff contract in the aforementioned project (CPI-22-735).

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.

Appendix A. Supplementary data

Supplementary data for this article are provided in Tables S1 for enzyme activity and S2-S4 for the tiger nut, carob, and rice beverages, respectively. Figs. S2-S4 show examples of chromatograms obtained for the tiger nut, carob, and rice beverages.

Data availability

Data will be made available on request.

References

- Alcorta, A., Porta, A., Tárrega, A., Alvarez, M. D., & Vaquero, M. P. (2021). Foods for plant-based diets: Challenges and innovations. *Foods*, 10(2), 293. <https://doi.org/10.3390/foods10020293>
- Al-Habsi, N., Al-Khalili, M., Haque, S. A., Elias, M., Al Olqi, N., & Al Uraimi, T. (2024). Health benefits of prebiotics, probiotics, Synbiotics, and postbiotics. *Nutrients*, 16 (22), 3955. <https://doi.org/10.3390/nu16223955>
- Ávila-Gálvez, M. A., Giménez-Bastida, J. A., Karadeniz, B., Romero-Reyes, S., Espín, J. C., Pelvan, E., & González-Sarrias, A. (2024). Polyphenolic characterization and anti-inflammatory effect of in vitro digested extracts of *Echinacea purpurea* L. plant parts in an inflammatory model of human Colon cells. *International Journal of Molecular Sciences*, 25(3), 1744. <https://doi.org/10.3390/ijms25031744>
- Badejo, A. A., Damilare, A., & Ojuade, T. D. (2014). Processing effects on the antioxidant activities of beverage blends developed from *Cyperus esculentus*, *Hibiscus sabdariffa*, and *Moringa oleifera* extracts. *Preventive Nutrition and Food Science*, 19(3), 227–233. <https://doi.org/10.3746/pnf.2014.19.3.227>
- Bocchi, S., Rocchetti, G., Elli, M., Lucini, L., Lim, C.-Y., & Morelli, L. (2021). The combined effect of fermentation of lactic acid bacteria and in vitro digestion on metabolomic and oligosaccharide profile of oat beverage. *Food Research International*, 142. <https://doi.org/10.1016/j.foodres.2021.110216>. Scopuss.
- Brodkorb, A., Egger, L., Alminger, M., Alvito, P., Assunção, R., Ballance, S., Bohn, T., Bourlieu-Lacanal, C., Boutrou, R., Carrière, F., Clemente, A., Corredig, M., Dupont, D., Dufour, C., Edwards, C., Golding, M., Karakaya, S., Kirkhus, B., Le Feunteun, S., ... Recio, I. (2019). INFOGEST static in vitro simulation of gastrointestinal food digestion. <https://doi.org/10.1038/s41596-018-0119-1>
- Buzzanca, C., D'Amico, A., Pistorio, E., Di Stefano, V., & Melilli, M. G. (2025). Carob-based functional beverages: Nutritional value and health properties. *Beverages*, 11 (1), Article 1. <https://doi.org/10.3390/beverages11010001>
- Carrasco-Chávez, J., Quintero-Soto, M. F., Velarde-Barraza, R., Rivero-Espejel, I. A., Díaz-Peña, I., Vázquez-Ontiveros, M. E., ... Félix-Medina, J. V. (2024). Changes in the profile of phenolic compounds and in the antioxidant, Hypoglycemic, and antidiabetic activities of a beverage based on sesame by-product caused by the simulated gastrointestinal digestion process. *Beverages*, 10(4), Article 4. <https://doi.org/10.3390/beverages10040115>
- Chait, Y. A., Gunenc, A. G., Bendali, F. B., & Hosseini, F. (2021). Functional fermented carob milk: Probiotic variability and polyphenolic profile. *Journal of Food Bioactives*, 14. <https://doi.org/10.31665/jfb.2021.14273>
- Chen, L., Hui, Y., Gao, T., Shu, G., & Chen, H. (2021). Function and characterization of novel antioxidant peptides by fermentation with a wild *Lactobacillus plantarum* 60. *LWT*, 135, Article 110162. <https://doi.org/10.1016/j.lwt.2020.110162>
- Cilla, A., Alegría, A., de Ancos, B., Sánchez-Moreno, C., Cano, M. P., Plaza, L., ... Barberá, R. (2012). Bioaccessibility of tocopherols, carotenoids, and ascorbic acid from milk- and soy-based fruit beverages: Influence of food matrix and processing. *Journal of Agricultural and Food Chemistry*, 60(29), 7282–7290. <https://doi.org/10.1021/jf301165r>
- Cilla, A., Perales, S., Lagarda, M. J., Barberá, R., Clemente, G., & Farré, R. (2011). Influence of storage and in vitro gastrointestinal digestion on total antioxidant capacity of fruit beverages. *Journal of Food Composition and Analysis*, 24(1), 87–94. <https://doi.org/10.1016/j.jfca.2010.03.029>
- Correa, V. G., Gonçalves, G. A., de Sá-Nakanishi, A. B., Ferreira, I. C. F. R., Barros, L., Dias, M. I., ... Peralta, R. M. (2017). Effects of in vitro digestion and in vitro colonic fermentation on stability and functional properties of yerba mate (*Ilex paraguariensis* A. St. Hil.) beverages. *Food Chemistry*, 237, 453–460. <https://doi.org/10.1016/j.foodchem.2017.05.125>
- Custódio, L., Escapa, A. L., Fernandes, E., Fajardo, A., Aligué, R., Alberício, F., ... Romano, A. (2011). Phytochemical profile, antioxidant and cytotoxic activities of the carob tree (*Ceratonia siliqua* L.) germ flour extracts. *Plant Foods for Human Nutrition (Dordrecht, Netherlands)*, 66(1), 78–84. <https://doi.org/10.1007/s11130-011-0214-8>
- Cuvas-Limon, R. B., Ferreira-Santos, P., Cruz, M., Teixeira, J. A., Belmares, R., & Nobre, C. (2022). Effect of gastrointestinal digestion on the bioaccessibility of phenolic compounds and antioxidant activity of fermented Aloe vera juices. *Antioxidants (Basel, Switzerland)*, 11(12), 2479. <https://doi.org/10.3390/antiox11122479>
- Da Silva, L. R., Velasco, J. I., & Fakhouri, F. M. (2023). Use of rice on the development of plant-based milk with antioxidant properties: From raw material to residue. *LWT*, 173, Article 114271. <https://doi.org/10.1016/j.lwt.2022.114271>
- Deziderio, M. A., de Souza, H. F., Kamimura, E. S., & Petrus, R. R. (2023). Plant-based fermented beverages: Development and characterization. *Foods*, 12(22), Article 22. <https://doi.org/10.3390/foods12224128>
- Dutra, M. D. C. P., Martins da Silva, A. B., de Souza Ferreira, E., Carvalho, A. J. d. B. A., Lima, M. d. S., & Telles Biasoto, A. C. (2023). Bioaccessibility of phenolic compounds from Brazilian grape juices using a digestion model with intestinal barrier passage. *Food Bioscience*, 52, Article 102501. <https://doi.org/10.1016/j.fbio.2023.102501>
- Ed Nigmpense, B., Francis, N., Blanchard, C., & Santhakumar, A. B. (2024). Effect of gastrointestinal digestion on the stability, antioxidant activity, and Caco-2 cellular transport of pigmented grain polyphenols. *Journal of Food Science*, 89, 2701–2715. <https://doi.org/10.1111/1750-3841.17009>
- Fiorino, G. M., Tlais, A. Z. A., Losito, I., Filannino, P., Gobetti, M., & Di Cagno, R. (2023). Triacylglycerols hydrolysis and hydroxy- and epoxy-fatty acids release during lactic fermentation of plant matrices: An extensive study showing inter- and intra-species capabilities of lactic acid bacteria. *Food Chemistry*, 412, 135552. <https://doi.org/10.1016/j.foodchem.2023.135552>
- Frühbauerová, M., Cervenka, L., Hájek, T., Pouzar, M., & Palářík, J. (2022). Bioaccessibility of phenolics from carob (*Ceratonia siliqua* L.) pod powder prepared by cryogenic and vibratory grinding. *Food Chemistry*, 377, Article 131968. <https://doi.org/10.1016/j.foodchem.2021.131968>
- Gaur, G., & Gänzle, M. G. (2023). Conversion of (poly)phenolic compounds in food fermentations by lactic acid bacteria: Novel insights into metabolic pathways and functional metabolites. *Current Research in Food Science*, 6, Article 100448. <https://doi.org/10.1016/j.crf.2023.100448>
- Goulas, V., Stylos, E., Chatziathanasiadou, M. V., Mavroumoustakos, T., & Tzakos, A. G. (2016). Functional components of carob fruit: Linking the chemical and biological space. *International Journal of Molecular Sciences*, 17(11), Article 11. <https://doi.org/10.3390/ijms17111875>
- Grau-Fuentes, E., Rodrigo, D., Garzón, R., & Rosell, C. M. (2023). Understanding the marketed plant-based beverages: From ingredients technological function to their nutritional value. *Journal of Functional Foods*, 106, Article 105609. <https://doi.org/10.1016/j.jff.2023.105609>
- Grzelczyk, J., Budryn, G., Sz wajgier, D., Baranowska-Wójcik, E., & Zaklos-Szyda, M. (2024). Evaluation of the effect of roasting and digestion on biological activity of compounds of coffee extracts—In vitro assessment of the bioavailability, cytoprotective properties and modulation of inflammatory response. *Food Chemistry*, 460, Article 140648. <https://doi.org/10.1016/j.foodchem.2024.140648>
- Inada, K. O. P., Silva, T. B. R., Lobo, L. A., Domingues, R. M. C. P., Perrone, D., & Monteiro, M. (2020). Bioaccessibility of phenolic compounds of Jaboticaba (*Plinia Jaboticaba*) peel and seed after simulated gastrointestinal digestion and gut microbiota fermentation. *Journal of Functional Foods*, 67, Article 103851. <https://doi.org/10.1016/j.jff.2020.103851>
- Jakobek, L. (2015). Interactions of polyphenols with carbohydrates, lipids and proteins. *Food Chemistry*, 175, 556–567. <https://doi.org/10.1016/j.foodchem.2014.12.013>
- Jiménez-Monreal, A. M., Cedeño-Pinos, C., Bañón, S., Muñoz, I., Guardia, M. D., Tahori, N., & Martínez-Tomé, M. (2025). Effect of in vitro gastrointestinal digestion on the antioxidant properties of fruit and vegetable powdered smoothies reinforced with WPC80. *LWT*, 215, Article 117301. <https://doi.org/10.1016/j.lwt.2024.117301>
- Liang, Z., Huang, Y., Zhang, P., & Fang, Z. (2023). Impact of fermentation on the structure and antioxidant activity of selective phenolic compounds. *Food Bioscience*, 56, Article 103147. <https://doi.org/10.1016/j.fbio.2023.103147>
- Llorens, P., Chiacchio, M. F., Tagliamonte, S., Juan-García, A., Pallarés, N., Moltó, J. C., ... Juan, C. (2024). Potential bioaccessibility and bioavailability of polyphenols and functional properties of tiger nut beverage and its by-product during in vitro digestion. *Food & Function*, 15(15), 8143–8152. <https://doi.org/10.1039/D4FO01537A>
- Méndez-Galarraga, M. P., Pirovani, M. E., García-Cayuela, T., & Van de Velde, F. (2025). Fruit and vegetable beverages fermented with probiotic strains: Impact on the content, bioaccessibility, and bioavailability of phenolic compounds and the antioxidant capacity. *Current Food Science and Technology Reports*, 3(1), 4. <https://doi.org/10.1007/s43555-024-00049-1>
- Méndez-Galarraga, M. P., Pirovani, M. E., Vinderola, G., & Van de Velde, F. (2025). Bioaccessibility of phenolic compounds in fermented strawberry-orange-apple-banana smoothies with lactobacilli. *Food Bioscience*, 65, Article 106074. <https://doi.org/10.1016/j.fbio.2025.106074>
- Mukherjee, A., Breselge, S., Dimidi, E., Marco, M. L., & Cotter, P. D. (2024). Fermented foods and gastrointestinal health: Underlying mechanisms. *Nature Reviews. Gastroenterology & Hepatology*, 21(4), 248–266. <https://doi.org/10.1038/s41575-023-00869-x>

- Naeem, M. M., & Youssef, G. M. (2022). Study the antioxidant and antimicrobial of tiger nut and the biological effects of some of its products. *International Journal of Family Studies, Food Science and Nutrition Health*, 3(2), 165–193. <https://doi.org/10.21608/ijfshh.2022.281747>
- Nicolescu, A., Babotă, M., Barros, L., Rocchetti, G., Lucini, L., Tanase, C., ... Crișan, G. (2023). Bioaccessibility and bioactive potential of different phytochemical classes from nutraceuticals and functional foods. *Frontiers in Nutrition*, 10, Article 1184535. <https://doi.org/10.3389/fnut.2023.1184535>
- Ozturk, T., Ávila-Gálvez, M.Á., Mercier, S., Vallejo, F., Bred, A., Fraisse, D., ... González-Sarriás, A. (2024). Impact of lactic acid Bacteria fermentation on (poly)phenolic profile and in vitro antioxidant and anti-inflammatory properties of herbal infusions. *Antioxidants*, 13(5), 562. <https://doi.org/10.3390/antiox13050562>
- Polat, Ş., Hamakhan, A., Kafkas, E., & Ali, M. A. (2023). Determination of sugars and organic acids in diverse carob genotypes using HPLC techniques. *International Journal of Agriculture, Environment and Food Sciences*, 7(4), 756–760. <https://doi.org/10.31015/iaefs.2023.4.4>
- Prior, R. L., Wu, X., & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry*, 53(10), 4290–4302. <https://doi.org/10.1021/jf0502698>
- Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology & Medicine*, 26(9–10), 1231–1237. [https://doi.org/10.1016/s0891-5849\(98\)00315-3](https://doi.org/10.1016/s0891-5849(98)00315-3)
- Salas-Millán, J.Á., & Aguayo, E. (2024). Bioaccessibility and unravelling of polyphenols, sulforaphane, and indoles biotransformation after in vitro gastrointestinal digestion of a novel lactofermented broccoli beverage. *Food & Function*, 15(24), 11949–11960. <https://doi.org/10.1039/D4FO03528C>
- Satir, G. (2022). The effects of fermentation with water kefir grains on two varieties of tigernut (*Cyperus esculentus* L.) milk. *LWT*, 171, Article 114164. <https://doi.org/10.1016/j.lwt.2022.114164>
- Sęczyk, Ł., Sugier, D., Świeca, M., & Gawlik-Dziki, U. (2021). The effect of *in vitro* digestion, food matrix, and hydrothermal treatment on the potential bioaccessibility of selected phenolic compounds. *Food Chemistry*, 344, Article 128581. <https://doi.org/10.1016/j.foodchem.2020.128581>
- Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, 16(3), 144–158.
- Turkmen, N., Akal, C., & Ozer, B. (2019). Probiotic dairy-based beverages: A review. *Journal of Functional Foods*, 53, 62–75. <https://doi.org/10.1016/j.jff.2018.12.004>
- United Nations. (2015). THE 17 GOALS | Sustainable Development. Retrieved March 31, 2025, from <https://sdgs.un.org/goals>.
- Vilas-Boas, A. M., Brassesco, M. E., Quintino, A. C., Vieira, M. C., Brandão, T. R. S., Silva, C. L. M., ... Pintado, M. (2022). Particle size effect of integral carob flour on bioaccessibility of bioactive compounds during simulated gastrointestinal digestion. *Foods*, 11(9), Article 9. <https://doi.org/10.3390/foods11091272>
- Vitali, M., Gandía, M., Garcia-Llatas, G., González-Sarriás, A., Vallejo, F., Cilla, A., & Gamero, A. (2025). Modulation of antioxidant capacity, nutritional composition, probiotic viability after digestion and sensory attributes of plant-based beverages through lactic acid fermentation. *Foods*, 14, 1447. <https://doi.org/10.3390/foods14091447>
- Vitali, M., Gandía, M., Garcia-Llatas, G., Tamayo-Ramos, J. A., Cilla, A., & Gamero, A. (2023). Exploring the potential of Rice, Tiger nut and carob for the development of fermented beverages in Spain: A Comprehensive Review on the Production Methodologies Worldwide. *Beverages*, 9(2), Article 2. <https://doi.org/10.3390/beverages9020047>
- Wang, Y., Wu, J., Lv, M., Shao, Z., Hungwe, M., Wang, J., ... Geng, W. (2021). Metabolism characteristics of lactic acid Bacteria and the expanding applications in food industry. *Frontiers in Bioengineering and Biotechnology*, 9. <https://doi.org/10.3389/fbioe.2021.612285>
- Wang, Y., Zhang, L., Xiao, H., Ye, X., Pan, H., & Chen, S. (2024). Revisiting dietary proanthocyanidins on blood glucose homeostasis from a multi-scale structural perspective. *Current Research in Food Science*, 9, Article 100926. <https://doi.org/10.1016/j.crfs.2024.100926>
- Yang, F., Chen, C., Ni, D., Yang, Y., Tian, J., Li, Y., ... Wang, L. (2023). Effects of fermentation on bioactivity and the composition of polyphenols contained in polyphenol-rich foods: A review. *Foods*, 12(17), Article 17. <https://doi.org/10.3390/foods12173315>
- Yeo, S. K., & Ewe, J. A. (2015). 5—Effect of fermentation on the phytochemical contents and antioxidant properties of plant foods. In W. Holzapfel (Ed.), *Advances in fermented foods and beverages* (pp. 107–122). Woodhead Publishing. <https://doi.org/10.1016/B978-1-78242-015-6.00005-0>
- Yu, J., Zheng, X., Zhu, D., Xu, Q., Xu, F., Chen, M., Meng, L., & Shao, Y. (2024). Changes of polyphenols and their antioxidant activities in non-pigmented, red and black rice during in vitro digestion. *Food Chemistry: X*, 24, Article 101821. <https://doi.org/10.1016/j.fochx.2024.101821>
- Zhao, J., Kong, X., Zhang, C., Hua, Y., Chen, Y., & Li, X. (2024). In vitro protein digestive properties and peptidomic characterization of five whole component plant protein beverages using a pepsin–pancreatin model. *Food Research International*, 196, Article 115076. <https://doi.org/10.1016/j.foodres.2024.115076>
- Zhu, F. (2015). Interactions between starch and phenolic compound. *Trends in Food Science & Technology*, 43(2), 129–143. <https://doi.org/10.1016/j.tifs.2015.02.003>