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Deciphering the interactions between lipids and red wine polyphenols through the gastrointestinal tract

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ABSTRACT

This paper investigates the mutual interactions between lipids and red wine polyphenols at different stages of the gastrointestinal tract by using the simgi® dynamic simulator. Three food models were tested: a Wine model, a Lipid model (olive oil + cholesterol) and a Wine + Lipid model (red wine + olive oil + cholesterol). With regard to wine polyphenols, results showed that co-digestion with lipids slightly affected the phenolic profile after gastrointestinal digestion. In relation to lipid bioaccessibility, the co-digestion with red wine tended to increase the percentage of bioaccessible monoglycerides, although significant differences were not found ($p > 0.05$). Furthermore, co-digestion with red wine tended to reduce cholesterol bioaccessibility (from 80 to 49 %), which could be related to the decrease in bile salt content observed in the micellar phase. For free fatty acids, almost no changes were observed. At the colonic level, the co-digestion of red wine and lipids conditioned the composition and metabolism of colonic microbiota. For instance, the growth [log (ufc/mL)] of lactic acid bacteria (6.9 ± 0.2) and bifidobacteria (6.8 ± 0.1) populations were significantly higher for the Wine + Lipid food model respect to the control colonic fermentation (5.2 ± 0.1 and 5.3 ± 0.2, respectively). Besides, the production of total SCFAs was greater for the Wine + Lipid food model. Also, the cytotoxicity of the colonic-digested samples towards human colon adenocarcinoma cells (HCT-116 and HT-29) was found to be significantly lower for the Wine and Wine + Lipid models than for the Lipid model and the control (no food addition). Overall, the results obtained using the simgi® model were consistent with those reported *in vivo* in the literature. In particular, they suggest that red wine may favourably modulate lipid bioaccessibility – a fact that could explain the hypocholesterolemic effects of red wine and red wine polyphenols observed in humans.

1. Introduction

In the context of a balanced diet and healthy lifestyle, it has been suggested that a moderate consumption of wine could have beneficial health effects, unlike other alcoholic beverages as proposed by several scientific studies (Castaldo et al., 2019; Pavlidou et al., 2018). This protective association reported for wine can be explained by components other than ethanol, namely wine is considered a dietary source of phytochemicals and, in particular, red wine is rich in a wide variety of polyphenolic compounds. The described effects attributed to wine polyphenols, include protection against non-communicable diseases such as cardiovascular diseases, metabolic syndrome and neurodegenerative diseases, among others (Artero et al., 2015; Levantesi et al., 2013; Moreno-Arribas et al., 2020; Sun et al., 2002; Tresserra-Rimbau et al., 2015). However, confidence in these associations is not high because the exact mechanisms by which red wine (or its polyphenols) may influence biological functions are not well understood. Specifically, in cardiovascular disease, the main health area investigated, one of the mechanisms proposed as being responsible for the protective effects of wine polyphenols includes changes in lipid profiles and the reduction of blood parameters such as LDL/HDL ratio and total cholesterol (Castaldo et al., 2019; Castro-Barquero et al., 2020).

The health-promoting effects of dietary polyphenols depend upon their bioavailability, absorption and metabolism (Cueva et al., 2017). In general, the bioavailability of polyphenols (the fraction that reaches the systemic circulation and tissues to exert biological action) is generally limited, which is closely linked to their poor bioaccessibility (the fraction that is released from the food matrix and is available for absorption in the gut). So, a key aspect of dietary polyphenols research is exploring the interactions with other food components during digestion (such as

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carbohydrates, lipids and proteins) that may affect their bioaccessibility and bioavailability (Jakobek, 2015). Polyphenol–lipid interactions have been studied using model compounds. It is known that these interactions are essential to the affinity of polyphenols for lipid membranes because these affect their biological activity. For example, hydrophobic polyphenols can penetrate more deeply into the lipid membranes (Karonen, 2022). These interactions have also been studied focused on the food matrix effect or related to plant oils (such as olive oil) (Jakobek, 2015; Kardum & Glibetic, 2018). *In vivo* studies have reported differences in polyphenol bioavailability in the presence of rich lipid foods as creams (Mullen et al., 2008; Sengul et al., 2014). However, the effect of co-digestion has been scarcely explored *in vitro*. Thus, a study of the *in vitro* digestibility of cocoa polyphenols suggested that the fat content of the cocoa samples might have a protective effect on cocoa polyphenols due to a better micellization, which enables better stability of polyphenols at intestinal level (Ortega et al., 2009). On the other hand, in regard to the effect of polyphenols on lipid digestion, it has been described that polyphenols are able to affect fat emulsification, causing, in addition, a decrease in the activity of the gastric and/or pancreatic lipases (Shishikura et al., 2006; Sugiyama et al., 2007), and can also create a positive antioxidant environment or react with harmful products of lipid peroxidation (Jakobek, 2015). Related to this, the *in vivo* study of Gorelik et al. (2013) demonstrated that the addition of red wine to meat meals prevented LDL modifications by lipid peroxidation products, which is consistent with the LDL/HDL ratio reduction noted in several studies (Apostolidou et al., 2015; Droste et al., 2013). Similarly, total cholesterol reductions were observed after a fatty meal accompanied by red wine (Natella et al., 2011), and in short (two weeks) (Rifler

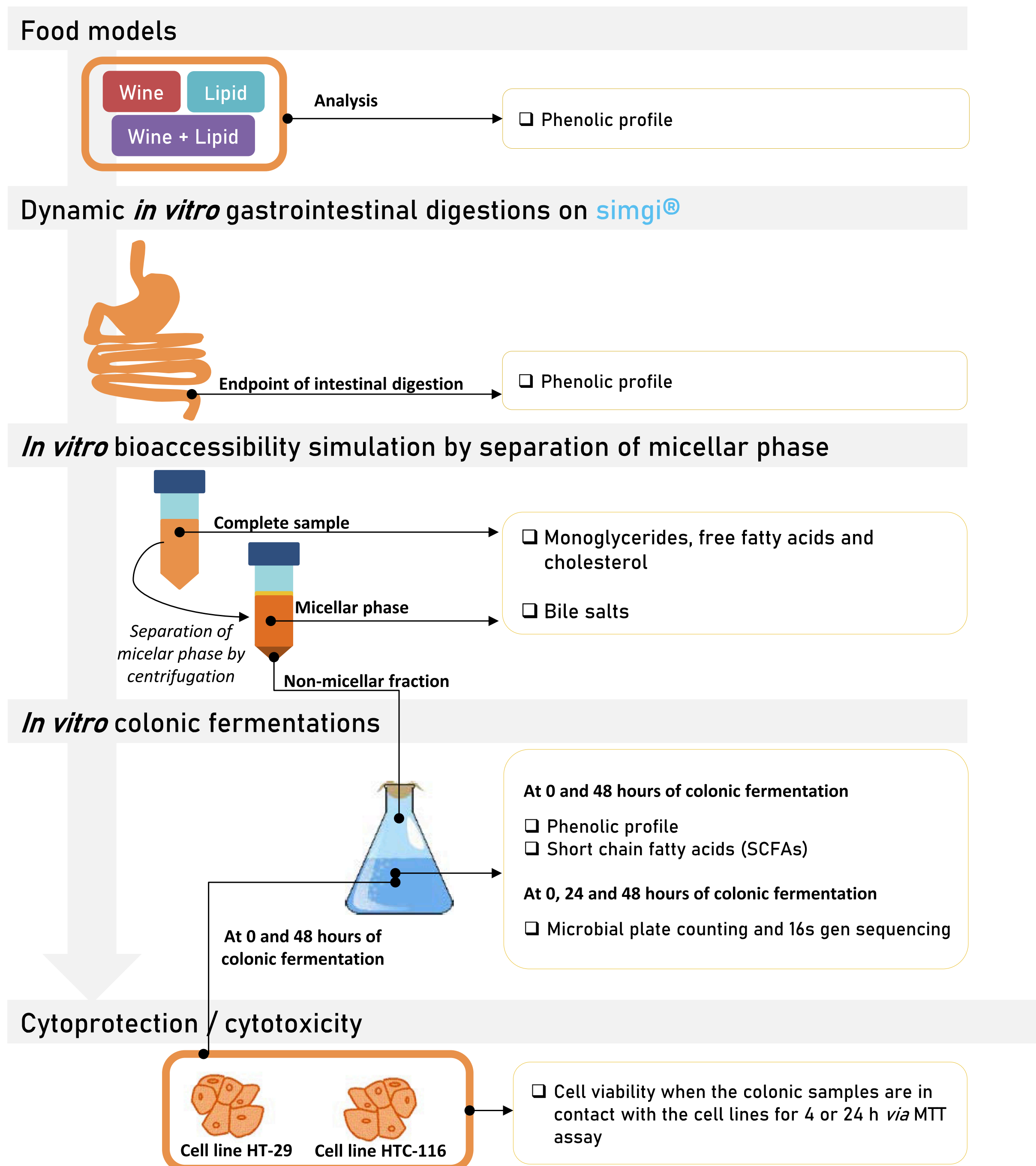

Fig. 1. Experimental set-up.

et al., 2012) and longer (one year) (Taborsky et al., 2017) dietary interventions with red wine. However, in order to assess the influence of co-digestion of dietary components on their bioaccessibility, food matrix compounds need to be evaluated individually (Lorenzo et al., 2021; Polia et al., 2022). In this sense, the interaction of wine polyphenols with lipids during gastrointestinal digestion has been only minimally studied.

In addition to the effects on the digestion process, the impact of polyphenol-lipid interactions can mediate their effects on gut microbiota composition and metabolic activity (Kardum & Glibetic, 2018). In the case of wine, the two-way interaction between polyphenols and gut microbiota is crucial in the polyphenols and health puzzle (Cueva et al., 2017; Moreno-Arribas et al., 2020; Nash et al., 2018). In terms of lipids, although there is less information in this regard, the latest scientific evidence indicates that their effect on the microbiota depends on the type of lipid. Likewise, it has been suggested that microbiota have the ability to contribute to lipid metabolism (Kardum & Glibetic, 2018). From a health point of view, this issue is highly relevant, especially if it is taken into account that, in the current framework of Western diets, it is associated with the development of a large number of chronic diseases (Medina-Remón et al., 2018). However, to the best of our knowledge, there are no studies that comprehensively evaluate the effect on the microbiota derived from a combined co-digestion of both types of compounds (wine polyphenols with lipids). Therefore, the aim of this paper was to investigate how the co-digestion of lipids and red wine polyphenols modifies their bioaccessibility and how this impacts on main lumen features such as colonic microbiota and gut epithelium. A dynamic simulator whose physiological relevance was previously validated in co-digestion studies of wine polyphenols and other nutrients (Tamargo et al., 2022) was used as a gastrointestinal digestion model for this study. After gastrointestinal digestion the, the micellar phase of the chemo was separated to address lipid bioaccessibility. Then, the non-micellar fraction was subjected to faecal fermentation under colon-simulated conditions. Changes in microbial communities and metabolic activities were monitored during colonic fermentation. In addition, fermented products were assessed for their cytoprotection/cytotoxicity towards two lines of colonic epithelium cells (Fig. 1).

2. Materials and methods

2.1. Formulation of food models

Three different food models (Wine, Wine + Lipid, Lipid) were prepared to evaluate the effects of the co-digestion of wine polyphenols and lipids (Table 1). A *reserva* red wine (Cabernet Sauvignon and Cabernet Franc, vintage 2006) was selected for this study. The ethanol content in the wine was 12%. A daily wine volume of 225 mL (Table 1) was considered based on previous studies (Cueva et al., 2015; Tamargo et al., 2022), always maintaining the daily dose within a moderate wine intake (Bucher et al., 2018).

The Lipid food model was established according to the daily reference intakes described in different nutritional guides, which reported an average of 2500 kcal/day (Grupo SENC, 2016) and a minimum of 7% kcal intake contributed by monounsaturated fatty acids (Grupo SENC, 2016). Thus, considering olive oil as a monounsaturated fatty acid source with 884 kcal/100 g, the recommended daily intake to reach a 7% kcal intake would result in 19.8 g of olive oil. Half of this daily consumption of olive oil was considered for the lipid model formulation (9.9 g, Table 1). In addition, cholesterol (343.8 mg, Table 1) was incorporated in the Lipid model in an amount similar to a commonly consumed food such as fresh eggs (2021). The lipid mix and emulsion for Wine + Lipid and Lipid food models were prepared following the procedure described on Tamargo et al. (2020).

Table 1
Food model composition for daily intake.

Food model	Components			
	Wine	SGF	Olive oil	Cholesterol
Wine	225 mL	–	–	–
Wine + Lipid	225 mL	–	9.9 g	343.8 mg
Lipid	–	225 mL	9.9 g	343.8 mg

SGF_Simulated gastric fluid.

2.2. Gastrointestinal digestion simulations

Gastrointestinal digestions of the three food models were carried out separately using the dynamic gastrointestinal model simgi® (Cueva et al., 2015). The simgi® system (https://www.cial.uam-csic.es/simgi/) is a computer-controlled gastrointestinal *in vitro* model designed to simulate the physiological processes taking place during digestion in the stomach and small intestine, while also reproducing the colonic microbiota responsible for metabolic bioconversions in the large intestine (Cueva et al., 2015). The flexible and modular design of the system allows continuous or staged simulation of its compartments. The gastrointestinal digestions in this study were performed by using just the stomach (ST) and small intestine (SI) compartments operating continuously.

Simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) were prepared as recommended earlier (Brodkorb et al., 2019) in sterile conditions. Simulated gastric juice is a solution comprised of SGF and pepsin (2,000 U/mL) (Sigma-Aldrich, Merck, USA), which was prepared daily and kept at 4 °C until gastric digestion. Simulated pancreatic juice consisted of Oxgall Dehydrated Fresh Bile (6 g/L) (Difco™ BD, USA) and pancreatin from porcine pancreas powder (9.2 U/mL) as described in Tamargo et al. (2022).

Bearing in mind that the daily food intake is distributed in three meals, the simgi® was fed with 80 mL of the different food models described in Table 1. Before each gastrointestinal digestion, the system was preconditioned: the stomach compartment was pre-filled with 65 mL of SGF (pH 2.0) and the small intestine with 55 mL of SIF (pH 7.0). Once the system was conditioned, the intake (80 mL) of each studied food model flowed to the ST, where the peristaltic movements mixed the food model content with the fasting content. Then, 15 mL of simulated gastric juice flowed to the ST. During gastric digestion, HCl was added gradually until the pH reached 1.8. To simulate physiological gastric emptying, the Elashoff function (Elashoff et al., 1982) was used to determine the gastric emptying flow to the SI. During SI digestion, the arriving chemo (95 mL) was mixed with 40 mL of simulated pancreatic juice and the fasting SI content. Anaerobic conditions – 150 rpm, 37 °C and pH 7.0 ± 0.2 – were maintained during the 120 min of intestinal digestion. For the three food models, gastrointestinal digestions were carried out in duplicate.

Samples of the food models after SI digestion were collected and kept at −20 °C until analysis of wine phenolic compounds (Section 2.6). Aliquots of the SI contents were also collected for further bioaccessibility studies (Section 2.3).

2.3. In vitro bioaccessibility of the digested food models

After gastrointestinal digestion, the micellar aqueous phase (considered as the bioaccessible fraction) of the digesta SI content (30 mL) was isolated by centrifugation at 4000 rpm for 40 min at room temperature (Martin et al., 2016) (Fig. 1). Non-micellar phase (considered as the non-absorbable fraction) was separated and kept at −80 °C for further colonic fermentation (Section 2.4). For the Wine + Lipid and Lipid food models, samples of the SI content before micellar separation and samples of isolated micellar phases were analysed for lipids (monoglycerides, free fatty acids and cholesterol) and bile salts (Section 2.7). Based on these contents, their bioaccessibility was calculated according to Eq. (1) (Martin et al., 2016):

$$pecentagebioaccessibilityofcompound = \frac{mgofcompoundinmicellarphase}{mgofcompoundintotalSIcontent} \times 100 \quad (1)$$

2.4. In vitro colonic fermentations

For each food model, non-absorbable fractions from the gastrointestinal digestion were subjected to static colonic fermentations (Fig. 1). Each flask contained the non-absorbable fraction from 30 mL (that supposed a total volume around 5 mL), 50 mL of colon nutrient medium (CNM) (Gil-Sánchez et al., 2017) and 5 mL of a faecal slurry following the procedure and proportions already used in Tamargo et al. (2022). Additionally, control fermentations, only with CNM and the faecal slurry, were carried out. All fermentation flasks were incubated for 48 h and 120 rpm, simulating the conditions of the distal region of the human large intestine (pH 6.8, 37 °C and anaerobic atmosphere) (Gil-Sánchez et al., 2017). Fermentations were carried out in triplicate and samples were collected at 0, 24 and 48 h. An immediately collected sample aliquot (1 mL) was used for microbial counts (Section 2.8). Other aliquots were centrifuged at 10,000 rpm at 4 °C for 10 min. Supernatants were separated and filtered through 0.22 μm PVDF filters (Symta, Spain) and kept at −80 °C until further assessment for cell cytoprotection/cytotoxicity (Section 2.5) and analysis of microbial-derived metabolites (Section 2.8). Pellets were kept at −80 °C until DNA extraction (Section 2.8).

2.5. Assessment of cell cytoprotection/cytotoxicity assay

Two human colon adenocarcinoma cell lines, HCT-116 (ATCC® CCL-247™) and HT-29 (ATCC® HTB-38™), cultured as described previously in Zorraquín-Peña et al. (2020), were used to evaluate cell cytoprotection/cytotoxicity.

Supernatants from colonic fermentations (0 and 48 h) were defrozen and diluted (10%, 25%, 50% and 75%, v/v) with serum-free cell culture medium. Then, cytoprotection/cytotoxicity towards HCT-116 and HT-29 cells was measured using the MTT cell viability assay for 4 and 24 h, as previously reported by Zorraquín-Peña et al. (2020). Results were expressed as cell viability (%) in comparison to the values in the absence of supernatants (control). Assays were performed in triplicate and two independent experiments were carried out.

2.6. Analysis of wine phenolic compounds by UPLC-MS

Samples from gastrointestinal digestions were defrosted and filtered through 0.22 μm PVDF filters (Symta, Spain). Analysis of wine non-anthocyanin phenolics was carried out by UPLC-ESI-MS/MS following a previously reported method (Muñoz-González et al., 2013), while wine anthocyanins were analysed following the method described in Sánchez-Patán et al. (2012b). The liquid chromatographic system was a Waters Acquity UPLC (USA) equipped with a binary pump, an autosampler thermostatic at 10 °C, and a heated column compartment (40 °C). The column employed was a BEH-C18, 2.1 × 100 mm and 1.7 μm particle size from Waters (USA). The gradient consisted of A (water/formic acid; 90:10, v/v) and B (acetonitrile) applied as follows: 0–1 min, 5–15% B; 1–5.25 min, 15–24% B; 5.25–5.88 min, 24–100% B; 5.88–7.05 min, 100–5% B; 7.05–9.38 min, 5% B. The flow rate was set constant at 0.5 mL/min and injection volume was 2 μL. The LC effluent was pumped to an Acquity TQD tandem quadrupole mass spectrometer equipped with a Z-spray electrospray ionization (ESI) source. The ESI parameters were set as follows: capillary voltage, 3 kV; source temperature, 130 °C; desolvation temperature, 400 °C; desolvation gas (N_2) flow rate, 750 L/h; cone gas (N_2) flow rate, 60 L/h. For the analysis of wine non-anthocyanin phenolics, the ESI operated in negative ionization mode. All compounds were quantified using commercial standards except for piceid (that was quantified using the external calibration curve of resveratrol), coutaric acid (that was quantified using the external calibration curve of *p*-coumaric acid) and caftaric acid (that was quantified using the external calibration curve of caffeic acid). For the analysis of anthocyanins, the gradient was the same, but the ESI was operated in positive mode using the same parameters reported above. All compounds were quantified using an external calibration curve of the malvidin-3-O-glucoside and analyses were carried out in duplicate.

2.7. Analysis of lipids and bile salts by GC-FID-MS

Extraction of lipids (monoglycerides (MGs), free fatty acids (FFAs) and cholesterol) and bile salts was carried out as described by Martin et al. (2016). The lipid phase of each sample was extracted with hexane: methyl *tert*-butyl ether (50:50 v/v) with a ratio of 3:1 (v/v) of solvent to sample. This mixture was vortexed for 1 min and centrifuged for 10 min at 3000 rpm. The upper organic phase was collected while the aqueous phase was extracted again with chloroform: methanol (2:1 v/v) at a ratio of 3:1 (v/v). The two organic phases obtained were mixed, and a rotary evaporator was used to remove the solvent.

Analysis and quantitation of MGs, FFAs, cholesterol and bile salts were performed by gas chromatography-flame ionization-mass spectrometry (GC-FID-MS) previous derivatization by silylation as described in Herrera et al. (2019). Analyses were carried out in triplicate. Briefly, the lipid extract was derived using bis(trimethylsilyl)trifluoroacetamide (BSTFA) at 75 °C for 1 h. The derivatized samples were analysed by gas chromatography-flame ionization mass spectrometry (GC-FID-MS) (Agilent 7890A, Agilent Technologies, USA), including a split/splitless injector, an electronic pressure control, a G4513A autoinjector, and a 5975C triple-axis mass spectrometer detector. The column used was an Agilent HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 μm phase thickness). Helium was used as carrier gas at 2 mL/min. The mass spectrometer ion source and interface temperatures were 230 and 280 °C, respectively. The sample injections (1 μL) were performed in splitless mode. The oven temperature at 50 °C was held for 3 min and increased at a rate of 15 °C/min to 310 °C, being held for 25 min. The mass spectra were obtained by electronic impact at 70 eV.

The scan rate was 1.6 scans/s at a mass range of 30–700 amu. Identification of compounds was performed by the NIST MS Data library, the mass spectra according to literature, or according to those of pure commercial compounds whenever possible. Quantitation of cholesterol, FFA, MG and bile salts was performed by the FID signal and using the calibration curve of commercial standards of cholesterol, oleic acid, monopalmitin and bile salts, which were derivatized under the same conditions as samples. Analyses were carried out in triplicate.

2.8. Microbial community analyses and colonic metabolic activity assessments

2.8.1. Microbial plate counting

Immediately after sampling, tenfold serial dilutions of the content of each colonic fermentation baker were plated on different types of selective media as described by Tamargo et al. (2018). Plate counting was done in triplicate and data were expressed as log of colony-forming units per millilitre (CFU/mL). Analyses were carried out in triplicate.

2.8.2. DNA extraction, sequencing and data processing

In order to ensure enough recovery, replicate pellets (n = 3) were pooled before DNA extraction. The QIAamp DNA Stool Mini Kit (Qiagen, Germany) was used for DNA extraction following the manufacturer's instructions.

As described by Taladrid et al. (2021), the V3-V4 region of the 16S ribosomal RNA gene was amplified, and a two-step Illumina® PCR protocol was followed to prepare the libraries. Samples were submitted to 2 × 300 bp paired-end sequencing utilizing an Illumina® MiSeq instrument (Illumina®, USA). RStudio v.1.3.1093 software was employed

to process the files with raw reads from the Illumina® instrument, using the DADA2 algorithm (Callahan, McMurdie et al., 2016; Callahan, Sankaran et al., 2016) to denoise joined paired-end reads and filter out chimaeras in the raw data. Silva v.138 was used as the reference database (Quast et al., 2013). A total of 533 amplicon sequence variants (ASVs) were found. Biodiversity, expressed in terms of alpha diversity, was estimated using the ASVs by calculating the Observed, Shannon and Simpson indices through the "Phyloseq" package. Beta-diversity was evaluated employing a Bray-Curtis dissimilarity matrix represented by non-metric multidimensional scaling (NMDS).

2.8.3. Phenolic metabolites analysis by UPLC-MS

Phenolic metabolites targeted in the study (mandelic acids, benzoic acids, phenols, hippuric acids, phenylacetic acids, phenylpropionic acids, cinnamic acids, 4-hydroxyvaleric acids and valerolactones) were analysed by UPLC-ESI-MS/MS operating with the parameters described in section 2.7 and following the method described by Gil-Sánchez et al. (2018). Analyses were carried out in duplicate.

2.8.4. Fatty acids analysis by GC–MS

Short-chain fatty acids (SCFAs) (acetic, propionic, butyric, isobutyric, valeric and isovaleric acids) and medium-chain fatty acids (MCFAs) (hexanoic, heptanoic, octanoic and decanoic acids) were analysed by SPME-GCMS as previously described (Cueva et al., 2015; Zorraquín-Peña et al., 2021). Briefly, filtered supernatants or calibration stock solutions (290 μL) were mixed with 10 μL of IS solution (2-methylvaleric acid, 1 g/L) and 30 μL of H_2SO_4 solution (0.9 N). Two aliquots of 100 μL of each treated sample were transferred to two 20 mL hermetically sealed vials. Analyses were carried out in duplicate.

2.9. Statistical analysis and data treatment

Differences in the bioaccessibility of lipids and bile salts were studied using the Mann-Whitney U paired-samples test. Analysis of variance (two-way ANOVA) was used to study the differences between food models and incubation times for plate counting and SCFA concentration in colonic-digested samples. Least significant differences were calculated using the Bonferroni test for SCFA production, and the Games-Howell test was carried out for plate counting. Analysis of variance (one-way ANOVA) was used to study the differences between food models for cell cytotoxicity data within each cell line, exposition time and dilution. Significant differences were calculated considering $p < 0.05$ for all the analyses. These statistical analyses were carried out with the XLSTAT Statistic for Microsoft Excel, version 2020.1 (Addinsoft-SARL, USA).

3. Results and discussion

3.1. Effect of wine and lipid co-digestion on phenolic profile

Recent evidence indicates that most dietary polyphenols are poorly absorbed in the small intestine, and they reach the large intestine where they suffer extensive metabolism by the gut microbiota. In the case of wine polyphenols, certain transformations during their passage through the stomach and small intestine have been described that might condition their bioactivity in the gut environment (Cueva et al., 2017).

The evolution of the main wine phenolic compounds in the Wine and Wine + Lipid food models after gastrointestinal digestion is shown in **Table S1**. For the Lipid food model, negligible contents of phenolic compounds were found. In general, intestinal digestion led to reductions (>50%) of most wine phenolic compounds, as seen for both Wine and Wine + Lipid food models. The disappearance of ellagic acid, tyrosol, esterified hydroxycinnamic acids (coutaric and caftaric acids), procyanidins and anthocyanins at the end point of the SI stage is worthy of note (**Table S1**). In contrast, concentrations of some phenolic acids such as protocatechuic, 4-hydroxybenzoic and vanillic, syringic and p-coumaric acids seemed slightly affected or almost not affected after gastrointestinal digestion (**Table S1**).

To better show the effect of lipid co-digestion on wine phenolic profile, Fig. 2 graphically compares values of those phenolic compounds detected after gastrointestinal digestion for both Wine and Wine + Lipid food models. For most of the phenolic compounds, concentrations after gastrointestinal digestion were lower in the Wine + Lipid model than in the Wine model, a finding that was also observed for the initial food models (Fig. 2). However, lipid co-digestion led to a relatively higher concentration of piceid (resveratrol-3-O-glucoside) in the Wine + Lipid food model (Fig. 2**b**, **Table S1**). This finding suggested that the hydrolysis of piceid into resveratrol that occurred through the gastrointestinal stage was aminorated in the presence of lipids. In the same way, gastrointestinal transformations of some phenolic acids such as vanillic, syringic and *p*-coumaric acids were delayed in the presence of lipids as concentrations of these compounds were higher in the Wine + Lipid model than in the Wine model after gastrointestinal digestion (Fig. 2**b**).

Interactions between polyphenols and dietary compounds such as proteins have been described previously (Ozdal et al., 2013), although less specific information about polyphenols and lipids during digestion is available in the scientific literature (Jakobek, 2015). In relation to wine polyphenols, Sun et al. (2020) recently carried out the *in vitro* gastrointestinal and colonic digestion of a red and a white wine in the absence and presence of an infant formula (20% turkey meat, 25% boiled corn paste, 10% boiled potato paste, 5% rice flour, 0.1% NaCl and 39.9% water) (Sun et al., 2020). After gastric digestion, samples were dialysed to obtain "serum-available" and "colon-available" fractions that were further subjected to colonic fermentation. The authors observed a general reduction in wine phenolic compounds (phenolic acids and flavan-3-ols) after gastrointestinal digestion (sum of "serum-available" and "colon-available" fractions); in particular, greater reductions were observed for caffeic acid, catechin, epicatechin and galloylated epicatechin. Interestingly, wine digestion in the presence of the infant formula attenuated the transformations of wine phenolic compounds during the gastric stage, and increased their concentration in the "serum-available" fraction, which was interpreted as an improvement in phenolic bioaccessibility (Sun et al., 2020). In spite of the differences in experimental materials and the *in vitro* digestion conditions, our findings were in agreement with those of Sun et al. (2020) and confirmed certain transformations of wine polyphenols during their *in vitro* gastrointestinal digestion that were lessened by co-digestion with food components (i.e. lipids). The extent of these transformations depends on the chemical structure of the phenolic compounds and might be related to the enzyme load of the simulated juices used in the *in vitro* simulation systems.

3.2. Effect of wine and lipid co-digestion on lipid bioaccessibility

Bioaccessible lipids are those available to be absorbed by intestinal enterocytes via mixed micelles. These micelles are formed during small intestinal digestion by lipid digestion products, cholesterol, phospholipids and bile salts. Hence, the micellar phase will contain the lipid and cholesterol fraction that could be considered bioaccessible in luminal media (Martin et al., 2010).

The bioaccessibility of the released lipid products from triglycerides of olive oil (mainly MGs and FFAs) together with cholesterol, as well as bile salts after simgi® gastrointestinal digestion is shown in Fig. 3. Although no significant differences ($p > 0.05$) in bioaccessibility between Lipid and Wine + Lipid food models for any of the lipids analysed were found, a remarkable increase in MGs was observed in the co-digestion with wine (from 13% to 39% bioaccessibility) (Fig. 3**a**). In contrast, a noticeable reduction in bioaccessibility was observed for cholesterol (from 80 to 49%) and bile salts (from 67 to 50%) when lipids were co-digested with red wine (Fig. 3**a** and **b**, respectively). However, FFAs showed similar mean values for both food models (42 and 44 %).

Previous studies using the simgi® system (Tamargo et al., 2020) reported values for lipid bioaccessibility close to those expected *in vivo*

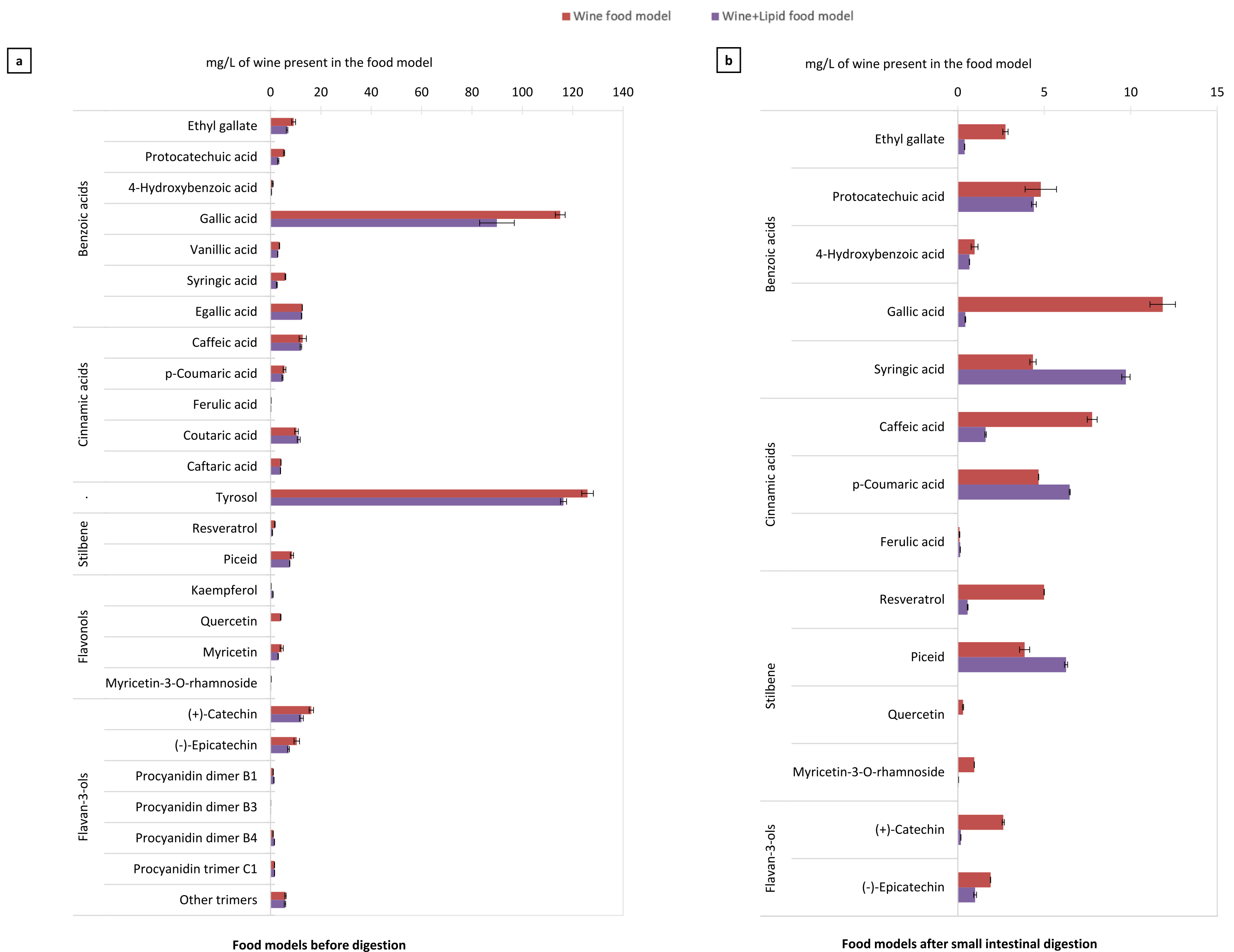

Fig. 2. Concentration (mg/L of wine present in the food model) of main phenolic compounds in the food model (a) and after intestinal digestion (b) for the Wine and Wine + Lipid food models. Data are expressed in mean values ± standard deviation.

(from 70 to 80% for MG and FFA, and from 20 to 80% for cholesterol (Ros, 2000)). The values found in this study for cholesterol bioaccessibility (Fig. 3**a**) were within the described range. However, values for MGs and FFAs were under the observed absorption range described *in vivo*. In considering these results, it is important to bear in mind that the wide range of *in vivo* absorption data is related to the presence in the intestinal lumen of other dietary components that can influence their availability (Moran-Valero et al., 2012), and the fact that the studied food models only included lipids or wine plus lipids, without the presence of other food constituents (such as proteins, fibre or other carbohydrates), which could influence the observed bioaccessibility results. The importance of the food matrix in lipolysis and the release and bioaccessibility of polyphenols has been recently reviewed (Paz-Yépez et al., 2019).

Although non-significant differences in cholesterol bioaccessibility between Wine + Lipid and Lipid models were observed, its reduction tendency might be supported by that observed in bile salt bioaccessibility (Fig. 3**a**). Ngamukote et al. (2011) hypothesized that polyphenols bound to bile acids reduced cholesterol micelle solubility. They reported that major polyphenols in grape seeds ((+)-catechin, (−)-epicathechin and gallic acid) were able to reduce the formation of cholesterol micelles (12, 20 and 27%, respectively, for a phenolic compound concentration of 200 mg/L) and showed partial binding to bile acids (taurocholic, taurodeoxycholic and glycodeoxycholic acids) (Ngamukote et al., 2011). The phenolic compounds referred to were present in red wine and detected in intestinal-digested samples for the Wine + Lipid food model (0.08, 0.21 and 0.09 mg/L of SI content, respectively). Additionally, the named bile acids were some of the main bile acids contained in the simulated pancreatic juice used in the present study (Hu et al., 2018). However, for further research it would be interesting to explore the hypothesis of Ngamukote et al. (2011) with a mixture of bile acids closer to human physiological conditions such as glycocholic acid, which was part of the simulated pancreatin juice and is the principal component of human bile salts (with a 96% contribution (Staggers et al., 1990)), but was not included in their *in vitro* study. Furthermore, reduced cholesterol micelle solubility and bile acid binding was also reported for other polyphenol-rich food such as black tea (Ikeda et al., 2010) or tea catechins and oolongtheanins (Ogawa et al., 2016).

Therefore, our results concerning lipid bioaccessibility may contribute elucidating the mechanisms underlying the hypocholesterolemic effects of red wine and red wine polyphenols observed *in vivo* (Apostolidou et al., 2015; Chiva-Blanch et al., 2013; Droste et al., 2013; Rifler et al., 2012; Taborsky et al., 2017), although further research is needed to better understand the interactions among wine components, cholesterol and bile salts during gastrointestinal digestion.

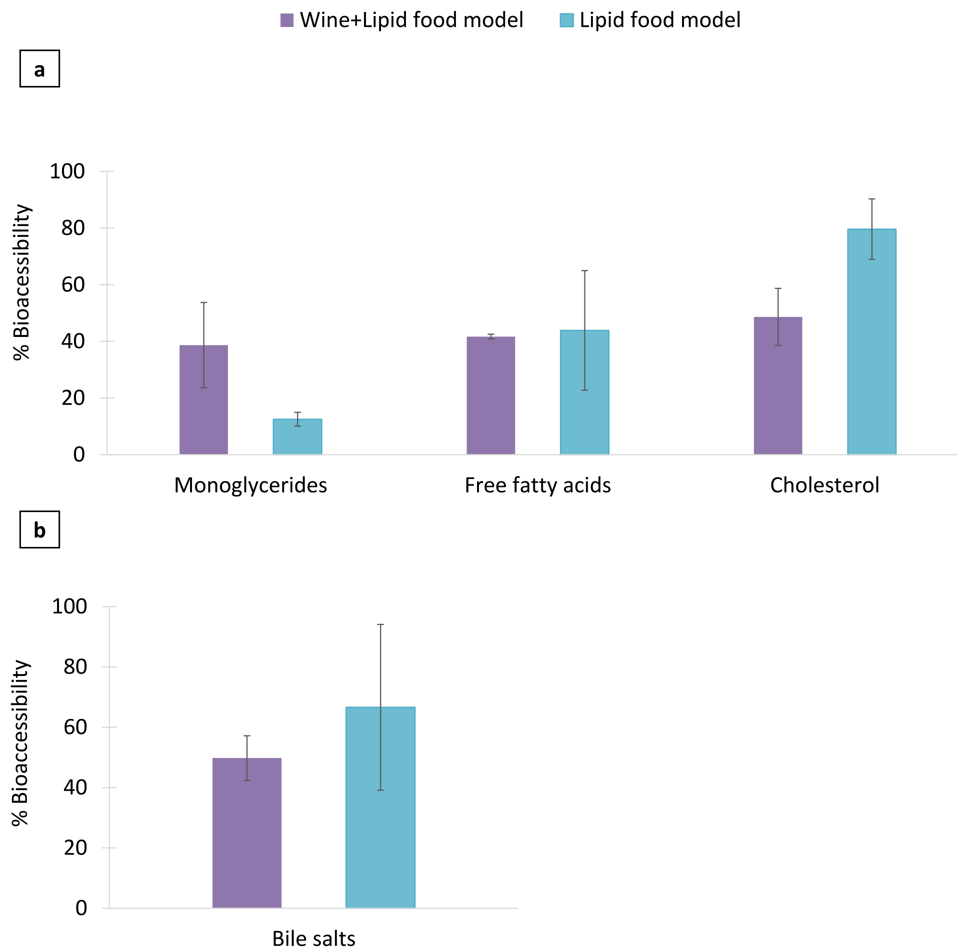

Fig. 3. Bioaccessibility (%) of free fatty acids, monoglycerides and cholesterol (a) and bioaccessibility of bile salts (b) for the Wine + Lipid and Lipid food models. Data are expressed in mean values ± standard deviation.

3.3. Effect of wine and lipid co-digestion on colonic microbiota composition and metabolism

To complete the digestion process, after gastrointestinal digestion, non-absorbable fractions from the food models were subjected to colonic fermentation (Fig. 1). Therefore, differences in previous intestinal stages (stomach and small intestine) among food models affected the composition of non-absorbable fractions and, consequently, colonic fermentations. Furthermore, given the bidirectional relationship between gut human microbiota and polyphenols (Fraga et al., 2019; Tomás-Barberán et al., 2016), the different phenolic profiles of the non-absorbable fractions at the start point of the colonic fermentations would

Table 2
Evolution of microbial growth of the selected bacterial groups' population during colonic fermentations, expressed as mean values of log(CFU/mL) ± standard deviation.

Food model	Time (h)	Total aerobic	Total anaerobic	*Enterobacteriae*	*Enterococcus* spp.	Lactic acid bacteria	*Clostridum* spp.	*Lactobacillus* spp.	*Bifidobacterium* spp.
Wine	0	6.0 ± 0.1 ^{bc}	6.2 ± 0.0^{c}	5.5 ± 0.1 ^{d}	5.8 ± 0.0 ^{cd}	6.4 ± 0.3 ^{bc}	5.1 ± 0.1 ^{d}	3.6 ± 0.3 ^{a}	6.6 ± 0.6 ^{a}
	24	7.6 ± 0.1 ^{a}	7.9 ± 0.1 ^{ab}	7.5 ± 0.2 ^{a}	7.3 ± 0.1 ^{a}	7.3 ± 0.0 ^{a}	6.6 ± 0.0 ^{a}	3.4 ± 0.5 ^{a}	7.1 ± 0.5 ^{a}
	48	5.4 ± 0.3^{c}	6.9 ± 0.3^{c}	5.4 ± 0.1 ^{d}	4.5 ± 0.3 ^{d}	4.1 ± 0.2 ^{d}	6.5 ± 0.0 ^{ab}	2.8 ± 0.5 ^{a}	4.3 ± 0.2^{b}
Wine + Lipid	0	6.1 ± 0.0 ^{bc}	5.8 ± 0.0^{c}	5.6 ± 0.6 ^{d}	6.1 ± 0.1^{c}	6.6 ± 0.1^{b}	5.0 ± 0.3 ^{d}	3.5 ± 0.1 ^{a}	6.3 ± 0.5 ^{a}
	24	7.2 ± 0.1 ^{a}	7.2 ± 0.1 ^{bc}	6.9 ± 0.1^{b}	7.0 ± 0.2 ^{ab}	7.1 ± 0.2 ^{ab}	6.5 ± 0.0 ^{a}	3.5 ± 0.1 ^{a}	6.7 ± 0.0 ^{a}
	48	7.0 ± 0.2 ^{ab}	7.9 ± 0.6 ^{ab}	5.2 ± 0.3 ^{d}	7.0 ± 0.2 ^{ab}	6.9 ± 0.2 ^{ab}	6.5 ± 0.1 ^{ab}	3.0 ± 0.2 ^{a}	6.8 ± 0.1^{c}
Lipid	0	6.0 ± 0.0 ^{bc}	6.2 ± 0.1^{c}	5.6 ± 0.0 ^{d}	5.9 ± 0.1 ^{cd}	6.6 ± 0.0^{b}	5.3 ± 0.9 ^{cd}	3.7 ± 0.4 ^{a}	6.8 ± 0.2 ^{a}
	24	7.5 ± 0.0 ^{a}	7.4 ± 0.1 ^{bc}	7.4 ± 0.6 ^{a}	6.8 ± 0.0^{b}	6.8 ± 0.0^{b}	6.3 ± 0.0 ^{bc}	2.9 ± 0.7 ^{a}	6.7 ± 0.1 ^{a}
	48	6.6 ± 0.0 ^{bc}	8.1 ± 0.1 ^{a}	6.5 ± 0.0^{c}	6.1 ± 0.2^{c}	5.7 ± 0.3 ^{bc}	6.0 ± 0.9 ^{cd}	2.7 ± 0.4 ^{a}	6.6 ± 0.2 ^{a}
Control	0	6.0 ± 0.1 ^{bc}	5.9 ± 0.1^{c}	5.6 ± 0.6 ^{d}	6.0 ± 0.2^{c}	6.6 ± 0.1^{b}	5.1 ± 0.1 ^{d}	3.4 ± 0.1 ^{a}	6.7 ± 0.1 ^{a}
	24	7.6 ± 0.0 ^{a}	7.8 ± 0.0^{b}	7.5 ± 0.1 ^{a}	6.6 ± 0.4 ^{bc}	6.9 ± 0.2 ^{ab}	6.1 ± 0.0 ^{bc}	3.4 ± 0.3 ^{a}	6.7 ± 0.3 ^{a}
	48	6.6 ± 0.1 ^{bc}	7.0 ± 0.3^{c}	6.2 ± 0.1 ^{cd}	5.9 ± 0.2 ^{cd}	5.2 ± 0.1^{c}	6.1 ± 0.1^{c}	2.8 ± 0.0 ^{a}	5.3 ± 0.2 ^{a}

^{a,b,c,d}Lower-case letters denote statistically significant differences between food models within time point and bacterial group. Microbiological significant differences, denoted as shaded cells, are considered when both statistically significant differences and Δ log > 1 take place.

modulate colonic microbial evolution (Cueva et al., 2017), and these different colonic microbiome populations would result in different microbial metabolite profiles at the end point of the colonic fermentations.

3.3.1. Impact on colonic microbiota

Plate counting is a first approximation to cultivable microorganisms present in colonic microbiota. The influence of time and the food model was evaluated considering the control (no food addition) as a guide of the colonic microbiota evolution during the *in vitro* colonic fermentations. Furthermore, and from a microbiological point of view, differences in values were considered significant when they were statistically significant and higher than Δ log (CFU/mL) ≥ 1 due to plate counting limitations (Gil-Sánchez et al., 2018).

At 0 h, there were no significant differences within food models for any tested bacterial groups (Table 2). After 48 h of colonic fermentation, *Enterococcus* spp., lactic acid bacteria and *Bifidobacterium* spp. presented lower values in the Wine food model than the control colonic fermentation, in contrast to the observed effect in other red wine colonic fermentations (Tamargo et al., 2022). However, the expected increase($p < 0.05$) was observed for these microbial groups in the Wine + Lipid food model, coinciding with the effects generally observed in red wine and grape seed extract colonic fermentations and *in vivo* intervention studies (Cueva et al., 2013; Dolara et al., 2005; Queipo-Ortuño et al., 2012), but also in several polyphenol supplementation studies (Ma & Chen, 2020).

Changes in microbial populations were also evaluated by 16S rRNA gene sequence analysis. **Fig. S1** shows the alpha-diversity analysis in terms of observed species and Shannon index. At the endpoint of colonic fermentation (48 h), the Wine and Wine + Lipid food models showed higher diversity values than the Lipid food model and the fermentation control (no food model addition) (**Fig. S1**). This observed effect could be related with the phenolic content after gastrointestinal digestion related with wine presence in both food models (**Table S1**), especially considering that red wine polyphenols have been extensively related with the increase of alpha-diversity (Barroso et al., 2017; Belda et al., 2021; Le Roy et al., 2020). The beta-diversity analysis did not reveal any changes between the different treatments, grouping the samples only for colonic fermentation time (data not shown). Otherwise, phylogenetic analysis at the phylum level is shown in Fig. 4. The Lipid food model slightly increased *Firmicutes* proportions, while the other phyla displayed the same trend as the control. Wine and Wine + Lipid food models showed a pattern similar to the control colonic fermentation, suggesting that the presence of wine in the food model could partially reverse the observed effect of the Lipid food model in the microbiota (Fig. 4). Furthermore, *Proteobacteria* proportions suffered a slower increase in the presence of wine. This effect is also in line with the observed decrease in *Escherichia/Shigella* proportions in Wine and Wine + Lipid food models (**Table S2**). Similar effects were reported for healthy subjects after red wine and dealcoholised red wine intake (Moreno-Indias et al., 2016).

Delving more deeply into bacterial communities with their key role in host health, *Akkermansia*, which is associated with the maintenance of the mucus layer of the colon (Geerlings et al., 2018), tended to increase during the first 24 h of colonic fermentation. However, its abundance decreased after 48 h in the Lipid food model (**Table S2**). This trend was not observed in Wine and Wine + Lipid food models, which maintained their abundance values. In the same line, wine content also favoured the presence of *Akkermansia* sp. in dynamic *in vitro* models after simulated digestion of red wine extract (Kemperman et al., 2013) and in human faecal microbiota after moderate and regular wine intake (Belda et al., 2021). Furthermore, an increase in lactic acid bacteria was detected in Wine and Wine + Lipid food models, while the Lipid food model displayed a result more similar to the control (**Table S2**).

Overall, the results show that the presence of wine in colonic media

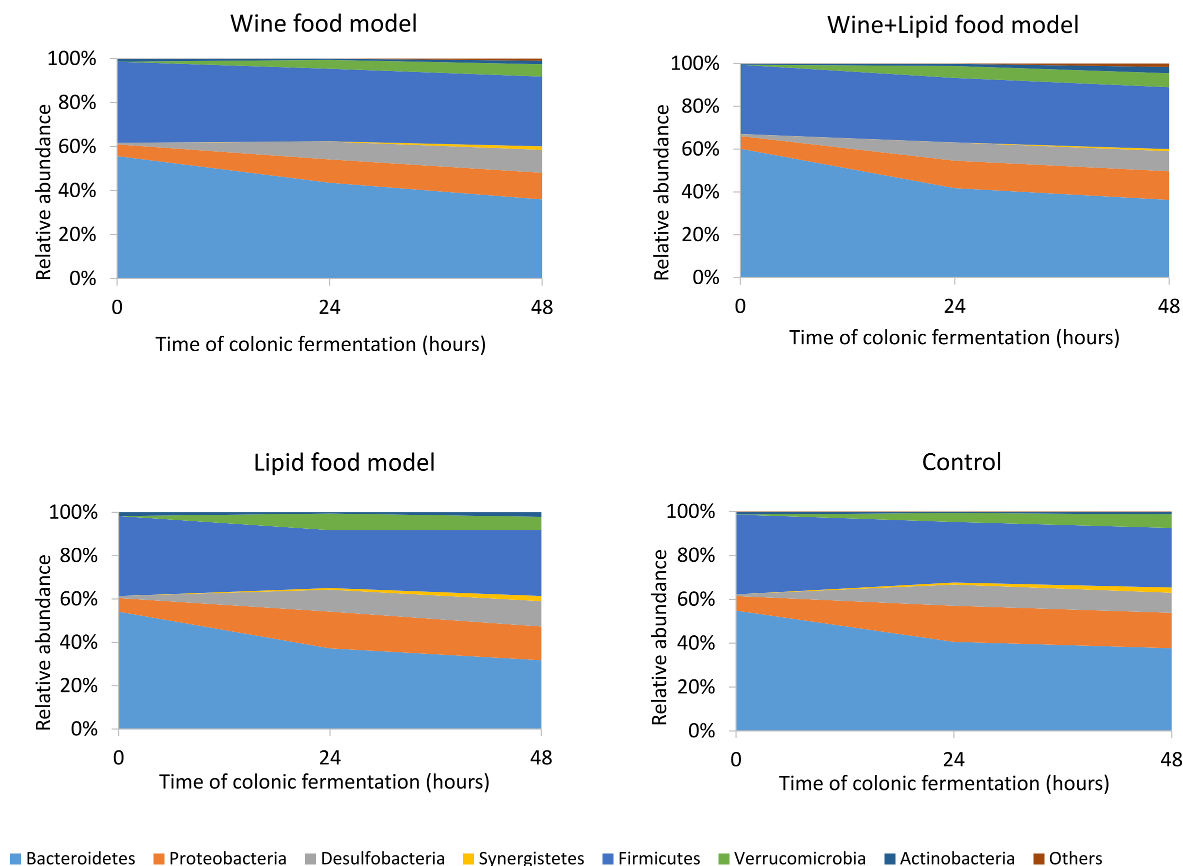

Fig. 4. Relative abundance at phylum level for the different food models during colonic fermentations. Data are expressed in mean values ± standard deviation. Graphs show the taxa with a relative abundance > 0.5%.

(Wine and Wine + Lipid food models) favours health-related taxa. Furthermore, these results are in accordance with the production of SCFAs (Section 3.3.2.) since several species from the detected microbial groups are related to butyric acid production (Markowiak-Kopeć & Śliżewska, 2020).

3.3.2. Impact on colonic microbiota metabolism

As a means of colonic microbiota metabolism, the production of fatty acids (SCFAs and MCFAs) and phenolic metabolites after 48 h of colonic fermentation was determined. The production (Δ mg/L) of butyric, isobutyric, isovaleric and pentanoic acids, among SCFAs, and hexanoic acid, among MCFAs, was found to be significantly ($p < 0.05$) lower for all of the food models with respect to the control (Fig. 5**a**). Among the food models, the Wine + Lipid food model showed a significantly higher production of total SCFA, due in particular to the contribution of butyric and pentanoic acids (Fig. 5**a**). Although Wine and Lipid food models presented no significant differences in total fatty acid production, butyric and pentanoic acid production in wine colonic fermentation was significantly higher for the Wine model (Fig. 5**a**). SCFA and MCFA production has a significant role in maintaining colonic homeostasis and host health, principally butyric acid, which is an energy source for intestinal epithelial cells and has shown anti-inflammatory effects, tumour cell inhibition and immunoregulatory effects (Markowiak-Kopeć & Śliżewska, 2020). The observed differences between food models could be related, at least in part, to the different microbial evolution during the colonic fermentation.

As regards phenolic metabolites, Fig. 5**b** and **c** display the production (Δ mg/L) of the detected phenylacetic and phenylpropionic acids as main microbial-derived metabolites of wine polyphenols (Cueva et al., 2015). Among them, the production of 3-(3-hydroxyphenyl)-propionic acid was found to be significantly ($p < 0.05$) higher for the Wine and Wine + Lipid food models, and the production of 3-(3,4-dihydroxyphenyl)-propionic acid was only observed in the Wine + Lipid food model (Fig. 5**b**). Both compounds come from the catabolism of different parent polyphenols present in red wine, and have been previously detected in the faecal and urine metabolomics fingerprint after red wine consumption (Esteban-Fernández, Ibañez, Simó, Bartolomé, & Moreno-Arribas, 2018, Esteban-Fernández, Ibañez, Simó, Bartolomé, & Moreno-Arribas, 2020; Jiménez-Girón et al., 2015). For the control and the three food models, a substantial increase in 4-hydroxyphenylacetic, phenylacetic and phenylpropionic during colonic fermentation was observed (Fig. 5**c**), which was mainly attributed to other pathways apart from wine polyphenol metabolism. This effect was previously described by other authors using the same colon nutrient medium after 48 h of fermentation (Gross et al., 2010; Sánchez-Patán et al., 2012a,2012b).

In summary, at the end point of colonic fermentation, it was observed that the Wine and Wine + Lipid food models exhibited some particular differences in colonic microbiota and phenolic metabolites that, albeit slightly, might favourably impact the gut environment as is investigated in Section 3.4.

3.4. Cytoprotection/cytotoxicity towards colon epithelial cells

As an approach to studying the role of the colonic metabolites inside

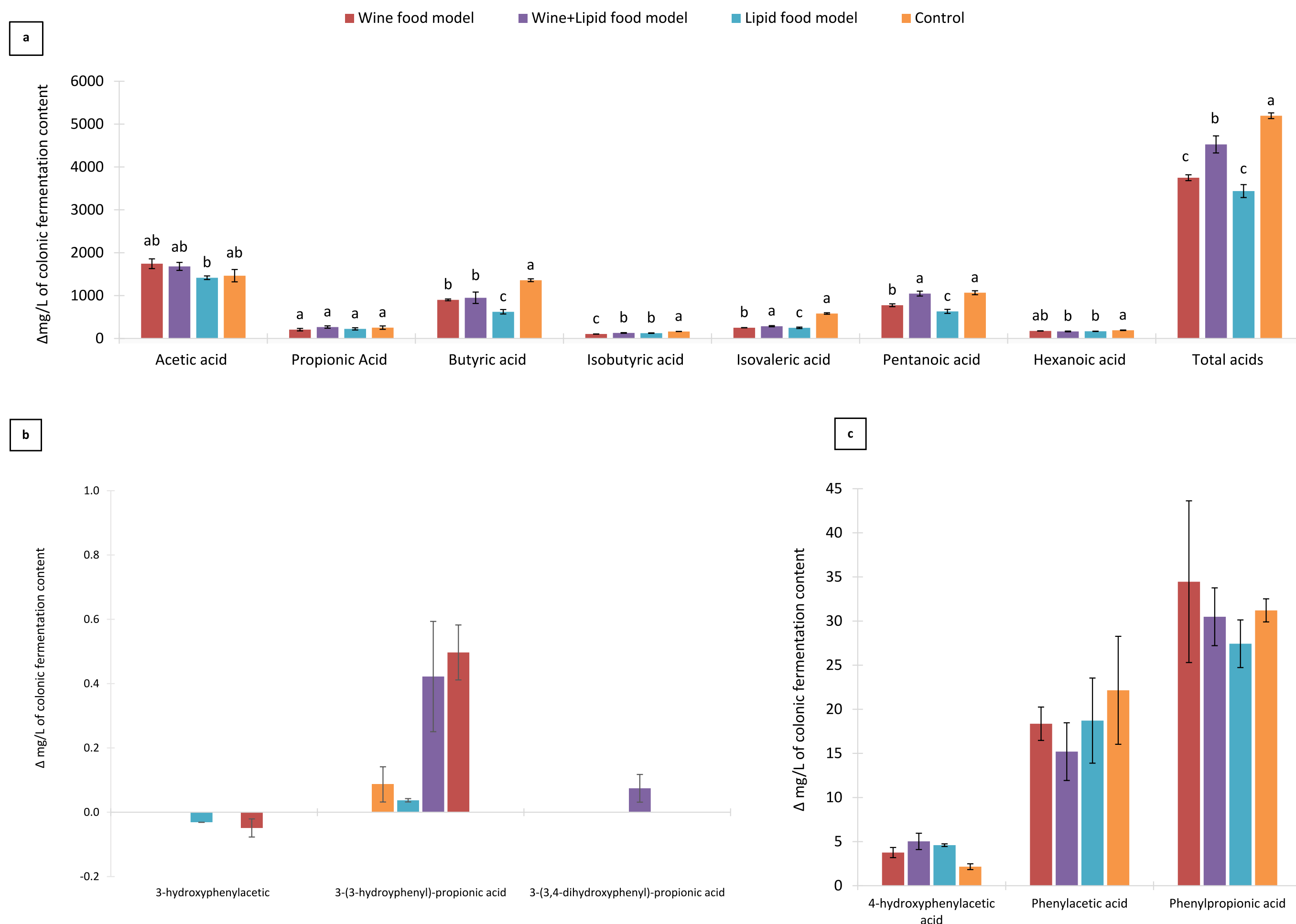

Fig. 5. Production (Δ mg/L) of SCFAs (a) and phenolic metabolites (b and c) after 48 h of colonic fermentation for the control and the Wine, Wine + Lipid and Lipid food models. Lower-case letters denote significant differences among control and food models.

the lumen, they are assayed for their protective/toxicity effects towards epithelial cell lines (Gill et al., 2007). Thus, Table 3 reports data on the % cell viability of the two cell lines tested (HCT-116 and HT-29) after incubation (4 h and 24 h) in the presence of the three colonic-digested food models collected at the beginning (0 h) and after 48 h of colonic fermentation, in comparison to the fermentation control (no food addition). For all the concentrations tested (10, 25, 50 and 75%, v/v), the control mixture (colon nutrient medium + faecal slurry) resulted in being toxic for both HCT-116 and HT-29 cell lines, although HCT-29 cells showed comparatively higher percentages of cell viability than HCT-116 cells (which were more sensitive) (Table 3). Loss of cell viability for the control mixture occurred proportionally to its concentration, being greater after 24 h than after 4 h of incubation with the cells. As can be seen, the presence of the intestinal-digested food models counterbalanced this loss of cell viability, and, in general, cytoprotective effects were in the following model order: Wine > Wine + Lipid > Lipid (Table 3). For instance, after 4 h of incubation with the cells, the data on cell viability corresponding to the time point of 48 h of colonic fermentation and 75% concentration were 49.4, 39.5, 35.3 and 26.5%, respectively, for the Wine model, Wine + Lipid model, Lipid model and control towards the HCT-116 cell line, and 69.7, 69.5, 63.1 and 46.8%, respectively, for the Wine model, Wine + Lipid model, Lipid model and control towards the HCT-29 cell line (Table 3).

As a biomarker of bowel health, numerous studies have assessed the cytotoxicity of what is called “faecal water” (FW), which is the aqueous phase obtained after faeces ultracentrifugation (Klinder et al., 2007). Thus, it has been shown that the intakes of prebiotics, probiotics and other dietary components are particularly effective in lowering the levels of FW cytotoxicity (Erba et al., 2014; Ślizewska et al., 2016; Windey et al., 2014). Recently, we assessed the FW cytotoxicity after moderate consumption of red wine in healthy volunteers (Zorraquín-Peña et al., 2020). Using HT-29 and HCT 116 cell lines, we found that the percentages of cell viability were higher (lower cytotoxicity) for faecal samples collected after the red wine intervention than for those collected before, although significant ($p < 0.05$) differences were only found in certain assay conditions for both cell lines (Zorraquín-Peña et al., 2020). The findings of the present study using *in vitro* colonic-digested wine confirmed these previous *ex vivo* results in that wine-derived metabolites might exhibit protective activities at the intestinal level. This protection seems to be related to the production of microbial-derived metabolites such as fatty acids and phenolic metabolites, whose faecal contents have been shown to increase after red wine consumption (Muñoz-González et al., 2013).

4. Conclusions

This study, using simgi® as a gastrointestinal simulation system, provides new evidence about the effects of the co-digestion of red wine and lipids at different stages of the gastrointestinal tract. Although no significant differences were found, the Wine + Lipid co-digestion tended to increase the percentage of bioaccessible monoglycerides and to reduce cholesterol and bile salt bioaccessibility, which was consistent with the hypocholesterolemic effects observed in intervention and epidemiological studies associated with moderate wine consumption. On the other hand, our results confirmed certain transformations of wine polyphenols during their *in vitro* gastrointestinal digestion that were lessened by co-digestion with lipids. At the colonic stage, the microbial community evolved differently when wine was co-digested with lipids, also leading to higher total SCFA production and, specifically, butyric acid. The colonic-digested samples derived from the Wine and Wine +

Table 3
Viability percentages of HCT-116 and HT-29 cells after exposure to colonic fermentation content at the start point (0 h) and end point (48 h) of colonic-stage simulations. Data represent mean values ± standard deviation for each food model colonic fermentation. Columns indicating 10%, 25%, 50% and 75% present the dilutions of the colonic media used for each cell viability assay. Lower-case letters denote statistically significant differences between food models within exposition time (4 or 24 h) and dilution.

HCT-116 cell line	Colonic fermentation time point 0 h				Colonic fermentation time point 48 h			
(4 h incubation)	10%	25%	50%	75%	10%	25%	50%	75%
Wine	89.9 ± 3.4 ^{a}	76.3 ± 12.6 ^{ab}	55.5 ± 6.0 ^{a}	36.1 ± 8.6 ^{a}	90.4 ± 3.1 ^{a}	82.5 ± 4.4 ^{ab}	58.3 ± 13.2 ^{a}	49.4 ± 9.3 ^{a}
Wine + Lipid	89.4 ± 2.9 ^{a}	87.5 ± 3.7 ^{a}	64.1 ± 13.5 ^{a}	38.8 ± 11.8 ^{a}	80.7 ± 3.7 ^{a}	73.2 ± 4.5 ^{ab}	64.8 ± 12.5 ^{a}	39.5 ± 8.7 ^{ab}
Lipid	92.3 ± 2.3 ^{a}	76.0 ± 5.4 ^{ab}	46.6 ± 4.9 ^{ab}	32.4 ± 10.8 ^{a}	93.3 ± 1.5 ^{a}	84.6 ± 0.5 ^{a}	55.9 ± 6.6 ^{a}	35.3 ± 2.9 ^{ab}
Control	91.5 ± 3.1 ^{a}	52.6 ± 9.5^{b}	34.8 ± 8.2^{b}	13.1 ± 5.3^{b}	80.9 ± 4.6 ^{a}	68.4 ± 8.1^{b}	46.4 ± 7.6 ^{a}	26.5 ± 10.2^{b}
HCT-116 cell line	Colonic fermentation time point 0 h				Colonic fermentation time point 48 h			
(24 h incubation)	10%	25%	50%	75%	10%	25%	50%	75%
Wine	91.3 ± 8.1 ^{a}	49.7 ± 7.9 ^{a}	6.8 ± 0.9 ^{a}	6.9 ± 1.0 ^{a}	70.9 ± 7 0.7 ^{a}	51.8 ± 4.1 ^{a}	7.8 ± 0.9 ^{a}	7.4 ± 1.0 ^{a}
Wine + Lipid	94.7 ± 1.5 ^{a}	57.2 ± 5.4 ^{a}	6.8 ± 0.9 ^{a}	6.8 ± 0.9 ^{a}	73.2 ± 3.2 ^{a}	48.2 ± 2.6 ^{a}	7.4 ± 0.7 ^{a}	7.2 ± 1.0 ^{a}
Lipid	94.1 ± 6.5 ^{a}	56.6 ± 17.7 ^{a}	7.7 ± 2.1 ^{a}	7.9 ± 1.6 ^{a}	73.1 ± 7.1 ^{a}	47.1 ± 17.9 ^{ab}	7.3 ± 0.9 ^{a}	7.2 ± 0.8 ^{a}
Control	88.6 ± 1.4 ^{a}	34.5 ± 11.2 ^{a}	7.4 ± 1.0 ^{a}	6.8 ± 0.9 ^{a}	72.4 ± 3.2 ^{a}	31.4 ± 13.3^{b}	9.3 ± 1.1 ^{a}	7.0 ± 1.0 ^{a}
HT-29 cell line	Colonic fermentation time point 0 h				Colonic fermentation time point 48 h			
(4 h incubation)	10%	25%	50%	75%	10%	25%	50%	75%
Wine	95.9 ± 4.6 ^{a}	93.6 ± 3.1 ^{a}	69.2 ± 9.1 ^{a}	52.8 ± 7.9 ^{a}	96.7 ± 2.9 ^{a}	98.4 ± 2.9 ^{a}	80.1 ± 4.7 ^{a}	69.7 ± 4.9 ^{a}
Wine + Lipid	97.1 ± 1.8 ^{a}	83.6 ± 0.9 ^{a}	76.5 ± 3.4 ^{a}	55.5 ± 6.1 ^{a}	90.9 ± 2.2 ^{a}	85.4 ± 1.5 ^{ab}	77.0 ± 6.4 ^{a}	69.5 ± 5.3 ^{a}
Lipid	95.9 ± 4.1 ^{a}	83.7 ± 13.3 ^{ab}	63.5 ± 11.5 ^{a}	51.7 ± 0.8 ^{a}	92.9 ± 6.7 ^{a}	74.6 ± 6.7^{b}	74.6 ± 6.3 ^{a}	63.1 ± 8.0 ^{a}
Control	93.8 ± 5.8 ^{a}	68.4 ± 5.4^{b}	45.2 ± 2.7^{b}	32.6 ± 5.0^{b}	89.8 ± 6.1 ^{a}	81.8 ± 1.2^{b}	62.4 ± 1.5^{b}	46.8 ± 0.5^{b}
HT-29 cell line	Colonic fermentation time point 0 h				Colonic fermentation time point 48 h			
(24 h incubation)	10%	25%	50%	75%	10%	25%	50%	75%
Wine	88.4 ± 3.9 ^{a}	54.4 ± 16.1 ^{a}	32.1 ± 9.7^{b}	5.2 ± 0.5 ^{a}	89.0 ± 9.6 ^{a}	39.9 ± 8.6 ^{ab}	15.1 ± 1.3 ^{a}	5.9 ± 0.9 ^{a}
Wine + Lipid	95.9 ± 3.9 ^{a}	72.6 ± 5.7 ^{a}	39.0 ± 7.8^{b}	8.3 ± 6.0 ^{a}	80.4 ± 7.8 ^{a}	37.9 ± 1.0^{b}	9.8 ± 1.1^{b}	4.9 ± 1.0 ^{a}
Lipid	88.2 ± 0.1 ^{a}	65.9 ± 8.8 ^{a}	18.5 ± 3.3 ^{a}	4.9 ± 1.9 ^{a}	68.5 ± 33.3 ^{a}	44.7 ± 8.4 ^{ab}	7.9 ± 6.4^{b}	3.8 ± 0.9 ^{a}
Control	91.9 ± 4.5 ^{a}	58.2 ± 6.3 ^{a}	17.2 ± 6.4^{a}	8.0 ± 2.3 ^{a}	79.6 ± 7.5 ^{a}	56.8 ± 5.6 ^{a}	10.6 ± 2.7^{b}	5.33 ± 1.5 ^{a}

Data represent mean values ± standard deviation for each food model colonic fermentation. Columns indicating 10%, 25%, 50% and 75% present the dilutions of the colonic media used for each cell viability assay. ^{a,b,c,d} Lower-case letters denote statistically significant differences between food models within exposition time (4 or 24 h) and dilution.

Lipid food models resulted in being less cytotoxic towards human colon adenocarcinoma cells (HCT-116 and HT-29) than the Lipid model and control (no food addition), indicating potential health-promoting activity in the colonic environment. Lastly, the *in vitro* results of this work indicate that the simgi® system constitutes a suitable tool for studying the co-digestion effect of polyphenol-rich foods, as it is able to mimic human digestion physiological conditions.

CRediT authorship contribution statement

Alba Tamargo: Conceptualization, Methodology, Software, Validation, Formal analysis, Writing – original draft, Writing – review & editing. **Dolores González de Llano:** Methodology. **Carolina Cueva:** Methodology. **Joaquín Navarro del Hierro:** Methodology, Software, Validation, Formal analysis. **Diana Martin:** Methodology, Software, Validation, Formal analysis, Writing – review & editing. **Natalia Molinero:** Software, Validation, Formal analysis, Writing – review & editing. **Begoña Bartolomé:** Resources, Writing – original draft, Project administration. **M. Victoria Moreno-Arribas:** Conceptualization, Resources, Writing – original draft, Project administration, Funding acquisition.

Declaration of Competing Interest

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

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2023.112524.

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