



# Release of adsorbed ferulic acid in simulated gastrointestinal conditions

Gonalo N. Martins<sup>1</sup> · V tor Sp nola<sup>1</sup> · Paula C. Castilho<sup>1</sup>

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## Abstract

Ferulic acid (FA) is a natural antioxidant with limited absorption when conjugated with biomolecules but whose free form is readily absorbed in the stomach and, to a lesser extent, in the ileum. The latter suffers from inflammation and oxidative stress, so a novel strategy for the delivery of FA in this compartment of the gastrointestinal tract was developed. Using the neutral un-functionalized resin Lewatit  VP OC 1064 MD PH, under optimized conditions, a loading of 144 mg FA/g of dry resin was obtained. By means of an in vitro simulated digestion, an average release of 32 mg FA/g of dry loaded resin (recovery of 22%) was observed in intestinal conditions. The incorporation/release of FA onto/from the resin was confirmed by ATR-FTIR spectroscopy and by HPLC-DAD. This work showed that the free form of FA can effectively be delivered in the small intestine, after immobilization in solid matrices.

**Keywords** Ferulic acid · Adsorption · Release · In vitro digestion simulation · Intestine

## Abbreviations

ATR-FTIR	Attenuated total reflectance-Fourier transform infrared
C <sub>0</sub>	Initial concentration
CA	Caffeic acid
C <sub>e</sub>	Concentration at equilibrium
ChA	5- <i>O</i> -Caffeoylquinic acid
C <sub>r</sub>	Concentration of released ferulic acid
FA	Ferulic acid
GI	Gastrointestinal
HCA(s)	Hydroxycinnamic acid(s)
HPLC-DAD	High-performance liquid chromatography with diode-array detection
Lewatit	Lewatit� VP OC 1064 MD PH
<i>m</i>	Mass of resin
pCA	<i>p</i> -Coumaric acid
<i>q<sub>e</sub></i>	Amount of compound adsorbed for unit mass of adsorbent

<i>q<sub>r</sub></i>	Amount of ferulic acid released from the resin
SA	Sinapic acid
<i>V</i>	Volume of solution
<i>V<sub>r</sub></i>	Volume of simulated medium

## Introduction

Ferulic acid (FA, 4-hydroxy-3-methoxycinnamic acid) is a phenolic compound, specifically a hydroxycinnamic acid. It is a potent antioxidant found in plant tissues mainly as a component of primary cell walls [1]. Among foodstuffs, cereals are the richest dietary source of FA, although it can be found in fruits, vegetables, and several beverages (e.g. coffee, beer) [2].

One of the most documented bioactivities of FA is its antioxidant capacity, resulting from the formation of a resonance-stabilized phenoxy radical structure [3, 4]. The health-beneficial effects of FA have been proposed against several acute and chronic pathologies, such as diabetes, intestinal ischemia, Alzheimer's disease, cancer, and cardiovascular diseases [4–8]. The ameliorative effects of FA in bowel diseases have been particularly studied. Administration of FA derivatives to colitis-induced rats showed positive effects, so these compounds were suggested as potential preventive or therapeutic agents for

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  Paula C. Castilho  
 pcastilho@staff.uma.pt

<sup>1</sup> CQM-Centro de Qu mica da Madeira, Universidade da Madeira, Campus da Penteada, 9020-105 Funchal, Portugal

gastrointestinal (GI) inflammatory diseases [9, 10]. The anti-inflammatory action was attributed to the mechanism of antioxidation mediated by the inhibition of NF- $\kappa$ B expression and arachidonic acid metabolism. This finding was later reinforced by other authors [11], who reported that FA upregulates the expression of HO-1 (heme oxygenase 1 mRNA) and Nrf-2.

The health benefits of FA depend on its absorption, which depends on its bioaccessibility. FA is found in nature in the free form or bound to carbohydrates and other biomolecules by etherification and esterification; and depending on its form, it can be either absorbed during the digestive process or metabolized by the microflora in the cecum [12–14]. Reportedly, 25% of FA present in foodstuffs such as tomatoes is effectively absorbed; however, the absorption of FA from cereals is very low [15]. The difference in the absorbance of FA is attributed to the presence of the free form of FA in the first case (e.g. tomatoes) and of the bound form in the latter (e.g. cereals). After the absorption of FA obtained from cereals, only 3% (of FA and its metabolites) was detected in urine, whereas 50% was detected when the free form was administered [16]. The free form is well absorbed in the small intestine and more extensively in the stomach [2, 17]. In food matrices, FA is mostly covalently bonded as side chains to carbohydrate molecules such as arabinoxylans, which compromise its absorption [14, 18]. Malunga and Beta [19] isolated feruloyl-arabinoxylans from maize and wheat and submitted their compounds to a simulated gastric digestion; they found out that the gastric medium can produce feruloyl arabinofuranose but not the free form of FA.

Most FA-containing foodstuffs are not capable of appropriately promoting the interaction between FA and the small intestine walls because free FA is absorbed in the stomach and FA bound to carbohydrates will only be released and absorbed when reaching the colon. To direct FA to the small intestinal mucosa, an alternative approach can be the encapsulation of free FA in matrices that resist the low pH of the stomach, thus retaining the compound during the gastric phase of the digestion, but are able to release FA once the pH rises in the small bowel [14]. Encapsulation systems for FA based on cyclodextrins, lipidic nanoparticles, micelles, microemulsions, and electrospun fibres have been developed [20–23]. The adsorption and desorption of FA on synthetic resins [24–26] and zeolites [27] have also been explored.

Lewatit® VP OC 1064 MD PH, an FDA-approved resin for the treatment of food products (e.g. juices), is a neutral adsorbent resin and is described as a material suited for the purification/extraction of a large variety of organic compounds, both natural and synthetic, highly stable over a wide pH range [28]. It has the capacity to selectively adsorb hydroxycinnamic acids (HCAs) from hydrolysed complex matrices without the co-adsorption of sugars. Here, we

evaluate the release of adsorbed FA from this resin when submitted to simulated GI conditions.

## Materials and methods

### Chemicals and reagents

All reagents and standards were of analytical reagent grade. Acetonitrile (99.9%) was purchased from Carlo Erba (Cornaredo, Italy). *p*-Coumaric ( $\geq 98\%$ ) and sinapic ( $\geq 99\%$ ) acids were acquired from Fluka (Lisbon, Portugal). Lewatit® VP OC 1064 MD PH was kindly provided by LANXESS (Germany).  $\alpha$ -amylase (porcine pancreas, type VI-B), caffeic ( $\geq 98\%$ ), chlorogenic (3-*O*-caffeoylquinic,  $\geq 95\%$ ), ferulic ( $\geq 99\%$ ), formic ( $\geq 98\%$ ) acids, mucin (type II; from porcine stomach), lipase (type II; from porcine pancreas), pepsin (porcine gastric mucosa) and porcine bile extract (contains glycine and taurine conjugates of hyodeoxycholic acid and other bile salts) were all purchased from Sigma-Aldrich (St. Louis, MO, USA). Other lab reagents were obtained from conventional commercial suppliers. Ultrapure water (Milli-Q Waters purification system; 18 M  $\Omega$  cm at 23 °C; Millipore; Milford, MA, USA) was also used in this study.

### Adsorption experiments

#### Selection, characteristics, and preparation of the adsorbent

The adsorbent resin Lewatit® VP OC 1064 MD PH (Lewatit) is a neutral un-functionalized crosslinked polystyrene resin with a wide range pH (0–14) and thermal (–20 to 120 °C) stability. The 0.44–mm–0.54–mm white, opaque, porous beads have a surface area of 800 m<sup>2</sup>/g, a pore volume of 1.2 cm<sup>3</sup>/g, and an average pore diameter of 5 nm–10 nm [28]. Preliminary data show that this resin is suitable for the adsorption of polyphenols such as HCAs. Before the experiments, the resin was preconditioned as recommended by the supplier and described elsewhere [29]. Briefly, the resin was washed consecutively with 6% HCl, distilled water and 4% NaOH until the pH of the cleaning water was close to that of distilled water. The water retention percentage of the resin was determined to be 64%, using a moisture analyser (DBS 60–3, Kern).

### Optimization of the adsorption experiments

The experimental conditions were optimized by mixing the resin with FA solutions in 2% ethanol:water. The effect of the amount of adsorbent used (5–20 mg resin/mL solution), FA's initial concentration (0.25–1.0 mg/mL), temperature (10–40 °C), and contact time on the efficiency of the adsorption was studied. A standard protocol, consisting of mixing

3 mL of a 0.5 mg/mL FA solution with 30 mg of resin for 5 h, at 23 °C (room temperature), was used as initial conditions, after which each parameter was changed while keeping the remaining constant. For the study of the contact time effect, a mixture of 250 mg of resin and 50 mL of a 0.5 mg/mL FA solution was placed in a water bath at room temperature with magnetic stirring at 100 rpm for 5 h, during which 1 mL aliquots were withdrawn, at certain times.

All adsorption experiments were performed in triplicate, protected from light, and with head-over-heels rotation (Reax 2, Heidolph) at 100 rpm. Controls without resin were also performed. After each assay, samples were centrifuged for 20 min at 4000 rpm and 10 °C and filtered with 0.45- $\mu$ m cellulose acetate filters (Frilabo).

After quantification of FA in solution by HPLC-DAD, the efficiency of the adsorption was determined by calculating the  $q_e$  value (mg FA/g dry resin):

$$q_e = \frac{(C_0 - C_e) \times V}{m}, \quad (1)$$

where  $q_e$  is the amount of compound adsorbed for unit mass of dry adsorbent (mg/g);  $C_0$  and  $C_e$  are the initial and equilibrium concentrations (mg/mL) of the adsorbate, respectively;  $V$  is the volume of solution used (mL);  $m$  is the mass of resin used (g, dry weight).

A final adsorption assay was performed, in triplicate, in larger quantities, combining the optimized parameters: 5000 mg resin was mixed with 1000 mL of a 0.5 mg/mL FA solution, for 2 h at 23 °C, with magnetic stirring at 100 rpm. After the adsorption, the loaded resin from the three assays was washed thrice with 10 mL of distilled water, lyophilized, and mixed. The samples were kept in the dark at 4 °C.

ATR-FTIR spectra of standard FA (powder) and unloaded and loaded resin were collected between the spectral range of 650 and 4000/cm, with a resolution of 4/cm and acquiring 36 scans per spectrum.

## Resin selectivity

FA was the main compound studied in this work; however, in a food matrix, several HCAs are usually present, albeit in very different amounts. Therefore, the selectivity of the resin towards the most common HCAs was evaluated by the resin's efficacy in removing each of the five compounds from a solution similar to that prepared for FA only and the experimental procedure for the adsorption studies were performed as previously stated.

To mimic a somewhat real matrix, a mixture containing FA, *p*-coumaric (pCA), caffeic (CA), sinapic (SA), and chlorogenic (5-*O*-caffeoylquinic acid, ChA) acids was prepared. For this, 25 mg of each was dissolved in 1000  $\mu$ L of ethanol, and the volume was made up to 50 mL with distilled water,

resulting in a concentration of 0.5 mg/mL of each HCA in a 2% ethanol solution. In the experiments, 3 mL of this solution and 15 mg of resin were used.

## HPLC-DAD analysis and FA quantification

Quantification of FA and other HCAs (CA, ChA, pCA and SA) was performed using high-performance liquid chromatography with diode-array detection (HPLC-DAD), on a Dionex UltiMate 3000 series instrument (Thermo Scientific Inc., California) equipped with a binary pump, an autosampler, and a column compartment at 30 °C. Elutions were carried out on a Phenomenex Gemini C<sub>18</sub> column (5  $\mu$ m, 250  $\times$  3.0 mm i.d.) with ultrapure water acidified with formic acid at 0.1% and acetonitrile. The DAD acquisition was done at 320 nm. All samples and standards were filtered with 0.45- $\mu$ m cellulose acetate filters (Frilabo) prior to their injection. Additional information of the chromatographic analysis can be found in Online Resource 1, namely the runs' conditions and the method's validation.

## Release of FA in simulated digestion conditions

Samples of loaded resin prepared according to the optimized experimental conditions were lyophilized prior to the assays and kept in the dark, at 4 °C.

### Simulated in vitro digestion

Briefly, 250 mg loaded resin was sequentially incubated at 37 °C with simulated juices whose pH and compositions reflected those of the GI environments of the mouth (6 mL of salivary juice, pH 6.8), the stomach (12 mL of gastric juice, pH 1.30), and the small intestine (12 mL of duodenal and 6 mL of bile juices, pH 8.1 and pH 8.2, respectively) [30, 31]. The composition of the juices (Online Resource 2) mimics that of each of the simulated GI compartments, with site-specific salts and enzymes. The amount of sample was adjusted from the 2 g used by Pinto et al. [30] to 250 mg because the resin used here is very similar to other resinate materials used in health applications, e.g. in drug delivery or as bile acid sequestrants, where the normal daily dosage in humans can range from 270 mg to 24 g [32, 33].

Samples were placed in 50-mL Falcon tubes, in a water bath at 37 °C, in the dark, and then GI-simulated juices were added and magnetically shaken at 500 rpm. To accurately assess the release of FA in each stage of the digestion, independent experiments were made ending the incubation either in the oral, gastric, or intestinal step, in triplicate. After each digestion, samples were recovered and centrifuged. The supernatant was filtered with 0.45- $\mu$ m cellulose acetate filters and analysed in the HPLC-DAD for quantification; and ATR-FTIR spectra of the solid residue, consisting of,

mainly, (un)loaded resin, were collected. A summary of the procedure is detailed in Fig. 1.

The amount of FA released from the resin,  $q_r$  (mg/g), was calculated, after determining FA's concentration ( $C_r$ , mg/mL) in each stage of the in vitro-simulated digestion, by the following equation, where  $V_r$  is the volume (mL) at each stage and  $m_r$  is the amount (g) of loaded resin used in the assay:

$$q_r = \frac{C_r \times V_r}{m_r} \quad (2)$$

## Statistical analysis

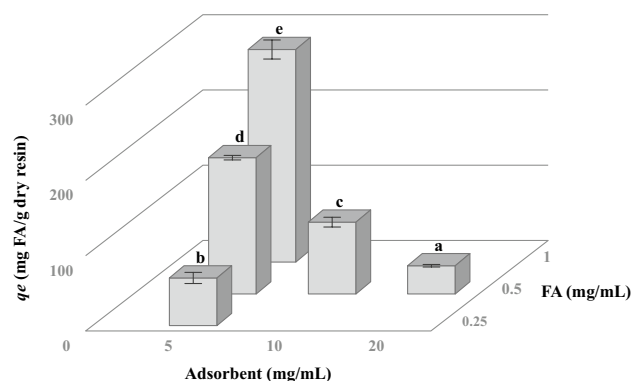
All assays were done in triplicate and the results are given as the mean for each experiment. Data analysis was carried out by means of a one-way ANOVA with Tukey's post hoc test using SPSS for Windows, IBM SPSS Statistics 20 (SPSS, Inc., USA). A value of  $p < 0.05$  was considered statistically significant.

## Results

### Adsorption experiments

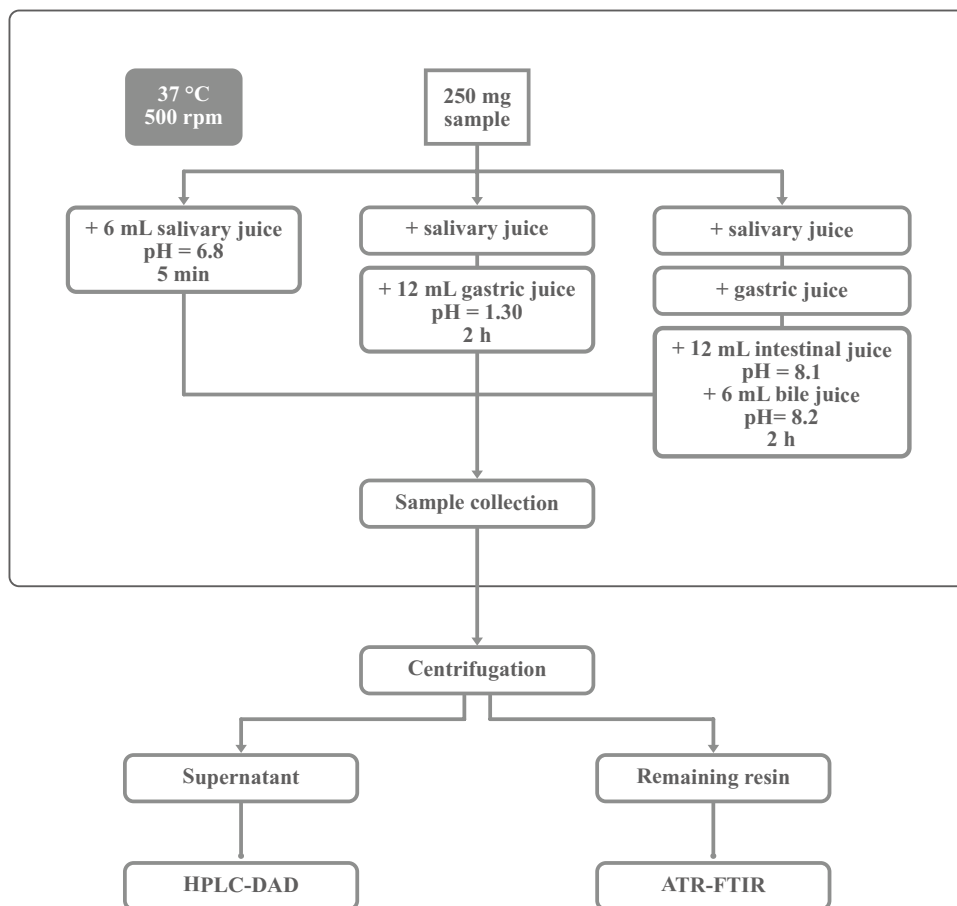
#### Optimization of the adsorption experiments

The results obtained during the adsorption experiments are shown in Fig. 2. The loading of FA onto the resin increased



**Fig. 2** Optimization of the adsorption of FA on Lewatit. Mean values not sharing the same letters are statistically different ( $p < 0.05$ )

**Fig. 1** Scheme of the in vitro-simulated digestion procedure



when using the lowest proportion of resin/FA solution (5 mg/mL), providing a  $q_e$  of 189 mg FA/g of dry Lewatit, so this ratio was used for the rest of the work. In the adsorption experiments with different initial FA concentrations, the highest loading efficiency was obtained after increasing the concentration of FA—with 1.0 mg/mL FA solutions, a  $q_e$  of 312 mg/g was registered.

There were no significant differences between the results obtained for the experiments at room temperature (23 °C), at 10 °C and 40 °C ( $p > 0.05$ ); the  $q_e$  ranged between 189 and 203 mg/g (Online Resource 3), so the following experiments were performed at room temperature.

The plot of the percentage of adsorption versus time was made to determine when the adsorption process of FA reached its equilibrium—when there are no noteworthy changes in the percentage of adsorption in time. From the experimental data, a model function was plotted. A good correlation was obtained between the experimental and calculated values  $r = 0.985$  ( $p = 0.01$ ) as shown in Fig. 3; the function was determined to have the equation:  $y = 52.0 \times (1 - e^{-t/30.7})$ , meaning that the system will evolve in time, as the percentage of adsorption stabilizes at 52.0%, after approximately 2 h.

The optimal conditions for the adsorption assays consisted of mixing 5 mg resin/mL of a FA solution with a concentration of 0.5 mg/mL for 2 h at room temperature. This experiment was carried out with larger quantities and provided a loading of 144 mg FA/g of dry resin (58%).

### ATR-FTIR spectroscopy

ATR-FTIR spectroscopy was used to confirm the loading of FA on the polystyrene resin. The unloaded and loaded

polystyrene resin's spectra showed some similarities (Fig. 4), although the incorporation of FA resulted in some differences. There is the appearance of new peaks in the Lewatit + FA spectrum, at 1685/cm, 1267/cm, and 1184/cm, resulting from the presence of oxygen-containing functional groups of FA, C=O, C–O–C, and C–O, respectively. In addition, some peaks became more intense after the incorporation of FA into the resin, such as those at 1602/cm, 1511/cm, and 702/cm. However, some of FA's characteristic peaks seem to be overlapped by those of the resin itself. Moreover, the free OH band (3430/cm), from the phenolic OH group of FA, and the two broad bands (3100–2500/cm), attributed to hydrogen-bonded (dimerized) carboxylic groups, seen in the FA spectrum later appear as a broad band around 3500/cm in the Lewatit + FA's spectrum.

### Resin selectivity

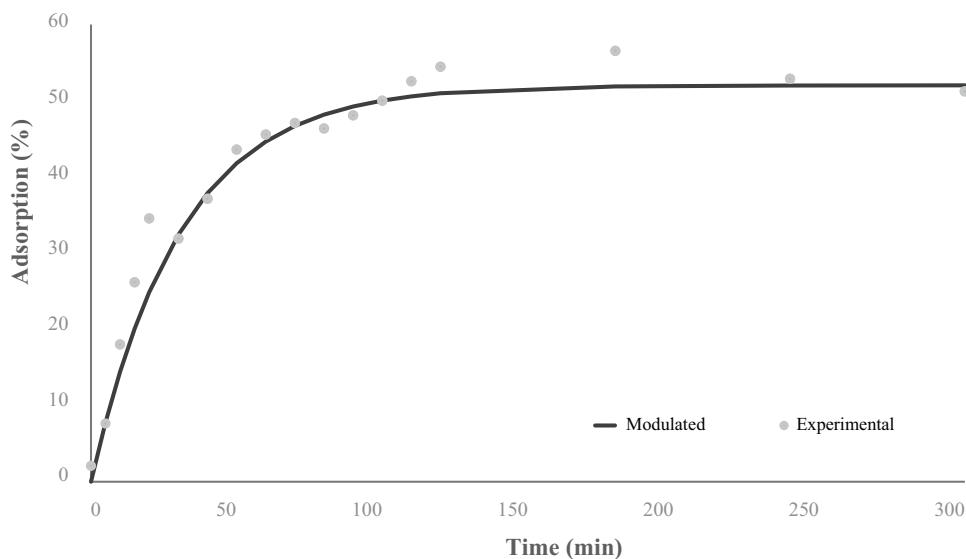
The resin's selectivity was studied using a mixture of the most common HCAs. ChA and CA were the least adsorbed compounds (0 mg/g and 4 mg/g, respectively) in the present conditions, while pCA, FA, and SA were the HCAs who showed the best results, with loadings of 77 mg/g, 109 mg/g and 179 mg/g Lewatit, respectively.

### Simulated digestion

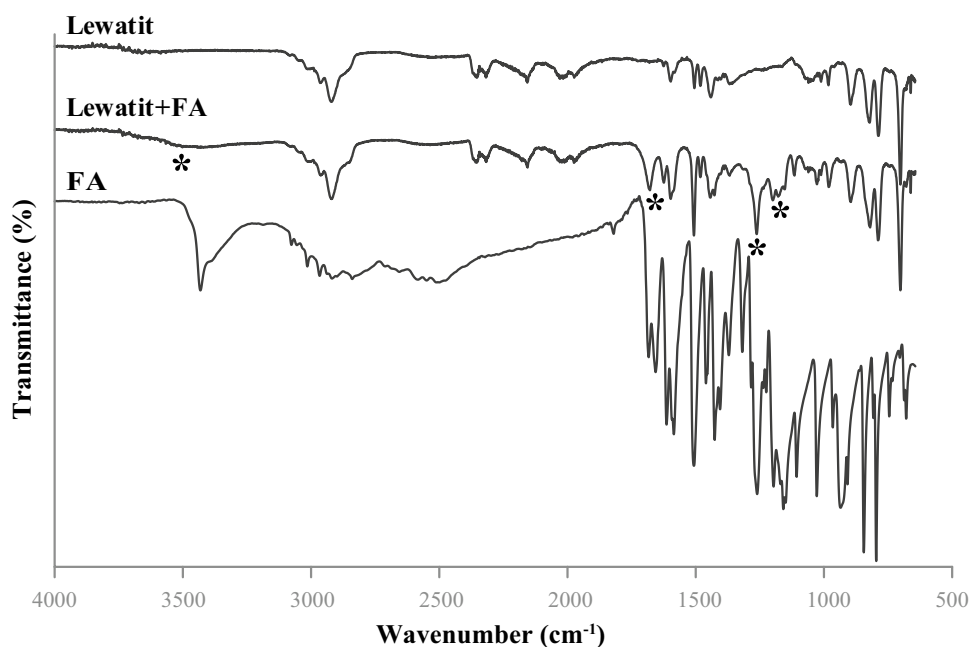
#### Release of FA

The complete simulated digestion provided the most extensive release of FA, with a  $q_r$  of 32 mg/g, followed by the oral incubation step (8 mg/g) and by the gastric step with the lowest average release (5 mg/g). The same trend was observed

**Fig. 3** Study of the effect of contact time over the adsorption



**Fig. 4** ATR-FTIR spectra of FA and Lewatit, before and after the adsorption of FA by the resin

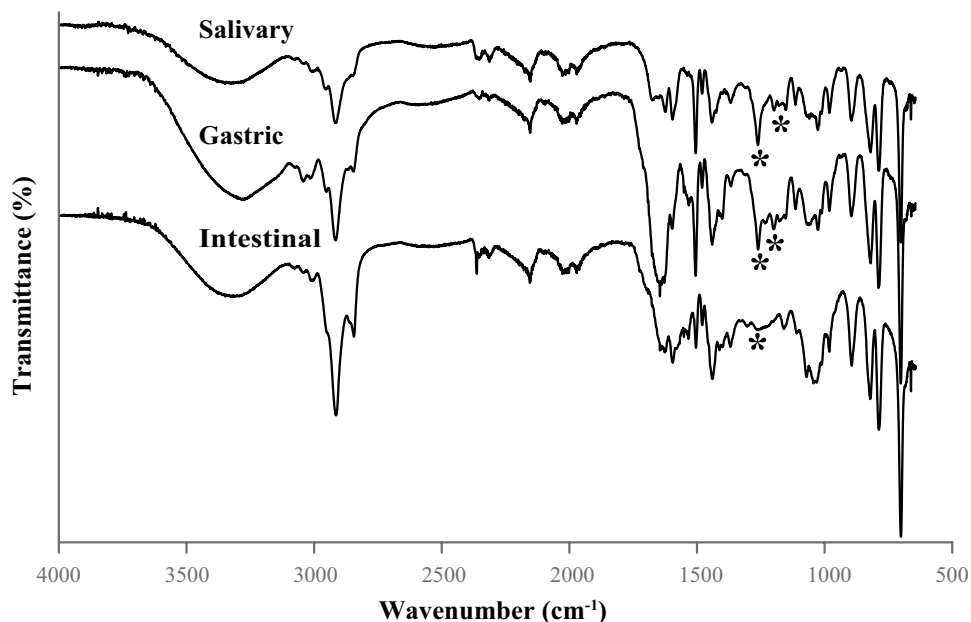


on a parallel experiment with the simulated GI juices in the absence of adjuncts and enzymes (data not shown), suggesting that pH and ionic strength have a greater influence in the release of FA than the activity of the enzymes present in the GI-simulated juices. No FA oxidation products were observed in the HPLC-DAD analysis.

#### ATR-FTIR spectroscopy

ATR-FTIR spectra of the resin were collected after each stage of the *in vitro* digestion simulation assays (Fig. 5). In general, all spectra still resemble that of the (un)loaded resin, after the simulation. The peaks at 1267/cm and 1205/cm, from FA adsorbed in the resin, are still present

**Fig. 5** ATR-FTIR spectra of Lewatit after the oral, gastric, and intestinal steps of the GI digestion simulation





after passing the oral and gastric media but are gone after the intestinal digestion.

## Discussion

### Adsorption experiments

#### Optimization of the adsorption experiments

The adsorption of FA into the resin over different conditions was studied (Fig. 2).

When studying the resin/solution ratio, the highest loading was obtained for the 5 mg/mL proportion, so this was the chosen condition for continuing the work. Dávila-Guzman et al. [24] obtained 133 mg FA/g on XAD-16, a divinylbenzene resin, using a higher proportion (25 mg resin/mL) and twice the initial concentration of FA. These differences are probably related to the nature (structure) of the adsorbents and their interactions with FA, suggesting FA has a higher affinity for polystyrene than divinylbenzene. In the work of Yang et al. [25], several resins were used for the adsorption of phenolic compounds and XAD-16 was one of the resins with the best recovery results, suggesting that the use of Lewatit for the adsorption of FA could be advantageous.

The amount of adsorbed FA increased when the initial concentration doubled: 189 mg/g was adsorbed from a 0.5 mg FA/mL solution, whereas with 1.0 mg/mL solutions, 312 mg/g was adsorbed. Despite this increase, the concentration of 0.5 mg/mL was used for the next experiments since 1.0 mg/mL is close to the limit of solubility of FA in the 2% ethanol: water mixture and stability was difficult to achieve.

Figure 3 shows the plot of the percentage of adsorption versus time. This type of curve can be divided in three sections related to (1) film diffusion, fast transport of molecules in the solution surrounding the resin—first 60 min; (2) intraparticle diffusion, diffusion of molecules inside the adsorbent 60–120 min; (3) stabilization of the adsorption process [24]. At 2 h, the percentage of adsorption was 51.5, very close to the 52.0%, at which the system is stable, so a separate adsorption experiment was performed with a contact time of 2 h and the  $q_e$  was found to be 156 mg/g. Although this value was lower than 189 mg/g previously obtained, it was still higher than the 133 mg/g obtained by Dávila-Guzman et al. [24] at 2.5 h.

#### ATR-FTIR spectroscopy

The overall similarities between the spectra of the unloaded and loaded resin indicate the structure of the

resin does not suffer any changes after the incorporation of FA but the appearance of new peaks corresponding to those of the FA's spectrum suggest FA was effectively adsorbed into the resin. This notion is further supported by the appearance of a single sharp peak at around 3500/cm instead of a broad band, like in the spectrum of FA, suggesting the interaction between the resin and FA is not a simple deposition of FA on the resin's surface and that FA is not dimerized.

#### Resin selectivity

For this work, the loading of the resin Lewatit was done by adsorption of FA from 2% (V/V) ethanol aqueous solutions of the pure compound. However, it was hypothesized that if the resin were selective towards FA, the option of loading it from a food matrix extract would save a purification step.

Since the solution used was a mixture of five compounds (CA, ChA, FA, pCA, and SA), the results may suggest a competition for the active sites of the resin by the analytes. SA appears as the HCA with the highest affinity for the neutral polymer, probably because the hydrophobic interactions increase with its extra methyl groups. Adsorption seems to be dependent on the compounds' hydrophobicity, i.e. the higher the hydrophobic nature of the acid, the higher the adsorption yield—hydrophobic forces are the main interactions in adsorption processes on neutral non-polar resins, such as Lewatit. This order of adsorption is the same as their elution from the reverse phase chromatographic column and the reverse to their solubility in water.

In food matrices from which FA is usually obtained, SA is often present in much smaller amounts (< 10% of FA) [34]. Nevertheless, these results show that using this method in real samples would involve the simultaneous adsorption of SA by the resin.

Kammerer et al. [26] performed recovery studies of various phenolic compounds from apple pomace extract using Lewatit and observed that ChA was well adsorbed in acid pH (pH 1). This contradictory result may be explained by differences in the experimental design since an 80% methanolic aqueous solution, a different pH, and a recirculatory packed-bed system were used. In the same work, the adsorption (and desorption) of other phenolic compounds (e.g. gallic acid, catechin) and at different pH values are also described; however, there is no mention of FA.

Kammerer et al. [35] highlighted the importance of studying the selectivity of resins, while working on the adsorption of phenolic compounds from apple juice onto the adsorbent resin Alimentech P-495, noting that the behaviour of individual compounds towards the adsorbent when dealing with complex solutions, like those found in nature, should be assessed. The bioactivity of FA depends on the natural

source from which it is recovered since it can be found in several forms and in the presence of different compounds (HCAs and others). It is, therefore, important to understand the selectivity of the resin towards FA while present in mixtures with other biomolecules.

## Simulated digestion

### Release of FA

32 mg/g of FA released is comparable to the quantities of FA found in different foodstuffs, as these range from  $0.19 \times 10^{-3}$  mg/g of their fresh weight in pot-grown lettuces to 33 mg/g of their fresh weight in refined corn bran [2]. Not all FA found in foods is bioaccessible, being bound to carbohydrates, but in this work, it is assumed all FA can effectively be absorbed since it is the free form that is being adsorbed and released. The present work demonstrated that this approach is a possible mean for delivering free FA in large quantities in the small intestine.

The obtained results show that the pH of the media influences the interactions between FA and the resin: acidic media promotes the adsorption of FA and prevents its release, whereas alkaline media causes its release from the adsorbent and hinders its adsorption. Dávila-Guzman et al. [24] noticed the adsorption of FA onto the neutral adsorbent resin XAD-16 was favoured at low pH and decreased in media with  $\text{pH} > 4$ , because the ionization of FA ( $\text{pK}_{\text{a}1} = 4.46$ ;  $\text{pK}_{\text{a}2} = 8.77$ ) in neutral and basic solutions weakens the hydrophobic interactions responsible for the adsorption. Also, in the work of Geerkens et al. [36], polyphenols were recovered from mango-peel extracts using Lewatit and their adsorption was much higher at pH 1 than at pH 7. In the present work, the amount of released FA during the gastric step ( $\text{pH} = 1.30$ ) is lower than the amount released in intestinal conditions ( $\text{pH} = 8.1$ ). This is probably caused by the pH difference of the media, i.e. the alkaline environment of the simulated intestinal juice promotes the release of FA by causing its ionization and subsequently weakening its interactions with the resin.

Adsorbent and ion-exchange resins have been used to concentrate and purify mixtures containing phenolic compounds obtained from natural sources, e.g. fruit juices or extracts. These resins are considered selective for these compounds and are used for the removal of undesired compounds, such as amino acids, that are not retained by the resin [26]. Polyphenols can, then, be recovered, usually, with alcoholic or hydro-alcoholic solutions [25, 26, 37–39]. Ethanol, for instance, is a common eluent because it is efficient, eco-friendly and non-toxic but the use of methanol is also common [40]. Since this work evaluates the release of FA in GI conditions, these solvents were not used.

When designing this experiment, the objective was the evaluation of the release of FA from the resin when the resin–FA complex was submitted to the conditions found in the GI tract, e.g. the pH shifts. Kammerer et al. [26] studied the adsorption of polyphenols from apple pomace and grape skin using Lewatit. When desorbing these compounds, two conditions were tested: one using methanol:water (30:70, V/V) and one with the same eluent but previously washing the resin with 2 M HCl. Although the acidic solution was an inefficient eluent, just as the gastric juice was in this work, this pre-step was beneficial for the recovery of phenols. The acidification followed by a sudden pH shift effectively weakened the interactions between the compounds and the resin, promoting the release of the polyphenols. This information suggests the passage through the gastric step, in this work, is essential for a more extensive release of FA in the intestinal media.

Moreover, during a real-life digestion process there are several enzymes and other compounds present in solution. Although the enzymes and other adjuncts used in these simulations were not expected to have any activity over the Lewatit + FA formulation, since adsorption resins are materials considered inert and resistant, their inclusion was important to assess the release of FA in these complex conditions. In fact, when the full digestion simulation process was assayed, there was a change in the resins' colour, from colourless/white into yellowish-brown (Online Resource 4)—the same colour as the bile-simulated juice. This change was attributed to a potential adsorption of bile acids from the bile-simulated juice (Online Resource 2), because adsorbent and ion-exchange resins are used as bile acid sequestrants, i.e. they are administered per os for the treatment of health conditions, e.g. intractable diarrhoea, and for lowering cholesterol levels through the adsorption of bile acids [32, 41, 42]. Since it has been reported that FA has anti-cholesterolemic activity, the concomitant use of both substances could have a beneficial synergetic effect for the reduction of cholesterol levels [5].

### ATR-FTIR spectroscopy

The ATR-FTIR spectra of the resin beads (Fig. 5) suggest the integrity and structure of the adsorbent were kept after being subjected to the simulated digestion media (different pH and enzyme activity). Additionally, the extensive release of FA during the intestinal step suggested by the HPLC-DAD results is supported by the disappearance of the peaks at 1267/cm and 1205/cm from the spectra between the gastric and intestinal steps. It was previously suggested that bile acids were adsorbed to Lewatit after the intestinal incubation step; however, the spectral analysis does not clearly reveal the peaks related to these



compounds, probably due to an overlap with the peaks from other substances.

## Conclusions

To the best of our knowledge, this is the first study of the in vitro release of FA from an adsorbent resin in simulated GI media. This work proved that free FA immobilized on a non-charged material can successfully overpass the low pH of the stomach and be released in the small intestine, where its action is much needed. The dietary intake of FA presents some limitations in terms of its bioaccessibility, and this study showed that using appropriate solid adsorbents for the delivery of the free compound, this problem can be potentially addressed.

## Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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**Author contributions** GNM did the experimental work. VS helped with the chromatographic analyses and with the gastrointestinal digestion simulation, statistical analysis, and manuscript revision. GNM and PCC contributed for the analysis and discussion of results and writing of the manuscript. PCC coordinated the work and did the final revision of the manuscript. All authors have approved the final version of the manuscript.

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## Compliance with ethical standards

**Conflict of interests** The authors declare no conflict of interest.

**Compliance with ethics requirements** No ethical issues derive from this work.

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