Effects of Cross-linking of Rice Protein with Ferulic Acid on Digestion and Absorption of Ferulic Acid

Xiao Ling
Guangxi University
Jiajia Zhang
Guangxi University
Jian-Wen Teng
Guangxi University
Li Huang
Guangxi University
Ning Xia (xianing@gxu.edu.cn)
Guangxi University

Research Article

Keywords: Ferulic acid, Rice protein, Antioxidative activities, Cell permeability

Posted Date: November 10th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-2222512/v1

Abstract

Though rice proteins have been applied to improve the stability of phenolic compounds, the effects of rice proteins on the digestibility and bioavailability of phenolic acid have not been clear. This study devoted to understanding the effects of protein interaction with ferulic acid on the digestion and absorption of ferulic acid in gastrointestinal environment. Ferulic acid were formed complexes with rice proteins with and without the presence of laccase at room temperature. It was found that rice protein could protect ferulic acid from degradation in simulated oral fluid and remain stable in gastrointestinal fluids. With the hydrolysis of pepsin and pancreatin, rice protein-ferulic acid complexes degraded and released ferulic acid in gastrointestinal environment. The DPPH scavenging activity digested rice protein-ferulic acid complexed was maintained while that of digested ferulic acid was significantly decreased. Moreover, the permeability coefficient of ferulic acid was not affected by rice peptides. Thus, rice protein is a promising food matrix to protect ferulic acid in digestion tract and maintain the antioxidant functions of ferulic acid.

1 Introduction

Ferulic acid (4-hydroxy, 3-methoxy cinnamic acid) is a monophenolic compound, which is widely present in plant-based food, such as grain bran, banana, eggplant, beer and coffee. There are intensive research work focusing on the bioactivity and health benefits of ferulic acid, such as antioxidant, anticancer, anti-inflammatory, antimicrobial, would healing, cholesterol lowering, blood sugar control. [1] Ferulic acid exists in free form and covalently bound to hemicellulosic polysaccharides or hydroxy acids. The antioxidant property of ferulic acid is mainly attributed to the phenolic nucleus and its unsaturated side chain conjugation, which readily forms a resonance stabilized phenoxy radical. [2] Thus, ferulic acid possesses ability to terminate free radical chain reactions. Ferulic acid has potential to be used in pharmaceutical, biomedical and food industries for photoprotection and lipid oxidation inhibition. However, the low water solubility, instability and low bioavailability hindered the application of ferulic acid. [3] An approach to improve these physicochemical properties of ferulic acid is encapsulating ferulic acid in biopolymers, such as proteins. [3–5]

Rice proteins have been a research interest in recent years due to its nutritive value, health benefits and hypoallergenic nature. Rice proteins have been intensively applied in gluten-free food products. Rice proteins can be extracted from broken or debris rice and rice brans which are abundant and affordable. [6, 7] According to the Osborne solubility-based classification, rice proteins can be categorized into four fractions: water-soluble albumin (5–10%), salt solution-soluble globulin (7–17%), alkali/acid-soluble glutelin (75–81%) and alcohol-soluble prolamin (3–6%). [8] It was reported that glutelin acidic subunits (34–37 kDa) and globulin (26 kDa) were the major rice proteins that interacted with ferulic acid. [9]

Phenolic compounds, including ferulic acid, can bind to proteins and form complex through covalent and non-covalent interaction, such as hydrogen bonds and hydrophobic interaction. Moreover, protein can interact with phenolic compound covalently with the assistant of Laccase. Laccase is a copper-

containing oxidase that oxidize substrates by a one-electron removal mechanism leading to the formation of free-radicals which can further induce reactions leading to polymerization, hydration and fragmentation. Laccase has capacity to oxidize a wide variety of phenolic compounds and further contributed to the phenolic compounds and protein interactions. [10, 11] The interaction between ferulic acid and rice protein with the assistant of Laccase has not been reported yet.

Some efforts have been made to improve the solubility, bioavailability and stability of phenolic compounds using protein as encapsulating materials and delivery carriers. [4, 5, 9, 12] Shi *et al.* (2017) found that binding tea catechins to rice bran protein isolate could protect catechins from pH shift and enzyme digestion through gastrointestinal tract and significantly increase the catechins recovery after *in vitro* intestinal digestion.[13] Our previous work has demonstrated that ferulic acid can interact with rice proteins through hydrophobic interactions and covalent binding. The ferulic acid-rice proteins complex had high DPPH and ABTS scavenging ability. Moreover, the emulsion prepared by ferulic acid-rice protein complex effectively restrained fat oxidative degradation. [9] However, the effects of rice protein-ferulic acid interaction on the digestibility and bioavailability of ferulic acid are not sufficiently studies.

Thus, this study aimed to investigate the effects of rice protein-ferulic acid interaction on the antioxidative activity, digestibility and absorption of ferulic acid. The rice protein-ferulic acid complexes were formed with and without the assistant of Laccase. The binding effects of rice protein and ferulic acid were evaluated in terms of free ferulic acid. The DPPH and ABTS radical scavenging capacities of ferulic acid and ferulic acid-rice protein complexes were measured. The cytotoxicity and transportation of ferulic acid and digested ferulic acid-rice protein complexes were analysis through Caco-2 cell model.

2 Materials And Methods

2.1 Materials

Feruli scid was purchased from Yuanchenggongchuang Technology CO., Ltd. (Wuhan, Hubei, China). Rice was mixed varieties purchased from a local market (Nanning, Guangxi, China). Laccase, pepsin and pancreatin and Hank's balanced salt solution (HBSS) were from Sigma-Aldrich Co. (St. Louis, Missouri, US). Dulbecco's Modified Eagle's Medium (DMEM), Fetal bovine serum (FBS), nonessential amino acids (NEAA), L-glutamine, trypsin-EDTA and phosphate-buffered saline and 12-well Transwell plates were purchased from Corning (Shanghai, China). Human colorectal adenocarcinoma cell line Caco-2 was purchased from the National Collection of Authenticated Cell Cultures (Shanghai, China). All other chemicals used in this work were reagent grade and used without further purification.

2.2 Rice protein isolation

The rice protein was isolated according to the method described by Xia et al. [6] with slight modification. Defatted rice was mix with water to a ratio of 1:10 (w/w) and soaked for 12 h before blending. The pH of mixture was adjusted to 10 with NaOH and stirred for 2h at room temperature (25°). Then, the supernatant was collected after centrifugation at 4,000g for 20 min. The pH of the supernatant was

adjusted 4.8 with 2 mol/L HCl. Protein was precipitated overnight before centrifuge at 4,000g for 20 min. The precipitate was washed three times with water and redispersed at pH 7. The protein was dialyzed against deionized water at 4°C for 24 h before freeze-drying.

2.3 Preparation of rice protein/ferulic acid complex with and without Laccase

Rice protein solution was mixed with ferulic acid solution at a ratio of 1:1 (v/v) and stirred for 4h. [9] For the rice protein/ferulic acid complex formed with Laccase, Laccase powder was added to ferulic acid solution to reach Laccase/ferulic acid = 0.9/1 (w/w) before mixing with rice protein. In the mixtures, the rice protein concentration was 10 mg/ml and the ferulic acid concentrations were 0.05, 0.1, 0.2 and 0.5 mg/ml, respectively. The rice protein/ferulic acid complexes were prepared before used. The rice protein-ferulic acid complexes without and with Laccase were coded as RF and RFL, respectively. The ferulic acid sample without rice protein was noted as FA. The concentration of ferulic acid added to each sample was added as suffix. For example, the rice protein-ferulic acid formed with Laccase at a ferulic acid concentration of 0.05 mg/ml was named as RFL-0.05.

2.4 Determination of ferulic acid

The contents of free ferulic acid in the samples were determined by reverse phase - high pressure liquid chromatography (RP-HPLC, waters 2695, Waters, US) equipped with Zorbax SB-C18 (4.6 × 250 mm, 5 μm). 0.1 ml sample was mixed with 0.9 ml methanol. The solvent A and B were 0.4% acetic acid solution and acetonitrile, respectively. The linear gradient extended from 25–70% solvent B with a runtime of 13 min at a flow rate of 1 mg/ml. Following each run, the percentage of solvent B decreased to 25% in 1 min and equilibrated with 3 min. Elution of ferulic acid was monitored at 320 nm. [14]

2.5 *In vitro* gastrointestinal (GI) digestion

The *in vitro* gastrointestinal digestion experiment was performed according to the methods described previously with slight modification. [15] The GI digestion was split into three stages: salivary, gastric and duodenal digestion. For the salivary digestion, 20 ml sample was mixed with 6 ml of stimulated saliva solution. The stimulated saliva included KCl (89.6 g/l), KSCN (20 g/l), NaH_2PO_4 (88.8 g/l), Na_2SO_4 (57.0 g/l), NaCl (175.3 g/l), $NaHCO_3$ (84.7 g/l), urea (25.0 g/l), and 290 mg α-amylase. The salivary digestion was performed at 37°C for 3 min with constant shaking. For the gastric digestion, pepsin (0.5g, 14,8000U) dissolved in 0.1 mol/l HCl was added to the mixture. The pH of the mixture was adjusted to 2 with 6 mol/l HCl before incubated at 37°C for 2 h with constant shaking. Following gastric digestion, the pancreatic digestion was simulated by adjusting the pH to 7 (10 mol/l NaOH) and adding pancreatin (8 mg/ml) and Bile salts (50mg/ml) solution. The pancreatic digestion was performed at 37°C for 2 h. The digestion was terminated by added 4-benzenesulfonyl fluoride hydrochloride (AEBSF). The collected samples were centrifuged at 10,000g for 5 min. 0.1 ml of supernatant was injected to HPLC to analyze the amount of ferulic acid. The relative ferulic acid content was calculated by the following equation:

$$Relative ferulic acid content\,(\%) = \frac{A_i}{A_0} \times 100$$

Where A_i is the peak area of ferulic acid in digested sample and A_0 is the peak area of ferulic acid in sample before digestion. The digested samples were coded with a suffix D. The digested ferulic acid, RF and RFL was named as F-D, RF-D and RFL-D, respectively.

2.6 Measurement of antioxidative activity

2.6.1 Measurement of DPPH radical scavenging activity

The DPPH radical scavenging activity was determined according to the method described by Wu *et al*. (2015) with slightly modification. [16] Briefly, digested samples collected as described in section 2.5 was centrifuged at 10,000g for 5 min at 4°C. 2 ml of supernatant was added to 2 ml DPPH• solution (0.2 mmol/l). The mixture was incubated in dark for 30 min at room temperature. The absorbance of the sample mixture was measured at 517 nm (A_i). Ascorbic acid was used as reference. DPPH• solution was replaced by ethanol as blank. For control, sample was replaced by same volume of ethanol. The DPPH scavenging activity was calculated by the following equation:

$$DPPH scavenging activity\,(\%) = \left(\frac{A_0 - A_i}{A_0}\right) \times 100$$

Where A_i is the absorbance of sample and A_0 is the absorbance of control.

2.6.2 Measurement of $ABTS^{+}$• radical scavenging activity

The ABTS assay was performed following the method described by Wu et al. [16] ABTS free radicals stock solution was prepared by mixing 10 ml of 7 mmol/l ABTS + solution with 178 µl of 140 mmol/l potassium persulfate ($K_2S_2O_8$) in dark at room temperature for 13 h. The ABTS stock solution was diluted by PBS buffer (pH 7.4) to an absorbance of 0.7 ± at 734 nm before use. An aliquot of 0.5 ml of sample was mixed with 3.5 ml of diluted ABTS and incubated in dark at room temperature for 6 min before recording the absorbance at 734 nm. ABTS solution was replaced by PBS as blank. Ascorbic acid was used as reference. For control group, sample was replaced by same volume of PBS. The ABTS scavenging activity was calculated by the following equation:

$$ABTS free radical scavenging activity\,(\%) = \left(\frac{A_0 - A_i}{A_0}\right) \times 100$$

Where A_i is the absorbance of sample and A_0 is the absorbance of control.

2.7 Stability of ferulic acid

A series concentration of ferulic acid (0.05 mg/ml 0.1 mg/ml and 0.2 mg/ml) were stored in incubator (37°C, 5%CO_2 and 95% humidity) for 0, 30, 60, 90 and 120 mins. The ferulic acid content was measured

by HPLC following the method mentioned in section 2.4.

2.8 *In vitro* cytotoxicity of ferulic acid

Caco-2 cells were grown in flasks at 37°C in a humidified atmosphere of 5% CO_2. The cells were cultured in DMEM supplemented with 10% FBS (v/v), 1% NEAA, 1% L-glutamine and 1% antibiotic-antimycotic. The medium was changed every other day until the cells reached 80% confluence when the cells were removed by 1 ml trypsin in 1 mM EDTA solution for 5 min and subcultured at a ratio of 1:3 with fresh medium.

The cytotoxicity of ferulic acid was determined by MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltertrazolium bromide) assay following the method described by Yang *et al.* with slight modification. [17] Caco-2 cells were transferred to 96-well plates with a cell density of 10,000 cells per well with 200 µl fresh medium. Cells were allowed to grow until the cell confluence reach 100%. Various concentrations of ferulic acid were added in culture medium and incubated with cells for 2h. After incubation, 20 µl MTT solution (5 mg/ml) and incubated for another 4h. Then the supernatant was removed, and the formazan crystals were dissolved in 150 µl DMSO. The absorbance was recorded at 490 nm using a microplate reader (UV-1601, Shimadzu, Japan). The viability was expressed by the percentage of living cells with respect to the control cells (without addition of ferulic acid).

2.9 Transport efficiency study

The transport efficiency of ferulic acid and rice protein-ferulic complexes were determined using Caco-2 cell monolayer. [18] Caco-2 cells were seeded onto a Transwell® support membrane with a density of 2.5 × 10^5 cells per insert. The cell culture medium was changed every two days. The cells were allowed to grow and differentiate for 21 days. On the day of experiment, four groups of samples were prepared: ferulic acid, ferulic acid digesta, RF digesta and RFL digesta. For each group of samples, four levels of ferulic acid (0.05, 0.1, 0.2 and 0.5 mg/ml) were prepared, respectively. Before experiment, the apical and basolateral sides of the Transwell® membrane were washed by HBSS for 3 times. The cells were incubated in HBSS for 30 mins in incubator. After removing the HBSS, 0.5 ml of sample was added to the apical side, and 1.5 ml of HBSS buffer was added to the basolateral chamber. The plate was incubated in the incubator. 0.5 ml of samples was taken from the basolateral chamber at 0, 30, 60, 90 and 120 min respectively to measure the ferulic acid content by HPLC following the method mentioned in section 2.4. Same volume of HBSS was added back to the basolateral chamber after sampling. The accumulated ferulic acid transport amount was calculated by the following equation:

$$Qr = 0.5 \times (C_{t1} + C_{t2} + \cdots + C_{ti-1}) + 1.5 \times C_{ti}$$

Where 0.5 is the sample volume (ml) and 1.5 is the volume of the buffer in basolateral chamber. C_{ti} represents the concentration of ferulic acid (µg/ml) in basolateral chamber at time point *i*.

The apparent permeability (P_{app}) values were calculated according to the following equation:

$$P_{app} = \left(\frac{dQ}{dt}\right) \times \frac{1}{C_0} \times \frac{1}{A}$$

Where dQ/dt is the permeability rate (mg/s), C_0 is the initial concentration of ferulic acid in the apical chamber, and A is the surface area of the Transwell® membrane (1.12 cm^2).

2.10 Statistical analysis

Experiments were performed in at least three independent batches. Data were represented as the mean ± standard deviation. Duncans multiple range test was employed in multiple comparisons (three or more groups). Statistical differences between samples were performed with a level of significance as $p < 0.05$.

3 Results And Discussion

3.1 Effects of rice proteins and laccase on the relative content of ferulic acid

HPLC was employed to determine the relative content of free ferulic acid before and after forming complexes with rice proteins. As shown in Fig. 1, for 0.05 to 0.5 mg/ml ferulic acid concentration, the relative contents of ferulic acid were about 100% without the addition of rice protein and laccase. After adding rice proteins, the relative contents of ferulic acid reduced to 81.8, 63.2, 71.2 and 82.6% for ferulic acid concentrations of 0.05, 0.1, 0.2 and 0.5 mg/ml respectively. This result indicated that ferulic acid and rice proteins interacted and formed complexes spontaneously, which was agreed with the previous studies that ferulic acid could bind to rice proteins through hydrophobic interactions and covalent bonds. [9, 19] The amounts of ferulic acid participated in the formation of RF complexes increased from 0.037 to 0.085 mg/ml as the ferulic acid concentration increased from 0.1 to 0.5 mg/ml, demonstrating that higher the spontaneous binding between ferulic acid and rice protein was not saturated even when the concentration of ferulic acid increased to 0.5 mg/ml. Ferulic acid may impact rice proteins conformation and allow rice proteins to expose more binding areas. [9]

Moreover, the relative contents of ferulic acid significantly decreased after adding laccase. The relative contents of ferulic acid decreased from about 41% to less than 5% when ferulic acid concentration increased from 0.05 to 0.5 mg/ml. Laccase can efficiently induce the oxidation and polymerization of ferulic acid. The ferulic acid oxidative derivates can interact with proteins. [10, 11] As the concentrations of ferulic acid and laccase increased, the RFL complexes presented a yellow-orange color (supplementary Fig. 2) which was caused by laccase-mediated ferulic acid oxidative derivatives.

3.2 Effects of rice proteins and laccase on the digestibility of ferulic acid

3.2.1 In vitro digestibility of ferulic acid, RF and RFL complexes in simulated oral fluid

The relative contents of ferulic acid after simulated oral digestion were shown in Fig. 2. As compared with Fig. 1, the relative contents of ferulic acid reduced from 100% to 93.5, 82.4, 86.1 and 91.4% for samples with ferulic acid concentration of 0.05, 0.1, 0.2 and 0.5 mg/ml respectively. Ferulic acid was not stable in the simulated saliva fluid. From 0.1 to 0.5 mg/ml, the higher ferulic acid concentration, the less amount of ferulic acid degraded during oral digestion.

Oppositely, as compared to the undigested samples, the relative contents of ferulic acid increased to 94.1, 71.1 and 86.4% for RF-0.05, RF-0.1 and RF-0.2, respectively, after oral digestion. This result indicated that the formation of rice protein-ferulic acid complexes partially protect ferulic acid from degradation in simulated saliva fluids. Moreover, rice protein-ferulic acid interactions were disrupted at pH 2. When the oral digestion was terminated by adjusting to pH 2, ferulic acid was partially released from RF complexes. The relative contents of ferulic acid in RF complexes with ferulic acid concentration of 0.5 mg/ml had no significant changes.

The relative contents of ferulic acid were significantly increased to 52.0, 27.8, 27.6 and 5.0% for RFL complexes with ferulic acid concentration of 0.05, 0.1, 0.2 and 0.5 mg/ml, respectively, after oral digestion. This result demonstrated that RFL complexes were not stable and release ferulic acid and/or its oxidative derivates under acid condition.

3.2.2 In vitro digestibility of ferulic acid, RF and RFL in simulated gastric fluid

As shown in Fig. 3, after incubating in simulated gastric fluid for 2 hours, the relative contents of ferulic acid had no significant changes in samples FA-0.1 to FA-0.5 as compared to those after oral digestion. Though ferulic acid was partially degraded when the oral digestion was terminated, the ferulic acid was relatively stable in simulated gastric fluid. The enzymes and salts in simulated gastric fluid had no significant impact on ferulic acid.

For RF complexes, the relative contents of ferulic acid significantly increased to 76.3, 90.1 and 88.6% for samples RF-0.1, RF-0.2 and RF-0.5, respectively, after gastric digestion. This result demonstrated that the RF complexes were not stable in gastric environment. Rice proteins were hydrolyzed by pepsin and the interaction of rice protein and ferulic acid may be interrupted at the low pH. Thus, the bonded ferulic acids were released to the simulated gastric fluid and be detected by HPLC. Previous study had also reported that phenols linked to biomacromolecules, such as proteins and carbohydrates, might be released by enzymatic hydrolysis, resulting in a significant increase in concentration after gastric digestion. [20]

For RFL complexes, the relative contents of ferulic acid have a slight increase to 53.6, 28.0, 29.8 and 5.2% for samples with ferulic acid concentration of 0.1, 0.2 and 0.5 mg/ml respectively. As compared to RF,

RFL complexes were relative more stable in gastric condition. The increase of relative contents of ferulic acid for RFL complexes was not as significant as in RF.

3.2.3 In vitro digestibility of ferulic acid, RF and RFL in simulated small intestinal fluid

By comparing Figs. 3 and 4, after intestinal digestion for 2 hours, the relative contents of ferulic acid had no significant changes in samples FA with ferulic acid concentration of 0.1, 0.2 and 0.5 mg/ml, respectively. This result indicated that ferulic acid was stable in simulated intestinal fluid. The neutral pH, pancreatin and bile salts in simulated small intestinal fluid did not show negative impact on the stability of ferulic acid. Overall, FA had about 10% degradation after the whole *in vitro* gastrointestinal digestion.

For RF complexes, the relative contents of ferulic acid were 96.3, 76.3, 99.9 and 87.7% for samples with ferulic acid concentration of 0.1, 0.2 and 0.5 mg/ml respectively. Except for RF-0.2, the other three RF complexes had no significant changes after intestinal digestion, indicating that RF complexes were resistant to the pancreatin hydrolysis and stable in simulated intestinal fluid. The digestion by pancreatin did not further destroy the interaction between ferulic acid and rice proteins. It has been reported that ferulic acid inhibited the activities of trypsin, which prevented rice protein from further degradation. [21]

For RFL complexes, the relative contents of ferulic acid were 52.1, 28.1, 30.9 and 5.2% for samples with ferulic acid concentration of 0.1, 0.2 and 0.5 mg/ml respectively. The changes were not significant between gastric and intestinal fluids, though the free ferulic acid content in RFL complexes samples were much lower than those of FA and RF. There might be two reasons. Firstly, the interactions between rice proteins and ferulic acid with the assistant of laccase were stable to pH shifting and proteases digestion. Secondly, after GI digestion, ferulic acid was released in the form of its oxidative derivates rather than free ferulic acid. Thus, the amounts of ferulic acid oxidative derivates in the RFL samples before and after GI digestion were determined. As shown in Fig. 5, the amounts of ferulic acid oxidative derivates significantly increase after oral and gastric digestion, indicating that the simulated oral and gastric fluids had destructive effects on the RFL structure and released the oxidized ferulic acid. However, after intestinal digestion, there was no significant increase of ferulic acid oxidative derivates, indicating that the RFL complexes were stable to the hydrolysis of pancreatin and neutral pH. Thus, both RF and RFL complexes had no significant increase of ferulic acid after intestinal digestion.

In summary, ferulic acid significantly decrease after oral digestion but it is relatively stable in both gastric and intestinal condition. On the other hand, RF and RFL protected ferulic acid from degradation in oral digestion and gradually released ferulic acid and/or ferulic acid oxidative derivates during oral and gastric digestion. Both RF and RFL were stable in intestinal digestion with no further increase of free ferulic acid. Moreover, this study showed that ferulic acid inhibited the activity of trypsin while pepsin was still active in the presence of ferulic acid.

3.3 Effects of rice proteins and laccase on the antioxidant activity of ferulic acid

Ferulic acid derives most of its antioxidant activity potentially from its radical scavenging ability.[2] DPPH and ABTS are two kinds of stable radicals that are used independently to evaluate the antioxidant activity of plant extract.[16] Thus, this study determined the antioxidant activities of ferulic acid, RF and RFL complexes by using both DPPH and ABTS radical scavenging assay. As mentioned above, the relative contents of ferulic acid in RF and RFL changed after digestion. Thus, the antioxidant activity of RF and RFL complexes after gastrointestinal digestion were also evaluated.

3.3.1 DPPH scavenging activity

The DPPH scavenging activities of FA, RF and RFL were showed in Table 1. For the FA samples, the DPPH scavenging activities increased as the ferulic acid concentration increased. The DPPH scavenging activities of FA were 38.0, 46.8, 48.1 and 94.6 with the ferulic acid concentration of 0.05, 0.1, 0.2 and 0.5mg/ml, respectively. The highest DPPH scavenging activities reached 95% at FA concentration of 0.5, which was the similar to that of the reference ascorbic acid (0.5 mg/ml, 96%). The DPPH scavenging activities of RF-0.05, RF-0.1, RF-0.2 and RF-0.5 were 43, 51, 54 and 55%, respectively. When the ferulic acid concentrations were from 0.05 to 0.2 mg/ml, the DPPH scavenging activities of RF complexes were higher than those of FA alone, though the relative ferulic acid content in RF complexes were lower than those of FA. This result demonstrated that both rice proteins and ferulic acid contributed to the DPPH scavenging activities of RF complexes by donating a hydrogen radical or an electron to DPPH free radicals and converting them into more stable molecules. In contrast, RFL had the lowest DPPH scavenging activities under all ferulic acid concentrations. The DPPH activities of RFL-0.05, RFL-0.1, RFL-0.2 and RFL-0.5 were 17, 18, 26 and 27%, respectively. The binding of ferulic acid to protein with the assistant of laccase resulted in the loss of antioxidative activities.

Table 1
DPPH radical scavenging activity of FA, RF and RFL before digestion

Ferulic acid (mg/mL)	DPPH scavenging activity (%)		
	FA	RF	RFL
0	$11.11 \pm 1.30^{*Aa}$	$12.37 \pm 0.86^{**Aa}$	$12.37 \pm 0.86^{**Aa}$
0.05	37.97 ± 1.94^{Ba}	42.68 ± 0.82^{Bb}	17.06 ± 0.77^{Bc}
0.1	46.80 ± 1.24^{Ca}	50.87 ± 0.44^{Cb}	18.25 ± 0.62^{Bc}
0.2	48.10 ± 0.98^{Ca}	53.85 ± 0.97^{Db}	26.41 ± 1.57^{Cc}
0.5	94.56 ± 0.65^{Da}	55.45 ± 1.60^{Db}	27.02 ± 1.88^{Cc}
*Control			
**Rice protein only			
Lower-case letters (expressed as a, b and c) showed significant differences ($p < 0.05$) among different samples at the same ferulic acid concentration. Upper-case letters (expressed as A, B, C and D) showed significant differences ($p < 0.05$) among the same type of sample with different ferulic acid concentrations.			

It has been intensively studied that the antioxidative activities of nutraceutical compounds may be damaged by some external factors, such as pH, oxygen, temperature, and lights. However, the effects of digestion process on the antioxidant activity have not been sufficiently investigated.[22] Thus, current study devoted to evaluating the antioxidant activity of ferulic acid and its rice protein complexes after *in vitro* gastrointestinal digestion. As illustrated in Table 2, the simulated gastrointestinal fluid without ferulic acid had a DPPH scavenging activity of about 65%. The DPPH scavenging activity of FA after digestion was not changed as the ferulic acid concentration increased. The DPPH scavenging activity of F-D-0.5 decreased to 64% after digestion as compared to 94% before digestion. This result demonstrated that the antioxidative activity of ferulic acid was damaged by digestion. The decreasing antioxidant activity after intestinal digestion might be caused by the fact that ferulic acid may be transformed into different structural forms with chemical properties due to its sensitivity to GI digestion fluids. [20]

Table 2
DPPH radical scavenging activity of FA, RF and RFL after intestinal digestion

Ferulic acid (mg/mL)	DPPH scavenging activity (%)		
	F-D	RF-D	RFL-D
0	$64.87 \pm 4.11^{*Aa}$	$62.75 \pm 1.79^{**Aa}$	$62.75 \pm 1.79^{**Aa}$
0.05	65.41 ± 1.52^{Aa}	69.58 ± 0.93^{Bb}	71.77 ± 1.91^{Bb}
0.1	64.39 ± 1.52^{Aa}	67.18 ± 1.16^{Bb}	72.58 ± 1.22^{Bc}
0.2	62.69 ± 2.02^{Aa}	68.72 ± 4.58^{Bb}	68.22 ± 0.30^{Cb}
0.5	63.87 ± 2.21^{Aa}	71.15 ± 0.65^{Bb}	72.38 ± 1.60^{Bb}
*Control			
**Rice protein only			
Lower-case letters (expressed as a, b and c) showed significant differences ($p < 0.05$) among different samples at the same ferulic acid concentration. Upper-case letters (expressed as A, B, C and D) showed significant differences ($p < 0.05$) among the same type of sample with different ferulic acid concentrations.			

Moreover, though it has been reported that peptides derived from rice proteins possessed high antioxidant activity[23], current study found that the DPPH scavenging activity of digested rice protein (63%) had no significant different from the simulated gastrointestinal fluid. The DPPH scavenging activities of RF and RFL after digestion were higher than that of digested rice proteins, simulated gastrointestinal fluids and FA samples. As mentioned above, ferulic acid was released from RF and RFL complexes during gastric digestion and was stable after intestinal digestion. The released ferulic acid participated in the DPPH scavenging activity, leading to a conclusion that rice proteins had protective effects on the antioxidant activity of ferulic acid. Aceituno-Medina *et al.* (2015) [22] had reported that encapsulation within food-grade electrospun fibers could maintain the antioxidant capacity of ferulic acid during intestinal digestion. Therefore, this study innovatively elaborated that rice proteins could act as a protective matrix to maintain the oxidative activity of ferulic acid through the digestion systems.

3.3.1 ABTS scavenging activity

Besides DPPH scavenging assay, the ABTS assay is another widely used colorimetric assay for the assessment of the antioxidant capacities of natural products based on quenching of stable radicals (ABTS•+). Table 3 showed that the rice protein possessed a ABTS scavenging activity of 22%, indicating that rice protein had the ability to neutralized ABTS free radical, which agreed with previous study. [24] The ABTS scavenging activities of FA-0.05 and RF-0.05 were 59 and 68%, respectively. Ferulic acid was well know for its free radical scavenging activity, which is attributed to its antioxidant activity. Though RF-0.05 had lower ferulic acid than FA-0.05, both rice protein and ferulic acid participated in the ABTS

scavenging activity of RF. Therefore, RF-0.05 had higher ABTS scavenging activity than FA-0.05, which was similar to DPPH scavenging activity. In contrast, RFL-0.05 had lowest ABTS scavenging activity of 36%, which was caused by its low ferulic acid content (40%) as compared to RF-0.05 and FA-0.05. When the ferulic acid concentration increased to 0.2, FA and RF could convert almost all the ABTS free radicals into stable molecules. For RFL, the ABTS scavenging activity increased as the ferulic acid concentration increased. However, RFL-0.5 had the lowest ABTS scavenging activity of 40%, since ferulic acid was oxidized by laccase and lost antioxidant activity.

Table 3
ABTS radical scavenging activity of FA, RF and RFL before digestion

Ferulic acid (mg/ml)	**ABTS scavenging activity (%)**		
	FA	**RF**	**RFL**
0	$7.31 \pm 0.81^{*Aa}$	$22.45 \pm 0.33^{**Ab}$	$22.45 \pm 0.33^{**Ab}$
0.05	58.63 ± 0.68^{Ba}	67.50 ± 0.62^{Bb}	36.01 ± 1.17^{Bc}
0.1	88.91 ± 0.41^{Ca}	89.30 ± 0.82^{Ca}	37.24 ± 1.03^{Bb}
0.2	99.60 ± 0.21^{Da}	99.79 ± 0.22^{Da}	42.05 ± 0.70^{Cb}
0.5	99.77 ± 0.04^{Da}	99.75 ± 0.31^{Da}	40.43 ± 0.82^{Db}
*Control			
**Rice protein only			
Lower-case letters (expressed as a, b and c) showed significant differences ($p < 0.05$) among different samples at the same ferulic acid concentration. Upper-case letters (expressed as A, B, C and D) showed significant differences ($p < 0.05$) among the same type of sample with different ferulic acid concentrations.			

As shown in Table 4, the simulated gastrointestinal fluid had high ABTS scavenging activity of 83%. Digested rice protein had significantly high ABTS scavenging activity of 86%, indicating that rice peptide had antioxidant activity. [23, 24] F-D-0.05 has a ABTS scavenging activity of 92%. This result demonstrated that ferulic acid still had ABTS scavenging activity after intestinal digestion, which were different from DPPH scavenging activity. RF-D and RFL-D also possessed high ABTS scavenging activities. RF-D-0.1 and RFL-D-0.2 reached 100% ATS scavenging activity.

Table 4
ABTS radical scavenging activity of FA, RF and RFL after intestinal digestion

Ferulic acid (mg/ml)	ABTS scavenging activity (%)		
	F-D	RF-D	RFL-D
0	$82.67 \pm 0.27^{*Aa}$	$85.88 \pm 0.34^{**Ab}$	$85.88 \pm 0.34^{**Ab}$
0.05	92.40 ± 0.46^{Ba}	94.43 ± 0.19^{Bb}	90.59 ± 0.26^{Bc}
0.1	99.44 ± 0.12^{Cac}	99.20 ± 0.10^{Ca}	94.40 ± 0.43^{Cb}
0.2	99.98 ± 0.07^{Da}	99.86 ± 0.03^{Db}	99.93 ± 0.07^{Dab}
0.5	99.71 ± 0.24^{CDa}	99.76 ± 0.06^{Da}	99.90 ± 0.04^{Da}
*Control			
**Rice protein only			
Lower-case letters (expressed as a, b and c) showed significant differences ($p < 0.05$) among different samples at the same ferulic acid concentration. Upper-case letters (expressed as A, B, C and D) showed significant differences ($p < 0.05$) among the same type of sample with different ferulic acid concentrations.			

3.4 Effects of rice proteins and laccase on bioavailability of ferulic acid

3.4.1 Cytotoxicity of ferulic acid

Ferulic acid is a common polyphenolic compound found in many foods, beverages and plants, which generally considered as safe. However, it has been reported that ferulic acid had some inhibitory effects on essential enzymes in the biosynthetic reactions at doses of 0.1–1.1 mM. [25] Thus, this study investigated the cytotoxicity of ferulic acid on Caco-2 cell line under a wide range of concentration. As showed in Table 5, the viability of Caco-2 cells was more than 100% when the ferulic acid concentration ranged from 0.025 to 2 mg/ml. The ferulic acid concentration used in this study (0.05 to 0.5 mg/ml) have almost no cytotoxic activity to Caco-2 cells. However, when the ferulic acid concentration increased to 5 mg/ml, it caused damage to Caco-2 cells.

Table 5
Effects of FA on Caco-2 viability (n = 6)

Ferulic acid (μg/mL)	Viability (%)	Ferulic acid (μg/mL)	Viability (%)
25	114.18 ± 9.88^{ab}	500	150.83 ± 26.76^{c}
50	119.43 ± 21.27^{ab}	1000	136.55 ± 17.04^{bc}
100	129.39 ± 11.25^{bc}	2000	105.61 ± 21.36^{a}
200	132.59 ± 8.46^{bc}	5000	66.30 ± 9.59^{d}
Lower-case letters (expressed as a, b and c) showed significant differences ($p < 0.05$) among different samples.			

3.4.2 *In vitro* cell evaluation

Intestinal epithelium is one of the barriers for oral nutraceutical delivery, thus many nutraceuticals suffer from low intestinal permeability and consequently low bioavailability.[26] Thus, the permeabilities of ferulic acid in RF and RFL through intestinal barrier were evaluated by the Caco-2 cell monolayer as an *in vitro* model of the small intestinal epithelium, and compared with pure ferulic acid. Both the time dependent transport amounts (Fig. 6) and the apparent permeabilities (Table 6) of FA, F-D RF-D and RFL-D had been measured.

Table 6
Apparent permeability coefficient (P_{app}) of FA F-D RF-D and RFL-D

Ferulic acid (mg/mL)	$P_{app} \times 10^{-5}$ (cm/s)			
	FA	F-D	RF-D	RFL-D
0.05	25.58 ± 0.44^{Aa}	24.92 ± 0.21^{Ab}	25.52 ± 0.14^{Aa}	24.09 ± 0.00^{Ac}
0.1	15.79 ± 0.24^{Ba}	15.78 ± 0.11^{Ba}	16.30 ± 0.48^{Ba}	14.59 ± 0.00^{Bb}
0.2	9.04 ± 0.18^{Ca}	9.98 ± 0.40^{Cb}	9.50 ± 0.28^{Cab}	9.14 ± 0.20^{Ca}
0.5	5.10 ± 0.17^{Da}	4.95 ± 0.32^{Da}	4.82 ± 0.14^{Da}	3.15 ± 0.00^{Db}
Lower-case letters (expressed as a, b and c) showed significant differences ($p < 0.05$) among different samples at the same ferulic acid concentration. Upper-case letters (expressed as A, B, C and D) showed significant differences ($p < 0.05$) among the same type of sample with different ferulic acid concentrations.				
Supplementary Information				

As showed in Fig. 6A, the transport of ferulic acid was rapid and linear up to 2 hrs for all the tested concentration. The accumulated transport amounts did not significantly increase when the ferulic acid concentration increased from 0.05 to 0.2 mg/ml; however, the cumulative transport amount significantly

grew when the ferulic acid concentration reached 0.5 mg/ml. This result indicated that ferulic acid was permeable through the intestinal epithelium as the free form. The transport was linear over time within two hours and was not saturated at 0.5 mg/ml, which was similar to the results reported previously through a Caco-2/HT29-MTX cells cocultures model. [27]

The intestinal digested ferulic acid samples (F-D) showed similar time dependent transport profile. At 30 min, the transport amounts of ferulic acid in F-D at all concentrations were similar to FA; nevertheless, the accumulated transport amounts of F-D were significantly higher than those of FA at 90 and 120 min. Although the relative ferulic acid in F-D-0.5 was less than free ferulic acid, Q_t of F-D-0.5 was approximately 5 μg more than that of FA at 120 min. This result suggested that the gastrointestinal fluids facilitated the transport of ferulic acid.

As showed in Fig. 6C, the transport amounts of digested RF (RF-D) were closed to those of F-D at all ferulic acid concentration. The transport of RF-D was also time-dependent and concentration dependent. Though the relative contents of ferulic acid in RF-D were lower than F-D, the transport amounts of RF-D were similar to those of F-D. In contrast, the transport amounts of digested RFL (RFL-D) were significantly lower than those of RF-D and F-D, which was caused by the lower content of ferulic acid in RFL-D samples. The transport of ferulic acid in RFL-D was time-dependent, but not concentration dependency.

Table 6 showed the apparent permeability of FA, F-D, RF-D and RFL-D. For all these four samples, the P_{app} decreased as the initial ferulic acid concentration increased. The permeability coefficients were higher than 1×10^{-6} cm/second; thus, ferulic acid could be considered as well-absorbed compounds as defined by Yee [28]. FA, F-D and RF-D showed similar P_{app} while that of RFL-D was slightly lower. Firstly, this result indicated that the digested rice protein, the rice peptides, did not obstacle the transport of ferulic acid. Secondly, the laccase or the oxidative derivatives acted antagonistically to the permeability of ferulic acid. This result indicated that digested RFL complexes or the oxidation of ferulic acid might obstacle the transport of ferulic acid. As the concentration of ferulic acid increased, the permeability coefficients of four samples decreased, suggesting that the transport of ferulic acid was concentration dependent and saturable. This result was in agreement with the finding of Konishi *et al*. (2003) that ferulic acid would be transported across Caco-2 cells monolayer with the assistant of monocarboxylic acid transporters (MCTs).[29, 30]

In summary, the *in vitro* evaluation through Caco-2 cells showed that ferulic acid had no significant cytotoxicity to Caco-2 cell viability. Moreover, ferulic acid, digested ferulic acid and digested RF had similar permeability coefficients which was concentration dependent. The digested RFL had slightly lower P_{app}. Rice peptides had no negative impact on ferulic acid bioavailability. The transport of ferulic acid involved transporters.

4 Conclusions

In this study, rice protein-ferulic acid complexes with and without the facilitation of laccase had been prepared. Laccase could efficiently induce the oxidation and polymerization of ferulic acid and promote the interactions with rice proteins. Ferulic acid was partially degraded in simulated oral fluid, but it was relatively stable in gastrointestinal fluids. On the other hand, RF and RFL had protection effects on ferulic acid in oral condition and release the ferulic acid in the gastrointestinal environment. Moreover, this study had found that the digestion in GI fluid had profoundly negative impacts on the DPPH scavenging activity of ferulic acid. The DPPH scavenging activity of FA-0.5 decreased from 94–64% after digestion. Forming complexes with rice proteins with or without laccase can maintain the antioxidative activity of ferulic acid. In contrast, the ABTS scavenging activity of ferulic acid was not affected by *in vitro* digestion. Both rice peptide and digested ferulic acid possessed strong ABTS scavenging activity.

The cytotoxicity and permeability of ferulic acid on intestinal cells were evaluated through *in vitro* Caco-2 cell line. Ferulic acid did not have significant cytotoxicity on Caco-2 cells in the concentration ranging from 0.025 to 2 mg/ml. This study also found that ferulic acid, digested ferulic acid (F-D) and the digested RF complex (RF-D) showed similar time-dependent transport profile and apparent permeability while those of RFL-D were slightly lower. FA, F-D, RF-D and RFL-D were considered as well-absorbed materials. This result demonstrated that rice peptides did not influence the transport of ferulic acid.

This study showed the protective effects of rice proteins on the antioxidant activities of phenolic acids through gastrointestinal environment. The knowledge generated by this study can be used to guide industry to develop functional food with natural phenolic acid for improved antioxidant activities.

Declarations

Funding

This research was financially supported by the Chinese National Natural Science Foundation of China (Serial No. 31760433), the Important Science and Technology Special Project Fund of Guangxi Province, China (Serial No. AA20302012-3)

Data Availability

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Competing interests The authors declare no competing interests.

Ethics Approval Not applicable.

Consent to Participate Not applicable.

Consent for Publication Not applicable.

Authors' contributions Xiao Ling: Methodology, Writing-Original draft, Formal analysis.Jiajia Zhang: Methodology, Writing-Original draft, Data curation. Jian-Wen Teng: Investigation. Li Huang: Reviewing. Ning Xia: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Critical Reviewing-Editing.

Conflict of Interest The authors declare no confict of interest.

References

1. Mancuso C, Santangelo R (2014) Ferulic acid: Pharmacological and toxicological aspects. Food Chem Toxicol 65:185–195. doi: https://doi.org/10.1016/j.fct.2013.12.024
2. Graf E (1992) Antioxidant potential of ferulic acid. Free Radic Biol Med 13(4):435–448. doi: https://doi.org/10.1016/0891-5849(92)90184-I
3. Yu JY, Roh SH, Park HJ (2021) Characterization of ferulic acid encapsulation complexes with maltodextrin and hydroxypropyl methylcellulose. Food Hydrocolloids 111:106390. doi: https://doi.org/10.1016/j.foodhyd.2020.106390
4. Kanakis CD, Hasni I, Bourassa P, Tarantilis PA, Polissiou MG, Tajmir-Riahi H-A (2011) Milk β-lactoglobulin complexes with tea polyphenols. Food Chem 127(3):1046–1055. doi: https://doi.org/10.1016/j.foodchem.2011.01.079
5. Tantoush Z, Stanic D, Stojadinovic M, Ognjenovic J, Mihajlovic L, Atanaskovic-Markovic M et al (2011) Digestibility and allergenicity of β-lactoglobulin following laccase-mediated cross-linking in the presence of sour cherry phenolics. Food Chem 125(1):84–91. doi: https://doi.org/10.1016/j.foodchem.2010.08.040
6. Xia N, Wang J, Yang X, Yin S, Qi J, Hu L et al (2012) Preparation and characterization of protein from heat-stabilized rice bran using hydrothermal cooking combined with amylase pretreatment. J Food Eng 110(1):95–101. doi: https://doi.org/10.1016/j.jfoodeng.2011.12.004
7. Xia N, Wang J-M, Gong Q, Yang X-Q, Yin S-W, Qi J-R (2012) Characterization and In Vitro digestibility of rice protein prepared by enzyme-assisted microfluidization: Comparison to alkaline extraction. J Cereal Sci 56(2):482–489. doi: https://doi.org/10.1016/j.jcs.2012.06.008
8. Amagliani L, O'Regan J, Kelly AL, O'Mahony JA (2017) The composition, extraction, functionality and applications of rice proteins: A review. Trends Food Sci Technol 64:1–12. doi: https://doi.org/10.1016/j.tifs.2017.01.008
9. Jia X, Zhao M, Xia N, Teng J, Jia C, Wei B et al (2019) Interaction between plant phenolics and rice protein improved oxidative stabilities of emulsion. J Cereal Sci 89:102818. doi: https://doi.org/10.1016/j.jcs.2019.102818
10. Cura DE, Lantto R, Lille M, Andberg M, Kruus K, Buchert J (2009) Laccase-aided protein modification: effects on the structural properties of acidified sodium caseinate gels. Int Dairy J 19(12):737–745. doi: 10.1016/j.idairyj.2009.06.007

11. Sato ACK, Perrechil FA, Costa AAS, Santana RC, Cunha RL (2015) Cross-linking proteins by laccase: Effects on the droplet size and rheology of emulsions stabilized by sodium caseinate. Food Res Int 75:244–251. doi: https://doi.org/10.1016/j.foodres.2015.06.010
12. Ozdal T, Capanoglu E, Altay F (2013) A review on protein–phenolic interactions and associated changes. Food Res Int 51(2):954–970. doi: https://doi.org/10.1016/j.foodres.2013.02.009
13. Shi M, Huang L-Y, Nie N, Ye J-H, Zheng X-Q, Lu J-L et al (2017) Binding of tea catechins to rice bran protein isolate: Interaction and protective effect during in vitro digestion. Food Res Int 93:1–7. doi: 10.1016/j.foodres.2017.01.006
14. Li Y, Bi K (2003) HPLC determination of ferulic acid in rat plasma after oral administration of Rhizoma Chuanxiong and its compound preparation. Biomed Chromatogr 17(8):543–546. doi: 10.1002/bmc.274
15. Tenore GC, Campiglia P, Giannetti D, Novellino E (2015) Simulated gastrointestinal digestion, intestinal permeation and plasma protein interaction of white, green, and black tea polyphenols. Food Chem 169:320–326. doi: https://doi.org/10.1016/j.foodchem.2014.08.006
16. Wu Z, Teng J, Huang L, Xia N, Wei B (2015) Stability, antioxidant activity and in vitro bile acid-binding of green, black and dark tea polyphenols during simulated in vitro gastrointestinal digestion. RSC Adv 5(112):92089–92095. doi: 10.1039/c5ra18784b
17. Yang J, Zhou Y, Chen L (2014) Elaboration and characterization of barley protein nanoparticles as an oral delivery system for lipophilic bioactive compounds. Food Funct 5(1):92–101. doi: 10.1039/C3FO60351B
18. Liu G, Huang W, Babii O, Gong X, Tian Z, Yang J et al (2018) Novel protein-lipid composite nanoparticles with an inner aqueous compartment as delivery systems of hydrophilic nutraceutical compounds. Nanoscale 10(22):10629–10640. doi: 10.1039/c8nr01009a
19. Kang J, Liu Y, Xie M-X, Li S, Jiang M, Wang Y-D (2004) Interactions of human serum albumin with chlorogenic acid and ferulic acid. Biochimica et Biophysica Acta (BBA) -. Gen Subj 1674(2):205–214. doi: https://doi.org/10.1016/j.bbagen.2004.06.021
20. Rodríguez-Roque MJ, Rojas-Graü MA, Elez-Martínez P, Martín-Belloso O (2013) Soymilk phenolic compounds, isoflavones and antioxidant activity as affected by in vitro gastrointestinal digestion. Food Chem 136(1):206–212. doi: https://doi.org/10.1016/j.foodchem.2012.07.115
21. Cirkovic Velickovic TD, Stanic-Vucinic DJ (2018) The Role of Dietary Phenolic Compounds in Protein Digestion and Processing Technologies to Improve Their Antinutritive Properties. Compr Rev Food Sci Food Saf 17(1):82–103. doi: https://doi.org/10.1111/1541-4337.12320
22. Aceituno-Medina M, Mendoza S, Rodríguez BA, Lagaron JM, López-Rubio A (2015) Improved antioxidant capacity of quercetin and ferulic acid during in-vitro digestion through encapsulation within food-grade electrospun fibers. J Funct Foods 12:332–341. doi: https://doi.org/10.1016/j.jff.2014.11.028
23. Wang Z, Li H, Liang M, Yang L (2016) Glutelin and prolamin, different components of rice protein, exert differently in vitro antioxidant activities. J Cereal Sci 72:108–116. doi:

https://doi.org/10.1016/j.jcs.2016.10.006
24. Li T, Wang L, Sun D, Li Y, Chen Z (2019) Effect of enzymolysis-assisted electron beam irradiation on structural characteristics and antioxidant activity of rice protein. J Cereal Sci 89:102789. doi: https://doi.org/10.1016/j.jcs.2019.102789
25. Adem S, Comakli V, Kuzu M, Demirdag R (2014) Investigation of the Effects of Some Phenolic Compounds on the Activities of Glucose-6-Phosphate Dehydrogenase and 6-Phosphogluconate Dehydrogenase from Human Erythrocytes. J Biochem Mol Toxicol 28(11):510–514. doi: https://doi.org/10.1002/jbt.21592
26. Heep G, Almeida A, Marcano R, Vieira D, Mainardes RM, Khalil NM et al (2019) Zein-casein-lysine multicomposite nanoparticles are effective in modulate the intestinal permeability of ferulic acid. Int J Biol Macromol 138:244–251. doi: https://doi.org/10.1016/j.ijbiomac.2019.07.030
27. Poquet L, Clifford MN, Williamson G (2008) Transport and Metabolism of Ferulic Acid through the Colonic Epithelium. Drug Metab Dispos 36(1):190. doi: 10.1124/dmd.107.017558
28. Yee S (1997) In Vitro Permeability Across Caco-2 Cells (Colonic) Can Predict In Vivo (Small Intestinal) Absorption in Man—Fact or Myth. Pharm Res 14(6):763–766. doi: 10.1023/A:1012102522787
29. Konishi Y, Hagiwara K, Shimizu M, Bioscience (2002) Biotechnol Biochem 66(11):2449–2457. doi: 10.1271/bbb.66.2449
30. Konishi Y, Shimizu M (2003) Transepithelial Transport of Ferulic Acid by Monocarboxylic Acid Transporter in Caco-2 Cell Monolayers. Biosci Biotechnol Biochem 67(4):856–862. doi: 10.1271/bbb.67.856

Figures

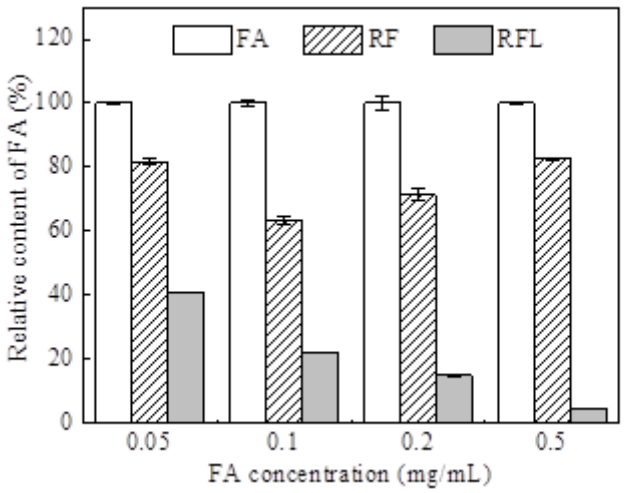

Figure 1

The relative contents of ferulic acid before digestion

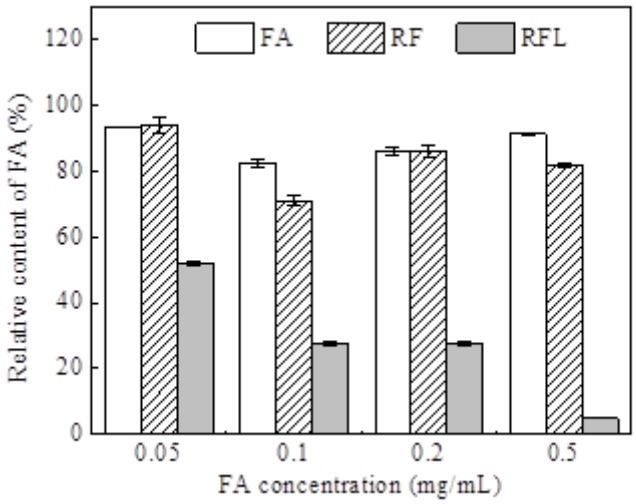

Figure 2

The relative contents of ferulic acid after oral digestion

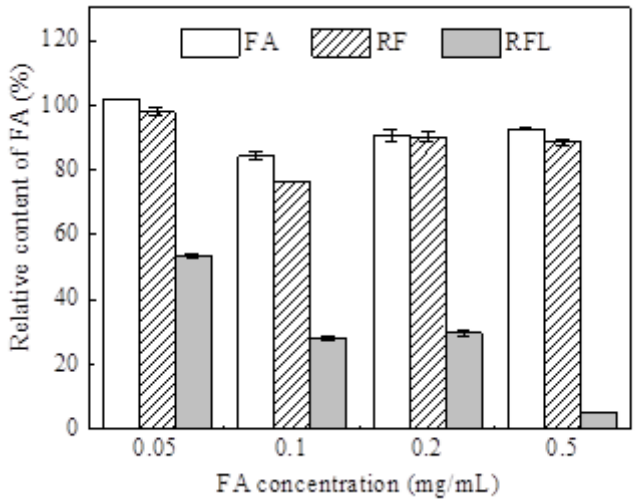

Figure 3

The relative content of ferulic acid after gastric digestion

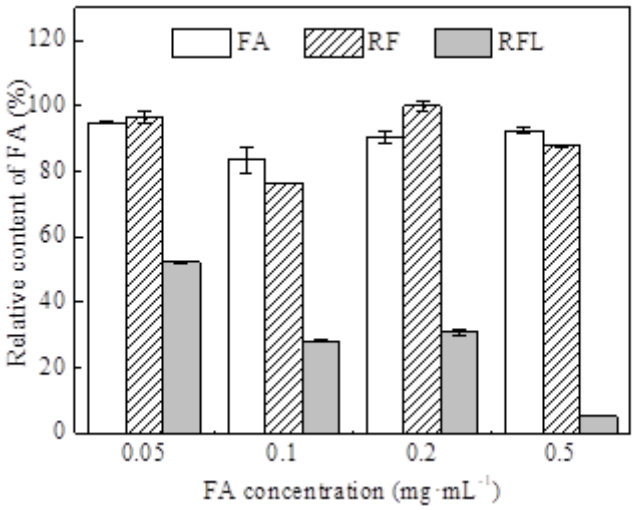

Figure 4

The relative content of ferulic acid after intestinal digestion

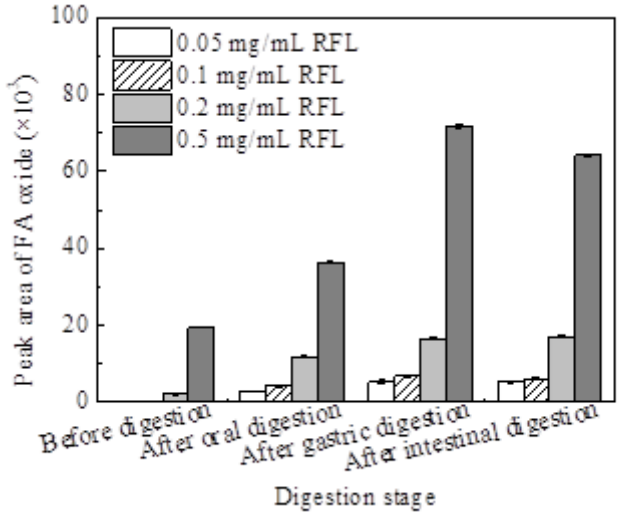

Figure 5

Peak area of ferulic acid oxidative derives during *in vitro* digestion

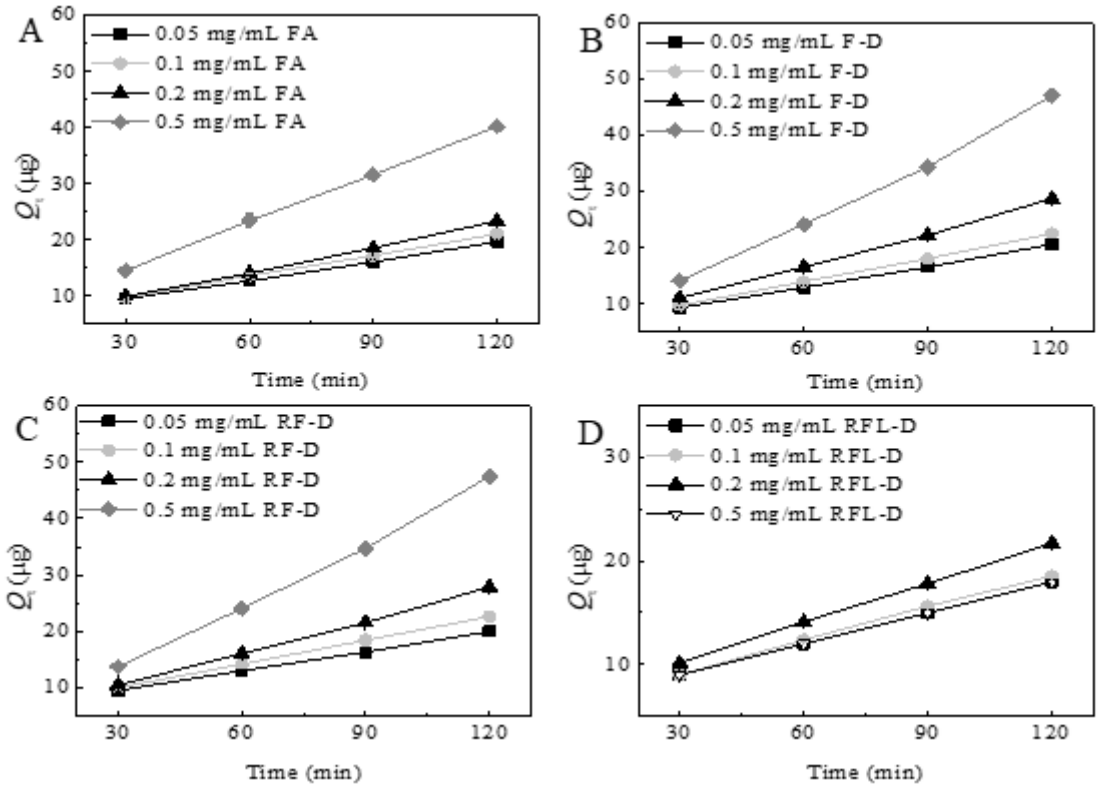

Figure 6

The time development of accumulated ferulic acid transport amount Q_t of FA F-D RF-D and RFL-D

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryInformation.docx