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ORIGINAL ARTICLE

Isovitexin, A New Metabolite, Was Found in the Metabolites of Co-cultured Five Flavonoids Isolated from *Ziziphus jujuba* Mill var. *spinosa* Seeds by Rat Intestinal Flora

Ying Liu, Junbo Xie[1], Xusheng Cui[2,3], Yanqing Zhang, Kunsheng Zhang

Department of Food Science, College of Biotechnology and Food Science, Tianjin University of Commerce, [1]Department of Chinese Materia Medica, School of Chinese Materia Medica, Tianjin University of Traditional Chinese Medicine, Tianjin, [2]Department of Medicinal Resources, Shijiazhuang Yiling Pharmaceutical Co. Ltd., Hebei, [3]Department of Chinese Medicinal Materials Research Center, China Agricultural University, Beijing, China

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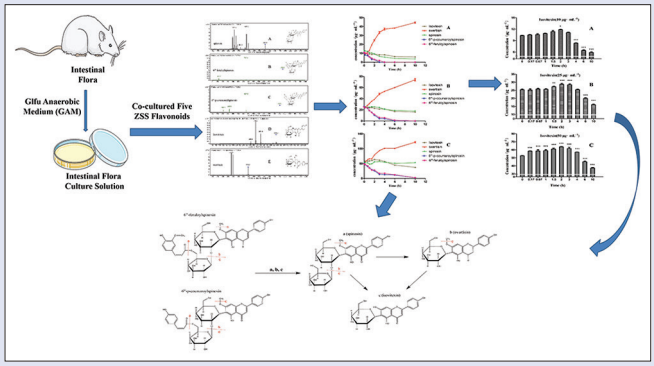
ABSTRACT

Background: *Ziziphus jujuba* Mill var. *spinosa* seeds (ZSS) is one of the most popular traditional Chinese herbs. It shows several pharmacological effects, such as anti-anxiety, antidepressant, neuroprotection, and cardiotonic, and prevents insomnia. The primary biologically active flavonoids derived from ZSS are 6′′′-feruloyl spinosin, 6′′′-*p*-coumaroyl spinosin, spinosin, swertisin, and isovitexin. **Objectives:** The primary objective of this study was to investigate the mechanism of degradation of co-cultured flavonoids, namely 6′′′-feruloyl spinosin, 6′′′-*p*-coumaroyl spinosin, spinosin, swertisin, and isovitexin, in the intestinal flora of rats under *in vitro* conditions. **Materials and Methods:** In this study, we co-cultured the five flavonoids in the intestinal flora of rats under *in vitro* conditions and determined the degradation of these five flavonoids via high-performance liquid chromatography with tandem mass spectrometry (MS/MS). **Results:** The degradation rate of the 6′′′-feruloyl spinosin, 6′′′-*p*-coumaroyl spinosin, and swertisin was affected by the concentration of the sample and conforms to the first-order kinetic model. According to our results, the 6′′′-feruloyl spinosin and 6′′′-*p*-coumaroyl spinosin may be degraded to spinosin and swertisin, and spinosin continued to decompose into swertisin. These results were consistent with their individual experiments. Moreover, by comparing the structures of isovitexin and swertisin, isovitexin was first found to be the product of the seventh demethylation reaction of the flavonoid core structure, demonstrating that isovitexin is a metabolite of ZSS flavonoids following spinosin and swertisin. **Conclusion:** Taken together, the results of this study explain the metabolic and interrelation of these five main flavonoids *in vitro*.

Key words: Degradation kinetics, flavonoids, high-performance liquid chromatography with tandem mass spectrometry, intestinal flora, *Ziziphi spinosae* semen

SUMMARY

- The degradation rate of the 6′′′-feruloyl spinosin, 6′′′-*p*-coumaroyl spinosin, and swertisin is affected by the concentration of the sample and conforms to the first-order kinetic model
- 6′′′-feruloyl spinosin and 6′′′-*p*-coumaroyl spinosin may be degraded to spinosin and swertisin, and spinosin continues to decompose into swertisin
- Isovitexin is a metabolite of ZSS flavonoids followed by spinosin and swertisin.



Abbreviations used: ZSS: *Ziziphus jujuba* Mill var. *spinosa* seeds; HPLC-MS/MS: High performance liquid chromatography-tandem mass spectrometry; SD: Sprague–Dawley rats; QC: Quality control; ESI: Electrospray ionization; MRM: Multiple reaction monitoring; LLOQ: Lower limit of quantification; LLOD: Lowest limit of detection; RSD: Relative standard deviation; S/N ratio: Signal-to-noise ratio.

Correspondence:

Prof. Yanqing Zhang,
College of Biotechnology and Food Science, Tianjin University of Commerce, Tianjin 300134, China.
E-mail: zhyqing@tjcu.edu.cn
Prof. Kunsheng Zhang,
College of Biotechnology and Food Science, Tianjin University of Commerce, Tianjin 300134, China.
E-mail: zhksheng@tjcu.edu.cn

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INTRODUCTION

Ziziphi spinosae semen is the dry mature seed of *Ziziphus jujuba* Mill. var. *spinosa* (Bunge) Hu ex. H.F. Chou (*Rhamnaceae*).[1] Studies have demonstrated that ZSS shows various pharmacological effects, such as anti-anxiety,[2] antidepressant,[3] neuroprotection,[4] and cardiotonic,[5] and prevents insomnia.[6] Flavonoids are one of the main biological components in ZSS. So far, more than 30 flavonoid compounds have been found in ZSS, and five among them are studied herein: 6′′′-feruloyl spinosin, 6′′′-*p*-coumaroyl spinosin, spinosin, swertisin, and isovitexin.[7-10] Previous studies have reported that these five flavonoids demonstrate significant beneficial activities: (1) improve learning and memory[11,12] and anti-inflammatory,[13] antioxidant,[14] and antidiabetic[15,16] and (2) ameliorate cognitive dysfunction.[17]

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It is well known that the first-pass metabolism of flavonoids commonly leads to its low bioavailability.[18] The interaction of flavonoids with intestinal flora can significantly affect the metabolic absorption of flavonoids.[19] Various bacterial groups in the intestine can produce different enzyme systems, mainly α-rhamnosidase, β-glucuronidase, α-glucosidase, β-galactosidase, and nitroreductase, which can transform the flavonoids by *O*-glycosylation, *C*-glycoside degradation, ester hydrolysis, and so on.[20] These enzyme systems transform the flavonoids into secondary metabolites that can be easily absorbed through the intestinal lumen. For example, baicalin cannot be directly absorbed into the blood but is metabolized into baicalein by the action of the intestinal flora, which is then the absorbed by the intestinal lumen and converted into baicalin.[21] Soy isoflavones are metabolized into equol by the intestinal flora.[22] The metabolites of quercetin-3-*O*-rutinoside (rutin) retain their antioxidant activity after being metabolized by the intestinal flora.[23] Moreover, the metabolites produced by the interaction of flavonoids with the intestinal flora have been shown to have better biological activity.[24] For example, oroxylin A, another metabolite of baicalin, has better anti-inflammatory effects than the prototype.[25] Proanthocyanidin metabolite 5-(3',4'-dihydroxy phenyl)-γ-valerolactone has better effect of preventing atherosclerosis than the prototype.[26]

Previous studies have shown that 6′′′-feruloyl spinosin and 6′′′-*p*-coumaroyl spinosin undergo significant degradation to yield spinosin and swertisin, respectively, by the action of intestinal flora.[27,28] Spinosin can be further degraded into swertisin.[29] However, to the best of knowledge, there are no studies on the co-culture effects of the five aforementioned flavonoids and their degradation products. Compared with the other four flavonoids, isovitexin is the product of demethylation reaction at the seventh position on the flavonoid core structure.[30] However, whether isovitexin is a degradation product of flavonoids in ZSS in the intestinal flora is still unknown.

In this study, we analyzed the flavonoids by high-performance liquid chromatography-coupled with tandem mass spectrometry (HPLC-MS/MS). The method was developed and validated for the simultaneous determination of 6′′′-feruloyl spinosin, 6′′′-*p*-coumaroyl spinosin, spinosin, isovitexin, and swertisin.

MATERIALS AND METHODS

Chemicals and materials

Spinosin and isovitexin (purity >98%) were purchased from Chengdu Cisco Hua Biotechnology Co., Ltd. (China). Chromatographic pure water, HPLC-grade acetonitrile, and methanol were purchased from JT Baker Chemicals (USA). Next, 6′′′-feruloyl spinosin, 6′′′-*p*-coumaroyl spinosin, and swertisin (purity >98%) were isolated in our laboratory and identified via ultraviolet, infrared, mass spectrometry (MS), and nuclear magnetic resonance. General anaerobic medium (GAM) was purchased from Aoboxing Biotech Co., Ltd. (Beijing, China).

Animals

Ten male Sprague – Dawley rats weighing 180–220 g (6–7 weeks) were provided by Tianjin Animal Epidemic Prevention and Quarantine Center (Tianjin, China). Rats were housed in squirrel cages at 22°C–24°C, light/dark cycle of 12 h, and relative humidity of 50%. Rats were free to enjoy the standard diet LAD3001M, a purified standard feed produced according to the US AIN93 standard for feeding adult rats or mice (TROPHIC Animal Feed High-tech Co., Ltd., China). One week after the rats were acclimatized to the new environment, the feces of the rats were collected at the same time of everyday for 1 week and stored at −80°C. The experiment was conducted under the National Guidelines for the Adequate Care and Use of Animals in Laboratory Studies.

Preparation of culture solution and degradation study

GAM (6.4 g) was dissolved in 200 mL of distilled water and subjected to autoclaving (121°C, 20 min). The feces of the rats were mixed and added to the GAM solution at a ratio of 1:15 (w/v), vortexed, and centrifuged at 8000 ×*g* for 5 min at 4°C, and the supernatant was taken as the intestinal flora culture solution for subsequent experiments.

In this study, 6′′′-feruloyl spinosin, 6′′′-*p*-coumaroyl spinosin, spinosin, isovitexin, and swertisin were dissolved in methanol to obtain a concentration of 10, 25, and 50 μg/mL for each individual flavonoid, respectively. Then, from each, a 100 μL sample was transferred to a 1.5 mL Eppendorf tube, shaken, and dried with N_2. The samples were reconstituted with 100 μL of the intestinal flora medium, filled with CO_2, and incubated at 37°C in a shaker. The samples were withdrawn at 0, 15, 30, and 45 min and at 1, 1.5, 2, 3, 4, 6, and 8 h and placed at −20°C to stop the reaction. The reaction solution was placed in the 1.5 mL Eppendorf tube, dried with N_2, reconstituted with the same volume of the mobile phase, and centrifuged at 13,000 ×*g* for 10 min, and the supernatant was withdrawn for use. The degradation kinetics of 6′′′-feruloyl spinosin, 6′′′-*p*-coumaroyl spinosin, spinosin, swertisin, and isovitexin were studied by measuring the changes in their concentrations at different times.

Chromatographic conditions and mass spectroscopic operational parameters

In this study, the flavonoids were analyzed using an Agilent Triple Quad LC/MS system, including G1312B Binary Pump, G1367D Autosampler SL Plus, G1322A Vacuum Degasser, G1316B Column Thermostat, and G6410B Triple Quadrapole Mass Spectrometer. YMC ODS-AQ™ column (2.0 mm × 250 mm, 3 μm) was used for the separation. The mobile phase constituted 35% acetonitrile (solvent A) and 65% water containing 0.1% formic acid (solvent B) and was kept at a flow rate of 0.3 mL/min. The column temperature was 30°C and the injection volume was 40 μL.

The ionization mode of MS detection is electrospray ionization(−), scanning mode: multiple reaction detection mode. The optimized operating parameters are as follows: electrospray voltage: 4000 V, atomizing gas: N_2, atomizing gas pressure: 35 psi, atomizing gas flow rate: 6 L/min, ion source temperature: 350°C, collision gas: nitrogen, and collision gas pressure: 0.15 MPa. The parameters of 6′′′-feruloyl spinosin used for quantitative analysis: *m/z* 783.0 → 427.2, fragmentor: 240 V, collision energy: 40 V; 6′′′-*p*-coumaroyl spinosin: *m/z* 753.3 → 427.0, fragmentor: 240 V, collision energy: 40 V; spinosin: *m/z* 607.0 → 427.0, fragmentor: 240 V, collision energy: 40 V; swertisin: *m/z* 445.2 → 281.5, fragmentor: 200 V, collision energy: 35 V; and isovitexin: *m/z* 431.0 → 311.0, fragmentor: 160 V, collision energy: 20 V; residence time is 200 ms.

Method validation

In this study, 6′′′-feruloyl spinosin, 6′′′-*p*-coumaroyl spinosin, spinosin, swertisin, and isovitexin are formulated with methanol to a series of solutions at concentrations of 1, 2.5, 5, 12.5, 25, and 50 μg/mL. The peak area of 6′′′-feruloyl spinosin, 6′′′-*p*-coumaroyl spinosin, spinosin, isovitexin, and swertisin was taken as the ordinate, and the concentration was used as the abscissa. The regression analysis was performed to obtain the standard curve equation.

Under the chromatographic conditions, the signal-to-noise ratio of lowest limit of detection and lowest limit of quantification (LLOQ) was 3 and 10, respectively. The effect of matrix was evaluated by comparing the peak area of the analyte in the quality control (QC) sample cultured in the intestinal flora with the peak area of the QC sample not cultured in the intestinal flora. The precision was evaluated by analyzing the QC samples at three different concentrations, and the experiment was repeated thrice. QC samples were mixed with the medium without the intestinal flora and were placed in an oven at 37°C for 0, 1, 2, 4, 8, 10, and 12 h to assess stability. The extraction recovery was determined by comparing the peak area of the QC sample extracted with the mobile phase with the peak area of the unextracted QC sample.

Statistical analysis

Data are expressed as mean ± standard deviation. Statistical analysis was performed using Microsoft Excel 2010 Edition. Statistical significance was assessed by one-way analysis of variance (ANOVA) using GraphPad Prism 5.01 (GraphPad Software Inc., San Diego, CA, USA). When the *P* value was less than 0.05, the results were considered to be statistically significant.

RESULTS AND DISCUSSION

Validation of the high-performance liquid chromatography-coupled with tandem mass spectrometry method

In this study, we determined the linear relation between 6′′′-feruloyl spinosin, 6′′′-*p*-coumaroyl spinosin, spinosin, isovitexin, and swertisin in the concentration range of 1–50 μg/mL. The linear equation for 6′′′-feruloyl spinosin, 6′′′-*p*-coumaroyl spinosin, spinosin, isovitexin, and swertisin is as follows: $y = 38.556x + 63.771$ ($R^2 = 0.9996$), $y = 38.556x + 63.771$ ($R^2 = 0.9991$), $y = 227.33x + 298.02$ ($R^2 = 0.9997$), $y = 585.16x + 857.98$ ($R^2 = 0.9995$), and $y = 123.91x + 198.77$ ($R^2 = 0.9997$), respectively, where y is the peak area of the sample and x is the concentration of the sample. The LLOQ of the analytes was 1 ng/mL, indicating that the method was sensitive.

Table 1 shows the matrix effect and sample recovery. According to the results, the matrix effect of 6′′′-feruloyl spinosin, 6′′′-*p*-coumaroyl spinosin, spinosin, isovitexin, and swertisin at three different concentrations was in the range of 85.66%–112.30%. The recovery rates were greater (range = 96.22%–104.94%) with relative standard deviation (RSD) no more than 15% (0.83%–2.98%). It indicates that the relative matrix effect of the analytical sample can be neglected, which means that the method of determination meets the experimental requirements.

Table 2 shows the precision and stability of 6′′′-feruloyl spinosin, 6′′′-*p*-coumaroyl spinosin, spinosin, isovitexin, and swertisin. The RSD of precision at three different concentrations ranged from 0.33% to 2.99%, and the RSD of stability ranged from 0.73% to 3.04%. The values of accuracy and stability were not more than 4%, indicating that the method of measurement meets the experimental requirements. Figure 1 shows the typical chromatograms of 6′′′-feruloyl spinosin, 6′′′-*p*-coumaroyl spinosin, spinosin, isovitexin, and swertisin, and Figure 2 shows the typical mass spectrogram.

Degradation kinetics

Flavonoids, in addition to a few free forms in plants, mostly bind to sugars to form glycosides. Flavonoid glycosides are hydrolyzed to form aglycones by the action of α-rhamnosidase, β-glucosidase, or β-glucuronidase that is produced by the human intestinal microbiota. Metabolic transformation is considered to be a major factor affecting the bioavailability of flavonoids.[31,32]

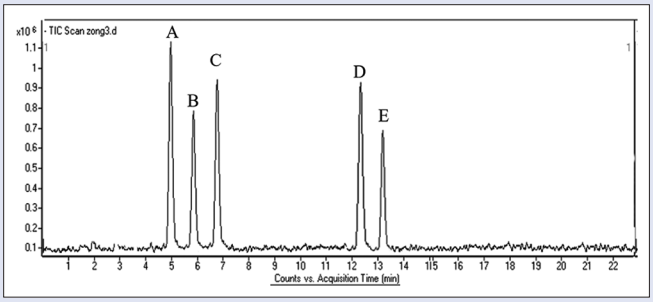

Figure 1: Representative chromatograms of the sample solution: (A) spinosin, (B) isovitexin, (C) swertisin, (D) 6‴-p-coumaroyl spinosin, and (E) 6‴-feruloyl spinosin

Table 1: The results of matrix effect and sample recovery rate (*n*=3)

	Mean±SD					
	Matrix effect			Sample recovery		
	10 (μg/mL)	25 (μg/mL)	50 (μg/mL)	10 (μg/mL)	25 (μg/mL)	50 (μg/mL)
Isovitexin	91.41±0.34	110.76±0.32	107.03±0.36	101.44±0.17	104.73±0.47	102.18±0.64
Swertisin	109.01±0.78	91.46±0.41	96.11±0.68	97.08±0.08	104.32±0.14	101.36±0.54
Spinosin	85.66±0.69	109.74±0.25	97.01±0.35	96.22±0.28	103.66±0.09	101.47±0.27
6‴-p-coumaroyl spinosin	86.10±0.53	84.27±0.16	112.30±0.24	103.80±0.23	104.94±0.13	98.89±0.73
6‴-feruloyl spinosin	86.79±0.49	88.89±0.28	91.73±0.29	103.96±0.14	98.60±0.41	102.72±0.49

SD: Standard deviation

Table 2: Precision and stability test results (*n*=3)

	Precision RSD (%)			Stability RSD (%)		
	10 (μg/mL)	25 (μg/mL)	50 (μg/mL)	10 (μg/mL)	25 (μg/mL)	50 (μg/mL)
Isovitexin	2.46	0.33	0.90	1.79	1.78	1.25
Swertisin	2.32	1.42	2.06	2.71	2.01	1.05
Spinosin	2.00	1.68	2.11	2.24	0.73	1.57
6‴-p-coumaroyl spinosin	2.99	1.30	1.03	3.04	1.05	2.63
6‴-feruloyl spinosin	2.46	0.68	0.70	2.62	1.46	1.37

RSD: Relative standard deviation

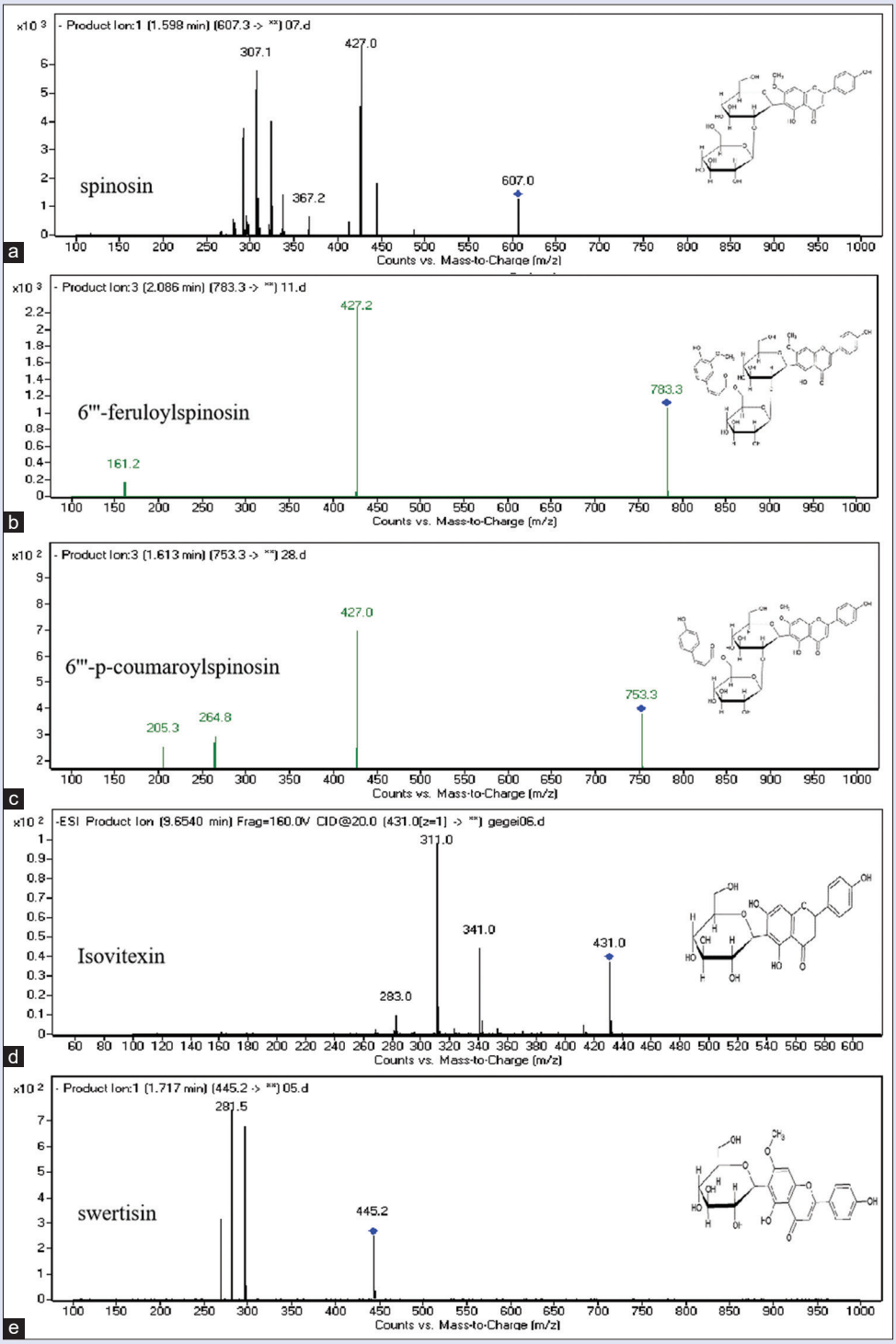

Figure 2: Corresponding product ion spectrum: (a) spinosin, (b) 6‴-feruloyl spinosin, (c) 6‴-p-coumaroyl spinosin, (d) isovitexin, and (e) swertisin

Intestinal flora participates in the decomposition of flavonoids in ZSS. Figure 3 shows the degradation kinetics of 6′′′-feruloyl spinosin, 6′′′-*p*-coumaroyl spinosin, spinosin, isovitexin, and swertisin at three different concentrations (10, 25, and 50 μg/mL). According to the results, 6′′′-feruloylspinosin, 6′′′-*p*-coumaroylspinosin, and spinosin showed a tendency to degrade and their concentration decreased with time, of which 6′′′-feruloyl spinosin and 6′′′-*p*-coumaroyl spinosin were completely degraded, whereas the concentration of swertisin increased with time. These results indicate that 6′′′-feruloyl spinosin, 6′′′-*p*-coumaroyl spinosin, and spinosin can be degraded into swertisin. According to our preliminary analysis,[27,28] both 6′′′-feruloyl spinosin and 6′′′-*p*-coumaroyl spinosin degrade into spinosin. However, spinosin does not show an increasing trend like swertisin. This is because spinosin can continue to degrade into swertisin and the degradation of 6′′′-feruloyl spinosin and 6′′′-*p*-coumaroyl spinosin to spinosin does not exceed the rate at which spinosin degrades to swertisin. Moreover, swertisin is the primary product of degradation of 6′′′-feruloyl spinosin and 6′′′-*p*-coumaroyl spinosin.

Although the degradation results were different, the degradation curves of 6′′′-feruloyl spinosin, 6′′′-*p*-coumaroyl spinosin, and swertisin were all finally determined to conform to the first-order kinetic model [Table 3]. According to the statistical analysis, the rate constant of each concentration and the rate constant of the logarithm of the concentration were analyzed by one-way ANOVA. As shown in Table 4, there was a significant difference between the three tested concentrations, indicating that the degradation rate of 6′′′-feruloyl spinosin, 6′′′-*p*-coumaroyl spinosin, and swertisin was affected by the level of the compounds in the intestine.

Table 3: 6‴-feruloyl spinosin 6‴-p-coumaroyl spinosin, and swertisin at three different concentrations, K, intercept (b), and *R* value (*n*=3)

C/ μg/ mL	6‴-feruloyl spinosin			6‴-p-coumaroyl spinosin			Swertisin		
	K	b	R	K	b	R	K	b	R
10	1.11	7.77	0.90	0.97	6.62	0.92	3.88	13.01	0.95
25	2.70	20.92	0.97	2.79	20.56	0.96	5.14	27.34	0.94
50	4.52	40.68	0.95	4.62	39.93	0.98	7.04	60.20	0.97

K: Degradation rate constants; b: Intercept; R: Linear correlation coefficient

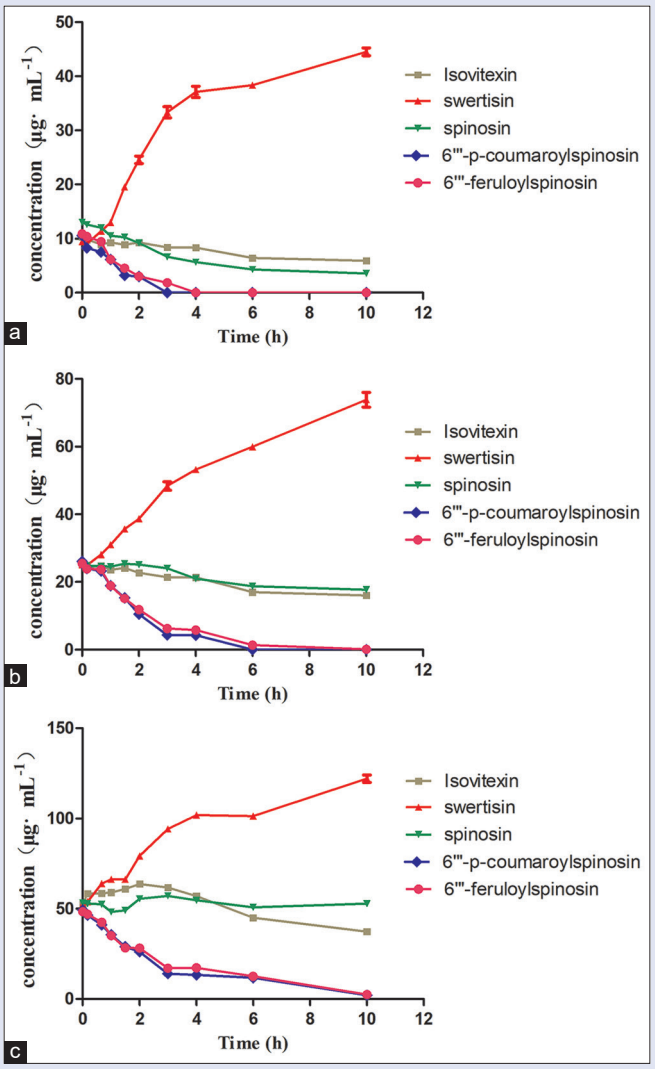

Figure 3: Degradation curves of different concentrations of 6‴-feruloyl spinosin, 6‴-p-coumaroyl spinosin, swertisin, spinosin, and isovitexin incubated with rat feces (*n* = 3): (a) 10 μg/mL, (b) 25 μg/mL, and (c) 50 μg/mL

It has been shown that the intestinal flora plays an important role in the body to transform flavonoids, which were demonstrated by the degradation characteristics of 6′′′-feruloyl spinosin, 6′′′-*p*-coumaroyl spinosin, spinosin, swertisin, and isovitexin in this study. Spinosin, a degradation intermediate of 6′′′-feruloyl spinosin and 6′′′-*p*-coumaroyl spinosin, not only increases pentobarbital-induced sleep but also upregulates adult hippocampal nerves, improves cognitive performance, and can be used to treat cognitive dysfunction in diseases, such as Alzheimer disease.[33] Swertisin can promote pancreatic islet regeneration in the pancreatic stem/progenitor cells via the p-38 MAP kinase-SMAD pathway and improve scopolamine-induced memory impairment in mice.[34,35] Consequently, 6′′′-feruloyl spinosin and 6′′′-*p*-coumaroyl spinosin may exert their pharmacological activities more efficiently through being converted to spinosin and swertisin under the action of intestinal flora.

Degradation process of isovitexin

As shown in Figure 4, the concentration of isovitexin was also altered during the degradation of the intestinal flora. The higher the initial

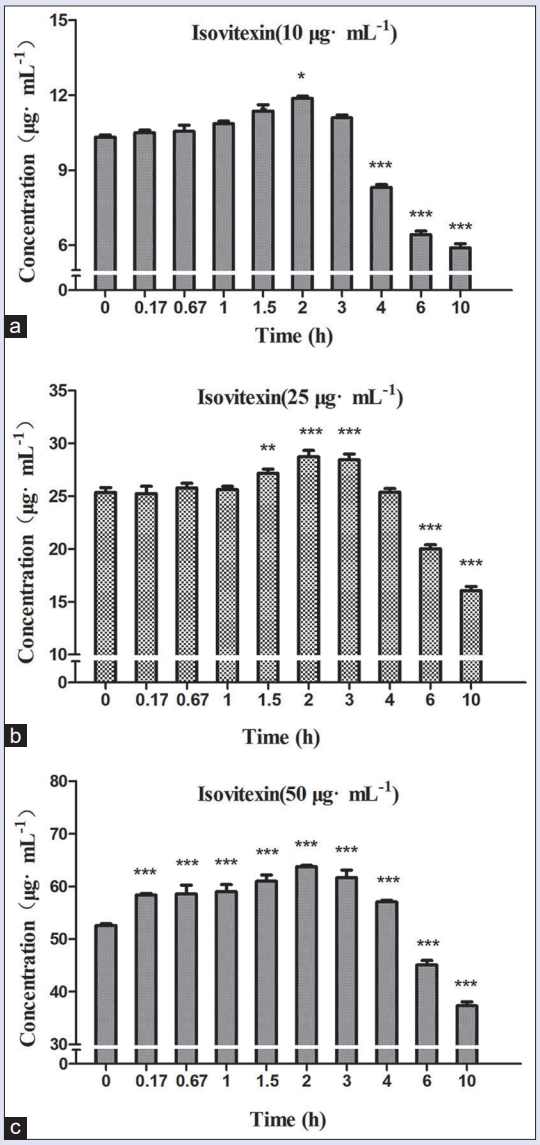

Figure 4: Degradation of isovitexin at different concentrations of rat feces (*n* = 3): (a) 10 μg/mL, (b) 25 μg/mL, and (c) 50 μg/mL (*$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ compared to the 0 h group)

Table 4: One-way analysis of variance was performed on the rate constants of the concentrations of 6‴-feruloyl spinosin, 6‴-p-coumaroyl spinosin, and swertisin and the logarithm of the concentration (*n*=3)

	6‴-feruloyl spinosin		6‴-p-coumaroyl spinosin		Swertisin	
	F-test	*P*	*F*-test	*P*	*F*-test	*P*
Concentration rate constant	2411.80	2×10^{-9}	18704.00	4×10^{-12}	165.80	6×10^{-6}
Concentration log rate constant	2037.00	3×10^{-5}	75.55	5.57×10^{-5}	465.00	2.63×10^{-7}

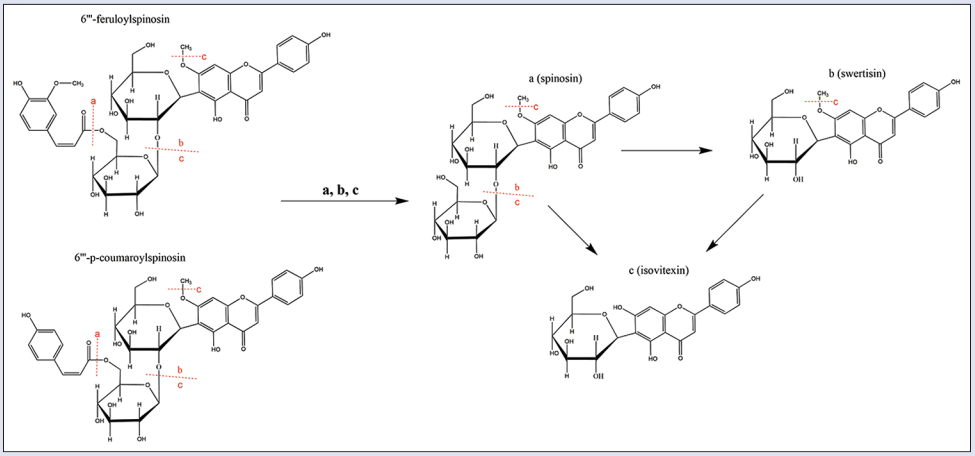

Figure 5: Formation process of ZSS flavonoid metabolites

concentration was, the more obvious was the trend. When the initial concentration was 50 μg/mL [Figure 4c], the subsequent concentration was significantly different from 0 h and the concentration of isovitexin increased first and then decreased. For experiments with initial concentrations of 10 [Figure 4a] and 25 μg/mL [Figure 4b], there was a significant change up to 1.5 and 2 h. Figure 3 shows that the concentration of isovitexin began to decrease significantly after 6 h, which is similar to the degradation curves of 6′′′-feruloyl spinosin and 6′′′-*p*-coumaroyl spinosin. Compared with the swertisin, isovitexin is the product of demethylation reaction at the seventh position on the flavonoid core structure. Therefore, we believe that isovitexin may be a metabolite of 6′′′-feruloyl spinosin, 6′′′-*p*-coumaroyl spinosin, spinosin, and swertisin.

It is well known that flavonoids have low bioavailability and are associated with extensive phase 2 metabolism and ATP-binding cassette (ABC) transporters, while phase 2 metabolism is associated with glucuronidation and sulfation of flavonoids, and the transport of flavonoids by ABC transporters is also related to the structure of flavonoids.[36] At present, it has been found that the C7-OH group in flavonoids can easily dock with the active site of SULT1A3 and produce the highest fitting and docking score.[37] Figure 5 shows that 6′′′-feruloyl spinosin, 6′′′-*p*-coumaroyl spinosin, spinosin, and swertisin may be finally degraded to isovitexin. Isovitexin has C7-OH group compared to other flavonoids. The ABC transporter acts as an ATP-dependent efflux pump found in the apical and basement membranes of the epithelial cells. The most relevant proteins for pharmacology are the P-glycoproteins (P-gps), namely MRP2 and BCRP, found in the apical membrane.[38] As we know, the transport of ZSS flavonoids across the membrane has MRP2 is involved. Hydroxylation at the C5 and C7 positions increases the inhibition of BCRP, whereas methylation at these positions decreases its activity, whereas hydroxyl groups at positions 5 and 3 inhibit the activity of P-gp.[39] A previous study has shown that hydroxymethylation of flavonols significantly promotes its passage through the intestinal cell model.[40] Moreover, isovitexin shows great potential in preventing the formation of atherosclerosis and is hepatoprotective, which can alleviate lipopolysaccharide/D-galactosamine-induced liver injury.[41,42] Furthermore, isovitexin demonstrates good antioxidant and anti-inflammatory activities.[43] The metabolism of flavonoids in ZSS leads to changes in its structure, which improves the bioavailability of flavonoids in ZSS.

CONCLUSION

In this study, we present the development and validation of a rapid and sensitive HPLC-MS/MS assay for the determination of 6′′′-feruloyl spinosin, 6′′′-*p*-coumaroyl spinosin, spinosin, swertisin, and isovitexin. The five flavonoids were co-cultured in the intestinal flora under *in vitro* conditions. According to our results, the degradation rate of 6′′′-feruloyl spinosin, 6′′′-*p*-coumaroyl spinosin, and swertisin was affected by the concentration of the sample, which was consistent with the first-order kinetic model. In addition, it was verified that 6′′′-feruloyl spinosin, 6′′′-*p*-coumaroyl spinosin degraded into spinosin and swertisin, whereas spinosin continued to degrade into swertisin. The possible degradation product isovitexin following swertisin was discovered.

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Conflicts of interest

There are no conflicts of interest.

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