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Pharmacokinetics of Quercetin Absorption from Apples and Onions in Healthy Humans

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ABSTRACT: A high-throughput method for the extraction and analysis of quercetin in human plasma using 96-well SPE and LC-(ESI)MS/MS (7 min/run) is described. Quercetin exists as a range of glycosides in foods. The dominant types of quercetin glycosides vary depending on genetics (i.e., species and cultivar). Dietary sources include onions and apples (i.e., the peel). Herein the quercetin glycoside composition was determined in a composite standard of dried apple peel and in onion powder. The predominant forms of quercetin in apple peel include quercetin *O*-arabinoside, 3-*O*-galactoside, 3-*O*-glucoside, and 3-*O*-rhamnoside. In the onion powder, quercetin occurred as the quercetin 3,4′-*O*-glucoside and 4′-*O*-glucoside. Pharmacokinetics relating to absorption (C_{max}, t_{max}, and $AUC_{0-24\,h}$) and elimination (k_{el} and $t_{1/2}$) were compared after the consumption of apple peel powder (AP), onion powder (OP), or a mixture of the apple peel and onion powder enriched applesauce (MP) by healthy volunteers (eight females and eight males). The enriched applesauce delivered ∼100 mg of quercetin aglycone equivalents. Consumption of the OP resulted in C_{max} = 273.2 ± 93.7 ng/mL, t_{max} = 2.0 ± 1.7 h, and $t_{1/2}$ = 14.8 ± 4.8 h, whereas the AP resulted in C_{max} = 63.8 ± 22.4 ng/mL, t_{max} = 2.9 ± 2.0 h, and $t_{1/2}$ = 65.4 ± 80.0 h. The MP resulted in an intermediate response with C_{max} = 136.5 ± 45.8 ng/mL, t_{max} = 2.4 ± 1.5 h, and $t_{1/2}$ = 18.7 ± 6.8 h. Consumption of the OP led to faster absorption, higher concentration, and greater bioavailability as compared to the AP. No significant gender-related differences were observed in the absorption of quercetin, whereas significant gender-related differences in the elimination half-time ($t_{1/2}$) were observed.

KEYWORDS: *apple, onion, quercetin, absorption, pharmacokinetics, bioavailability, gender, LC-(ESI)-MS/MS*

INTRODUCTION

Flavonoids are the most abundant subclass of plant-derived polyphenolics and are of interest because of their apparent beneficial effects on human health, including antioxidant, anti-inflammatory, and antiviral activities.[1−3] Quercetin is one of the most abundant flavonoids in fruits and vegetables and the most studied of the flavonoids. In addition to its anti-inflammatory and antioxidant activities, quercetin prevents platelet aggregation and promotes relaxation of cardiovascular smooth muscle.[4] *o*-Dihydroxy substitution of the B-ring (catechol) and 2,3-unsaturation in the C-ring and 4-carbonyl group uniquely contribute to the hydrogen-donating antioxidant activity of quercetin.[5] Onions and apples (i.e., the peel) are primary sources of dietary quercetin. Typical levels in onions range from 28.4 to 48.6 mg/100 g, whereas levels in apples including apple peels as a fruit range from 2.1 to 7.2 mg/100 g.[6] In plants, quercetin exists as more water-soluble glycosides. The preferred site of glycosylation is the 3-position, and D-glucose is the most frequent sugar residue, but D-galactose, L-rhamnose, L-arabinose, D-xylose, D-apiose, and D-glucuronic acid are found as well.[7] The dominant types of quercetin glycoside vary among species and cultivars.[8]

Although the food matrix influences nutrient absorption, it appears that the glycosidic moiety is a key determinant for influencing the absorption of flavonoids.[9,10] For example, enzymatic conversion from hesperidin (hesperetin 7-*O*-glucose-rhamnose) to hesperetin 7-glucoside increased bioavailability 2-fold.[9] The consumption of a purified quercetin 4′-*O*-glucoside or onion dominating in quercetin 4′-*O*-glucoside resulted in the same pharmacokinetic parameters over 24 h[10] but differed from quercetin aglycone over 13 h.[11] The wide variation in chemical structure of quercetin in foods (i.e., glycosides) has important consequences on its bioavailability (i.e., the amount absorbed into the bloodstream) and other pharmacokinetic parameters because the hydrolysis of the glycosidic moiety is requisite for intestinal absorption.[12] Flavonoid glycosides are substrates for enterobacterial β-glucosidases and lactase phlorizin hydrolase present in enteric membranes. The affinity of this enzyme for various forms of quercetin glycosides is likely to affect their absorption and, thus, bioavailability. Carrier-mediated transport of quercetin glycosides via the sodium-dependent glucose transporter-1 (SGLT1) has also been suggested.[13,14] However, it has been reported that flavonoids absorbed by this transporter are pumped back to the intestinal lumen by the multidrug resistance-associated protein 2.[15]

To date, only the aglycone form of quercetin is available to manufacturers for supplementation of food products and, therefore, it is most frequently used in human clinical studies. However, Hollman et al.[11] demonstrated that the absorption of the glycosides surpasses that of the aglycone (52% of the glycosides is absorbed versus 24% of the aglycone) in ileostomy patients. Most human feeding trials relating quercetin (or other flavonoids) intake with biomarkers of health, inflammation, or athletic performance have either not included quantitative information on the composition of quercetin glycosides used in

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the test diets or have used the quercetin aglycone. This may in part explain discrepancies in outcomes found among human feeding trials that compare the effects of quercetin, but utilize different foods or supplements as a source of the quercetin. On average, the oral absorption of quercetin aglycone ranges between 36 and 53% and the $t_{1/2}$ between 20 and 72 h.[16] Upon absorption, quercetin undergoes extensive first-pass metabolism in the small intestine, colon, liver, and kidney. Metabolites formed include the methylated, sulfated, and glucuronidated forms of quercetin.[17] At least 21 quercetin-related metabolites were identified after the ingestion of quercetin glycosides from onion.[17] Typical concentrations of quercetin in human plasma are in the low nanomolar range, but upon quercetin supplementation, they may increase to the high nanomolar or low micromolar range.

Understanding which forms of quercetin are bioavailable and which foods provide these forms is essential for formulating true value-added products based on a platform of intelligent ingredient choices. Currently, there is little information evaluating how the glycoside composition of a whole food influences pharmacokinetics. Hollman et al.[18] demonstrated that the level of quercetin absorbed from fried onions was 2-fold higher than that absorbed from raw apple peels in ileostomy patients. However, it is not clear if the differences were the result of the sample preparation method (e.g., frying) or food matrix interactions. In studies using a porcine model, quercetin bioavailability positively correlated with the amount of fat in the diet.[19] The pharmacokinetics of quercetin absorption from apples in healthy volunteers has not previously been examined.

To the best of our knowledge, this is the first reported investigation of the influence of the glycoside composition from mixtures of real foods on the pharmacokinetics of quercetin. To date, studies investigating the pharmacokinetics of flavonoids are performed using a single food, and investigations of the pharmacokinetics from mixtures of foods have not been characterized. However, foods are consumed not as a single item, but typically as mixtures. Understanding how mixture sources of flavonoids ultimately influence plasma levels of flavonoids is critical for systematically pairing foods together to achieve desirable results.

The current study compares the pharmacokinetics of quercetin in 16 healthy human volunteers (8 males and 8 females) after the consumption of a quercetin-free applesauce enriched with either apple peel (AP), onion powder (OP), or a mixture of the apple peel and onion powder standards (MP). Gender differences in the pharmacokinetics of quercetin were also investigated.

MATERIALS AND METHODS

Chemicals and Reagents. Isorhamnetin, quercetin dehydrate, quercetin 3-*O*-rutinoside (rutin), and sulfatase type H-1 powder from *Helix pomatia* were purchased from Sigma-Aldrich (St. Louis, MO, USA). Fisetin was obtained from Indofine Chemical Co. (Hillsborough, NJ, USA). Quercetin 3,4′-*O*-diglucoside, quercetin 3-*O*-galactoside, quercetin 3-*O*-glucoside, quercetin 3-*O*-rhamnoside, and quercetin 4′-*O*-glucoside were purchased from Extrasynthese (Genay, France). HPLC-grade methanol was purchased from Fisher Scientific (Fair Lawn, NJ, USA). ACS-grade formic acid (96%) and L-(+) ascorbic acid were purchased from Acros Organics (Geel, Belgium).

Study Design. Twenty-one individuals were initially recruited for the study, but five subjects failed to complete the food intake protocol. A total of 16 individuals (8 men and 8 women) successfully completed the three dietary treatment groups (i.e., apples, onions, or a mixture of the two). A description of the study population is shown in Table 1. Mean age was 23.3 ± 2.8 years, and mean body mass index was 21.5 ± 2.0 kg/m^2. The subjects were healthy, nonpregnant, nonsmoking adults who were not taking medications other than oral contraceptives. Potential candidates were screened by a study questionnaire about health, age, body mass index, and dietary habits. Individuals with active disease were excluded. Subjects drinking >2 cups/day of coffee and/or >5 alcoholic drinks/week were also excluded. The study protocol was approved by the Institutional Review Board of the University of California, Davis, and written informed consent was obtained from all participants.

Table 1. Description of the Study Population

	males	females	total study population
mean age (years)	24.3 ± 3.5	22.3 ± 1.5	23.3 ± 2.8
height (cm)	175.8 ± 7.7	159.0 ± 3.8	167.4 ± 10.5
weight (kg)	66.5 ± 12.0	54.9 ± 4.6	60.7 ± 10.6
body mass index (kg/m^2)	21.4 ± 2.2	21.7 ± 1.9	21.5 ± 2.0

A randomized crossover study was performed to evaluate the pharmacokinetics of quercetin from apples, onions, or a mixture of the two. All subjects completed all three study treatments, acting as their own controls, and treatments were administered 3 weeks apart (Figure 1).

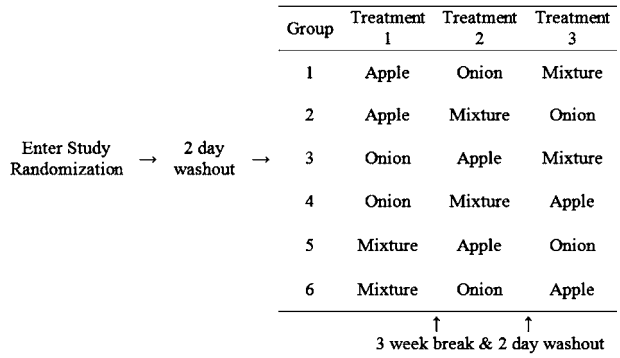


Figure 1. General design of the clinical study.

Subjects were randomly assigned to one of three groups, and each group had a different treatment order. The foods were provided as breakfast. Subjects were asked to abstain from eating quercetin-containing foods for 2 days prior to the study (washout period) and to fast for 12 h before the study. Subjects also consumed low-flavonoid (quercetin-free) diets during the treatment day. Caffeine consumption was not allowed on the treatment day and for 1 day prior to the treatment day. Any dietary supplements or any food products enriched with vitamin A, C, or E, plant/herbal extracts of flavonoids, fish oil, omega-3 fatty acids, or antioxidants were prohibited for a week prior to the treatment day. On the treatment day, a quercetin-free lunch and a snack were provided at 4 and 8 h after consumption of each treatment, respectively. The amount of water consumption was limited to 1 L on treatment day.

Venous blood was collected before (baseline) administration of the foods and 0.5, 1, 2, 4, 6, 8, and 24 h after administration. Blood was obtained by venous catheterization up to 8 h and by venipuncture at 24 h. Plasma was prepared within 15 min by centrifugation at 4 °C at 1200*g* for 15 min. Aliquots of plasma were stored at −80 °C until analysis.

Preparation of Foods. Appropriate cultivars of apples and onions rich in specific quercetin glycosides (i.e., onions with only quercetin

4′-glucoside and apples with 3-glucoside and no 4′-glucoside) were identified. Apple peels and onions (i.e., outer layers of onions after removal of the paper layer) were freeze-dried or air-dried, respectively, and micronized for human consumption. Apple peels and the outer layers of onions were used as a source of quercetin as they are the primary sources of flavonoids in these foods.

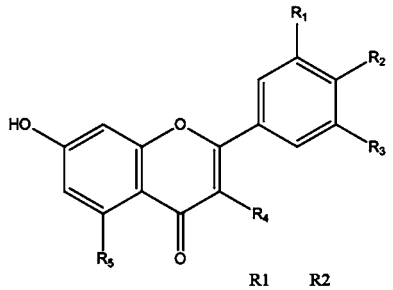
Materials were supplied and prepared by Gills Onions, Cadbury Schwepps, and Oregon Freeze-Dry, Inc. Apples (*Malus domestica* var. Macintosh) and onions (*Allium cepa* L. var. Gobi) were identified as the best sources of quercetin glycosides for these studies. Quercetin treatment was standardized to deliver approximately ∼100 mg of quercetin aglycone equivalents. The dried apple peels and/or onion powders (85 g of apples, 47.5 g of onions, and 42.5 g of apples and 23.7 g of onions for a mixture) were mixed with 100 g of applesauce and 1 cup of water. The processed applesauce was chosen as a vehicle because of the absence of quercetin, pleasant flavor, and property of mixing well with apple peel and onion powder. The food was prepared fresh daily.

Determination of Quercetin Glycosides in Foods. The quercetin glycoside composition was characterized in the freeze-dried apple, onion, and applesauce. Quercetin glycosides were not detected in the applesauce. The total extraction process was done in triplicate, and the average was used to calculate the amounts of quercetin glycosides in each treatment dose.

The extraction method was modified from the method of Lee et al.[20] Extraction was performed as follows: quercetin glycosides and quercetin aglycone were extracted from 2 g of a ground dried apple sample, onion sample, or applesauce using 20 mL of 80% aqueous methanol. The mixture was sonicated for 20 min and filtered through Whatman no. 2 filter paper (Whatman International Limited, Kent, U.K.) using a chilled Buchner funnel. The residue was rinsed with 10 mL of 100% methanol. The extraction process was repeated on the residue (filter cake), and the two filtrates were combined, mixed with 10 mL of methanol, and evaporated using a rotary evaporator at 40 °C. The remaining concentrate was mixed with 10 mL of 100% methanol and diluted to a final volume of 20 mL using distilled deionized water. The concentrate was centrifuged (12000*g*) at 4 °C for 20 min, and the supernatant was filtered through a 0.45 μm PTFE membrane filter. The extracts were stored at −20 °C until analysis.

Chromatographic separation was carried out according to the method of Hong et al.,[17] with slight modification. Qualitative analysis of extracts was performed by LC-(ESI)MS/MS using an HPLC system (Shimadzu Scientific, Columbia, MD, USA) interfaced to a Quattro LC triple-quadrupole tandem mass spectrometer (Micromass, Altrincham,U.K.) equipped with an SIL-10A autosampler, binary LC 10AD pumps, and Z spray. MassLynx software (v. 3.5) was used for data acquisition and processing. Separations of flavonol glycosides were performed on an Agilent Zorbax XDB C_{18} column (4.6 × 250 mm, 5 μm) with a C_{18} guard column (4.6 × 12.5 mm, 5 μm). The flow rate was 1.0 mL/min, and the HPLC eluent was split in a ratio of 1:1. Injection volume was 20 μL. The mobile phase consisted of a linear gradient of 1% formic acid in water (A) and 1% formic acid in acetonitrile (B) as follows: 5% B, 0−10 min; 5−20% B, 10−30 min; 20% B, 30−50 min; 20−21% B, 50−70 min; 21% B, 70−80 min; 21−60% B, 80−90 min; 60% B, 90−95 min; and 60−100% B, 95−100 min. The column was re-equilibrated between injections using 10 mL of the initial mobile phase. A full scan over a mass range of m/z 250−800 was performed to identify all possible flavonoid glycosides in positive mode, and data-dependent MS^2 scanning was further investigated with positive LC-(ESI)MS/MS by applying optimum collision energy for different analytes. The source temperature and the desolvation gas temperatures were 145 and 400 °C, respectively, and the capillary, cone, and extractor voltages were 3.0 kV, 30 V, and 2 V, respectively. Nitrogen was used with a flow rate of 550 L/h.

Quercetin glycosides were quantified using a Hewlett-Packard series 1200 liquid chromatograph (Agilent, Palo Alto, CA, USA) equipped with a multiple-wavelength detector (G1365B) monitoring at 370 nm. The same HPLC condition was used as above without split. Quantification of quercetin 3,4′-*O*-diglucoside, quercetin 3-*O*-rutinoside (rutin), quercetin 3-*O*-galactoside, quercetin 3-*O*-glucoside, quercetin 3-*O*-rhamnoside, and quercetin 4′-*O*-glucoside was achieved using authentic standards (Figure 2). The adjacent chromatographic peaks of quercetin glycoside standards were selected for relative quantification. Quercetin *O*-xyloside and quercetin *O*-arabinoside (*O*-arabinofuranose and *O*-arabinopyranose) were quantified using quercetin 3-*O*-glucoside and quercetin 3-*O*-rhamnoside, respectively. The detection limits of flavonol glucosides and quercetin aglycone were all 0.5 μg/mL. The linear ranges of quantification of flavonol glycosides and quercetin aglycone were 1−100 μg/mL. Extracts that exceeded the concentration range of the standard curve were diluted before injection.



	R1	R2	R3	R4	R5	M.W.
quercetin 3,4′-*O*-diglucoside	OH	O-glucose	H	O-glucose	OH	626.5
quercetin 3-*O*-rutinoside (rutin)	OH	OH	H	O-rutinoside	OH	610.5
quercetin 3-*O*-galactoside	OH	OH	H	O-galactoside	OH	464.4
quercetin 3-*O*-glucoside	OH	OH	H	O-glucoside	OH	464.4
quercetin 3-*O*-rhamnoside	OH	OH	H	O-rhamnoside	OH	448.4
quercetin 4′-*O*-glucoside	OH	O-glucose	H	OH	OH	464.4
quercetin aglycone	OH	OH	H	OH	OH	302.2
isorhamnetin aglycone	OCH_3	OH	H	OH	OH	316.3
fisetin	H	OH	OH	OH	H	286.2

Figure 2. Molecular structures and molecular weights of quercetin glycosides, quercetin aglycone, isorhamnetin, and fisetin (internal standard).

Determination of Total Quercetin Species in Plasma. The absolute recovery (100%) was improved compared with those for other published methods (50%) when the absolute recovery was measured after the addition of quercetin aglycone (final concentrations of 10 and 100 ng/mL plasma) without internal standard addition.[21,22] To improve the extraction recovery, the four major factors enzyme reaction, solid phase extraction (SPE), evaporation, and measurement with LC-(ESI)MS/MS, which could contribute to a decrease in extraction recovery, were modified from the method of Ishii et al.[21] For example, phosphoric acid used in SPE was changed to acetic acid, and the SPE wash solvent was also changed from 50% methanol to 40% methanol. Retaining a small amount of solvent in the evaporation step also enhanced the extraction recovery. A rapid and sensitive method was developed for quantifying total quercetin species (i.e., quercetin metabolites) in plasma using 96-well SPE and LC-(ESI)MS/MS. Extraction time was rapid (2 h/96 samples) compared with traditional method extraction time (20 h/96 samples). Limit of detection (<5 ng/mL) represented a significant improvement over other methods for the analysis of quercetin in biological samples. Rapid measurement (7 min/analysis) by the LC-(ESI)MS/MS method allowed 96 samples to be run in <2 h. The specific method is described below.

Quercetin glucuronide and/or sulfate conjugates and isorhamnetin glucuronide and/or sulfate conjugates were enzymatically hydrolyzed to quercetin aglycone and isorhamnetin. Quercetin and isorhamnetin analysis was performed as follows: a 300 μL aliquot of plasma from human subjects was mixed with 30 μL of 0.1 M ascorbic acid and 100 μL of sodium acetate buffer (pH 5.5) containing 150 U of sulfatase and 17000 U of glucuronidase. The plasma−enzyme mixture was incubated at 37 °C for 6 h, and 0.07 μg of fisetin was added as an internal standard (I.S.).

After the SPE plate had been preconditioned with 1 mL of methanol and 1 mL of water, the sample was loaded onto a 60 mg 96-well OASIS HLB SPE plate (Waters Corp., Milford, MA, USA).

The plate was washed with 2 mL of 40% aqueous methanol in 2% acetic acid. Low vacuum was applied for 5 min after the plate had been washed to remove residual liquid from the sorbent bed. Quercetin and isorhamnetin with the I.S. were eluted with 3 mL of methanol. The extract was evaporated using a speed-vac until ~100 μL of solvent remained, then it was mixed for 30 s using a vortex mixer. The supernatant was analyzed by negative LC-(ESI)MS/MS on the same day of extraction.

LC-(ESI)MS/MS analyses of extracts were carried out with a HPLC system (Shimadzu Scientific) interfaced to a Quattro LC triple-quadrupole tandem mass spectrometer (Micromass) controlled by MassLynx software (v. 3.5) for data acquisition and processing. Additionally, an SIL-10A autosampler, binary LC 10AD pumps, and Z spray were equipped with the mass spectrometer. The sample components were separated on a Waters Xterra RP 18 (15 cm × 4.6 mm, 3.5 μm) with an isocratic mobile phase of 0.2% formic acid in 90% methanol/10% water (v/v). The flow rate was 0.5 mL/min, and the injection volume was 20 μL.

Electrospray ionization (ESI) achieved better sensitivity than atmospheric pressure chemical ionization. The negative ESI mode showed better sensitivity than the positive ESI mode; thus, the negative ESI mode was used. The capillary, cone, and extractor voltages were set to 3.0 kV, 35 V, and 2 V, respectively, and dwell time was 500 ms. The source temperature and the desolvation gas temperatures were 140 and 400 °C, respectively. Nitrogen was used with a flow of 610 L/h. The multiple reaction monitoring (MRM) mode was utilized for data acquisition. Quantification of quercetin and isorhamnetin was achieved using a calibration curve prepared by plotting the ratio of the analyte response to that of the I.S. MRM was used to analyze quercetin, isorhamnetin, and fisetin (I.S.) by measuring areas of m/z 301 (parent ion) to m/z 151 (product ion) for quercetin, m/z 315 (parent ion) to m/z 300 (product ion) for isorhamnetin, and m/z 285 (parent ion) to m/z 135 (product ion) for fisetin, respectively. Two transitions were monitored for quercetin and isorhamnetin, and the respective m/z were as follows: m/z 301 (parent ion) to m/z 151 and m/z 179 (product ions) for quercetin, and m/z 315 (parent ion) to m/z 151 and m/z 300 (product ions). The limit of detection of quercetin and isorhamnetin was below 5 ppb, and linear ranges of quercetin and isorhamnetin were 10−500 and 10−100 ppb, respectively.

Recovery was accessed by observing the amounts of added authentic standards recovered from a sample matrix. Quercetin aglycone and isorhamnetin standards were added to 0 h plasma (final concentration of 100 ng/mL plasma). The experiment, which was repeated three times, indicated that the recoveries of quercetin aglycone and isorhamnetin from plasma were 103.5 ± 4.3 and 81.3 ± 3.6%, respectively. The reproducibility of the method was followed with analysis of a pooled plasma sample in each run. Day-to-day variations (CV (%)) of quercetin and isorhamnetin in the reference (96.5 ± 8.9 ng/mL of quercetin and 7.8 ± 1.1 ng/mL of isorhamnetin) were 9.3 and 13.5%, respectively.

Pharmacokinetic Methods. Pharmacokinetic variables included peak plasma quercetin concentration (C_{max}), time to reach C_{max} (t_{max}), elimination half-time ($t_{1/2}$), elimination constants (k_{el}), and area under the plasma concentration−time curve from 0 to 24 h ($AUC_{0-24\,h}$). $t_{1/2}$ and k_{el} were calculated by regression of the semilogarithmic concentration versus time data, and $AUC_{0-24\,h}$ was calculated using trapezoidal rule. The pharmacokinetic parameters were calculated using PK Function add-ins (Allergan, Irvine, CA, USA) for Microsoft Excel (Microsoft Corp., Redmond, WA, USA).

Statistical Analysis. Statistical analysis was performed using SPSS software (v. 16.0, SPSS, Inc., Chicago, IL, USA). Significant differences of plasma pharmacokinetic parameters among food treatments were determined using one-way ANOVA followed by Duncan's multiple-range test at $p < 0.05$. Gender difference of plasma pharmacokinetic parameters was evaluated with an independent t test at $p < 0.05$.

RESULTS AND DISCUSSION

Flavonoid glycosides in freeze-dried apple peels and onion powder were characterized by LC-(ESI)MS/MS and quantified using HPLC (Table 2). Typical chromatograms of an apple peel extract and an onion extract are given in Figure 3. Two quercetin diglucosides were identified by monitoring ions corresponding to the $[M + H]^+$ of quercetin 3,4′-*O*-diglucoside

Table 2. Identification of Flavonoid Glycosides in Apple Peel and Onion Powder by LC-(ESI)MS/MS

peak	RT (min)	possible compound	$[M + H]^+$ (m/z)	major fragment ions (m/z)
1	32.2	quercetin 3,4′-*O*-diglucoside	627	465, 303
2	35.6	quercetin 3-*O*-rutinoside (rutin)	611	465, 303
3	36.1	quercetin 3-*O*-galactoside	465	303
4	36.6	quercetin 3-*O*-glucoside	465	303
5	37.8	quercetin *O*-xyloside	435	303
6	38.7	quercetin *O*-arabinofuranoside	435	303
7	39.7	quercetin *O*-arabinopyranoside	435	303
8	40.6	quercetin 3-*O*-rhamnoside	449	303
9	42.5	quercetin 4′-*O*-glucoside	465	303

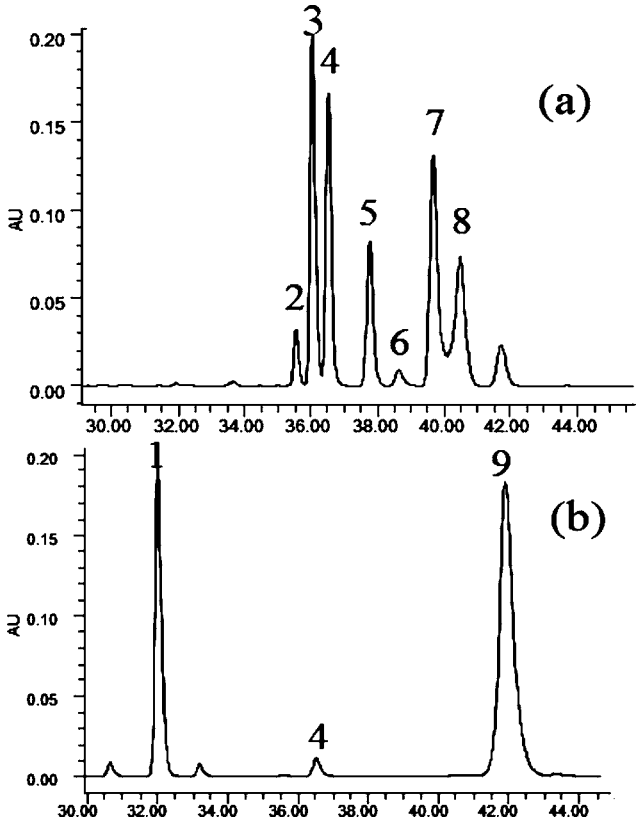


Figure 3. HPLC chromatogram of (a) apple peel extract and (b) onion extract monitored at 370 nm. Peaks: 1, quercetin 3,4′-*O*-diglucoside; 2, quercetin 3-*O*-rutinoside (rutin); 3, quercetin 3-*O*-galactoside; 4, quercetin 3-*O*-glucoside; 5, quercetin *O*-xyloside; 6, quercetin *O*-arabinofuranoside; 7, quercetin *O*-arabinopyranoside; 8, quercetin 3-*O*-rhamnoside; 9, quercetin 4′-*O*-glucoside.

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Table 3. Quercetin Glycoside Content (Milligrams) of the Three Treatment Foods[a]

		food		
peak	quercetin glycoside	apples	onions	mixture
1	quercetin 3,4′-*O*-diglucoside	0 Aa	44.9 ± 1.3 Cc	22.5 ± 0.7 Gb
2	quercetin 3-*O*-rutinoside (rutin)	6.5 ± 0.1 Cc	0 Aa	3.3 ± 0.1 Bb
3	quercetin 3-*O*-galactoside	16.8 ± 0.2 EFc	0 Aa	8.4 ± 0.1 Db
4	quercetin 3-*O*-glucoside	16.1 ± 0.1 Ec	4.9 ± 0.1 Ba	10.5 ± 0.0 Eb
5	quercetin *O*-xyloside	9.3 ± 0.1 Dc	0 Aa	4.7 ± 0.0 Cb
6	quercetin *O*-arabinofuranose	3.0 ± 0.2 Bc	0 Aa	1.5 ± 0.1 Ab
7	quercetin *O*-arabinopyranose	17.6 ± 0.1 Fc	0 Aa	8.8 ± 0.1 Db
8	quercetin 3-*O*-rhamnoside	30.6 ± 2.4 Gc	0 Aa	15.3 ± 1.2 Fb
9	quercetin 4′-*O*-glucoside	0 Aa	48.7 ± 0.8 Dc	24.4 ± 0.4 Hb
	total	99.9 ± 2.3	98.5 ± 2.1	99.2 ± 2.2

[a]Values are the mean ± SD. Mean values followed by different capital letters within each column in the same treatment food for different quercetin glycosides are significantly different at $p < 0.05$. Mean values followed by different lower case letters in different treatment foods for the same quercetin glycoside are significantly different at $p < 0.05$.

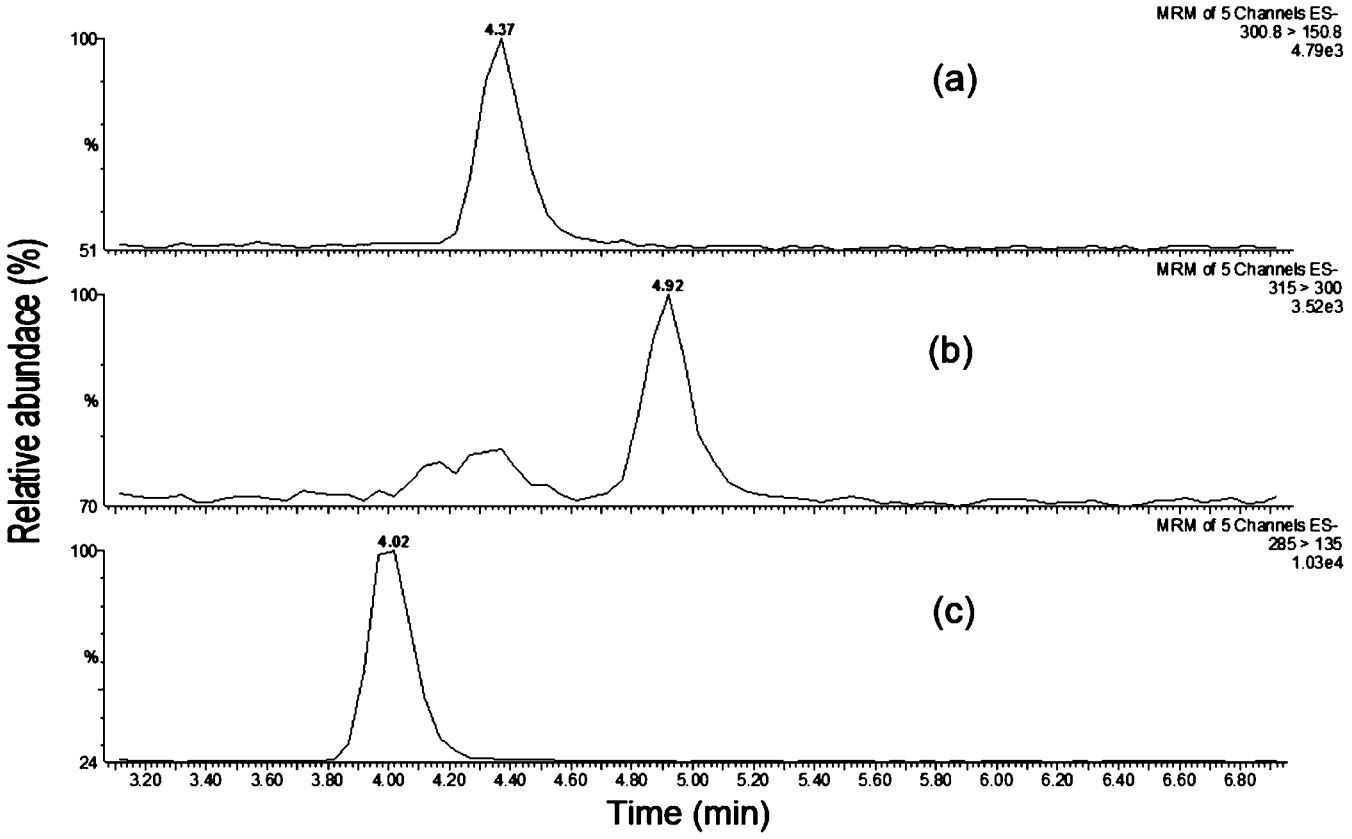

Figure 4. MRM peaks for quercetin obtained from a plasma sample 0.5 h after applesauce containing apple peel: (a) quercetin; (b) isorhamnetin; (c) internal standard (I.S.).

(peak 1) at m/z 627 and the $[M + H]^+$ of quercetin 3-*O*-rutinoside (rutin) (peak 2) at m/z 611. The precursors produced fragment ions at m/z 465 and 303, corresponding to loss of a rhamnoside and a glucoside and loss of two glucosides, respectively. MS spectra of peaks 3, 4, and 9 indicated that these peaks are monoglycosides. MS/MS spectra demonstrated a product ion of m/z 465 at m/z 303 $[M + H - 162]^+$, corresponding to the loss of a glycosyl group. Authentic standards indicate that these peaks correspond to quercetin 3-*O*-galactoside, quercetin 3-*O*-glucoside, and quercetin 4′-*O*-glucoside. Peak 8 was identified as quercetin 3-*O*-rhamnoside with a $[M + H]^+$ of m/z 449 and MS/MS fragment ion of m/z 303 $[M + H - 146]^+$, corresponding to the loss of a rhamnoside. The t_R matched that for the quercetin 3-*O*-rhamnoside standard. Peaks 5−7 had the same mass spectral pattern with a $[M + H]^+$ at m/z 435 and MS/MS fragmentation of m/z 303 $[M + H - 132]^+$. The peaks were tentatively identified as quercetin *O*-xyloside, quercetin *O*-arabinofuranose, and quercetin *O*-arabinopyranose on the basis of MS spectra and reported elution order of these compounds in an apple extracts.[20,23]

The contents of individual and total quercetin glycosides in the three food treatment groups are given in Table 3. Each treatment provided ∼100 mg of total quercetin glycosides.

In apples, quercetin is present as a mixture of *O*-arabinofuranose, *O*-arabinopyranose, 3-*O*-galactoside, 3-*O*-glucoside, 3-*O*-rhamnoside, 3-*O*-rutinoside (rutin), and *O*-xyloside, whereas it occurs primarily as the 4′-*O*-glucoside and 3,4′-*O*-diglucoside in onions.[8,24]

Quercetin and its methyl conjugate (isorhamnetin) were monitored in plasma for 24 h after the subjects consumed the AP, OP or MP. Quercetin was recovered as quercetin and isorhamnetin after the enzymatic cleavage of the sulfate and glucuronide metabolites by sulfatase and glucuronidase. Figure 4 shows the representative MRM peaks for quercetin (a), isorhamnetin (b) and the I.S. (c) in plasma. The time course of the total quercetin (sum of quercetin and isorhamnetin) in plasma after consumption of AP and OP by human subjects is shown in Figure 5. Consumption of OP led to a rapid rise of plasma quercetin, whereas consumption of the AP led to a gradual absorption. After administration of OP, considerable amounts of quercetin were present in plasma at 30 min. The fast absorption of quercetin glycosides (quercetin 3,4′-*O*-diglucoside and quercetin 4′-*O*-diglucoside) from onions implies that they were absorbed in the stomach and/or upper part of the intestine. Bacterial glucosidases in the stomach might also be involved in quercetin glycoside absorption as well as human glucosidases such as lactase phlorizin hydrolase.[25,26] The plasma concentration of quercetin was consistently higher after the consumption of OP than after that of AP. The absorption rate following consumption of the MP was intermediate between the absorption rates of AP and OP. After 24 h, some quercetin remained in plasma, but the concentration was not significantly different from that at 0 h (before oral administration).

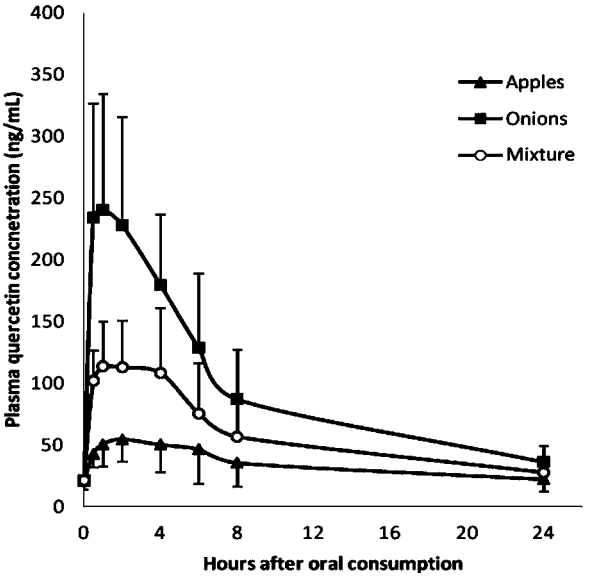

Figure 5. Total plasma quercetin concentration time profiles after consumption of applesauce containing apple peel, onion, or a mixture of the two in a randomized crossover study (n = 16). Error bars represent the SD.

The pharmacokinetic parameters after administration of AP and OP were significantly different (Table 4). The mean peak plasma quercetin concentration (C_{max}) was 63.8 g ng/mL after consumption of AP and 4.3- and 2.1-fold higher after consumption of OP and a MP, respectively ($p < 0.05$). C_{max} after consumption of AP (63.8 ng/mL) was less than reported for apples (92 ng/mL), whereas C_{max} after consumption of OP (273.2 ng/mL) was higher than that reported for onions (224 ng/mL) by Hollman et al.[18] The peak plasma quercetin concentration (t_{max}) was reached 2.9, 2.0, and 2.4 h after consumption of AP, OP, and MP, respectively. t_{max} was not significantly different after consumption of the three treatments. This is not in good agreement with reported values for t_{max} (0.7 and 2.5 h after consumption of onions and apples, respectively).[18] However, in the previous study the onions were fried in oil prior to consumption, which may have increased the absorption rate.[19]

The elimination half-time ($t_{1/2}$) of quercetin following consumption of OP (14.8 h) for all subjects was significantly faster than that following consumption of AP (65.4 h). The results show that circulating quercetin metabolites in human blood after consumption of apples and onions may not be identical. Large individual differences in $t_{1/2}$ of quercetin following consumption of apples may have resulted in the increased average of $t_{1/2}$.

The total absorption of quercetin glycosides as determined from the area under the plasma concentration−time curve (AUC) was significantly greater in OP ($AUC_{0-24\ h}$ of 2340 ng·h/mL) than that of AP ($AUC_{0-24\ h}$ of 843 ng·h/mL) ($p < 0.05$). This value is in good agreement with previously reported values ($AUC_{0-36\ h}$ of 2330 ng·h/mL following consumption of onions and $AUC_{0-36\ h}$ of 1061 ng·h/mL following consumption of apples).[18] The bioavailability of quercetin glycosides from the MP ($AUC_{0-24\ h}$ of 1415 ng·h/mL) was intermediate between the bioavailability values for AP and OP. This result indicates that mixing food sources of glycosides can alter pharmacokinetic parameters as well as circulating metabolites. This may be an important consideration when foods are formulated to have specific functional attributes.

Table 4. Plasma Pharmacokinetic Parameters for Total Quercetin after Consumption of Applesauce Containing Apple Peel, Onion Powder, or a Mixture of the Two (n = 16)[a]

	food		
parameter	apples	onions	mixture
C_{max} (ng/mL)	63.8 ± 22.4 a	273.2 ± 93.7 a	136.5 ± 45.8 b
t_{max} (h)	2.9 ± 2.0	2.0 ± 1.7	2.4 ± 1.5
$t_{1/2}$ (h)	65.4 ± 80.0 b	14.8 ± 4.8 a	18.7 ± 6.8 a
k_{el}	0.025 ± 0.017 a	0.051 ± 0.016 b	0.041 ± 0.012 b
$AUC_{0-24\ h}$ (ng·h/mL)	843 ± 371 a	2340 ± 713 c	1415 ± 580 b

[a]Values are the mean ± SD. Mean values followed by different letters within a row are significantly different at $p < 0.05$. C_{max}, peak plasma quercetin concentration; t_{max}, time to reach C_{max}; $t_{1/2}$, elimination half-time; k_{el}, elimination constants; $AUC_{0-24\ h}$, area under the plasma concentration−time curve from 0 to 24 h.

Over the exposure period post administration of all treatments, the ratio of AUCs for isorhamnetin/(total quercetin) ranged between 7.3 and 10.8. The mean peak plasma isorhamnetin concentration was 3.5-fold higher after consumption of OP than after consumption of AP ($p < 0.05$). The rate of methylation (C_{max} of isorhamnetin/C_{max} of quercetin) was similar regardless of the treatment (7−9%), and the peak plasma isorhamnetin concentrations were reached at almost the same time (~3 h after intake of AP and/or OP).

The pharmacokinetics of orally administered quercetin were described by a two-compartment model with two- or three-sequential absorption phases (Figure 6). Six of 16 subjects showed three-sequential absorption phases. This finding implies that absorption did not undergo a first-order process. Enterohepatic recirculation may indicate that quercetin already eliminated was reabsorbed in the colon, resulting in the second rise. The second rise might be due to colonic bacteria liberating quercetin glycosides bound to the food matrix such as dietary fiber. The released quercetin glycosides may be hydrolyzed to quercetin by flavonoid glycoside-hydrolyzing enzymes in the colon.[7] In the colon, bacterial glucosidase cleave the flavonoid ring, resulting in ring fission products such as hydroxyl phenylacetic acids.[7,25] The different absorption phases among subjects may have been the result of carrier-mediated transport processes, physiological differences such as pH changes along the intestinal tract, and distinct transit times.

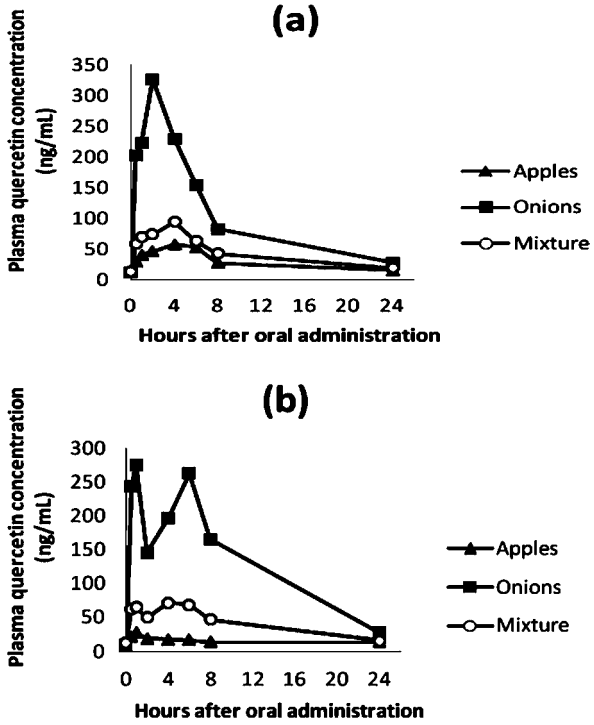

Figure 6. Total quercetin concentration−time profiles after consumption of applesauce containing apple peel, onion, or a mixture of the two: (a) representative two-absorption phase curve; (b) representative three-absorption phase curve.

Notable differences in the total quercetin concentration−time profiles between subjects were apparent and demonstrated the potential of individual differences in the pharmacokinetic parameters. For example, the C_{max} of quercetin for the 16 subjects ranged between 52.8 and 74.7 ng/mL (95% confidence interval) after AP consumption, between 227.2 and 319.1 ng/mL after OP consumption, and between 114.1 and 158.9 ng/mL after MP consumption. In 25% of the subjects, the peak concentrations were achieved by 0.5 h after consumption of OP, which was the first data time point; in 13% subjects, the peak concentrations were reached by 4 h (data not shown). C_{max} of a high absorber subject was 2.2-fold higher than that of a low absorber subject after consumption of OP.

The pharmacokinetics of quercetin were not significantly different in females ($n = 8$) and males ($n = 8$) after consumption of the OP or AP. However, elimination half-time ($t_{1/2}$) of females (93.8 h for AP and 15.2 h for OP) was much higher than that of males $t_{1/2}$ of (29.9 h for AP and 13.4 h for OP). $t_{1/2}$ times were not significantly different because of high variation among female subjects. This finding may imply that quercetin was more slowly eliminated in females than in males and, in turn, significantly prolonged the half-life. Females appear to empty food from their stomachs more slowly than males do; although the mechanism is unknown, sex hormones are thought to have an influence on gastrointestinal emptying time.[27] In this population, gender did not affect the relative bioavailability of quercetin ($AUC_{0-24\ h}$) when given as the AP (868 ± 311 ng·h/mL for females and 932 ± 641 ng·h/mL for males) or the OP (2260 ± 425 ng·h/mL for females and 2681 ± 977 ng·h/mL for males). In a previous study, gender was reported to affect the AUC of quercetin from rutin (quercetin rutinoside), but not when given as quercetin aglycone. Quercetin from rutin was more bioavailable in women than in men.[28]

In conclusion, this is the first report of a high-throughput method for the extraction of quercetin in plasma and for its rapid analysis by LC-(ESI)MS/MS. In apples, the predominant forms of quercetin were 3-*O*-galactoside, 3-*O*-glucoside, and 3-*O*-rhamnoside, and in onions, quercetin occurred as the 3,4′-*O*-diglucoside and 4′-*O*-glucoside. Consumption of the OP led to faster absorption, higher concentrations, and greater bioavailability as compared to the AP. Importantly, we have demonstrated that mixing food sources of quercetin can alter the pharmacokinetics of quercetin. This result can help direct intelligent formulation of products for enhanced and prolonged delivery of flavonoids such as quercetin based on glycoside patterns.

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Notes

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■ ABBREVIATIONS USED

AP, apple (peel) powder enriched applesauce; OP, onion powder enriched applesauce; MP, mixture of apple peel and onion powder enriched applesauce; SPE, solid phase extraction; I.S., internal standard; ESI, electrospray ionization; MRM, multiple reaction monitoring.

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