Human Gut Microbial Degradation of Flavonoids: Structure–Function Relationships

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The relationship between chemical structure and gut microbial degradation rates of 14 flavonoids, flavone, apigenin, chrysin, naringenin, kaempferol, genistein, daidzein, daidzin, puerarin, 7,4′-dihydroxyflavone, 6,4′-dihydroxyflavone, 5,4′-dihydroxyflavone, 5,3′-dihydroxyflavone, and 4′-hydroxyflavone, was investigated by anaerobically fermenting the flavonoids with human gut microflora ($n = 11$ subjects). Degradation rates for the 5,7,4′-trihydroxyl flavonoids, apigenin, genistein, naringenin, and kaempferol, were significantly faster than the other structural motifs. Puerarin was resistant to degradation by the gut microflora. Extensive degradation of flavonoids by gut microflora may result in lower overall bioavailability than those flavonoids that are slowly degraded because rapidly degrading flavonoids are less likely to be absorbed intact.

KEYWORDS: Flavonoids; microbial degradation; structure−activity

INTRODUCTION

Flavonoids are a large group of polyphenolic compounds that are widely distributed in all plants. Fruits, vegetables, and beverages (fruit juices, wine, tea, and coffee) are major sources of flavonoids in the human diet, and over 4000 of these compounds have been reported to date (*1*). Adequate intakes of fruits and vegetables are reported to be associated with reduced risks of cardiovascular disease (*2*) and cancer (*3*). These observations may be attributed, in part, to the antioxidant effects of flavonoids (*4−7*).

Flavonoids are diphenylpropanes consisting of two phenolic rings, A and B, connected by a three carbon unit, which along with an oxygen atom, forms the heterocyclic C ring. Flavonoids are systematically classified into subgroups including flavones, isoflavones, flavonols, and flavanones, which are characterized by differences in their C ring structure (*8*). Differences within these flavonoid subgroups are characterized by substitutions of hydroxyl, methoxyl, methyl, and glycosidic groups on the A, B, and C rings (**Figure 1**). Flavonoids are found in foods mainly as *O*-glycosides. Glucose is the most common sugar moiety, but other glycosidic units can include galactose, rhamnose, arabinose, and xylose. The *O*-β-glucosidic bonds of flavonoids including the isoflavones daidzin (daidzein-7-*O*-β-D-glucopyranoside), genistin, and glycitin are hydrolyzed in the gut by microbial and mammalian β-glucosidases to their aglucons, daidzein, genistein, and glycitein, respectively (*9−13*).

The absorption and metabolism of flavonoid aglucons in humans are not fully understood, but the aglucons may be absorbed, undergo first pass hepatic metabolism (*14*), and be excreted in the urine or bile (*15*). Intestinal bacteria can further catabolize the flavonoid aglucons into smaller phenolic compounds that can be reabsorbed by enterohepatic recirculation via the bile duct or catabolized completely for energy (*13*, *15−19*).

The chemical structure of flavonoids determines the extent and rate of absorption in the gut as reflected in the amounts of flavonoids found intact in the urine and plasma (*20−23*). Previous studies by Xu et al. (*20*) have shown that the rate of isoflavone degradation by human gut microflora depends on the structure of the isoflavone. Genistein, which possesses a 5-hydroxyl, was rapidly degraded as compared to daidzein, which does not have a 5-hydroxyl group. Lin et al. (*24*) have reported that flavonoids with methoxyl groups, such as diosmetin (5,7,3′-trihydroxy-4′-methoxyflavone), hesperetin (5,7,3′-trihydroxy-4-methoxyflavanone), and wogonin (5,7-dihydroxy-8-methoxyflavone), were less rapidly degraded as compared to flavonoids without methoxyl groups.

Microbial degradation of flavonoids with C−C-linked glucose groups, such as puerarin (daidzein-8-*C*-β-D-glucopyranoside), has not been well-studied. Puerarin is structurally similar to daidzin but with the glucose group bound directly to the C-8 position of the A ring. Puerarin is the major isoflavone found in kudzu (*Pueraria lobata*), a plant used in traditional Chinese medicine and as a nutraceutical (*25*). Puerarin can be found in commercial isoflavone dietary supplements where kudzu was used as the sole source or in combination with soy isoflavones.

The purpose of this study was to determine the structural characteristics of flavonoids that are important for optimal degradation by the human gut microflora. This study compared

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Structure	Flavonoid Subgroup	Flavonoid	R_2	R_3	R_5	R_6	R_7	R_8	$R_{3'}$	$R_{4'}$
	Flavone	Flavone		H	H	H	H	H	H	H
		4'-hydroxyflavone		H	H	H	H	H	H	OH
		5,4'-dihydroxyflavone		H	OH	H	H	H	H	OH
		6,4'-dihydroxyflavone		H	H	OH	H	H	H	OH
		7,4'-dihydroxyflavone		H	H	H	OH	H	H	OH
		5,3'-dihydroxyflavone		H	OH	H	H	H	OH	H
		Apigenin		H	OH	H	OH	H	H	OH
		Chrysin		H	OH	H	OH	H	H	H
	Isoflavone	Daidzein	H		H	H	OH	H	H	OH
		Daidzin	H		H	H	Ogl	H	H	OH
		Puerarin	H		H	H	OH	Cgl	H	OH
		Genistein	H		OH	H	OH	H	H	OH
	Flavonol	Kaempferol		OH	OH	H	OH	H	H	OH
	Flavanone	Naringenin		H	OH	H	OH	H	H	OH

Figure 1. Flavonoid subgroup structures and substitution patterns. Apigenin, 5,7,4′-trihydroxyflavone; chrysin, 5,7-dihydroxyflavone; daidzein, 7,4′-dihydroxyisoflavone; daidzin, daidzein-7-*O*-β-D-glucopyranoside; puerarin, daidzein-8-*C*-β-D-glucopyranoside; genistein, 5,7,4′-trihydroxyisoflavone; kaempferol, 3,5,7,4′-tetrahydroxyflavone; naringenin, 5,7,4′-trihydroxyflavanone; Ogl, 7-*O*-β-D-glucopyranose; and Cgl, 8-*C*-β-D-glucopyranose. (**A**) A ring, (**B**) B ring, and (**C**) C ring.

the relationship of the chemical structure of 14 flavonoids, flavone, apigenin, chrysin, naringenin, kaempferol, genistein, daidzein, daidzin, puerarin, 7,4′-dihydroxyflavone, 6,4′-dihydroxyflavone, 5,4′-dihydroxyflavone, 5,3′-dihydroxyflavone, and 4′-hydroxyflavone, with their degradation rate by gut microflora from human subjects.

MATERIALS AND METHODS

Chemicals. Genistein was synthesized according to modification of Chang et al. (*26*). Daidzein and 2,4,4′-trihydroxydeoxybenzoin (THB) were synthesized using the method of Song et al. (*27*). Flavone, apigenin, chrysin, naringenin, kaempferol, puerarin, 7,4′-dihydroxyflavone, 6,4′-dihydroxyflavone, 5,4′-dihydroxyflavone, 5,3′-dihydroxyflavone, and 4′-hydroxyflavone were from Indofine Chemical Co., Inc. (Hillsborough, NJ). Daidzin was purchased from LC Labs (Woburn, MA). High-performance liquid chromatography (HPLC) grade acetonitrile, methanol, acetic acid, dimethyl sulfoxide (DMSO), and all other chemicals were from Fisher Scientific (Fairlawn, NJ). Milli-Q system (Millipore Co., Bedford, MA) HPLC grade water was used to prepare all solutions.

Subject Protocol. Approval of the study design was obtained from the Iowa State University Human Subjects Research Committee in 2003. Three men and eight women volunteered from Iowa State University and the surrounding Ames area. The selection criteria required that the subjects be in good health and not taking any medication. The subjects' ages ranged from 24 to 53 years (mean age = 33.8 ± 3.2 years) with a body mass index (BMI) of 20.9−25.8 kg/m^2 (mean BMI = 23.9 ± 0.9 kg/m^2), respectively. The ethnicities of the subjects included five Caucasians, three African Americans, one Chinese immigrant, one Asian-Indian, and one Latino. All subjects provided one fresh fecal sample in sealed sterile containers (Sage Products Inc., Crystal Lake, IL) that was used immediately.

Flavonoid Fermentation. Brain−heart infusion (BHI) broth media (Difco Laboratories, Detroit, MI) was prepared according to Zheng et al. (*21*). All flavonoid aglucons were dissolved in 100% DMSO. One and a half grams of freshly voided feces was transferred to incubation test tubes (Fisher Scientific) containing 25 mL of BHI. The flavonoids flavone, 4′-hydroxyflavone, 5,4′-dihydroxyflavone, 6,4′-dihydroxyflavone, 7,4′-dihydroxyflavone, 5,3′-dihydroxyflavone, chrysin, apigenin, genistein, naringenin, kaempferol, and daidzein were added to the incubation test tubes for a final concentration of 78.7 μmol/L. The fermentations were performed in duplicate. The incubation test tubes were flushed with CO_2, sealed with rubber stoppers and autoclave tape, and then vortexed for 5 s. One milliliter was taken anaerobically from each test tube immediately for time 0 and frozen on dry ice. The tubes were placed in a 37 °C incubator. One milliliter aliquots were sampled from the incubation test tubes at 3, 6, 9, 12, and 24 h and frozen. Negative controls consisted of the fecal suspension without flavonoids. Microbial degradation by the fecal suspension was confirmed by positive controls, which consisted of BHI media and flavonoids without the fecal suspension.

Isoflavone Glucoside Fermentation. Stock solutions of 10 mmol/L puerarin and 5.4 mmol/L daidzin were prepared in 80% methanol. Two grams of fresh feces from two subjects was transferred to incubation test tubes with 27 mL of BHI. Puerarin and daidzin stock solutions were each added to give a final concentration of 4.8 μmol/L. The control incubation contained no isoflavones. Incubations were performed according to the method stated above for flavonoids.

Flavonoid Extraction. THB, as an internal standard, was added at 100 μmol/L to the thawed fermentation sample and slowly loaded onto preconditioned C18 solid phase extraction cartridges (Waters Corporation, Milford, MA). The cartridge was washed twice with 2 mL of Milli-Q system water. The flavonoids were eluted with 1 mL of 80% methanol, filtered through 0.45 μm filters, and analyzed directly by HPLC.

HPLC Analysis. The HPLC system consisted of a Hewlett-Packard 1050 Series. Twenty microliters of sample was injected onto a reversed-phase, 5 μm, C18 AM 303 column (250 mm × 4.6 mm) (YMC Co. Ltd., Wilmington, NC). The mobile phase consisted of 0.1% glacial acetic acid in water (A) and 0.1% glacial acetic acid in acetonitrile (B). Solvent B increased from 30 to 50% in 8 min, increased to 100% in 8 min, and was held for 3 min. The gradient was recycled back to 30% in 1 min for the next run. The flow rate was 1 mL/min. The wavelengths used for the preparation of standard curves, detection, and quantification of flavonoid peaks were 254 and 292 nm. The minimum

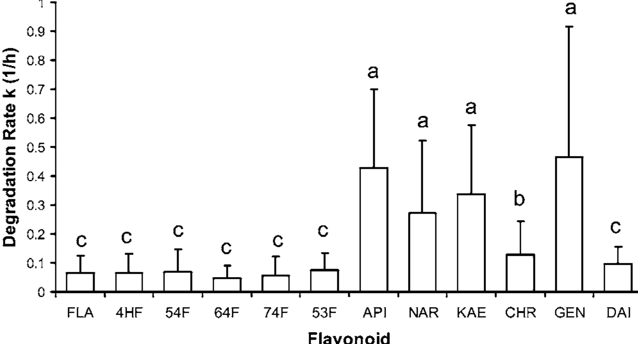


Figure 2. In vitro human microbial degradation rates of flavonoids. FLA, flavone; 4HF, 4′-hydroxyflavone; 54F, 5,4′-dihydroxyflavone; 64F, 6,4′-dihydroxyflavone; 74F, 7,4′-dihydroxyflavone; GEN, genistein; API, apigenin; NAR, naringenin; KAE, kaempferol; 53F, 5,3′-dihydroxyflavone; CHR, chrysin; and DAI, daidzein. Bars with different letters are significantly different ($p < 0.05$, $n = 11$). Error bars represent standard deviation from the mean.

detection limit of all flavonoids ranged from 5 to 7 nM. The gradient elution used to separate puerarin and daidzin was of the method of Song et al. (*27*). Chem station^{3D} software (Hewlett-Packard Company, Scientific Instruments Division, Palo Alto, CA) was used to integrate the peak area responses and to evaluate the ultraviolet absorbance spectra.

Data Analysis. The ratio of peak area of a flavonoid to THB (100 μmol/L) vs the flavonoid concentration was used as an internal standard curve to estimate the concentration of flavonoids in the fecal fermentations. The rate of disappearance of flavonoids in fecal fermentation mixtures was estimated by plotting ln(% remaining flavonoid) vs time. The negative slope of this line was the apparent first-order degradation rate constant. Statistical evaluation of degradation rate differences was performed using the SAS system (version 8.1, SAS Institute., Cary, NC). Differences between the overall and the individual degradation rates of flavonoids were estimated using one-way analysis of variance. Flavonoid degradation phenotypes were identified using cluster analysis (*28*). The statistical significance of all analyses was set at $\alpha = 0.05$.

RESULTS AND DISCUSSION

The structures of four flavonoid subgroups, including the A, B, and C rings and the substitution patterns of each flavonoid analyzed, are shown in **Figure 1**. Degradation rate differences due to different A ring substitution patterns were investigated by comparing the microbial degradation rates of 4′-hydroxyflavone, 5,4′-dihydroxyflavone, 6,4′-dihydroxyflavone, and 7,4′-dihydroxyflavone. There were no differences when the degradation rates of flavonoids with A ring variations were compared across all subjects with an average $k = 0.060 \pm 0.053$ h^{-1} ($p = 0.30$) (**Figure 2**).

Degradation rate differences due to different hydroxylation patterns on the B ring of flavonoids were analyzed by pairwise comparison of flavone and 4′-hydroxyflavone, 5,3′-dihydroxyflavone, and 5,4′-dihydroxyflavone and chrysin (5,7-dihydroxyflavone) and apigenin (5,7,4′-trihydroxyflavone) degradation rate constants. There were no differences between the degradation rates of flavone and 4′-hydroxyflavone with an average of $k = 0.065 \pm 0.061$ h^{-1} ($p = 0.83$) and between 5,4′-dihydroxyflavone and 5,3′-dihydroxyflavone with an average of $k = 0.071 \pm 0.067$ h^{-1} ($p = 0.42$). However, the degradation rate of apigenin with an average $k = 0.43 \pm 0.27$ h^{-1} was significantly faster than chrysin with an average $k = 0.13 \pm 0.11$ h^{-1} ($p = 0.01$) suggesting that the hydroxyl group at the 4′-position was important for rapid microbial degradation but only if additional hydroxyl groups were present at the 5- and 7-positions (**Figure 2**).

The degradation rate differences due to C ring substitution were compared by analyzing the degradation rates of apigenin (5,7,4′-trihydroxyflavone), genistein (5,7,4′-trihydroxyisoflavone), naringenin (5,7,4′-trihydroxyflavanone), and kaempferol (3,5,7,4′-tetrahydroxyflavone). There were no differences between the degradation rates of apigenin, genistein, naringenin, and kaempferol with an average $k = 0.38 \pm 0.25$ h^{-1} ($p > 0.05$) (**Figure 2**). These data suggested that the absence of the 2−3 double bond, as found in naringenin, or the addition of a 3-OH group, as in kaempferol, did not affect the rate of microbial degradation.

Apigenin (5,7,4′-trihydroxyflavone) and 7,4′-dihydroxyflavone are the flavone analogues to genistein (5,7,4′-trihydroxyisoflavone) and daidzein (7,4′-dihydroxyisoflavone), respectively. The average degradation rate of genistein was not different from apigenin with an average $k = 0.45 \pm 0.29$ h^{-1} ($p = 0.13$), and the average degradation rate of daidzein was not different from 7,4′-dihydroxyflavone with an average $k = 0.076 \pm 0.063$ h^{-1} ($p = 0.72$) (**Figure 2**), suggesting that the attachment of the B ring at the C-3-position for isoflavones, instead of at the C-2-position for flavones, does not affect the rate of bacterial degradation.

Genistein, apigenin, kaempferol, and naringenin were the most rapidly degraded flavonoids as compared to all other flavonoids examined ($p < 0.0001$) (**Figure 2**). These flavonoids all have a common structure with hydroxyl groups at the 5-, 7-, and 4′-positions. This observation suggested that these three hydroxyls were important for optimal flavonoid degradation. It is evident from the results reported here that any flavonoid missing any one of the 5-, 7-, or 4′-hydroxyls degraded slower than genistein, apigenin, naringenin, and kaempferol.

Lin et al. (*24*) was the only other investigation of the relationship between chemical structures and microbial degradation of flavonoids (*24*). Thirteen flavonoids were analyzed including genistein, apigenin, naringenin, kaempferol, and daidzein. The other flavonoids included morin (3,5,7,2′,4′-pentahydroxyflavone), luteolin (5,7,3′,4′-tetrahydroxyflavone), quercetin (3,5,7,3′,4′-pentahydroxyflavone), wogonin (5,7-dihydroxy-8-methoxyflavone), baicalein (5,6,7-trihydroxyflavone), hesperetin (5,7,3′-trihydroxy-4-methoxyflavanone), diosmetin (5,7,3′-trihydroxy-4′-methoxyflavone), and neophellamuretin (3,5,7,4′-tetrahydroxy-8-isoprenylflavanone). Fecal incubations from rabbits, rats, and three humans were used. Lin et al. (*24*) observed that wogonin and diosmetin, which possess methoxyl groups, were the least degraded flavonoids in all three species and concluded that the presence of methoxyl groups on the A or B ring rendered the flavonoid resistant to microbial degradation (*24*). Our preliminary experiments have shown similar results in which glycitein (7,4′-dihydroxy-6-methoxyisoflavone), which possesses a 6-methoxyl group, was degraded at a slower rate than genistein, $k = 0.30 \pm 0.21$ h^{-1} vs 0.43 ± 0.44 h^{-1} ($p < 0.18$) in fecal fermentations from 12 human subjects. Additional evaluation of the Lin et al. data, however, revealed that all of the flavonoids with 5-, 7-, and 4′-hydroxyl groups degraded faster than the flavonoids that were lacking all of these hydroxyls in all three species, which is in agreement to our study. The only exception to this generalization was observed with baicalein (5,6,7-trihydroxyflavone), which had no hydroxyl groups at the 4′-position. Baicalein was found to be extensively degraded by human fecal microorganisms but not rabbit or rat fecal microorganisms. Similarly, we found that chrysin (5,7-dihydroxyflavone), which has no 4′-hydroxyl group, degraded significantly faster than flavonoids lacking all three 5-, 7,- and 4′-hydroxyl groups (**Figure 2**). We speculate that flavonoids

Table 1. Cluster Analysis of Subjects' Degradation Rates and Segregation into Degradation Phenotypes Groupings[a]

flavonoid	degradation rate k (1/h)[b]			subject ID[c]		
	high	moderate	low	high	moderate	low
flavone	0.18 ± 0.02	0.06 ± 0.02	0.02 ± 0.01	13, 26	5, 8, 9, 18	2, 3, 4, 6, 17
4′-hydroxyflavone	0.16 ± 0.03	0.06 ± 0.02	0.02 ± 0.01	9, 13, 26	5, 18	2, 3, 4, 6, 8, 17
5,3′-dihydroxyflavone	0.16 ± 0.03	0.06 ± 0.02	0.01 ± 0.01	13, 17, 26	4, 5, 8, 9, 18	2, 3, 6
5,4′-dihydroxyflavone	0.21 ± 0.04	0.10 ± 0.03	0.02 ± 0.02	17, 26	5, 13	2, 3, 4, 6, 8, 9, 18
6,4′-dihydroxyflavone	0.11 ± 0.02	0.04 ± 0.01	0.02 ± 0.01	13, 17, 26	2, 5, 18	3, 4, 6, 8, 9
7,4′-dihydroxyflavone	0.18 ± 0.05	0.08 ± 0.03	0.02 ± 0.01	17, 26	4, 13	2, 3, 5, 6, 8, 9, 18
chrysin	0.28 ± 0.06	0.15 ± 0.03	0.03 ± 0.02	9, 13, 26	4, 5, 8	2, 3, 6, 17, 18
daidzein	0.17 ± 0.02	0.12 ± 0.01	0.04 ± 0.01	9, 13, 26	2, 4, 17	3, 5, 6, 8, 18
genistein	1.54 ± 0.00	0.77 ± 0.01	0.18 ± 0.08	13	2, 3, 26	4, 5, 6, 8, 9, 17, 18
apigenin	0.77 ± 0.01	0.53 ± 0.03	0.17 ± 0.07	9, 13, 26	4, 5, 8	2, 3, 6, 17, 18
naringenin	0.77 ± 0.01	0.19 ± 0.01	0.08 ± 0.04	13, 26	4, 5, 6, 8, 9, 17, 18	2, 3
kaempferol	0.70 ± 0.10	0.47 ± 0.07	0.15 ± 0.07	13, 26	2, 8, 9	3, 4, 5, 6, 17, 18

[a] Degradation phenotypes of subjects. Subjects separated into three significantly different groups for each flavonoid, named high, moderate, and low degraders ($p < 0.0001$). [b] Degradation rates expressed as average ± standard deviation from the mean. [c] Whole numbers shown are subjects' identification numbers.

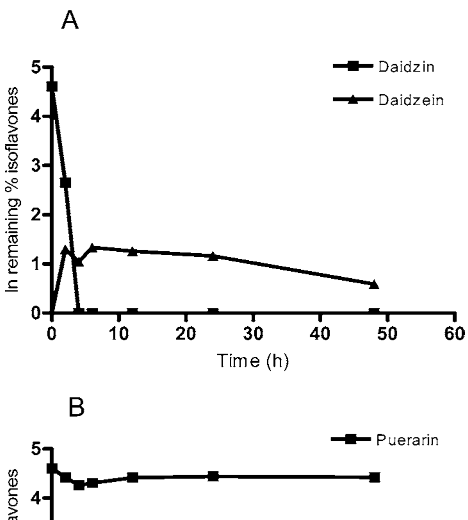
with 5- and 7-hydroxyls are moderately degraded by human gut microorganisms, but the addition of the 4′-hydroxyl significantly enhances the microbial degradation rate.

Naringenin and kaempferol are structurally similar to apigenin except that naringenin lacks the 2−3 double bond in the C ring and kaempferol has an additional hydroxyl group attached to the C-3-position of the C ring. The lack of significant differences between the average degradation rates of apigenin, naringenin, and kaempferol suggests that the 2−3 double bond and a 3-OH group on the flavonoid structure were not necessary for microbial degradation as long as the flavonoid possessed hydroxyl groups at the 5-, 7-, and 4′-positions. Our results support the work of Lin et al. (*24*), who reported that these features did not affect the degradation rate.

Puerarin (daidzein-8-C-β-D-glucopyranoside) and daidzin (daidzein-7-*O*-β-D-glucopyranoside) are the two predominant isoflavones found in kudzu root. Daidzin was rapidly hydrolyzed to daidzein with a $k = 1.15 \pm 0.01$ h^{-1} and disappeared from the fecal fermentation mixture within 4 h of incubation with fecal microorganisms of two subjects. Daidzein was detected at 2 h after incubation with daidzin (**Figure 3A**). In contrast, puerarin was not hydrolyzed after 48 h with no daidzein being detected in the puerarin incubation (**Figure 3B**). These results suggest that *C*-glucosides are resistant to β-glucosidase activity as compared to *O*-glucosides. It is possible that the position and type of glycosidic linkages of flavonoid glycosides alter their rate of hydrolysis by the gut microflora.

Kim et al. (*29*) reported that puerarin was converted to daidzein after incubation with fresh feces from a single subject after 48 h, which is in contrast to our observations. No concentration of daidzein formed from puerarin in the Kim et al. study was reported, however. Yasuda et al. (*30*) reported that the urine of rats orally dosed with 100 mg/kg of pure puerarin contained unchanged puerarin, daidzein, and the glucuronide and sulfate conjugates of daidzein and puerarin. The total amount of urine metabolites excreted in 48 h was 3.63% of the puerarin administered, with daidzein comprising less than 0.5%. These data suggest that puerarin might be hydrolyzed to daidzein but not in significant amounts based on the percentage recovered in urine. Less than 1% of unchanged puerarin was recovered in the urine and bile suggesting that puerarin is minimally absorbed intact in the gut (*30*, *31*). The Yasuda et al. (*30*) data conflict other studies reporting that flavonoid glucosides must be hydrolyzed before absorption in the gut (*32*−*36*).

There was considerable variability among the subjects in their degradation rates for each flavonoid ($p < 0.0001$). All subjects'



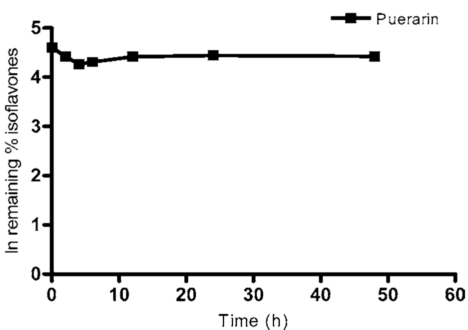


Figure 3. Human microbial degradation of isoflavone glucosides vs time. (**A**) Disappearance of daidzin and appearance of daidzein. (**B**) Stability of puerarin over time. Standard deviation from the mean at each time point was <0.07.

degradation rates for each flavonoid were analyzed using cluster analysis. Cluster analysis is a statistical program that is able to group together similar degradation rates. The subjects segregated into three different degradation rate groupings for each flavonoid. We described these groups as phenotypic differences in the subjects and called them high, moderate, and low flavonoid degraders (**Table 1**). Most of the subjects remained in their respective flavonoid degradation phenotype groups for all flavonoids examined suggesting that each phenotype may exist as a stable characteristic in these subjects (**Table 1**). These phenotypes may represent differences in gut microbial populations or enzyme activities. High flavonoid degraders would be more likely to produce flavonoid metabolites as a result of anaerobic metabolism in the gut and thus absorb less intact flavonoids across the intestinal epithelium. On the other hand, low flavonoid degraders may produce fewer flavonoid metabo-

lites but have greater probability to absorb intact flavonoids. Additionally, low flavonoid degraders may experience greater "in situ" activity from flavonoid aglucons as compared to high flavonoid degraders.

We have not determined why the human gut microorganisms prefer to degrade 5,7,4′-trihydroxylflavonoids. Synthesis of flavonoids in plants comes from resorcinol or phloroglucinol synthesis from the acetate pathway. This pathway produces the 5,7-hydroxylation pattern in the A ring. The shikimate pathway produces the B ring and results in 4′-, 3′,4′-, and 3′,4′,5′-hydroxylation patterns (*37*). Because genistein, apigenin, naringenin, and kaempferol are the flavonoid aglucons found predominantly in the food supply, we believe that flavonoids with 5,7,4′-hydroxylation patterns dominate in nature, and the human gut microflora are more exposed to these dietary compounds and have adapted to metabolize them.

We observed that hydroxyl groups at the 5-, 7,- and 4′-positions of flavonoids are important structural characteristics for optimal flavonoid degradation by human gut microflora. The flavonoid degradation rate ranking is as follows: genistein = apigenin = kaempferol = naringenin > chrysin > daidzein = 5,3′-dihydroxyflavone = 5,4′-dihydroxyflavone = 6,4′-dihydroxyflavone = 7,4′-dihydroxyflavone = 4′-hydroxyflavone = flavone. These observations have great implications for evaluating the potential bioavailability of flavonoids. A prediction of the rate of flavonoid degradation by human gut microorganisms can be made by evaluating the structure of a flavonoid. Genistein, apigenin, naringenin, and kaempferol were degraded more rapidly than the other flavonoids without hydroxyl groups at the 5-, 7-, and 4′-positions. Therefore, genistein, apigenin, naringenin, and kaempferol may not be as bioavailable in the colon as compared to more slowly degraded flavonoids, because they have less time to be absorbed before they are degraded by the gut microflora. However, the degradation products of genistein, apigenin, naringenin, and kaempferol may potentially be bioactive metabolites of interest. The other slowly degraded flavonoids examined may be more bioavailable because the gut microflora degraded them at a slower rate, which gives these flavonoids a greater opportunity to be absorbed.

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