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Improving Bioavailability and Stability of Genistein by Complexation with High-Amylose Corn Starch

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ABSTRACT: Genistein, like other phytochemicals, has beneficial health effects, but its bioavailability is limited. This research studied the effect of complexation of genistein with starch on genistein bioavailability. Genistein release from these complexes was tested in vitro under simulated intestinal conditions and in vivo in rats fed high-amylose corn starch (HACS)—genistein complexes (experimental group) as compared to those fed a physical mixture of HACS and genistein (controls). In vitro results showed that genistein release is sustained and fits the normal transit time of food in the intestine. The genistein concentration in the plasma was twice as high in the experimental group versus controls; the genistein concentration in the urine was also higher in the experimental group but lower in the feces. These results indicate that starch—genistein complexes increase genistein bioavailability and suggest that starch can affect the bioavailability of additional food components.

KEYWORDS: Amylose, bioavailability, genistein

INTRODUCTION

The high consumption of isoflavones from soy products in Asian countries is assumed to contribute to their lower rates of prostate and breast cancers as compared to western populations.[1] There is now much evidence to support the hypothesis that genistein (4′,5,7-trihydroxyisoflavone), one of the main aglycone forms of isoflavones found in soybean and soy products, is responsible for many of these health-promoting effects, such as reduced risk of cardiovascular diseases, lowered rate of prostate, breast, and colon cancers, improved bone health, and attenuated postmenopausal symptoms.[2−4] Some of the health benefits of genistein are related to its structural similarity to estradiol-17β and to its antioxidant activity.[4−6] Calculation of the genistein dose required to exert its beneficial health effects is based on the consumption of soy products in Asian countries, giving a recommended isoflavone dose of ∼40−50 mg daily.[4,7] This amount is not consumed by most people in western countries, so it would be a great advantage to add genistein to food products.

The introduction of nutraceuticals into food products poses a number of technological challenges due to their sensitivity to light, heat, and oxidation.[8] In addition, genistein has a bitter taste and low water solubility, limiting its applicability in the food industry. Moreover, genistein's low water solubility, low partition coefficient in oil/water, poor absorption, and significant first-pass metabolism (glucuronidation and sulfation) strongly limit its bioavailability.[9−11] Genistein bioavailability is also determined by gut transit time.[9] Many polyphenolic bioactives share the same problems, and a generic solution is expected to have wide application. Therefore, there is a need for a cost-effective delivery system for genistein that preserves its chemical stability, controls its release in the food product and through the gastrointestinal tract, and improves its sensory properties.

Amylose, the linear fraction of starch, is known to form inclusion complexes with a variety of hydrophobic guest molecules, such as iodine and fatty acids.[12−16] Amylose has also been shown to form inclusion complexes with bulkier molecules, such as aroma compounds and genistein, and to modulate the guest's release under specific conditions.[17,18] Amylose—genistein complexes characterized by X-ray diffraction (XRD) form a $V6_{III}$ crystalline structure.[19] These complexes show very good stability under simulated stomach conditions and release the entrapped genistein under simulated intestinal conditions using α-amylase. In addition, high-amylose corn starch (HACS) can also form complexes with genistein with only a slight difference in genistein content (∼9.3% in HACS complexes as compared to ∼11.3% in amylose complexes).[19] The significance of this complexation has only been investigated in vitro.

The aim of the present study was to create a delivery system for genistein to test its bioavailability in a complex with HACS and to compare it to a physical mixture of genistein and HACS. Genistein bioavailability was tested in vitro under simulated intestinal conditions for 24 h periods and assessed in vivo in rats.

MATERIALS AND METHODS

Materials. Potato amylose type III (av. DP 900, essentially free of amylopectin) and pancreatin (amylase activity of 41 USP units/mg, from porcine pancreas) were obtained from Sigma Chemical Co. (St. Louis, MO). HACS was obtained from National Starch (HYLON VII; Bridgewater, NJ). Genistein was purchased from LC Laboratories (Woburn, MA), and daidzein was purchased from Acros Organics BVBA (Geel, Belgium). All other reagents were of analytical grade.

Preparation of HACS—Genistein Complexes. Complexation was carried out via acidification of an alkali solution (KOH/H_3PO_4) as previously described.[19] Briefly, amylose or HACS was dissolved in a 0.1 N KOH solution (60 mL, 10 mg/mL of 0.1 N KOH, pH 12.5) at 90 °C.

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The genistein solution was prepared separately at 30 °C (40 mL, 1.5 mg/mL of 0.1 N KOH, pH 12.5). The solutions were mixed at 30 °C, and the mixture was precipitated by adjusting the pH to 4.7 (±0.5) with 2% (w/v) H_3PO_4 and holding for 24 h under gentle stirring. All samples were then centrifuged (20000*g*, 25 min, 4 °C), the supernatant was discarded, and the precipitate was washed twice with ethanol/water mixture (50/50 w/w) and centrifuged as before. The complexes were then freeze-dried and crushed into a fine powder. Genistein analysis in the complex (see below) showed no structural difference from genistein during the complexation process.

XRD. XRD measurements were carried out in a Philips PW 3020 powder diffractometer equipped with a graphite crystal monochromator. The operating conditions were as follows: Cu Kα1 radiation (0.154 nm), 40 KV, and 40 mA. Samples were scanned over 5−30° 2θ in steps of 0.02° 2θ/s, and the crystalline nature of the complex was determined by the position of the XRD peaks.

Genistein Loading in Amylose−Genistein Complexes. The genistein content in the complex was tested by full dissolution of the complexes in 0.1 N KOH for release of the guest molecule. The complex (15 mg) was incubated in 1 mL of 0.1 N KOH solution at 37 °C for 24 h. PBS (159 mL, 20 mM phosphate pH 6.9, and 10 mM NaCl) was added to the solution, which was then centrifuged at 5500*g* for 15 min. Genistein was quantified from the supernatant by high-performance liquid chromatography (HPLC) using an HP 1100 equipped with a DAD and autosampler and controlled by ChemStation software (Hewlett-Packed, Wilmington, DE). HPLC analysis was carried out in a reverse-phase C18 column, 250 mm × 4 mm with 5 μm packing. Samples were eluted at a flow rate of 1 mL/min by solvent A (0.1% v/v acetic acid in water) and solvent B (acetonitrile). The gradient elution was from 5 to 35% B in a linear gradient over 33 min, washing with 100% B for 5 min, and then equilibrated for 10 min between runs with 5% B. The injection volume was 20 μL, and detection was performed by UV absorbance at 254 nm.[20] The amount of genistein in the complex was quantified by a calibration curve using pure genistein as a standard. The genistein content in the complex was calculated per 100 mg complex using eq 1.

$$\text{genistein content in the complex (\%)} = \frac{\text{genistein in the complex (mg)} \times 100}{\text{complex weight (mg)}} \quad (1)$$

Genistein Release from the Complex under Enzymatic Digestion. The release of genistein by enzymatic digestion was measured by exposing the complex to pancreatic amylase, with PBS used as a control. The complex (15 mg) was incubated in 1 mL of pancreatic amylase or PBS at 37 °C. The pancreatic solution was prepared by dissolving 0.177 g of pancreatin in 20 mL of PBS. The complexes were incubated at different time points: 0, 0.3, 0.6, 1, 2, 4, 6, 12, and 24 h for amylose complexes and 0, 1, 2, 4, 6, 12, and 24 h for HACS complexes. PBS (159 mL) was added to the complex and centrifuged (5500*g*, 15 min), and the genistein released from the complexes was quantified by HPLC as already described. The amount of genistein released from the complex was calculated based on the total genistein content in the complex.

Animals, Diets, and Treatment. Sprague−Dawley rats (10 male, 220−250 g) were obtained from Harlan Laboratories (Jerusalem, Israel). Rats were housed under standard conditions at a temperature of 23 °C, a controlled relative humidity of 50%, a 12 h light/dark cycle, and free access to powdered rodent diet (see below) and water. The experimental protocol was in compliance with the relevant laws and institutional guidelines and was approved by the Hebrew University Animal Care Committee (AG-01.10-4). Rats were weighed 12 h before starting the experiment, and each individual rat was placed in a metabolic cage and fasted overnight with free access to water. Following the 12 h fast, the rats were randomly divided into two groups, and each rat was given a single oral dose of 5 g of powdered rodent diet (AIN-76A) with the treatment. Briefly, the rodent diet contained 49.9% sucrose, 20.0% vitamin-free casein, 15.0% corn starch, 5.0% powdered cellulose, 5.0% corn oil, 3.5% AIN-76 mineral mix, 1.0% AIN-76A vitamin mix, 0.3% DL-methionine, 0.2% choline bitartrate, and 0.001% ethoxyquin (a preservative). The AIN-76A rodent diet has been recently shown to be devoid of isoflavones.[21] The control group (5 rats) was given HACS mixed with genistein, and the experimental group (5 rats) was given HACS−genistein complexes. The dose of genistein in each sample was 30 mg/kg body weight (BW). After they finished the first dose, the rats were allowed to eat ad libitum and were given free access to water. Individual blood samples were taken from the tail vein in heparinized tubes at six time points after ingesting the 5 g of ground food with the treatment. Blood samples were centrifuged at 2000*g* for 10 min, and plasma samples were stored at −80 °C until analysis. Stool and urine samples were collected at 0 and 24 h after treatment administration. NaN_3 (0.003 M) and ascorbic acid (1 g/L) were added to the urine storage bottle to prevent oxidation of genistein. All of the samples were stored at −80 °C until analysis. Twenty-four hours after administration, the animals were anesthetized using sodium barbital (8 mg/100 g BW) and then killed by cervical dislocation.

Genistein Extraction from Plasma. Plasma samples (100 μL) were first hydrolyzed using 100 μL of acetate buffer (0.1 M, pH 5) containing 0.2 U/mL β-glucuronidase from *Escherichia coli* K12 obtained from Roche (Basel, Switzerland) and 2 U/mL sulfatase from *Helix pomatia* obtained from Sigma Chemical Co. Samples were mixed and incubated overnight at 37 °C. For the recovery calculation, 100 μL of [6,7]-^{3}H-estradiol-17-glucuronide was added to controls, and samples were incubated for 30 min at room temperature before hydrolysis.[22] After hydrolysis, 1.5 mL of diethylether was added to the hydrolyzed samples, which were then shaken vigorously for 3 min. The water phase was frozen in an ethanol−ice bath, the ether phase was transferred into a disposable glass tube, and the water phase was re-extracted with the same amount of ether. The ether phases were combined and evaporated to dryness. Then, 100 μL of assay buffer (0.5% BSA-Tris pH 7.76) was added.

Genistein Extraction from Urine Samples. Urine (50 μL) samples were first hydrolyzed using 450 μL of acetate buffer (0.1 M, pH 5) containing 0.2 U/mL β-glucuronidase and 2 U/mL sulfatase. Samples were mixed and incubated overnight at 37 °C. After hydrolysis, 750 μL of assay buffer was added to the urine samples.

Genistein Concentration in Plasma and Urine Samples. The genistein concentration in plasma and urine samples was determined with a time-resolved fluoroimmunoassay (TR-FIA) genistein kit from Labmaster Co. (Turku, Finland) as described previously.[22,23] Briefly, 20 μL of standard, plasma, or urine samples (in duplicate) was pipetted into prewashed goat antirabbit IgG microtiter-plate strips. To each well, 100 μL of antiserum in 0.5% BSA-Tris buffer and 100 μL of europium-labeled genistein were added. After incubation and shaking the strips slowly at room temperature for 90 min using a DELFIA plate shaker (Wallac Oy, Turku, Finland), the strips were washed. An enhancing solution (200 μL) was added to each well, and the strips were gently shaken for an additional 5 min. The enhanced fluorescence was measured in a VICTOR 1420 multilabel counter, and the final result was calculated based on eq 2. Samples giving a value outside the range of the standard curve were diluted with the assay buffer.

$$\text{genistein final concentration} = \frac{\text{concentration (read)} \times \text{dilution factor}}{\text{recovery (\%)}} \quad (2)$$

For plasma samples, the area under the concentration−time curve during the experiment, $AUC_{(0-24h)}$, was calculated using the linear

trapezoidal rule. For urine samples, the genistein recovery rate was calculated based on eq 3.

$$\text{genistein recovery rate } (\%) = \frac{\text{genistein in urine samples } (\mu \text{ mol}) \times 100}{\text{genistein in the food intake } (\mu \text{ mol})} \quad (3)$$

Genistein Concentration in Feces. Feces were collected before the experiment and 24 h after treatment administration. Samples were freeze-dried, ground, and weighed. Analysis of genistein in the feces was based on the method described by Poulsen et al. and Steensma et al.[24,25] Fecal samples (250 mg, in duplicate) were mixed with 2 mL of sodium acetate buffer (0.125 M, pH 5) and incubated overnight at 37 °C, and then, excess anhydrous Na_2SO_4 was added. Samples were acidified with 800 μL of 5 N HCl, and as an internal standard, 200 μL of daidzein (1 mg/mL) was added. Samples were extracted three times by ethyl acetate (6 mL) and diethyl ether (3 mL), vortexed for 30 s, and then centrifuged (4000*g*, 5 min, 4 °C). The solutions were dried under nitrogen stream at 37 °C, and 6 mL of ammonium acetate (10 mM, pH 6.5) and 4 mL of methanol were added. The samples were further extracted by passage over an activated Oasis HLB-30 cartridge (Waters, Milford, MA). Methanol and double-distilled water (3 × 1 mL) were used to pre-equilibrate the cartridge. Samples were added, and the cartridge was washed with 3 × 1 mL of 10 mM ammonium acetate (pH 6.5) and 3 × 1 mL of 20% (v/v) methanol in 10 mM ammonium acetate (pH 6.5), and then, 4 mL of methanol was added to elute the genistein. The methanol fraction was evaporated to dryness under nitrogen stream at 37 °C, and 1 mL of methanol was added to each sample for HPLC analysis as already described. The genistein concentration was corrected based on daidzein recovery rate in each sample. The genistein recovery rate in the feces was calculated based on eq 4.

$$\text{geniatein recovery rate } (\%) = \frac{\text{genistein in feces sampels } (\mu \text{ mol}) \times 100}{\text{genistein in the food intake } (\mu \text{ mol})} \quad (4)$$

UPLC/MS. To verify our HPLC results and to identify expected genistein metabolites, reprehensive feces samples were analyzed by ACQUITY Ultra Performance Liquid Chromatography (UPLC) system (Waters) coupled to a LCT Premier orthogonal accelerated TOF-MS (Waters Corp., Manchester, United Kingdom).

Feces samples were first extracted as described earlier, and 4 μL of the extracted sample was injected into the UPLC equipped with a binary solvent-delivery system, an autosampler, and a UV detector. Chromatographic separation was performed on a Kinetex C18 column (1.7 μm, 100 A, 50 mm × 2.1 mm). Samples were eluted at a flow rate of 0.2 mL/min by solvent A (0.1% v/v trifluoroacetic acid in water) and solvent B (acetonitrile). The gradient elution was from 80 to 20% A in a linear gradient over 5 min, maintained for 2 min at 20% A, and then to 80% A for 1 min. Detection was performed by UV absorbance at 254 nm. The column eluate was directed to the MS without split.

MS was performed with an electrospray ionization (ESI) source operating in positive-ion mode and a scan range from 100 to 1000 m/z. The capillary voltage and cone voltage were set at 3000 and 116 V, respectively. Nitrogen was used as the drying gas. The desolvation gas rate, and the cone gas rate were set to 560 L/h at a temperature of 150 °C.

Statistical Analysis. Statistical analysis was performed with SAS 9.2 software (SAS System for Windows, version 9; SAS Institute, Inc., Cary, NC). Results were expressed as means ± standard deviations (SDs) and were analyzed using two-way analysis of variance involving two main effects (treatment and time). The interaction between the two main effects (treatment × time) was also analyzed. The significance of the differences between groups was tested with posthoc analysis using Student's t test. Statistical significance was set at $p < 0.05$.

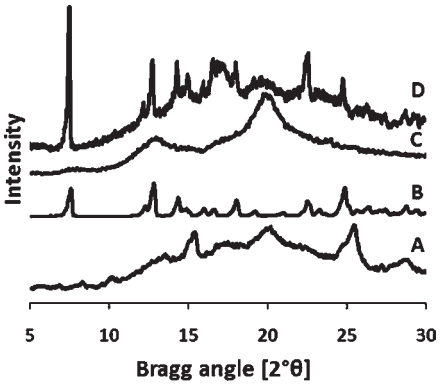

Figure 1. XRD patterns of (A) HACS−genistein complexes, (B) pure genistein, (C) HACS without genistein, and (D) HACS−genistein physical mixture.

RESULTS AND DISCUSSION

XRD Analysis. The crystalline nature of the HACS−genistein complex was determined by the position of the XRD peaks. This diffraction pattern was compared to that of HACS processed in a similar way but without a guest molecule, pure genistein, and a physical mixture of HACS and genistein. XRD examination of the complexes (Figure 1A) showed a diffraction pattern different from those obtained with pure genistein (Figure 1B), HACS with no guest (Figure 1C), and the HACS−genistein mixture (Figure 1D). The diffraction pattern of the HACS−genistein mixture was a superposition of those of pure genistein and pure HACS. However, when genistein was complexed to HACS, a new diffraction pattern emerged, which was practically independent of pure genistein, pure HACS, and the HACS−genistein mixture. The diffraction pattern of HACS−genistein complexes was characterized by main peaks at Bragg angle (2θ) 7°, 8°, 10°, 14°, 15.5°, 17°, 20°, 25.5°, and 29° (Figure 1A), typical of the previously suggested $V6_{III}$ diffraction pattern.[19] These results indicate that HACS forms new interactions in the presence of genistein, which can be used for the latter's complexation. It also provides the basis for the discussion, suggesting that experiments were conducted with complexes versus mixtures.

Genistein Loading in HACS−Genistein Complexes. The total amount of genistein in the HACS−genistein complexes was measured by total dissolution of the complexes in KOH solution and quantification by HPLC. HACS−genistein complexes contained 9.4 ± 0.7 mg genistein (35 μmol) per 100 mg complex (n = 10).

Genistein Release from the Complexes under Enzymatic Digestion. V-amylose complexes can be digested by α-amylase, thereby releasing the bioactive molecule entrapped in the polysaccharide helical structure.[12,13] This could confer an advantage for the complexed form: Not only will the guest molecule be delivered in a chemically stable form to the intestine, it can also be released in the intestine where α-amylase is secreted. Targeting the release of the guest molecule to the small intestine and colon opens the way for applications using V-amylose compositions. The release of genistein from amylose and HACS complexes was tested in vitro by pancreatic digestion over a period of 24 h. Genistein release in the pancreatic solution was significantly

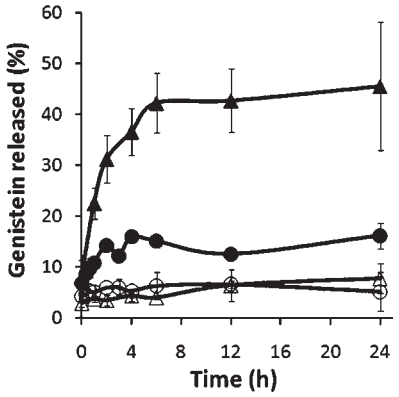

Figure 2. Release of genistein from amylose−genistein complexes (circles) and from HACS−genistein complexes (triangles) by pancreatic amylase (solid triangles or circles) over 24 h, compared to release in PBS (open triangles or circles).

higher than that in PBS for both amylose and HACS complexes from 1 h into the incubation ($p < 0.05$) (Figure 2). The maximal level of genistein release occurred after 4 (for amylose complexes) and 6 h (for HACS complexes). This period fits well with the normal transit time of food through the small intestine. However, not all of the complexed genistein was recovered by α-amylase. This can be attributed to the helical configuration of the amylose complex and the interactions stabilizing it. Because the V-amylose helices are stabilized by intramolecular binding with the guest, they become less susceptible to α-amylolysis.[16,26] Thus, entrapment of genistein in the crystalline segment leads to its slow release. It was interesting to note that although the total amount of genistein entrapped in the amylose complexes was higher than that entrapped in the HACS complexes,[19] the amount of genistein released by enzymatic digestion was significantly higher in the latter. This appears to be because genistein in HACS helices is less trapped in the crystalline segments, suggesting a less stable or ordered crystalline phase, as compared to genistein in amylose complexes. Thus, α-amylase is better able to digest HACS−genistein complexes than amylose−genistein ones, leading to higher amounts of genistein recovered from the former.

HACS−genistein complexes have proven to be stable in the acidic environment of the stomach.[19] Digestion of these complexes is controlled by the amylase-digesting enzyme α-amylase, resulting in slow release of genistein. Therefore, HACS−genistein complexes are expected to contribute to an increase in genistein bioavailability. To verify this, the effect of HACS−genistein complexes on genistein bioavailability was tested in vivo in rats.

Animal Weight and Food Intake. There were no statistically significant differences in rat BW or food intake between the control and the experimental groups ($p > 0.05$). The average BW of rats in the experimental group was 234.8 ± 10.0 g, and in the control group, 241.6 ± 3.0 g. Food residues (food not consumed by the rats) were lower than 5% of the given dose for all animals. Genistein was extracted from the residues and quantified by HPLC, and the amount of genistein in the residues was not significantly different between the groups ($p > 0.05$) (not shown).

Genistein Concentration in the Plasma. Table 1 summarizes the pharmacokinetic parameters for genistein concentration, measured at different time points in the plasma. The basal level of genistein in the plasma was tested 24 h after its administration, based on Uehara et al.'s study,[27] which suggested

Table 1. Pharmacokinetic Parameters of Genistein Concentration in the Plasma for the Experimental (HACS−Genistein Complexes) and Control (HACS−Genistein Mixture) Groups[a]

	experimental group[b]	control group[b]
$C_{t=24h(\mu M)}$[c]	0.41 ± 0.13	0.35 ± 0.07
t_{max} (h)[d]	1−2	
C_{max} (μM)[e]	12.59 ± 6.48	8.15 ± 1.19
AUC_{0-24h} (μM h)[f]	126.96 ± 47.54	81.21 ± 28.26

[a] The dose of genistein was 30 mg/kg BW. [b] Values are means ± SDs, n = 5 rats in each treatment group. [c] $C_{t=24h}$, genistein basal level concentration. [d] t_{max}, time to reach to the maximum concentration. [e] C_{max}, genistein concentration at t_{max}. [f] AUC_{0-24h}, area under the concentration-time curve.

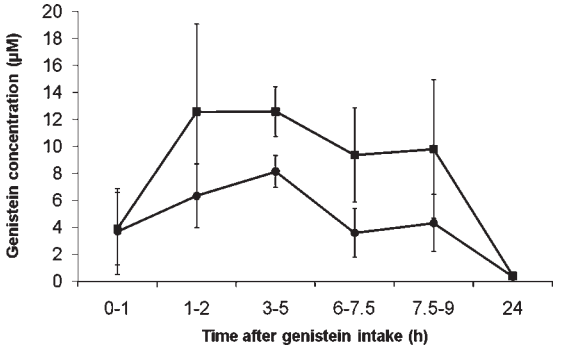

Figure 3. Concentration of genistein in the plasma at different time points. The rats were orally administered HACS−genistein complexes (squares) or HACS mixed with genistein (circles) at a dose of 30 mg/kg BW.

that, in rats, genistein disappears from the blood and body 20−30 h after treatment (similar to intestinal transit time). In our study, there was no significant difference in basal plasma levels of genistein between the experimental group given HACS−genistein complexes (0.41 ± 0.13 μM) and the control group given free genistein mixed with HACS (0.35 ± 0.07 μM) ($p > 0.05$, Figure 3). These results were in the range reported by Piskula et al.[28] for total isoflavone concentration in the plasma (~0.8 μM).

The plasma genistein concentration was significantly higher in the experimental group than in the control group 1−2 h after treatment administration ($p < 0.05$) (Figure 3). This concentration did not decrease for the first 9 h of incubation in the experimental group, and the maximum concentration (C_{max}) was 12.59 ± 6.48 μM observed at 1−2 h after genistein administration (t_{max}) (Table 1). In the control group, plasma genistein concentration decreased 6−7.5 h after incubation, and C_{max} was 8.15 ± 1.19 μM. However, it was difficult to determine t_{max} for this group since there was no significant difference in genistein concentration for the first 5 h ($p > 0.05$). These results show that in the presence of HACS, genistein concentration in the plasma increases and remains at a higher level for a longer time.

Kwon et al.[29] showed that for an oral dose of 20 mg genistein/kg BW, t_{max} is 0.5 h, but for an oral dose of 40 mg/kg BW, t_{max} is 2 h. In their study, C_{max} for both doses was moderately higher (for 20 mg/kg BW, C_{max} = 12.3 ± 2.4 μM and for 40 mg/kg BW, C_{max} = 18.04 ± 0.07 μM) than that measured in our experiment for the control group (Table 1). King et al.[30] reported a genistein

concentration of about 5 μM 2 h after administration. Sepehr et al.[31] reported a t_{max} of ~6.5 h for genistein after oral administration of 59% genistein, 29% daidzein, and 12% glycitein (20 mg/kg BW) and a C_{max} of ~3.53 ± 1.03 μM, which, 24 h after administration, decreased to almost basal levels. These results are similar to ours, taking into account the different genistein doses between the experiments. It is important to note that increasing the administered genistein dose has been related to a nonlinear increase in genistein concentration in the plasma.[11]

The AUC_{0-24h} was calculated for each group for the 24 h experimental period (Table 1). The mean AUC_{0-24h} for the HACS−genistein complex-administered group was significantly higher than that for the control group 126.96 ± 47.54 (μM h) and 81.21 ± 28.26 (μM h), respectively (Table 1, $p < 0.01$). This suggests that complexation of genistein with HACS increased its bioavailability. It has been reported that complexation of isoflavone extract with β-cyclodextrin increases aglycone bioavailability:[32] Genistein complexation with β-cyclodextrin increased C_{max} almost 4-fold, and the AUC after 6 h was 180% higher for the group that was given the complexes as compared to the controls. In our experiment, C_{max} was only 50% higher in the experimental group as compared to the control group. However, the total AUC was ca. 200% higher in the experimental group. In addition, t_{max} in our experiment was higher than t_{max} reported for β-cyclodextrin complexes, which may suggest a different release mechanism. Steensma et al.[25] showed that the mean AUC_{0-24h} for rats given an oral genistein dose of 15 mg/kg BW is 44.6 μM h. Kwon et al.[29] reported about 44 μM h for rats given genistein orally at 20 mg/kg BW and about 116 μM h for rats given 40 mg genistein/kg BW. The AUC_{0-24h} in our experiment for the control group (Table 1), which was given 30 mg genistein/kg BW, fits these reports, strengthening the validity of our results.

Our hypothesis was that the release of genistein is controlled by digestion of the complexes by α-amylase, which cleaves amylose. Consequently, genistein is slowly released to the intestine, thereby increasing its absorption in the body. The plasma genistein concentration in the first hour may result from genistein found at the surface of the particles or entrapped in the amorphous parts of the complexes. We have previously shown that these different forms of entrapment are important in genistein release from complexes.[19]

Genistein Concentration in the Urine and Feces. The genistein basal level in the urine and feces was measured from samples that were collected shortly before the experiment. The genistein concentration in the urine was tested using the TR-FIA genistein kit and in the feces by HPLC. The basal level of genistein in the urine was similar between the experimental group (5.07 ± 1.58 μM) and the control group (3.28 ± 2.04 μM) ($p > 0.05$) (Figure 4). The basal level of genistein in the feces was also similar between the experimental and the control groups (0.012 ± 0.018 and 0.004 ± 0.004 μmol, respectively) ($p > 0.05$) (Figure 5).

The genistein concentration in the urine 24 h after administration was significantly higher in the experimental group, given the HACS−genistein complex (658.1 ± 325.7 μM), than in the control group, given the physical mixture (169.9 ± 62.2 μM) ($p < 0.05$) (Figure 4). The total amount of genistein in the urine, collected 24 h after the start of the experiment, was 4.10 ± 2.40 μmol for the experimental group and 1.20 ± 0.40 μmol for the control group (Table 2). However, the genistein amount in the feces collected 24 h after administration was significantly higher in the control group (0.803 ± 0.401 μmol) than in the experimental group (0.291 ± 0.209 μmol) ($p < 0.05$) (Table 2). These results indicate that HACS complexes increase genistein bioavailability.

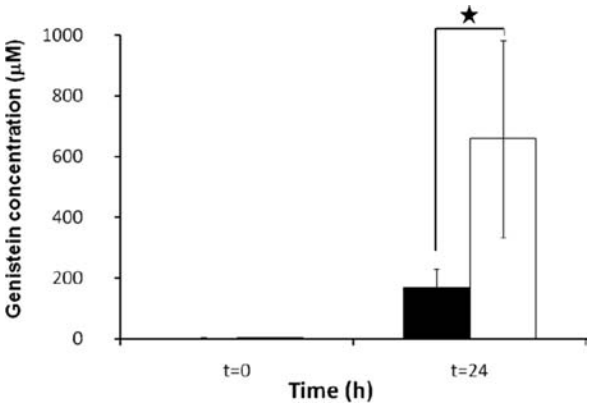

Figure 4. Concentration of genistein in the urine before the experiment ($t = 0$) and 24 h after genistein administration. The rats were orally administered HACS−genistein complexes (white bars) or HACS mixed with genistein (black bars) at a dose of 30 mg/kg BW. The star indicates a significant difference at $p < 0.05$.

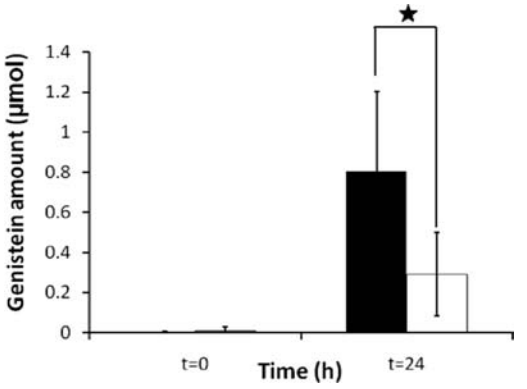

Figure 5. Genistein amount in the feces before administration ($t = 0$) and 24 h after genistein administration ($t = 24$). Rats were orally administered HACS−genistein complexes (white bars) or HACS mixed with genistein (black bars) at a dose of 30 mg/kg BW. The star indicates significant difference at $p < 0.05$.

The genistein recovery rate in the urine was 15 ± 9% for the experimental group and 4 ± 1% for the control group (Table 2), similar to that reported previously.[27,33] However, the recovery rates from the feces, which were 1.0 ± 0.7% for the experimental group and 2.9 ± 1.4% for the control group (Table 2), were lower than those reported by King et al. (~20%)[33] and Steensma et al. (~29%).[25] The lower recovery rates of genistein in our experiment may result from the fact that genistein can be degraded by gut microflora to a series of metabolic products;[30] therefore, in the feces, most of the genistein might appear as its metabolites or not at all, rather than as pure genistein.[34] The lower recovery of genistein in the feces may also result from differences in the populations of gut bacteria, arising from the use of rats from different sources.[30]

It has been reported that in the feces, genistein is likely to appear as its metabolites: 2-(4-hydroxyphenyl)-propanoic acid (HPPA), 1,3,5-trihydroxybenzene (THB), 6′-OH-*O*-desmethylangolensin, dihydrogenistein, and 3′,4′,5,7-tetrahydroxyisoflavone, which are the end products of genistein intestinal metabolism.[29,35,36]

Table 2. Summary of Genistein Amount and Recovery Rates in Urine and Feces Samples from Experimental (HACS−Genistein Complexes) and Control (HACS−Genistein Mixture) Groups

	urine[a]				feces[a]			
	experimental group		control group		experimental group		control group	
time (h)	amount (μmol)	R^b (%)	amount (μmol)	R^b (%)	amount (μmol)	R^b (%)	amount (μmol)	R^b (%)
0	0.06 ± 0.02		0.05 ± 0.02		0.012 ± 0.018		0.004 ± 0.004	
24	4.10 ± 2.40	15 ± 9	1.20 ± 0.40	4 ± 1	0.291 ± 0.209	1.0 ± 0.7	0.803 ± 0.401	2.9 ± 1.4

[a] Values are means ± SDs, n = 5 rats in each treatment group. [b] R = recovery rate.

UPLC/MS of the feces samples extracted before genistein intake (t = 0) did not detect either genistein or its metabolites. In addition, in the extracted feces samples collected during the experiment (t = 24 h), a peak related to genistein was observed, but none of the metabolites were detected. One possible explanation to the low recovery rate of genistein in the feces is that most of the fecal genistein is fully degraded by the microbiota under this experimental setup.

The data obtained in this study demonstrate that complexation with amylose changes the bioavailability of genistein in the body. This study may also suggest that starch, via a similar mechanism, can affect the bioavailability of many other food components. Thus, the results presented here are extremely important since it can be suggested that complexation of food components with starch might provide a solution for the better assimilation and stability of various food components, under various food-processing operations. The interactions of starch with a large range of food components might play a key role in their bioavailability and should be further studied.

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ABBREVIATIONS USED

HACS, high-amylose corn starch; XRD, X-ray diffraction; BW, body weight; AUC, area under the concentration−time curve

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