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Hydrolysis of Soybean Isoflavonoid Glycosides by *Dalbergia* β-Glucosidases

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Two β-glucosidases from the legumes *Dalbergia cochinchinensis* and *Dalbergia nigrescens* were compared for their ability to hydrolyze isoflavonoid glycosides from soybean. Both *D. nigrescens* and *D. cochinchinensis* β-glucosidases could hydrolyze conjugated soybean glycosides, but *D. nigrescens* β-glucosidase hydrolyzed both conjugated and nonconjugated glycosides in crude soybean extract more rapidly. The kinetic properties K_m, k_{cat}, and k_{cat}/K_m of the *Dalbergia* β-glucosidases toward conjugated isoflavonoid glycosides, determined using high-performance liquid chromatography, confirmed the higher efficiency of the *D. nigrescens* β-glucosidase in hydrolyzing these substrates. The *D. nigrescens* β-glucosidase could also efficiently hydrolyze isoflavone glycosides in soy flour suspensions, suggesting its application to increase free isoflavones in soy products.

KEYWORDS: Isoflavones; β-glycosidases; *Dalbergia*; β-glucosidase; soy

INTRODUCTION

Isoflavones are a group of diphenolic secondary metabolites produced in a very limited distribution of higher plants, most frequently in the Leguminosae (*1*). Among the common dietary legumes, soybean contains the highest level of isoflavones. From the natural food sources, soybeans and soy foods contain the most dietary isoflavones, which provide many health benefits. The major types of isoflavones in soybean are daidzein, genistein, and glycitein. Most of these isoflavones in soybean seeds are conjugated with glucose or malonylglucose (**Figure 1**). In addition, acetylglucose conjugates are also detected in small amounts in soy products, but it appears that acetyldaidzin and acetylgenistin may be generated from daidzin and genistin during their heat-induced decompositions (*2*).

There are many reports that soybean isoflavones may have potential benefits for reducing the occurrences of diseases afflicting humans, such as certain types of breast, prostate, and colon cancer (*3*, *4*). Besides being anticarcinogenic, they exhibit antiatherosclerotic, blood glucose lowering, antibacterial (*5*), and antioxidative (*6*) properties. With the discovery of increased cancer risks associated with estrogen-based hormone replacement therapy, the use of isoflavones as an alternative for menopausal women has received much public and scientific interest (*7*, *8*). In addition, isoflavones may reduce low-density lipoproteins and increase high-density lipoproteins, which help to prevent coronary heart disease (*9*). Because of these health benefits, there is interest in increasing the amounts of free isoflavones in soy products.

Hessler et al. (*10*) reported that β-glucosidases from *Saccharopolyspora erythraea* could hydrolyze genistin during fermentation of soy-based media, and β-glucosidase from *Bifidobacteria* in soy milk was capable of converting glucosides to their aglycones (*11*). Pandjaitan et al. (*12*) treated soy protein isolate with almond β-glucosidase to convert most of its isoflavone glucosides to their aglycones. However, it has been shown that *Escherichia coli* β-glucosidase was more effective than almond emulsin β-glucosidase, and neither of these enzymes could effectively hydrolyze malonylglucosyl isoflavone conjugates, even at high concentrations and extended times (*13*). In 2001, Hsieh et al. (*14*) partially purified and characterized β-glucosidase from soybean that could hydrolyze isoflavone conjugates. Recently, Suzuki et al. (*15*) reported the purification and characterization a β-glucosidase (GmICHG) from the roots of soybean seedling with high specificity toward conjugated isoflavones.

Dalbergia is a large genus of small- to medium-size leguminous trees native to the tropical regions of Central and South America, Africa, and southern Asia (*16*). Isoflavonoid β-glycosidases have been described from the seeds of *Dalbergia cochinchinensis* Pierre (Thai rosewood) and *Dalbergia nigrescens* Kurz (Thai blackwood) (*17*−*19*). These enzymes had high hydrolytic activity on isoflavonoid glycosides from the same seeds. *D. cochinchinensis* β-glucosidase had high substrate specificity toward dalcochinin-8′-*O*-β-glucoside (**Figure 1**),

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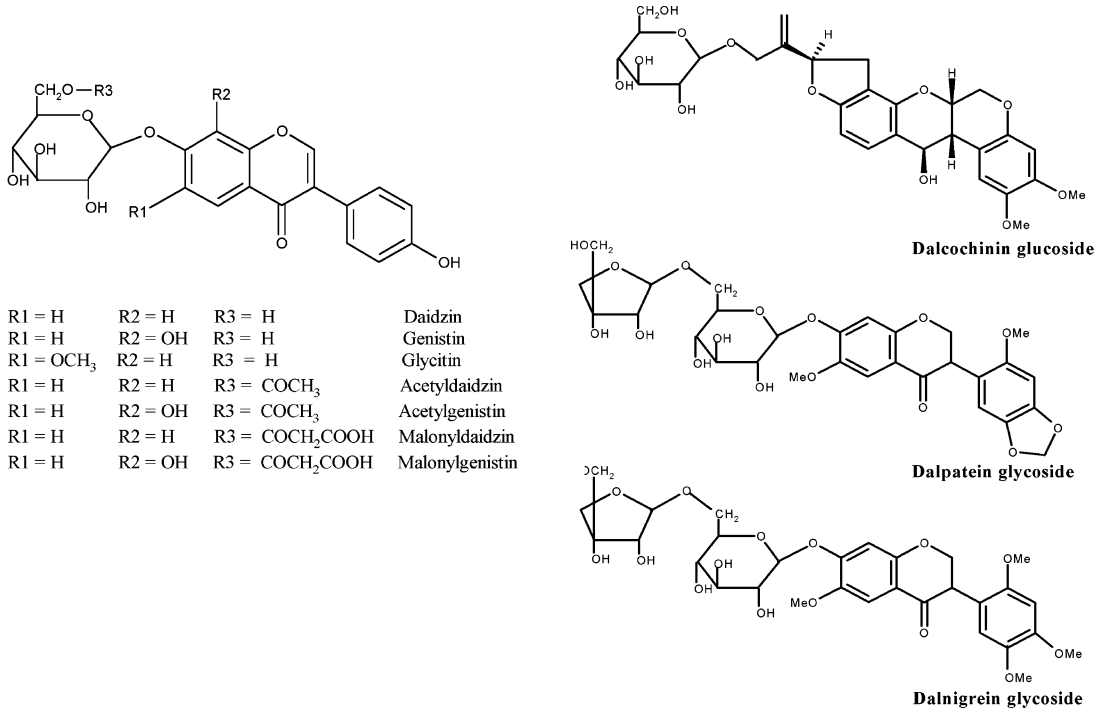

Figure 1. Structures of soybean isoflavonoid glycoside natural substrates of *D. nigrescens* and *D. cochinchinensis* β-glucosidase.

which has an aglycone structure similar to that of rotenone, a natural insecticide and pesticide (*18*). *D. nigrescens* β-glucosidase efficiently hydrolyzed its diglycoside natural substrates dalpatein 7-*O*-β-D-apiofuranosyl-(1,6)-β-D-glucopyranoside and dalnigrein 7-*O*-β-D-apiofuranosyl-(1,6)-β-D-glucopyranoside (**Figure 1**) to release a β-1,6-apiosylglucose (acuminose) disaccharide unit and the aglycones (*19*). It could also hydrolyze genistin and daidzin, which are isoflavonoid 7-*O*-β-D-glucosides. In this study, we evaluate the potential of *Dalbergia* β-glucosidases for release of isoflavones in soy products by comparing their ability to hydrolyze conjugated and nonconjugated isoflavonoid glycosides in soybean extracts.

MATERIALS AND METHODS

Materials. High-performance liquid chromatography (HPLC) grade methanol, acetonitrile, and water were purchased from Fisher Scientific (Hanover Park, IL). Isoflavone standards of daidzin, daidzein, genistin, genistein, malonylgenistin, and glycitin were purchased from LC Laboratories (Woburn, MA). Glycitein standard was produced by digestion of glycitin with *D. nigrescens* β-glucosidase. All other chemicals and reagents were of analytical grade.

Crude Soy Flour Extraction. Ten grams of defatted soybean flour from ADM Protein Specialties (Decatur, IL) was extracted with 40 mL of 80% methanol by stirring overnight at room temperature. The solid was removed from the extract supernatant by centrifugation at 12000 rpm for 15 min.

***β*-Glucosidase Enzymes.** *Dalbergia* β-glucosidases were purified from *D. cochinchinensis* Pierre and *D. nigrescens* Kurz and seeds as previously described (*17*, *19*). Almond β-glucosidase was purchased from Sigma Fine Chemicals (St. Louis, MO). Quantification of protein was by Coomassie brilliant blue staining (Bio-Rad Protein Assay, Bio-Rad Laboratories, Hercules, CA) with bovine serum albumin (BSA, frac 5, GE Healthcare, Uppsala, Sweden) as a reference, and enzyme activity was quantified by *p*-nitrophenol release from *p*-nitrophenyl β-D-glucoside (*p*NPG), as previously described (*19*). One unit was defined as the amount of enzyme that hydrolyzes 1 μmol of *p*NPG per minute at 1 mM *p*NPG and 30 °C in 0.1 M sodium acetate, pH 5.5.

HPLC Analysis of Soy Isoflavones. HPLC analysis was used to measure the amounts of daidzein, genistein, daidzin, genistin, and malonylgenistin to quantify changes in isoflavone content of crude extracts treated with the *Dalbergia* β-glucosidases. Separation and quantification of isoflavonoids were achieved with an Eclipse XDB-C18 (4.6 × 250 mm, 5 μm) reverse phase column on an HP series 1100 HPLC (Agilent Corp., Palo Alto, CA) with the UV detector set at a wavelength of 260 nm, in a manner similar to that previously described for the separation of soy isoflavones (*20*). Solvent A was 0.1% phosphoric acid in water, and solvent B was acetonitrile. The sample was injected in 10% solvent B, which was held for 5 min and then increased in a linear gradient from 10 to 35% B over 45 min. The flow rate was 0.8 mL/min.

Peaks of soy isoflavone glucosides and aglycones were identified by matching retention times with isoflavonoid standards. Malonyldaidzin, acetyldaidzin, and acetylgenistin were tentatively identified by comparison of the relative retention times of the peaks with those in published HPLC methods (*20*). Relative amounts were calculated from relative peak areas, because all peaks were well within the linear range of the instrument.

Hydrolysis of Crude Soy Flour Extract. To compare the hydrolysis efficiencies of *D. nigrescens* β-glucosidase, *D. cochinchinensis* β-glucosidase, and almond β-glucosidase toward the isoflavonoid glycosides in crude soy flour extract, 10 μL aliquots of crude soybean extract were hydrolyzed with 0.001 unit of each β-glucosidase in 100 μL of 0.1 M sodium acetate, pH 5.5. The reaction mixtures were incubated at 37 °C for 10 min or 16 h, and the reaction was stopped by boiling for 5 min. The stopped reactions were dried by speed vacuum and resuspended in 100 μL of 10% acetronitrile in 0.1% phosphoric acid/water. A control reaction of crude extract without enzyme was set up in the same manner.

Hydrolysis of Soy Flour Suspension. The defatted soybean flour (0.15 g/mL) was suspended in 0.1 M sodium acetate, pH 5.5, and 200 μL aliquots of the suspension were incubated with and without 0.01 unit of *D. nigrescens* β-glucosidase. The reaction mixtures were incubated at 37 °C for 10 min, and the reaction was stopped by boiling for 5 min. The reactions were centrifuged at 12000 rpm for 5 min to remove the supernatant, the solid was extracted with 80% methanol, and the methanol extract was removed by centrifugation at 12000 rpm for 5 min. The reaction supernatant and methanol extract were dried by speed vacuum and resuspended in 20 μL of 10% acetronitrile in 0.1% phosphoric acid/water. The hydrolysis of daidzin and genistin in the soy flour suspension supernatant and particles was evaluated by thin-layer chromatography on analytical silica gel 60 F_{254} aluminum plates (Merck, Darmstadt, Germany) with ethyl acetate/methanol/acetic

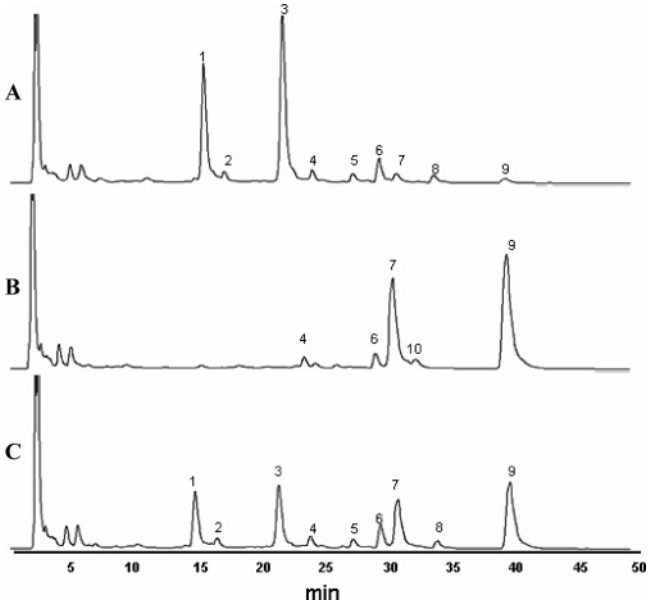

Figure 2. HPLC separation of isoflavonoids in crude soybean flour methanol extract: (**A**) crude soybean flour methanol extract (peaks: 1, daidzin; 2, glycitin; 3, genistin; 4, malonyldaidzin;* 5, acetyldaidzin;* 6, malonylgenistin; 7, daidzein; 8, acetylgenistin;* 9, genistein; 10, glycitein); (**B**, **C**) crude soybean flour methanol extract after 10 min of hydrolysis reaction with 0.001 unit of *D. nigrescens* β-glucosidase (**B**) or *D. cochinchinensis* β-glucosidase (**C**) in 0.1 M sodium acetate, pH 5.5. The digested and undigested methanol extracts were separated on analytical C_{18} reverse phase HPLC as describedunder Materials and Methods. * These peaks were provisionally identified on the basis of their relative elution times in comparison to a previous study that used a similar solvent system and HPLC column (*20*).

acid/water (15:1:2:2) (v/v) as solvent. Isoflavonoids were visualized by absorbance under UV light and identified by comparison to commercial standards.

Kinetic Studies. The kinetic properties of both *Dalbergia* β-glucosidases toward soybean isoflavonoid glycosides were determined by incubating genistin, malonylgenistin, and daidzin at five or six concentrations in the range of 0.035−1 mM, depending on the K_m seen in preliminary experiments and whether substrate inhibition was seen at higher concentrations, in 50 μL reactions containing 5% DMSO in 0.1 M sodium acetate, pH 5.5, with 0.45 ng of *D. nigrescens* or 10 ng of *D. cochinchinensis* β-glucosidase at 30 °C. These enzyme concentrations were determined to convert <10% of the substrates to products, in order to ensure initial reaction rates were observed. All reactions were done in duplicate. After 10 min, the reactions were stopped by boiling for 5 min. The reactions were dried and resuspended in 50 μL of 0.1% phosphoric acid in water (solvent A), and then 20 μL of each sample was injected for HPLC analysis. The column was equilibrated with 85% solvent A. After the sample was injected, ACN was increased from 15 to 35% as a linear gradient over 45 min. The aglycone product of each soybean isoflavonoid glycoside substrate was detected by measuring the absorbance at 260 nm. The amounts of liberated aglycones were calculated from standard curves of aglycone (genistein and daidzein) peak areas over the 0.7−10 mmol range. Because substrate inhibition was seen at some higher concentrations and detection limits prevented the use of very low concentrations, nonlinear regression of the Michaelis−Menten equation curves could not be used to estimate the K_m and k_{cat}, so calculation of kinetic parameters with standard errors were obtained from linear regression of the double-reciprocal (Lineweaver−Burk) plots with the Enzfitter 1.05 program (Elsevier Biosoft, Cambridge, U.K.).

Table 1. Comparison of the Effects of 10 min and Prolonged Incubation of *Dalbergia* β-Glucosidases and Almond β-Glucosidase on Soy Flour Extract Isoflavones[a]

	% hydrolysis activity of β-glucosidases						
	control		*D. nigrescens*		*D. cochinchinensis*		almond
soybean glycoside	10 min	16 h	10 min	16 h	10 min	16 h	16 h
daidzin	1	9	100	100	52	100	74
glycitin	9	10	100	100	5	67	33
genistin	1	8	100	100	62	98	70
malonyldaidzin	12	12	8	60	0	10	12
acetyldaidzin	0	3	100	100	0	44	14
malonylgenistin	0	14	63	96	0	12	12
acetylgenistin	4	7	100	100	0	47	18

[a] The crude soy flour methanol extract was hydrolyzed with 0.001 unit of the β-glucosidases for the indicated times at 37 °C in 0.1 M sodium acetate, and the amounts of isoflavones were quantified by analytical reverse phase HPLC, as described under Materials and Methods.

RESULTS AND DISCUSSION

Hydrolysis of Crude Soy Flour Extract. To evaluate the usefulness of *D. cochinchinensis* and *D. nigrescens* β-glucosidases for the hydrolysis of soy isoflavone glycosides, the enzymes were compared for digestion of the glycosides in soy flour extracts. Defatted soy flour was extracted with methanol, and the crude methanol extract was separated and analyzed by HPLC, as shown in **Figure 2A**. The peaks of daidzin, genistin, malonylgenistin, glycitin, daidzein, and genistein were identified in the crude extract by comparison with commercial standards, whereas glycitein aglycone was identified by digestion of glycitin with *D. nigrescens* β-glucosidase, which gave complete digestion (data not shown). Three additional isoflavonoid glycosides (malonyldaidzin, acetyldaidzin, and acetylgenistin) could be provisionally identified by comparison of their relative elution positions with those in a previous paper (*20*). Genistin and daidzin were the most predominant isoflavonoids in the crude soy flour extract, whereas the peaks for their aglycones were very small. Actually, malonylglucosides are expected as the predominant isoflavone in unprocessed soybean, but in the processed flour evaluated here, the glucosides were present in higher amounts. The content and composition of isoflavones can be changed by various processing conditions, and previous studies suggest that malonylglucosides can easily be converted to less conjugated forms depending on the thermal conditions of processing and preparation (*22*, *23*).

As shown in **Figure 2B**, 0.001 unit of *D. nigrescens* β-glucosidase hydrolyzed nearly all soybean isoflavonoid glycosides that appeared in the crude extract within 10 min. Daidzin, glycitin, genistin, acetyldaidzin, and acetylgenistin were completely hydrolyzed by *D. nigrescens* β-glucosidase, although only approximately 8% of malonyldaidzin and 60% of malonylgenistin could be hydrolyzed in this time. *D. cochinchinensis* β-glucosidase could also hydrolyze 52% of the daidzin, 5% of the glycitin, and 60% of the genistin within the 10 min, but it showed less hydrolysis of the conjugated forms of genistin and daidzin (**Figure 2C**). When the reactions were extended up to 16 h to see whether the enzymes could hydrolyze the remaining glycosides, *D. nigrescens* β-glucosidase hydrolyzed 60% of the malonyldaidzin and achieved nearly complete hydrolysis of malonylgenistin. By 16 h, *D. cochinchinensis* β-glucosidase had hydrolyzed essentially all of the daidzin and genistin, but it still showed little hydrolysis of the malonylated forms of daidzin and genistin (**Table 1**). Essentially no hydrolysis was seen in control reactions without added enzymes, demonstrating no

Table 2. Kinetic Properties of Purified *D. nigrescens* β-Glucosidase[a]

substrate	*D. nigrescens* β-glucosidase			*D. cochinchinensis* β-glucosidase		
	K_m (mM)	k_{cat} (s^{-1})	k_{cat}/K_m (M^{-1} s^{-1})	K_m (mM)	k_{cat} (s^{-1})	k_{cat}/K_m (M^{-1} s^{-1})
genistin	$(6.7 \pm 0.3) \times 10^{-2}$	700 ± 15	$(1.05 \pm 0.03) \times 10^{7}$	$(1.26 \pm 0.69) \times 10^{-1}$	208 ± 99	$(1.7 \pm 0.8) \times 10^{6}$
daidzin	$(1.44 \pm 0.11) \times 10^{-1}$	300 ± 13	$(2.08 \pm 0.03) \times 10^{6}$	1.57 ± 0.09	50 ± 3	$(3.22 \pm 0.18) \times 10^{4}$
malonylgenistin	$(5.2 \pm 1.1) \times 10^{-2}$	21 ± 2	$(4.1 \pm 0.4) \times 10^{5}$	$(5.10 \pm 1.09) \times 10^{-2}$	1.88 ± 0.18	$(3.7 \pm 0.4) \times 10^{4}$

[a] Reactions were done in the standard reaction at 37 °C in 0.1 M acetate and stopped at 10 min when <10% of the substrate had been hydrolyzed. The isoflavone aglycones were quantified by HPLC, as described under Materials and Methods, and the rates of product formation were calculated. Kinetic parameters and standard errors were calculated from the linear regression of the Lineweaver–Burk plots with the Enzfitter program.

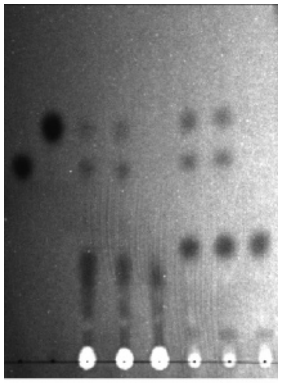

Figure 3. TLC analysis of hydrolysis of soybean flour suspension in 0.1 M sodium acetate buffer with 0.01 unit of *D. nigrescens* β-glucosidase. Standards and samples were separated on an analytical silica gel 60 F_{254} aluminum plate and isoflavones visualized by absorbance of UV light, as described under Materials and Methods. Lanes: 1, daidzin; 2, genistin; 3, suspension reaction without enzyme at time 0; 4, suspension reaction without enzyme after 10 min; 5, suspension reaction with 0.01 unit of enzyme after 10 min; 6, methanol extract of the pellet from the suspension reaction without enzyme at time 0; 7, methanol extract of the pellet from the suspension reaction without enzyme after 10 min; 8, methanol extract of the pellet from the suspension reaction with 0.01 unit of enzyme after 10 min.

Table 3. Relative Levels of Soy Glycosides and Aglycones before and after 10 min of Digestion of Suspended Soybean Flour with or without 0.01 Unit of *D. nigrescens* β-Glucosidase[a]

glycoside or aglycone	relative amount of isoflavone after reaction					
	0 min		10 min with *D. nigrescens* β-glucosidase		10 min control	
	supernatant	pellet	supernatant	pellet	supernatant	pellet
daidzin	100	100	0	0	90	92
glycitin	100	100	0	0	109	103
genistin	100	100	0	0	88	93
malonyldaidzin	100	100	12	54	109	115
acetyldaidzin	100	100	0	0	71	79
malonylgenistin	100	100	3	0	110	124
acetylgenistin	100	100	0	0	77	89
daidzein	100	100	1710	383	230	160
genistein	100	100	467	488	212	143
glycitein	100	100	1118	670	229	156

[a] Reactions were done in 0.1 M sodium acetate buffer with incubation at 37 °C, and relative amounts were determined by HPLC, as described under Materials and Methods. The amounts in the 0 time were set at 100%, and relative levels (relative HPLC peak areas) at other times are reported.

active endogenous isoflavonoid β-glycosidases were present in the methanolic extract.

Ismail and Hayes (*13*) reported that high amounts of *E. coli* and almond β-glucosidases could hydrolyze genistin and daidzin, but those enzymes showed little hydrolysis of conjugated glycosides, even when the amount of the enzyme was increased up to 6 units and the digestion time to 24 h for *E. coli* β-glucosidases and to 30 units and 4 h for almond β-glucosidases. For comparison, we also tested almond β-glucosidase at the prolonged time and found it could hydrolyze most of the daidzin and genistin, but hydrolyzed little of the conjugated forms (**Table 1**). Although conversion between the conjugated and nonconjugated forms can occur during processing (*24*), the activity of the *D. nigrescens* β-glucosidase toward conjugated forms is a useful property. However, both *Dalbergia* β-glucosidases had highest activity toward daidzin and genistin, as previously reported for almond, *E. coli*, and soybean root β-glucosidases (*13*, *15*). Although both *Dalbergia* enzymes had much higher activity than almond β-glucosidase toward both daidzin and genistin, *D. nigrescens* β-glucosidase showed higher activity than *D. cochinchinensis* β-glucosidase, for which two small peaks of daidzin and genistin were still observed after the 10 min digestion (**Figure 1C**). This experimental result could be confirmed by the kinetic properties of these enzymes toward isoflavone glycosides.

The kinetic properties of *D. nigrescens* and *D. cochinchinensis* β-glucosidases toward daidzin, genistin, and malonylgenistin were determined using HPLC to quantify the amounts of products (**Table 2**). Previously, we had determined provisional kinetic parameters of *D. nigrescens* β-glucosidase for daidzin and genistin by the glucose oxidase assay (*19*). Because daidzein and genistein are antioxidants that interfere with that assay, the kinetics were repeated by HPLC. *D. nigrescens* β-glucosidase has K_m values approximately 6 times lower than those of *D. cochinchinensis* β-glucosidase for daidzin and genistin. Although *D. cochinchinensis* β-glucosidase has a lower K_m value for malonylgenistin than *D. nigrescens* β-glucosidase, its k_{cat} value was 200 times lower than that of *D. nigrescens* β-glucosidase. Therefore, *D. nigrescens* β-glucosidase hydrolyzed soybean isoflavonoid glycosides more quickly than *D. cochinchinensis* β-glucosidase (**Table 2**), which correlates with the results in **Figure 2**, where Thai rosewood β-glucosidase hydrolyzed daidzin and genistin more slowly than *D. nigrescens* β-glucosidase and had little effect on malonylgenistin.

Hydrolysis of Soy Flour Suspension. The hydrolysis of isoflavonoid glycoside in methanolic extracts of soy flour by *D. nigrescens* β-glucosidase was promising, but it is not directly relevant to food and feed processing. Therefore, hydrolysis was evaluated in a suspension of soy flour in aqueous buffer. The hydrolysis of soy flour extract was analyzed by thin-layer chromatography as shown in **Figure 3**. The two most intense spots, daidzin and genistin, were completely digested with *D. nigrescens* β-glucosidase, and daidzin and genistin were not detected after digestion in either the aqueous solution or the

methanol extract of the flour particles left after aqueous extraction. The hydrolysis of isoflavonoids in the flour particles (lanes 8) indicated that *D. nigrescens* β-glucosidase could efficiently hydrolyze these isoflavonoid glycosides inside the particles or that they are in equilibrium with those free in solution. The hydrolysis of genistin and daidzin could also be seen to a lesser extent in controls, which can occur when soybean flour is soaked in water, probably by an endogenous soybean β-glucosidase (*27*). This result was confirmed by HPLC of the reactions with and without *D. nigrescens* β-glucosidase (**Table 3**). *D. nigrecsens* β-glucosidase (0.01 unit) completely hydrolyzed almost all isoflavonoid glycosides in the suspended soy flour within 10 min at 37 °C, including both those in solution and those associated with the insoluble particles. Only malonyldaidzin remained in significant amounts of 12 and 54% of the initial levels in the solution and particles, respectively. This hydrolysis resulted in increases of approximately 17-fold in daidzein, 5-fold in genistein, and 11-fold in glycitein in the solution and in approximately 4-, 5-, and 6-fold increases of daidzein, genistein, and glycitein in the particles, respectively. The soy flour also appeared to have endogenous activity, which hydrolyzed up to 29% of acetyldaidzin, 23% of acetylgenistin, 12% of genistin, and 10% of daidzin in the solution and lower amounts in the particles, but did not affect malonylglucoside levels. This hydrolysis in the control resulted in increases of <2.5-fold of each isoflavone aglycone in the solution and <2-fold in the particles. Thus, addition of *D. nigrescens* β-glucosidase greatly increased the release of free isoflavones in the suspended soy flour.

It is noteworthy that *D. nigrescens* β-glucosidase is able to hydrolyze almost all isoflavonoid conjugated and nonconjugated glycoside forms in the crude soy flour extract and suspended soy flour in a short time. *D. cochinchinensis* β-glucosidase could also hydrolyze some of these glycosides, although less rapidly than *D. nigrescens*. This may correlate with the differences in their natural substrates, because *D. nigrescens* are 7-*O*-glycosides, as are the soybean isoflavonoids, whereas *D. cochinchinensis* hydrolyzes a rotenoid glycoside. The hydrolysis of isoflavonoid glycosides may increase their conversion to isoflavones (aglycone), which have bioavailability and health benefits (*26*−*29*). *D. nigrescens* β-glucosidase is a very stable enzyme with a temperature optimum of 65 °C, so it should be useful for industrial processes. Some evidence of endogenous soybean isoflavonoid β-glucosidase was evident in the processed soy flour, which suggests that it is also somewhat heat stable, but this residual activity was much less than that of the small amount of *D. nigrescens* enzyme added to the digest. This research suggests that *D. nigrescens* β-glucosidase may be useful for processing of soy foods to enhance their nutritional and economic value.

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