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Kinetic Modeling of Malonylgenistin and Malonyldaidzin Conversions under Alkaline Conditions and Elevated Temperatures

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The conversion and degradation of malonylglucosides were kinetically characterized under elevated pH/heat conditions. Malonylgenistin and malonyldaidzin were heated at 60, 80, and 100 °C and pH values of 8.5, 9, and 9.5. A simple kinetic model was developed, which adequately predicted the conversion and degradation reactions. The conversion and degradation rates increased as temperature and pH increased. The rates of conversion of both malonylglucosides into their respective β-glucosides were comparable under all pH/heat treatments. However, at 100 °C, the rates of degradation of malonyldaidzin were approximately double those of malonylgenistin, under all pH treatments. When malonlydaidzin was heated at 100 °C and pH 9.5, degradation of the produced daidzin occurred. Therefore, an alternative kinetic model was developed to better predict the conversion and degradation of malonyldaidzin occurring at 100 °C and pH 9.5. The models developed provide soy food manufacturers with guidelines for better control of the profile and level of isoflavones.

KEYWORDS: Isoflavones; β-glucosides; malonylglucosides; kinetics; isoflavone conversions and degradations

INTRODUCTION

Research is still ongoing to investigate the health benefits, toxicity level, and minimum requirements as well as the best biologically available form of isoflavones in soybeans. While all of these issues are being resolved, isoflavones' stability remains a key factor in the determination of processing conditions of soy-based products. The stability of the various chemical forms of isoflavones is affected to a great extent by processing conditions. As a result of variable processing conditions, interconversions as well as a significant amount of loss/degradation of isoflavones have been witnessed by several researchers, in real and model systems. The amount of isoflavone lost is often attributed to loss in the waste stream (*1*−*3*), binding to the protein matrix (*4*−*8*), and/or degradation (*9*, *10*). The literature still lacks a complete understanding of the chemical modification of isoflavones as affected by key processing factors, namely, pH, temperature, and time. Particularly, little is known about the effect of pH on the stability of isoflavones in heated systems. Recent work has shown that different types/forms of isoflavones vary in stability under different pH/heat treatments in model systems (*10*). Conversions of conjugated glucosides accelerated as pH increased, with loss of isoflavones (on a mole basis) reaching up to 30% in model systems that did not involve a waste stream or binding to proteins (*10*).

In raw soybeans, malonylgenistin and malonydaidzin are the most abundant forms (*11*), and both are known to be thermally unstable (*6*). To date, kinetic modeling of malonylglucosides has focused mostly on the effect of temperature on malonylgenistin. Kinetic modeling of malonylgenistin heated at 100 °C in a model system, with no protein present, showed loss of malonylgenistin to unknown products with a first-order rate constant of 0.422/h (*9*). However, the concentrations reported were in micrograms per milliliter and not on a molar basis; therefore, the loss of isoflavones measured included loss due to degradation as well as unreal loss due to differences in molecular weights of the products and reactants. Heating soy milk, in a closed system at 80 °C for 3 h, showed significant conversion of malonylgenistin to genistin without significant loss (*6*). Heat would cause denaturation and thus unfolding of the proteins, which might affect the extraction rates of the different isoflavone forms and, consequently, the measured loss.

Alkaline treatments have been used in the production of soy protein concentrates (*12*) and soy protein isolates (*13*) and also have been included in an AOAC method to simplify the analysis of conjugated isoflavones, namely, malonylglucosides and acetylglucosides, by converting them to their respective β-glucosides (*5*). When the stability of conjugated forms was studied under various pH/heat treatments, it was observed that under alkaline conditions malonyl- and acetylglucosides were con-

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Table 1. pH of 0.01 M Borax Buffers at 21.5 °C and at Treatment Temperatures and Molarity of HCl Used To Reduce the pH after Each pH/Heat Treatment

treatment temperature (°C)	pH at 21.5 °C	treatment pH ±0.1	concentration of HCl[a] (M)
100	11.03	9.5	0.25
	9.70	9.0	0.22
	9.08	8.5	0.21
80	10.16	9.5	0.22
	9.46	9.0	0.22
	8.94	8.5	0.21
60	9.85	9.5	0.22
	9.31	9.0	0.21
	8.84	8.5	0.205

[a] Fifty microliters of HCl at indicated molarity for each pH treatment was used to reduce the pH to 1.65.

verted to their respective β-glucosides as well as to unknown degradation products and that the reactions were driven by temperature (*10*). During the production of soy slurry, increasing the temperature from 25 to 60 °C and the pH from 8.5 to 10.5 caused an increase in the loss of total isoflavones (*13*). However, the calculations for the loss were not monitored over time.

In summary, considerable conversions and degradation of conjugated isoflavones are prone to occur under the elevated pH and temperature conditions that are often used in the processing of soybean products. Kinetically characterizing the interconversions and degradation of malonylglucosides, the most abundant isoflavones in raw soybeans, would provide soy food manufacturers with guidelines for better control of the profile and level of isoflavones. Therefore, the objective of this work was to kinetically characterize the interconversions and degradation of malonylglucosides, in closed model systems, under elevated pH/heat conditions similar to those applied in the industry.

MATERIALS AND METHODS

Materials. High-performance liquid chromatography (HPLC) grade acetonitrile and methanol (99.95%) were purchased from Fisher Scientific (Hanover Park, IL). Isoflavone standards, malonylgenistin, genistin, daidzin, genistein, and daidzein, were purchased from LC Laboratories (Woburn, MA). Malonydaidzin was purchased from Wako Chemicals, USA, via Fisher Scientific. Standard solutions (500 ppm) were prepared using 80% aqueous methanol solutions. All nine borax buffers used in the different pH/heat treatments were made up to the same concentration (0.01 M), and the pH of each buffer was adjusted using either 0.2 N NaOH or 0.1 N HCl solutions, as necessary.

Heat and pH Treatments of Malonylgenistin and Malonyldaidzin. Because the pH of the buffers changed upon heating, nine different borax buffers (0.01 M) were prepared to carry out nine different pH/heat treatments. The pH of each buffer was adjusted prior to heating to result in the desired pH for each heat treatment, as illustrated in **Table 1**. The pH of the buffers was monitored using a Corning Pinnacle 540 pH-meter with a Cole Palmer electrode (reference cell Ag/AgCl) that had an error of ±0.05 at elevated temperatures. An aliquot (192.5 μL) of each borax buffer at a particular pH was placed in 2-mL clear borosilicate HPLC screw-cap vials and preheated at temperatures of 60, 80, or 100 °C for 1 min. An aliquot (7.5 μL) of malonylgenistin solution (500 ppm) was added to each preheated buffer, and the vials were closed with a screw cap, covered with Parafilm, and placed into a water bath at 60, 80, or 100 °C for three different time intervals as shown in **Table 2**. After each time interval, the vials were removed from the water bath and placed on ice for 5 min (the average time needed to get to 22 °C, as monitored using a thermocouple). When room temperature (22 °C) was resumed, and prior to the addition of 1 mL of methanol (99.95%), the pH was reduced to 1.65 by adding 50 μL of 0.205−0.25 M HCl (**Table 1**). The solutions were filtered through 0.45 μm filters and stored at −20 °C until analysis by HPLC (storage time ranged between 12 and 24 h). A control (concentration T_0) was prepared for each pH/heat treatment following the same procedure as above; however, the pH of the buffers was adjusted to 1.65 before the 7.5-μL aliquot of the malonylgenistin solution was added. All treatments were performed in triplicate. The above procedure was repeated using 10-μL aliquots of malonyldaidzin solution (500 ppm) and 190-μL aliquots of the different borax buffer solutions.

Table 2. Time Intervals for Each pH/Heat Treatment of Malonylgenistin and Malonyldaidzin

		malonylgenistin			malonyldaidzin		
temperature (°C)	pH	T1[a]	T2	T3	T1	T2	T3
100	9.5	0.50	1.00	1.50	0.50	1.00	3.00
	9.0	1.00	1.75	3.25	0.50	1.00	3.00
	8.5	1.50	4.00	9.00	1.00	3.00	10.00
80	9.5	2.00	5.00	8.00	2.00	5.00	8.00
	9.0	3.00	7.00	14.00	5.00	8.00	11.00
	8.5	8.00	16.00	36.00	10.00	20.00	30.00
60	9.5	5.00	15.00	36.00	7.50	15.00	30.00
	9.0	30.00	60.00	120.00	30.00	60.00	150.00
	8.5	60.00	165.00	285.00	60.00	150.00	300.00

[a] Time intervals for each treatment in minutes.

HPLC Analysis. A Shimadzu HPLC system, equipped with a SIL-10A VP autoinjector, a SPD-10A VP UV detector set at a wavelength of 256 nm, and a 250 mm × 4.6 mm, 5 μm, YMC pack ODS AM-303 C18 reverse phase column was used for analysis. Separation and quantification of isoflavones was achieved using the HPLC method described by Ismail and Hayes (*14*), with one modification. Each set of daidzein and genistein derivatives was calibrated separately, using their respective aglycone, glucoside, and conjugated glucosides.

Kinetics Analysis. On the basis of the general behavior that emerged from the experimental results, alternative reaction mechanisms were proposed and criticized. For discriminating among the competing kinetic models resulting from the alternative reaction mechanisms we used analysis of residuals and regression analysis. The numerical values of the model's parameters (i.e., the reaction rate constants) were estimated by fitting the models to the experimental data using nonlinear regression. The nonlinear regression was carried out using the Levenberg−Marquardt method (*15*) as implemented in the function NonlinearRegress in Mathematica 5 (Wolfram Research, Inc.). The sum of squared deviations from the mean (sum of squares) provided by the function NonlinearRegress was used for defining a parameter r^2, so that the fit of a kinetic model can be compared to the fit of the other competing models (*16*). The parameter r^2 for a nonlinear regression was defined as the ratio of the difference between the corrected sum of squares and the model sum of squares and the corrected sum of squares, in a similar manner to their correlation coefficient counterparts used for linear regression. Nonlinear regression was preferred in every case, even if linear regression was possible, to avoid transformation of the raw data, for example, taking logarithms for first-order reactions, which might violate critical assumptions for regression such as the assumption of normal distribution of errors (*17*). Finally, the activation energies of the conversion reactions for each malonylglucoside were calculated by assuming an Arrhenius relationship between the rate constants and the absolute temperature.

RESULTS AND DISCUSSION

Kinetic Model for Malonylgenistin and Malonydaidzin Conversions and Degradation. A kinetic model was developed and discriminated to describe the conversion of malonydaidzin and malonylgenistin into their respective derivatives, as well as their degradation, over the experimental range of temperature

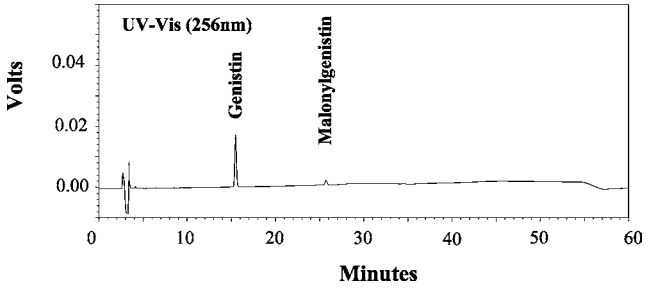

Figure 1. Example of a HPLC chromatogram of treated malonylgenistin, showing the conversion into β-glucoside genistin. The chromatogram shows absorption at 256 nm and was obtained following the method indicated under HPLC Analysis.

and pH. Upon various pH/heat treatments of both malonylglucosides, respective nonconjugated β-glucosides were detected by HPLC analysis, whereas acteylglucosides and aglycones were not (**Figure 1**). Therefore, it was most likely that each malonylglucoside was de-esterified into its respective β-glucoside by giving up a malonate, as illustrated in eq 1. Under the tested conditions, the production of β-glucosides was not equimolar to the initial concentration of malonylglucosides. Thus, in parallel to conversions, degradation into unknown products (D) occurred, as is illustrated in eq 2. On this basis, a first-order rate equation corresponding to total structural change (conversions plus degradation) with a combined rate constant of $k_{12} \equiv k_1 + k_2$ was used (eq 3). Equation 4 shows the integration of eq 3, where [malonylglucoside0] represents the initial concentrations of the corresponding malonylglucoside. The production of β-glucoside was predicted using the rate equation (eq 5). Integration of eq 5 provided the concentration of the β-glucoside with time, as shown in eq 6.

$$\text{malonylglucoside} \xrightarrow{k_1} \beta\text{-glucoside} + \text{malonate} \quad (1)$$

$$\text{malonylglucoside} \xrightarrow{k_2} \text{D} \quad (2)$$

$$\frac{\text{d[malonylglucoside]}}{\text{d}t} = -(k_1 + k_2)[\text{malonylglucoside}] = -k_{12}[\text{malonylglucoside}] \quad (3)$$

$$[\text{malonylglucoside}] = [\text{malonylglucoside0}]\, e^{-k_{12}t} \quad (4)$$

$$\frac{\text{d}[\beta\text{-glucoside}]}{\text{d}t} = k_1[\text{malonylglucoside}] = k_1([\text{malonylglucoside0}]\, e^{-k_{12}t}) \quad (5)$$

$$[\beta\text{-glucoside}] = \frac{k_1[\text{malonylglucoside0}](1 - e^{-k_{12}t})}{k_{12}} \quad (6)$$

Determination of Rate Constants for the Conversions and Degradation of Malonylgenistin and Malonydaidzin. Using nonlinear regression, as explained under Kinetic Analysis, the rate constant k_{12} for the total structural change of each malonylglucoside and the rate constant k_1 for the conversion of each malonylglucoside into their respective β-glucoside were estimated from eqs 4 and 6, respectively. Although eq 6 includes two parameters (k_1 and k_{12}), only one (k_1) is a free parameter. The free parameter k_1 in eq 6 is estimated after the parameter k_{12} has been estimated using eq 4. The rate constant k_2 for the degradation of each malonylglucoside was calculated as $k_2 = k_{12} - k_1$. Equations 4 and 6 demonstrated a good fit (r^2 ranged from 0.961 to 0.999) and thus would adequately predict the conversions and degradation of both malonylgenstin and malonyldaidzin (**Table 3**), within the range of temperatures and pH values studied. To demonstrate the adequacy of the model, predicted results from eqs 4 and 6 for all pH treatments at 60 °C and for all heat treatments at pH 9.5 were plotted (**Figures 2** and **3**). All other fits for the different pH values and temperatures showed a similar trend (graphs not shown).

The rate constants of both the conversion and degradation reactions increased as temperature increased at a given pH (**Table 3**). Similarly, the rate constants increased as pH increased at a constant temperature (**Table 3**). De-esterification increased with higher pH because more hydroxyl groups, from the buffer,

Table 3. Rate Constants for the Conversion (k_1) and Degradation (k_2) of Malonylgenistin and Malonyldaidzin at Different pH/Heat Treatments

malonylglucoside	temperature (°C)	pH	k_1 (min^{-1})	r^2	k_2 (min^{-1})	r^2
malonylgenistin	100	9.5	1.27 ± 0.03[a]	0.995	0.325 ± 0.166	0.979
		9.0	0.570 ± 0.011	0.996	0.159 ± 0.018	0.998
		8.5	0.191 ± 0.005	0.993	0.042 ± 0.012	0.994
	80	9.5	0.339 ± 0.003	0.999	0.049 ± 0.025	0.994
		9.0	0.111 ± 0.007	0.967	0.033 ± 0.009	0.991
		8.5	0.037 ± 0.001	0.993	0.016 ± 0.005	0.981
	60	9.5	0.039 ± 0.001	0.995	0.016 ± 0.007	0.966
		9.0	0.012 ± 0.0004	0.992	0.004 ± 0.001	0.990
		8.5	0.006 ± 0.0001	0.997	0.002 ± 0.001	0.979
malonyldaidzin	100	9.5	1.30 ± 0.074	0.961	0.669 ± 0.193	0.988
		9.0	0.592 ± 0.028	0.980	0.354 ± 0.086	0.984
		8.5	0.217 ± 0.006	0.994	0.132 ± 0.024	0.993
	80	9.5	0.263 ± 0.011	0.978	0.058 ± 0.024	0.990
		9.0	0.104 ± 0.006	0.961	0.016 ± 0.008	0.984
		8.5	0.041 ± 0.002	0.985	0.013 ± 0.004	0.983
	60	9.5	0.037 ± 0.001	0.988	0.029 ± 0.006	0.975
		9.0	0.012 ± 0.0002	0.990	0.007 ± 0.001	0.988
		8.5	0.005 ± 0.0001	0.993	0.002 ± 0.0004	0.993

[a] Standard error, $n = 3$.

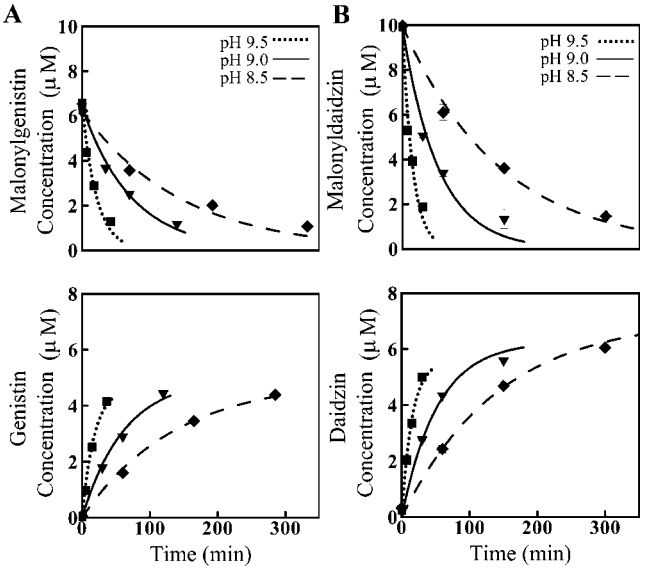

Figure 2. Influence of pH on (**A**) the total conversion of malonylgenistin and production of genistin and (**B**) the total conversion of malonydaidzin and production of daidzin, as predicted by eqs 4 and 6, along with the corresponding experimental data points (■, 9.5 pH; ▼, 9.0 pH; ◆, 8.5 pH) at 60 °C. Each symbol represents the average of three experimental measurements. Error bars were calculated using the standard error of the mean; in some cases they are not visible as the errors are smaller than the size of the symbol.

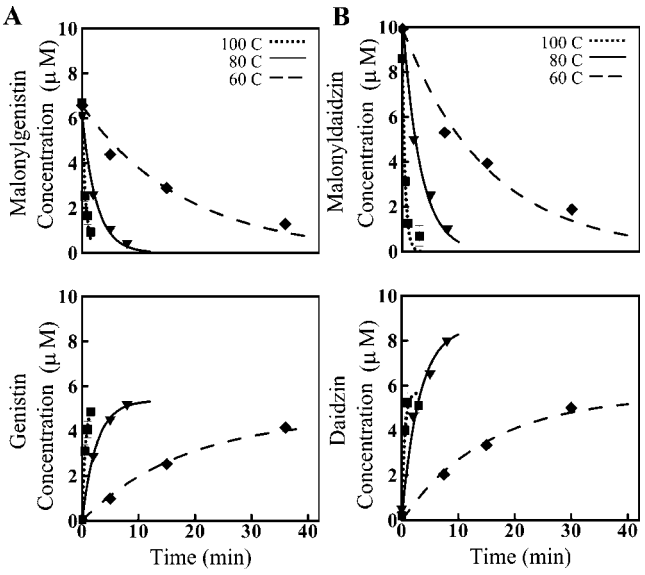

Figure 3. Influence of temperature on (**A**) the total conversion of malonylgenistin and production of genistin and (**B**) the total conversion of malonyldaidzin and production of daidzin, as predicted by eqs 4 and 6, along with the corresponding experimental data points (■, 100 °C; ▼, 80 °C; ◆, 60 °C) at pH 9.5. Each symbol represents the average of three experimental measurements. Error bars were calculated using the standard error of the mean; in some cases they are not visible as the errors are smaller than the size of the symbol.

were available to attack the carbonyl carbon participating in the ester bond of the malonylglucosides (**Figure 4**). The rate constants k_1 for both malonylgenistin and malonydaidzin were comparable, suggesting that the extra hydroxyl group at position 5 of the A ring in malonylgenistin (**Figure 4**) did not have a significant effect on the rate of de-esterification under the conditions tested. However, at 100 °C, under all pH treatments, the k_2 values of malonyldaidzin were approximately double those

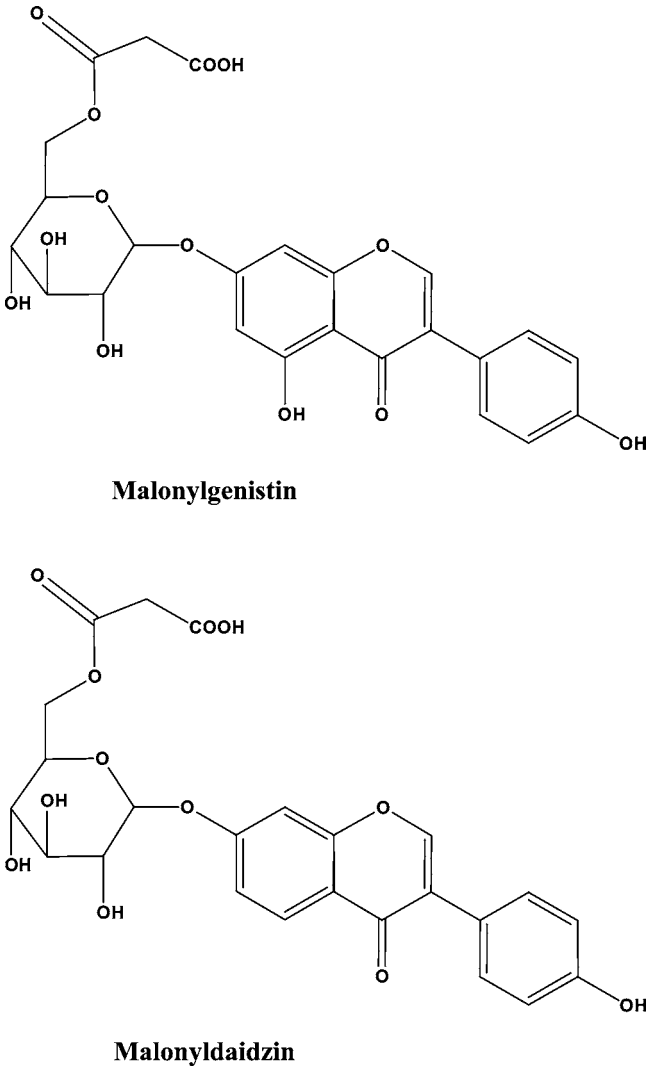

Figure 4. Molecular structures of malonylgenistin and malonyldaidzin.

of malonylgenistin (**Table 3**), suggesting that the extra hydroxyl group in malonylgenistin might have had a significant effect on the rate of degradation. These results are comparable to our previously reported findings (*10*), where 30% loss was observed in malonyldaidzin as compared to only 15% loss in malonylgenistin with treatment at pH 10 and 100 °C.

Arrhenius Modeling. The mechanisms of de-esterification in an aqueous base suggests that eq 1 can be extended to represent the conversion of the malonylglucosides to β-glucosides and malonate by explicitly including the hydroxyl group, from the buffer, as shown in eq 7. The chief distinction between eqs 1 and 7 is that the rate constant k, in this case, is independent of the pH. A first-order rate equation corresponding to the conversion of malonylglucoside, with the new rate constant, was used (eq 8). Both eqs 5 and 8 were used to calculate the new rate constant k, where rate constant k_1 (from eq 5), of each treatment, was divided by the constant hydroxyl concentration of either 31.62, 10.00, or 3.162 corresponding to the buffer systems at pH values of 9.5, 9, or 8.5, respectively. At every temperature studied, the new k values calculated, after accounting for the different hydroxyl concentrations of the various pH treatments, were relatively the same, as would be expected, with minor variations due to experimental error. Therefore, the three k values obtained for every heat treatment were averaged and used to configure an Arrhenius plot that related the rate constants to the absolute temperatures. The r^2 for the Arrhenius plots

configured for malonylgenistin and malonyldaidzin were 0.9979 and 0.9989, respectively.

$$\text{malonylglucoside} + \text{OH} \xrightarrow{k} \beta\text{-glucoside} + \text{malonate} + \text{H}_2\text{O} \quad (7)$$

$$\frac{\text{d}[\beta\text{-glucoside}]}{\text{d}t} = k[\text{malonylglucoside}][\text{OH}^-] \quad (8)$$

The Arrhenius activation energies calculated for the conversion of malonylgenistin to genistin and malonyldaidzin to daidzin were 22.23 ± 1.403 and 23.30 ± 1.164 kcal/mol, respectively. The similarity of the Arrhenius activation energies of the two malonylglucosides is attributed to similarity in structure and the reaction site. The extra hydroxyl group at position 5 of the A ring (**Figure 4**) did not contribute to a change in the activation energy. Chien et al. (*9*) reported a different activation energy for the conversion of malonylgenistin to genistin (18.9 kcal/mol); this difference could be attributed mainly to differences between the proposed reaction models. They included conversion of malonylgenistin to acetylgenistin; however, that particular reaction was not detected under the conditions used in our experiments. Furthermore, their model systems were in 100% methanol, whereas our reactions were carried out in buffered systems.

Including the hydroxyl group in the degradation equation used (eq 2) was not possible because the exact mechanism for participation of hydroxyl groups in the conversion of malonylglucosides to unknown products was unclear. Therefore, the Arrhenius activation energies for the degradation of the two malonylglucosides were not computed. The degradation reaction is thought to be a more complex reaction than conversion, and the k_2 rate constants were likely to include multiple unknown reactions. Further research is needed to identify degradation mechanisms and resulting products, in order to formulate a more complete model for the degradation of malonylglucosides, in particular, and isoflavones in general.

Alternative Kinetic Model for the Conversion and Degradation of Malonyldaidzin at 100 °C and pH 9.5. Results showed that when malonlydaidzin was heated at 100 °C and pH 9.5, degradation of the produced β-glucoside occurred. This phenomenon was not observed in malonylgenistin treated under similar conditions. All derivatives of daidzein were previously shown to be less stable than the genistein derivatives under heated alkaline conditions (*10*, *18*). Therefore, an alternative kinetic model was proposed to describe the conversion and degradation occurring when malonyldaidzin was heated at 100 °C and pH 9.5. As illustrated in eq 9, malonyldaidzin was de-esterified to daidzin by giving up a malonate. The production of daidzin was not equimolar to the initial concentration of malonyldaidzin, due to degradation of malonyldaidzin to an unknown compound (D) (eq 10) as well as the degradation of the produced daidzin into further unknown compounds (D_1) (eq 11). The total structural change (conversion plus degradation) of malonyldaidzin is illustrated by eq 12, which is a simple first-order reaction with a combined rate constant of $k'_{12} \equiv k'_1 + k'_2$. Equation 13 is the integration of eq 12, where [malonyldaidzin0] represents the initial concentration of malonyldaidzin. The production plus degradation of daidzin is illustrated by eq 14. The total production of daidzin was predicted by eq 15, the integrated form of eq 14.

$$\text{malonyldaidzin} \xrightarrow{k'_1} \text{daidzin} + \text{malonate} \quad (9)$$

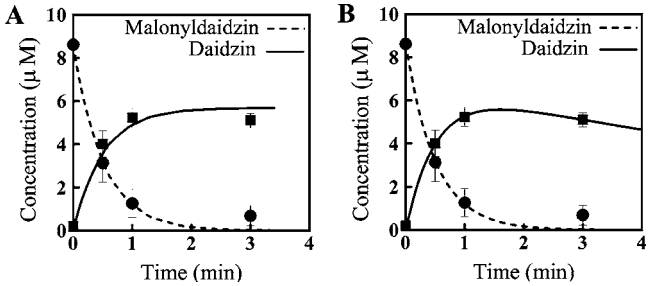

Figure 5. Concentration changes of malonyldaidzin and daidzin during heating at 100 °C and pH 9.5, as predicted by (**A**) the two-parameter model and (**B**) the alternative three-parameter model. Experimental data points are represented by symbols (●, malonyldaidzin; ■, daidzin) with error bars representing standard error of the mean of three determinations.

$$\text{malonyldaidzin} \xrightarrow{k'_2} \text{D} \quad (10)$$

$$\text{daidzin} \xrightarrow{k'_3} \text{D}_1 \quad (11)$$

$$\frac{\text{d}[\text{malonyldaidzin}]}{\text{d}t} = -k'_{12}[\text{malonyldaidzin}] \quad (12)$$

where $k'_{12} = k'_1 + k'_2$.

$$[\text{malonyldaidzin}] = [\text{malonyldaidzin0}]\, \text{e}^{-k'_{12}t} \quad (13)$$

$$\frac{\text{d}[\text{daidzin}]}{\text{d}t} = k'_1[\text{malonyldaidzin}] - k'_3[\text{daidzin}] = k'_1([\text{malonyldaidzin0}]\, \text{e}^{-k'_{12}t}) - k'_3[\text{daidzin}] \quad (14)$$

$$[\text{daidzin}] = \frac{\text{e}^{-(k'_{12}+k'_3)t}(\text{e}^{k'_{12}t} - \text{e}^{k'_3 t})k'_1[\text{malonyldaidzin0}]}{k'_{12} - k'_3} \quad (15)$$

The rate constant k'_{12} for the total structural change of malonyldaidzin was estimated from experimental HPLC data corresponding to the change in malonyldaidzin concentration with time, using eq 13. The rate constants k'_1 and k'_3 for the conversion of malonyldaidzin to daidzin and that of daidzin to D_1, respectively, were estimated using eq 15 and the k'_{12} value estimated from eq 13 along with the experimental HPLC data corresponding to the concentration of daidzin produced over time. Finally, the rate constant k'_2 for the degradation of malonyldaidzin was calculated as $k'_2 = k'_{12} - k'_1$. The rate constant k'_1 (1.494 ± 0.039/min, $r^2 = 0.988$) obtained using this alternative model was higher than that obtained in the previously discussed model under similar conditions ($k_1 = 1.3$/min, **Table 3**), thus predicting more conversion of malonyldaidzin into daidzin. Part of the daidzin produced, however, underwent subsequent degradation ($k'_3 = 0.097 \pm 0.017$/min, $r^2 = 0.997$). In the previous model degradation of daidzin was included in the total degradation of malonyldaidzin, resulting in a higher k_2 (0.669/min, **Table 3**) value that overestimated degradation of malonyldaidzin, as compared to the k'_2 value (0.481 ± 0.198/min, $r^2 = 0.988$) obtained using the alternative model. For the treatment of malonyldaidzin at 100 °C and pH 9.5, the alternative model provides a better fit than the first one, as illustrated in **Figure 5**, where the fit for daidzin concentration passed through all experimental data points. Further kinetic work is necessary to characterize the rate of conversion and degradation resulting upon treatment of malonyldaidzin at 100 °C/pH 9.5 and beyond.

The relative simplicity of the kinetic equations, the accuracy of the fittings, the trends in reaction rates, and the similarities in activation energies as analyzed above suggested that the proposed kinetics adequately described the chemical reactions involved in the conversion of malonylglucosides, with a low number of parameters. Results indicated that the small difference in molecular structure of both malonylglucosides did not affect the rate of conversion into β-glucosides. However, as pH and heat increase, the difference in molecular structure affected significantly the rate of degradation of each malonylglucoside and its respective β-glucoside. This work confirmed that, along with conversion, degradation occurs, resulting in loss. In real heated systems, similar degradation might be occurring, leading to loss that is independent of waste streams and protein matrices. To confirm this assertion, complementary kinetic work ought to be done in real systems, taking into account the protective effect of proteins on isoflavone chemical modification, as well as the effect of protein denaturation (due to pH and temperature) on the rate of extraction of each type/form of isoflavone.

Overall, the findings of this work highlighted the importance of the chemical structure of isoflavone with respect to stability and provided useful information to manufacturers aiming to minimize loss of isoflavones to enhance the nutritional value of their soy products, without sacrificing the quality characteristics. Therefore, while nutritionists investigate the biological significance of each type/form of isoflavone, it is a necessity to fully understand their distinct reaction to typical processing conditions and thus minimize loss.

LITERATURE CITED

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