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# Kinetic model for studying the conversion and degradation of isoflavones during heating

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**Abstract**

The conversion and degradation of isoflavones during dry or moist heating at 100, 150 and 200 °C, for varied lengths of time, were kinetically studied. Results showed that, at the early stage, all the reaction rates of malonylgenistin (MG), acetylgenistin (AG), genistin (G), and genistein (Ge) increased with increasing temperature and fitted a first-order model, when the concentration changes during heating were analyzed using HPLC. For dry heating, the conversion of MG to G exhibited the highest rate constant ($h^{-1}$), followed by MG to AG, AG to G, AG to Ge, G to Ge and MG to Ge. Moist heating showed the same phenomenon; however, the last three conversions were not observed. In addition, MG had the highest degradation rate, followed by G, Ge and AG during dry heating, while the reversed trend occurred for moist heating. Moist heating was more susceptible to conversion and degradation of isoflavones than dry heating. The correlation coefficients ($r^2$) ranged from 0.664 to 0.987 for moist heating and 0.688–0.960 for dry heating. The kinetic model developed in this study can be used to predict the concentration changes of isoflavones during dry heating and moist heating.
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*Keywords:* Isoflavone stability; HPLC; Heating; Kinetics

## 1. Introduction

Isoflavones, a class of flavonoids that are mainly present in soybean, have been demonstrated to possess high biological activity (Birt, Hendrich, & Wang, 2001; Brouns, 2002; Fitzpatrick, 2003). Many epidemiological studies have shown that the consumption of diets rich in isoflavones was associated with lower rates of cancers, such as breast, prostate and colon (Birt et al., 2001; Wu et al., 1998; Zheng et al., 1999). Of the various isoflavones, genistein and daidzein have been studied extensively for anti-cancer activity because of their estrogen receptor antagonist and agonist activities (Birt et al., 2001). However, several investigators have suggested that the inhibition of human cancer cell growth by genistein was unrelated to the estrogenic activity of this compound (Constantinou, Krygier, & Mehta, 1998).

The bioavailability of isoflavones can be affected by their chemical forms in foods and their stabilities during processing (Kao & Chen, 2002; Wang, Ma, Pagadala, Sherrard, & Kishnan, 1998). For instance, the major type of isoflavone in soybean is 6″-*O*-malonylgenistin, which can be converted to 6″-*O*-acetylgenistin or genistin after drying or hot water extraction, respectively (Barnes, Coward, Kirk, & Sfakianos, 1998). Interestingly, the formation of genistein was observed after fermentation (Barnes et al., 1998). Thus, the conversion among various isoflavones, as affected by different heating treatments, has to be investigated. Many authors have studied the concentration change of isoflavones during processing of soybean-based products (Coward, Smith, Kirk, & Barnes, 1998; Murphy, Barua, & Hauck,

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2002; Wang et al., 1998). However, the conversion mechanism among various isoflavones remains uncertain. Furthermore, it would be easier to explore the stability of isoflavones in model systems than in food systems because they reduce the number of system variables to more manageable levels. In addition, in a complicated food system, the interaction caused by the presence of components such as protein and lipid may interfere with data-fitting for nonlinear differential equations, which in turn results in a decrease of $r^2$ value and an increase of $P$ value of least square difference. For kinetic study of the isoflavone stability in a model system, it is essential that the key variables should not be eliminated and the number of variables should be carefully controlled to avoid interference. Thus, the most important variables in this study are "temperature" and "time", and the oxygen concentration was assumed to be constant during heating. The objectives of this study were to develop a kinetic model for studying the conversion and degradation of four isoflavones, as affected by various heating treatments.

## 2. Materials and methods

### 2.1. Materials

A Vydac 201TP54 C18 column (250 × 4.6 mm i. d., 5 μm particle) used for separation of isoflavones was purchased from Vydac Co. (Hesperia, CA, USA). Isoflavone standards, including acetylgenistin, genistin and genistein were from LC Laboratories Co. (Woburn, MA, USA), while 6″-*O*-malonylgenistin was from Fujico Co., (Kobe, Japan). Internal standard formononetin was from Fluka Co. (Switzerland). The HPLC-grade solvents, methanol and acetonitrile, were from Mallinckrodt Co. (Paris, KY, USA) and degassed by sonication prior to use. Deionized water was made using a water purification system by Millipore Co. (Bedford, MA).

### 2.2. Instrumentation

The HPLC instrument consisted of a Jasco MD-915 photodiode-array detector (Tokyo, Japan), a Jasco 970 UV detector (Tokyo, Japan), two Jasco pumps (model PU-980, Tokyo, Japan), a Rheodyne injector (model 7161, CA, USA) and a Phenomenex degassing system (model DG-440, Torrance, CA, USA); a BORWIN computer software system was used for processing data. The high-speed Sorvall centrifuge (RC5C) was from Du Pont Co. (Wilmington, Delaware, USA). The N-1 rotary evaporator was from Eyela Co. (Tokyo, Japan). The oil-bath heater (model B503) was from I-Seng Co. (Taipei, Taiwan). The sonicator (model 2210R-DTH) was from Branson Co. (Danbury, CT, USA).

### 2.3. Heating of isoflavone standards

Four isoflavone standards, malonylgenistin, acetylgenistin, genistin and genistein, were each dissolved in methanol, separately to give concentrations of 50 μg/ml. Each isoflavone (50 μl) was poured into a 2-ml ampoule and sealed with a propane flame for heating. An oil-bath heater (40 × 30 × 20 cm) containing 12 l silicone oil was preheated to 100, 150 or 200 °C, and then the ampoules were immersed in the oil for the heating times of 0, 5, 15, 30, 60, 120 or 180 min. The temperatures 100, 150 and 200 °C were selected because they are similar to processing conditions of soybean products such as soymilk, yuba and tempeh. In Asian countries, soymilk is often processed at 100 °C for 10 or 20 min, which should resemble the heating condition of a moist system in this study. Yuba, a popular fried soybean product in Taiwan, is often processed at 150 °C (low-temperature -frying) or 200 °C (high-temperature -frying). Similarly, tempeh, a popular fermented soybean product in Indonesia, is fried at 200 °C for 3 min or boiled at 100 °C for 10 min. Therefore, the stability of isoflavones observed in this study may be similar to that in a food system. After heating, the ampoules were inserted into an ice bath to terminate the reaction. Each isoflavone in the ampoule was transferred to a vial and 25 μl internal standard, formononetin (200 μg/ml), were added and diluted to 1 ml with methanol. The isoflavone solution was filtered through a 0.2-μm membrane filter and 20 μl were injected into the HPLC. Three injections and triplicate analyses were carried out for each moist heating treatment and, in total, 63 ampoules were used. For dry heating treatment, the same procedure was applied with the exception that the isoflavone solution in the ampoule was evaporated to dryness under nitrogen prior to sealing so that a thin film of isoflavone was formed on the inner surface of the ampoule. Similarly, the ampoules were sealed with a flame and subjected to heating, with a total of 60 samples used.

### 2.4. HPLC analysis of isoflavone

A fast HPLC method developed in our lab was used to separate the various isoflavones (Hsieh, Kao, & Chen, 2004). A gradient binary solvent system of acetonitrile (A) and water (B) was employed: 8% A and 92% B initially, increased to 10% A in 2 min, 12% A in 4 min, 22% A in 10 min, 23% A in 11 min, 35% A in 12 min, 50% A in 13 min, maintained for 3 min and returned to 8% A in 20 min. The flow rate was 2 ml/min, with column temperature at 35 °C and UV detection at 262 nm. All the four isoflavones were adequately resolved within 15 min. Each isoflavone was quantified using an internal standard method, as described in a previous study (Kao & Chen, 2002). The data were subjected to analysis of variance and Duncan's multiple range test using a statistical analysis system (SAS, 2000).

### 2.5. Statistical analysis for kinetic data

The various concentration changes of isoflavones during dry and moist heating at 100, 150 and 200 °C were subjected to statistical analyses using linear and nonlinear regression procedures (SAS, 2000). The parameters of the linear models were estimated by using the least square difference method. All the rate constants in each nonlinear model were estimated by computing least squares with a nonlinear regression (NLIN) procedure-Marquardt iterative methods until the convergence criteria of the best-fitted parameters were met. The rate constants and correlation coefficients of each linear or nonlinear equation were determined from three data points. The precision of the parameters of the kinetic equations was also assessed.

## 3. Results and discussion

### 3.1. Stability of isoflavone during dry heating at 100 °C

When malonylgenistin was heated alone at 100 °C for 120 min, acetylgenistin began to form. The level of acetylgenistin increased further, by 7.0 μg/ml, after 180 min heating (Fig. 1(a)). Barnes et al. (1998) also reported that when soybean-based foods were dry heated at 100 °C, acetylgenistin could be formed from malonylgenistin.

### 3.2. Stability of isoflavone during moist heating at 100 °C

When acetylgenistin was heated alone at 100 °C, genistin was formed at levels of 6.8, 8.3, 10.2, 16.0, 18.9 and 20.9 μg/ml after 5, 15, 30, 60, 120 and 180 min, respectively (Fig. 1(b)). Meanwhile, the acetylgenistin concentration showed a decreasing trend. A similar outcome was observed for malonylgenistin during moist heating, which could be converted to acetylgenistin or genistin, depending on heating time. Acetylgenistin was not formed until heating time reached 15 min, and the amounts increased by 9.5, 8.7 and 8.5 μg/ml after 30, 60 and 120 min, respectively (Fig. 1(c)). The further decrease of acetylgenistin after 60 min heating is probably due to conversion to genistin. In contrast, genistin began to form after 5 min, and a large increase of 14.2, 20.3, 26.7 and 28.2 μg/ml occurred for heating times of 15, 30, 60 and 120 min, respectively (Fig. 1(c)). At the same time, the malonylgenistin level decreased with increasing heating time and was not detected after 60 min of heating. This result further demonstrated that malonylgenistin could be converted to acetylgenistin or genistin during moist heating at 100 °C, and genistin was generated at a higher level than acetylgenistin. It has been well documented that, during heating, both the malonyl and acetyl ester groups can be cleaved to form genistin (Barnes et al., 1998).

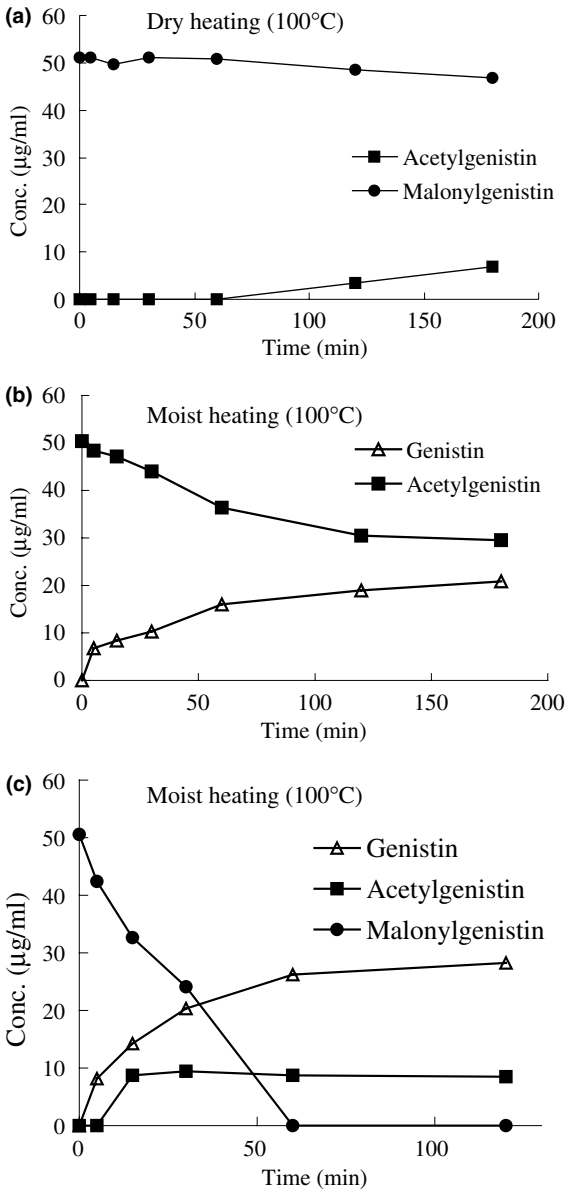



Fig. 1. Formation curves of Acetylgenistin (a), Genistin (b) and Acetylgenistin or Genistin (c) in the presence of Malonylgenistin (a) or (c) or Acetylgenistin (b) during dry or moist heating at 100 °C.

### 3.3. Stability of isoflavone during dry heating at 150 °C

As described above, the loss of acetylgenistin and malonylgenistin is probably due to formation of genistin from the former and acetylgenistin or genistin from the latter (Fig. 2). When acetylgenistin was heated alone at 150 °C, genistin was not detected until heating time was 5 min and reached a plateau (14.9 μg/ml) after 180 min (Fig. 2(a)). Likewise, concentrations of both acetylgenistin and genistin increased following the decrease of malonylgenistin (Fig. 2(c)). After heating for 5 min, acetylgenistin and genistin began to form and a maximum was attained after 60 min, which amounted to 15.5 and 15.0 μg/ml, respectively (Fig. 2(c)). However,

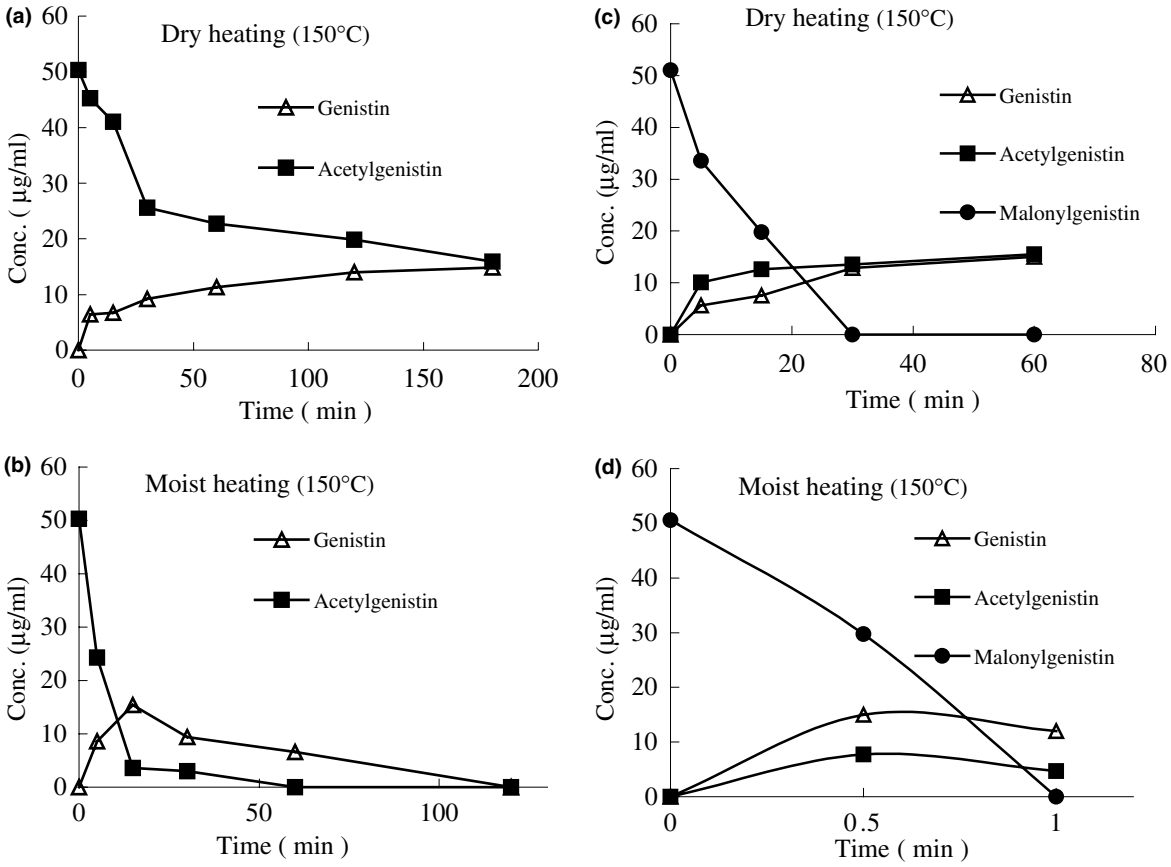



Fig. 2. Formation curves of Genistin (a,b) and Acetylgenistin or Genistin (c, d) in the presence of Acetylgenistin (a or b) or Malonylgenistin (c or d) during dry or moist heating at 150 °C.

in the first 15 min, acetylgenistin (12.6 μg/ml) was formed at a much higher concentration than genistin (7.6 μg/ml), implying that the conversion from malonylgenistin to acetylgenistin was faster than to genistin. Interestingly, the content of genistin was greater than acetylgenistin after heating for 30 min and above, which could be attributed to conversion from acetylgenistin or malonylgenistin. No malonylgenistin was detected after 30 min. A similar report was shown when soybean powder was heated at 150 °C for 4 h, with acetylgenistin being the major product and genistin the minor product from malonylgenistin (Murphy et al., 2002).

### 3.4. Stability of isoflavone during moist heating at 150 °C

Similar to moist heating at 100 °C, genistin was formed following the decrease of acetylgenistin and reached the highest level (15.4 μg/ml) after 15 min of heating. However, the amount of acetylgenistin dropped sharply to 3.1 μg/ml after 30 min and resulted in a decrease of genistin (Fig. 2(b)). This outcome showed that, in addition to conversion, genistin may undergo degradation after 30 min of heating. The formation curve of acetylgenistin and genistin in the presence of malonylgenistin during moist heating at 150 °C is shown in Fig. 2(d). Both acetylgenistin and genistin were formed at levels of 7.8 and 15.0 μg/ml, respectively, after heating for 30 s, whereas malonylgenistin was completely degraded in 1 min. Genistin was more readily formed than acetylgenistin since it could be generated from malonylgenistin or acetylgenistin. Theoretically the decrease of acetylgenistin could result in an increase of genistin. However, in our study, this phenomenon did not occur, mainly because the degradation of genistin may proceed rapidly after 30 s of heating. The same trend also applied to acetylgenistin. By comparing the results shown above, the malonyl type of isoflavone was found to be preferentially converted to the β-glucoside type under moist heating while the acetyl type of isoflavone was favoured under dry heating.

### 3.5. Stability of isoflavones during dry heating at 200 °C

Fig. 3(a) shows the formation curve of genistein in the presence of genistin during dry heating at 200 °C. Genistein began to form after 15 min heating and a maximum of 8.8 μg/ml was accomplished in 120 min. However, the slow formation of genistein after 30 min

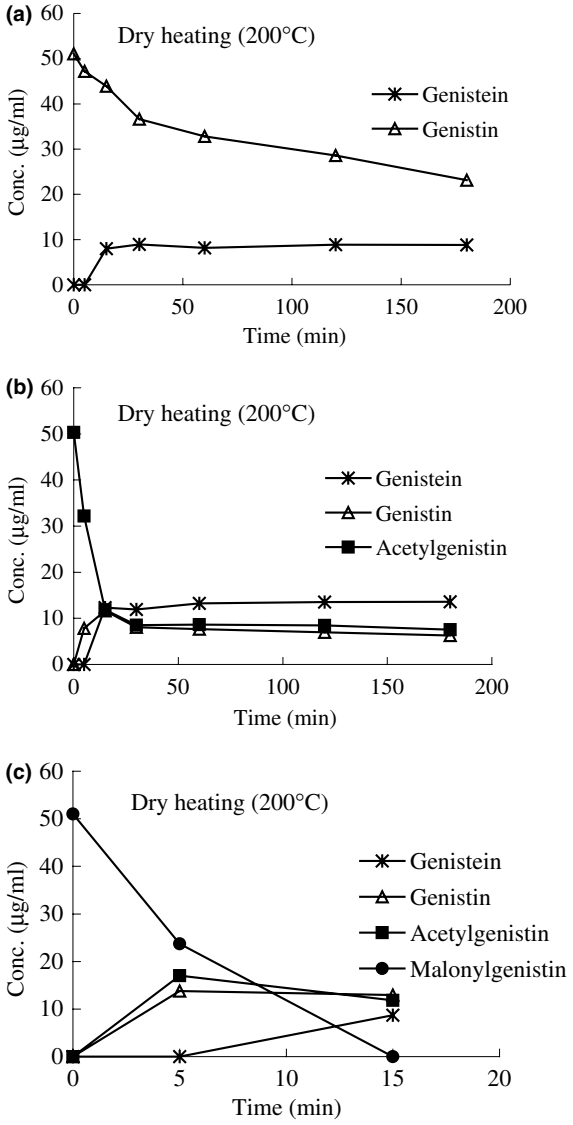



Fig. 3. Formation curves of Genistein (a), Genistin or Genistein (b) and Genistin, Genistein or Acetylgenistin (c) in the presence of Genistin (a), Acetylgenistin (b) or Malonylgenistin (c) during dry heating at 200 °C.

of heating revealed that the degradation of genistein may proceed rapidly thereafter because genistin continued to decrease at the same time. Similarly, with a heating time of 5 min, genistin was formed and a peak (11.6 μg/ml) was attained after 15 min in the presence of acetylgenistin (Fig. 3(b)). However, a declining tendency occurred thereafter, indicating that genistin may be converted to genistein or undergo degradation. For genistein, it did not form until heating time reached 15 min; however, no significant change was found afterwards. Genistein was formed at a higher level than genistin, probably because the former could be produced from acetylgenistin or genistin. Nevertheless, with a heating time of 30 min and above, a final equilibrium may be achieved as no significant changes were shown for acetylgenistin, genistin and genistein. When malonylgenistin was dry-heated alone at 200 °C, it could be converted to acetylgenistin, genistin and genistein (Fig. 3(c)). The largest amount (17.0 μg/ml) of acetylgenistin was formed after 5 min heating, followed by genistin (13.8 μg/ml) and genistein (0 μg/ml). Meanwhile, acetylgenistin began to decline, probably because of continued degradation of malonylgenistin or formation of genistin from acetylgenistin. The formation of genistin was not so pronounced, which could be due to its conversion to genistein. Nevertheless, a higher content (13.0 μg/ml) of genistin was found when compared to genistein (8.7 μg/ml), showing that genistin was the major product formed after 15 min of heating. Coward et al. (1998) heated a mixture of soybean powder and flour in an oven at 190 °C and also found that malonylgenistin could be simultaneously degraded to form acetylgenistin, genistin or genistein.

### 3.6. Stability of isoflavones during moist heating at 200 °C

Similar to the treatment of moist heating at 150 °C, genistin was formed after heating acetylgenistin at 200 °C for 5 min (Fig. 4(a)). Interestingly, both were completely degraded after 15 min of heating. The same trend also applied to malonylgenistin, which was not detected after 1 min when heated alone (Fig. 4(b)). Conversely, with a heating time of 30 s, the amounts of acetylgenistin and genistin formed were 9.2 and 10.8 μg/ml, respectively, after which both began to decline. Genistin was formed at a higher level than acetylgenistin, which could be attributed to the formation of the former from the latter proceeding faster than the latter from malonylgenistin.

On comparison of the results shown above, with the exception of dry heating at 100 °C, all other treatments resulted in formation of genistin from acetylgenistin (Fig. 5). However, the major product formed from conversion of malonylgenistin was acetylgenistin during dry heating. On the other hand, the primary product formed from malonylgenistin during moist heating was genistin. Mahungu et al. (1999) also reported that malonylgenistin could be deacylated to form acetylgenistin during heating of soy products. In addition to genistin, genistein was also formed by dry heating at 200 °C (Fig. 5). However, with moist heating at 200 °C, no genistein formation was found, probably because of fast degradation as soon as it was formed at elevated temperature.

### 3.7. Complex consecutive reaction models

For kinetic studies, only four isoflavones, malonylgenistin, acetylgenistin, genistin and genistein, were selected, and one should first examine the order of each reaction before developing the complicated kinetic equations. Each isoflavone may undergo consecutive

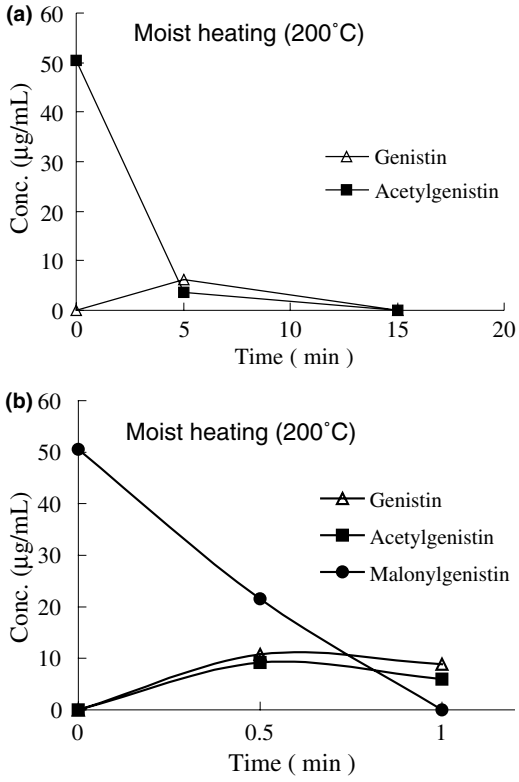



Fig. 4. Formation curves of Genistin (a), Acetylgenistin or Genistin (b) in the presence of Acetylgenistin (a) or Malonylgenistin (b) during moist heating at 200 °C.

conversions (MG → AG → G → Ge) or degradation to other products ($D_n$) when all the possible reactions are taken into consideration during heating. Fig. 5 shows the major pathways of intermolecular conversion and degradation of isoflavones. For kinetic study of consecutive reactions, changes in concentration of each isoflavone were monitored to determine the rate constants. If each conversion or degradation reaction is proved to be first-order, the consecutive kinetics can be divided into four major categories as shown below:

1. *Kinetic model for isoflavone conversion during dry heating at 100 °C*

According to the concentration change in Fig. 1(a) and the pathway in Fig. 5, acetylgenistin (AG) is the only product formed from malonylgenistin (MG) during dry heating at 100 °C. The corresponding pathway can be simplified as follows:

$$\mathrm{MG} \xrightarrow{k_1} \mathrm{AG}.$$

The above pathway is similar to the simple first-order reaction model. Thus, the following rate equation can be proposed

$$-\frac{\mathrm{d}[\mathrm{MG}]}{\mathrm{d}t} = k_1[\mathrm{MG}]. \tag{1}$$

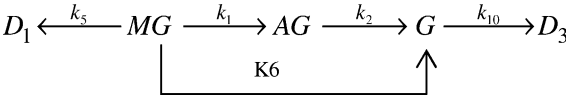
The integration of Eq. (1) gives

$$\ln\left(\frac{[\mathrm{MG}]_t}{[MG]_0}\right) = -k_1 t \quad \text{or} \quad [\mathrm{MG}]_t = [\mathrm{MG}]_0 \mathrm{e}^{-k_1 t} \tag{2}$$

where [MG] and $[MG]_0$ are concentrations of malonylgenistin at time $t$ and 0; $k_1$ is the reaction rate constant $t$ is time.

2. *Kinetic model for isoflavone conversion and degradation during moist heating at 100 °C*

According to the concentration change in Fig. 1(b) and (c) as well as the pathway in Fig. 5, formation of acetylgenistin and genistin (G) and the degraded product ($D_1$) from malonylgenistin in the early stage of moist heating at 100 °C can be demonstrated as follows:




The degradation of genistein (Ge) may also occur during moist heating at 100 °C, and thus the corresponding pathway can be represented as follows:

$$\mathrm{Ge} \xrightarrow{k_4} \mathrm{D}_4.$$

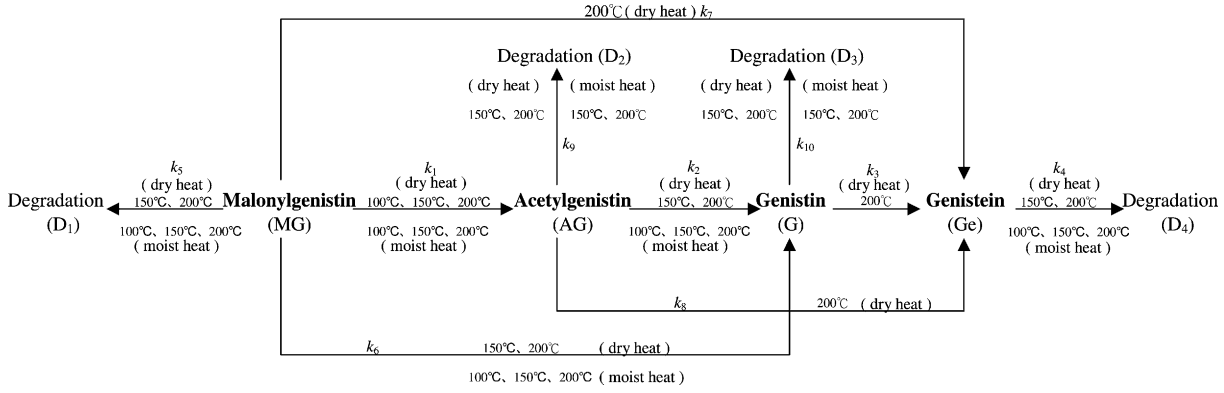



Fig. 5. Influence of dry or moist heating on conversion and degradation of 4 isoflavones.

From the above two pathways, the rate Equations (3)–(6) could be written mathematically as follows:

$$\frac{d[MG]}{dt} = -(k_1 + k_5 + k_6)[MG] = -K_1[MG], \quad (3)$$

$$\frac{d[AG]}{dt} = k_1[MG] - k_2[AG], \quad (4)$$

$$\frac{d[G]}{dt} = k_2[AG] + k_6[MG], \quad (5)$$

$$\frac{d[Ge]}{dt} = -k_4[Ge], \quad (6)$$

where $K_1 = k_1 + k_5 + k_6$

Eqs. (3) and (6) can be integrated into (7) and (8), respectively,

$$[MG]_t = [MG]_0 e^{-K_1 t}, \quad (7)$$

$$[Ge]_t = [Ge]_0 e^{-k_4 t}. \quad (8)$$

Substituting Eq. (7) into Eq. (4), Eq. (9) can be obtained by integration as follows:

$$[AG]_t = \frac{k_1[MG]_0}{k_2 - K_1}\left(e^{-K_1 t} - e^{-k_2 t}\right). \quad (9)$$

Likewise, Eq. (10) can be acquired by integration with substituting Eqs. (7) and (9) into Eq. (5):

$$[G]_t = [G]_0 + \frac{k_6[MG]_0}{K_1} + \frac{k_1[MG]_0}{K_1 - k_2}\left(1 - \frac{k_2}{K_1}\right) + \frac{[MG]_0}{K_1}\left(\frac{k_1 k_2}{K_1 - k_2} - k_6\right)e^{-K_1 t} + \frac{k_1[MG]_0}{k_2 - K_1}e^{-k_2 t}, \quad (10)$$

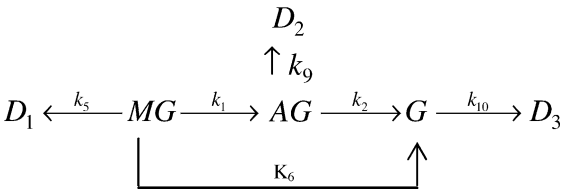
where $[AG]_t$ and $[AG]_0$ are concentrations of acetylgenistin at time $t$ and 0; $[G]_t$ and $[G]_0$ are concentrations of genistin at time $t$ and 0; $[Ge]_t$ and $[Ge]_0$ are concentrations of genistein at time $t$ and 0; $k_5$ and $k_6$ are the reaction rate constants; $t$ is time.

Of course, the above differential equations (3)–(5), may also simultaneously be solved by using the Laplace transform method.

3. *Kinetic model for isoflavone conversion and degradation during dry heating at 150 °C and moist heating at 150 and 200 °C*

I. Thermal conversion and degradation from malonylgenistin

According to the pathway in Fig. 5, formation of acetylgenistin, genistin and genistein from malonylgenistin and their degradation in the early stage of heating could be summarized as follows:




Likewise, the degradation of genistein may also be observed in our study as previously described

$$Ge \xrightarrow{k_4} D_4.$$

The following rate equations could be proposed from the two pathways shown above:

$$-\frac{d[MG]}{dt} = K_1[MG], \quad (11)$$

$$\frac{d[AG]}{dt} = k_1[MG] - (k_2 + k_9)[AG] = k_1[MG] - K_2[AG], \quad (12)$$

$$\frac{d[G]}{dt} = k_2[AG] + k_6[MG] - k_{10}[G], \quad (13)$$

where $K_1 = k_1 + k_5 + k_6$ and $K_2 = k_2 + k_9$

$$\frac{d[Ge]}{dt} = -k_4[Ge]. \quad (14)$$

The integration of Eqs. (11) and (14) gives the same mathematic models as Eqs. (7) and (8), as previously described

$$[MG]_t = [MG]_0 e^{-K_1 t}, \quad (7)$$

$$[Ge]_t = [Ge]_0 e^{-k_4 t}. \quad (8)$$

After substituting Eq. (7) into Eq. (12), integration gives the following equation:

$$[AG]_t = \frac{k_1[MG]_0}{k_2 - K_1}\left(e^{-K_1 t} - e^{-k_2 t}\right). \quad (15)$$

Similarly, Eq. (16) could be derived from integration by substituting Eqs. (7) and (15) into (13) as previously described

$$[G]_t = \frac{[MG]_0}{K_1 - K_2}\left[\frac{k_1 k_2 - k_6(k_1 - k_2)}{K_1 - k_{10}}\left(e^{-K_1 t} - e^{-k_{10} t}\right) - \frac{k_1 k_2}{K_2 - k_{10}}\left(e^{-k_2 t} - e^{-k_{10} t}\right)\right]. \quad (16)$$

II. Thermal conversion and degradation from acetylgenistin

According to the pathway in Fig. 5, in addition to malonylgenistin, both genistin and genistein may also be formed from acetylgenistin in the early stage of heating. The corresponding pathway could be written as follows:

$$\begin{array}{l} D_2 \\ \uparrow k_9 \\ AG \xrightarrow{k_2} G \xrightarrow{k_{10}} D_3 \end{array}$$

The following rate equations could then be extracted from the pathway shown above

$$\frac{d[AG]}{dt} = -(k_2 + k_9)[AG] = -K_2[AG], \tag{17}$$

$$\frac{d[G]}{dt} = k_2[AG] - k_{10}[G]. \tag{18}$$

The integration of Eq. (17) gives Eq. (19)

$$[AG]_t = [AG]_0 e^{-K_2 t}. \tag{19}$$

Substituting Eq. (19) into Eq. (18), Eq. (20) could be obtained from integration

$$[AG]_t = \frac{k_1[MG]_0}{k_{10} - K_2}\left(e^{-K_2 t} - e^{-k_{10} t}\right). \tag{20}$$

4. *Kinetic model for isoflavone conversion and degradation during dry heating at 200 °C*

According to the pathway in Fig. 5, formations of acetylgenistin, genistin and genistein from malonylgenistin and their degraded products in the early stage of dry heating at 200 °C are as follows:

$$\frac{d[MG]}{dt} = K_1[MG], \tag{21}$$

$$\frac{d[AG]}{dt} = k_1[MG] - K_3[AG], \tag{22}$$

$$\frac{d[G]}{dt} = k_6[MG] + k_2[AG] - K_4[G], \tag{23}$$

$$\frac{d[Ge]}{dt} = k_7[MG] + k_8[AG] + k_3[G] - k_4[Ge], \tag{24}$$

where $K_3 = k_2 + k_8 + k_9$ and $K_4 = k_3 + k_{10}$.

Since the above rate equations are linear with respect to the reactant concentrations, the differential equations (21)–(24), could be simultaneously solved by using the Laplace transform method in order to find the time-dependent concentrations for [MG], [AG], [G], and [Ge]. The following equations (25)–(28) could thus be obtained by performing the Laplace transformation:

$$[MG]_t = [MG]_0 e^{-K_1 t}, \tag{25}$$

$$[AG]_t = \frac{k_1[MG]_0}{K_3 - K_1}\left(e^{-K_1 t} - e^{-K_3 t}\right), \tag{26}$$

$$\begin{aligned}[G]_t = {} & \frac{(k_6(K_3 - K_1) + k_1 k_2)[MG]_0}{(K_3 - K_1)(K_4 - K_1)} e^{-K_1 t} \\ & - \frac{k_1 k_2 [MG]_0}{(K_3 - K_1)(K_4 - K_3)} e^{-K_3 t} \\ & + \frac{k_1 k_2 (K_1 - K_3) + k_6(K_1 K_3 - K_1 K_4 + K_3 K_4) - k_3 K_3^2}{(K_1 - K_3)(K_1 - K_4)(K_3 - K_4)} \\ & \times [MG]_0 e^{-K_4 t}, \end{aligned} \tag{27}$$

$$\begin{aligned} Ge = {} & \frac{[MG]_0}{(K_1 - K_3)}\left[\frac{\Delta_1 e^{-K_1 t}}{(K_1 - K_4)(K_1 - k_4)} + \frac{\Delta_2 e^{-K_3 t}}{(K_3 - K_4)(K_3 - k_4)}\right. \\ & - \frac{\Delta_3 e^{-K_4 t}}{(K_1 - K_4)(K_3 - K_4)(K_4 - k_4)} + \left(\frac{\Delta_1}{(K_1 - K_4)(K_1 - k_4)}\right. \\ & \left.\left. - \frac{\Delta_2}{(K_3 - K_4)(K_3 - k_4)} + \frac{k_3 \Delta_3}{(K_1 - K_4)(K_4 - k_4)(K_3 - K_4)}\right) e^{-k_4 t}\right], \end{aligned} \tag{28}$$

where

Table 1
Conversion and degradation rate constants of the four isoflavones during dry heating

| Rate equation | | Heating Temperature (°C) | | | | | | Ea[c] |
|---|---|---|---|---|---|---|---|---|
| | | 100 | | 150 | | 200 | | |
| | | $k$ ($h^{-1}$) | $r^2$ | $k$ ($h^{-1}$) | $r^2$ | $k$ ($h^{-1}$) | $r^2$ | |
| MG[a] → AG[a] | $k_1$ | 0.0445 ± 0.0032[b] | 0.96 | 1.43 ± 0.006 | 0.849 | 7.86 ± 0.015 | 0.900 | 18.3 |
| MG → G[a] | $k_6$ | –[d] | | 2.31 ± 0.014 | 0.849 | 9.71 ± 0.059 | 0.887 | 11.95 |
| MG → Ge[a] | $k_7$ | – | | – | | 0.000 ± 0.099 | 0.887 | |
| MG → $D_1$[a] | $k_5$ | – | | 0.206 ± 0.000 | 0.849 | 5.808[e] | | 27.76 |
| AG → G | $k_2$ | – | | 0.221 ± 0.0013 | 0.688 | 0.585 ± 0.0177 | 0.900 | 8.1 |
| AG → Ge | $k_8$ | – | | – | | 0.2766[e] | | |
| AG → $D_2$ | $k_9$ | – | | 0.219 ± 0.0013 | 0.688 | 0.00[e] | | |
| G → Ge | $k_3$ | – | | – | | 0.0157 ± 0.003. | 0.918 | |
| G → $D_3$ | $k_{10}$ | – | | 0.0368 ± 0.0026 | 0.912 | 4.61 ± 1.000 | 0.918 | 40.15 |
| Ge → $D_4$ | $k_4$ | – | | 0.0338 ± 0.0022 | 0.922 | 0.853 ± 0.065 | 0.925 | 26.84 |

[a] MG, malonylgenistin; AG, acetylgenistin; G, genistin; Ge, genistein; $D_1$, $D_2$, $D_3$ and $D_4$, degraded products.
[b] Means ± standard deviations.
[c] Activation energy (kcal/mole).
[d] Not detected by HPLC.
[e] Calculated from the other rate constants.

$$\Delta_1 = k_3k_6(K_1 - K_3) - k_1k_8(K_1 - K_4) - k_7(K_1 - K_4)(K_1 - K_3) - k_1k_2k_3,$$

$$\Delta_2 = k_1((K_3 - K_4)k_8 - k_2k_3),$$

$$\Delta_3 = k_6(K_1 - K_3)(K_3 - K_4) - k_1k_2(K_1 + K_3),$$

Table 1 shows the rate constants of the four isoflavones during dry heating. The first-order rate constants, at three heating temperatures, were estimated by the Marquardt iterative method until the convergence of the best-fitted parameters of a non-linear regression model was found. All the correlation coefficients ($r^2$) for the reactions of dry heating ranged from 0.688 to 0.96. This result implied that the equations shown above fitted the first-order model and could be used to predict the concentration changes of four isoflavones during dry heating. The conversion of MG to G ($k_6$) was found to have the highest rate constant, followed by MG to AG ($k_1$) and AG to G ($k_2$), while the MG to Ge ($k_7$), G to Ge ($k_3$) and AG to Ge ($k_8$) showed the lowest rate constant. With the exception of degradation of MG to D1 ($k_5$), the conversion rate constants were substantially higher than the degradation rate constant. This phenomenon could be inferred from Figs. (1–4), showing that both acetylgenistin or genistin dominated during dry heating of isoflavones. It was also found from Table 1 that $k_1$ and $k_6$ values were very large when compared to the other rate constants, revealing that the conversion of malonylgenistin is fast during heating.

Activation energy for each reaction during dry heating could be estimated from rate constants at various temperatures. Results showed that the order of activation energy (kcal/mole) was: G → $D_3$($k_{10}$) > MG → $D_1$($k_5$) > Ge → $D_4$($k_4$) > MG → AG($k_1$) > MG → G($k_6$) > AG → G($k_2$), indicating that the conversion reactions of MG to AG or G as well as AG to G occurred more readily than the degradations of MG, AG, G and Ge. This is because a low rate constant generally accompanies a high activation energy during dry heating, with the exception of conversion of AG to G.

Table 2 depicts the rate constants of the four isoflavones during moist heating. Likewise, the first-order rate constants at different temperatures were estimated by the Marquardt iterative method, as shown for dry heating. All the correlation coefficients ($r^2$) for the reactions of moist heating ranged from 0.664 to 0.987. This result also implied that the equations shown above fit, the first-order model. Similar to the result for dry heating, the orders of rate constant ($h^{-1}$) were: MG → G ($k_6$) > MG → AG ($k_1$) > AG → $D_2$ ($k_9$) > Ge → $D_4$ ($k_4$), G → $D_3$ ($k_{10}$) > AG → G ($k_2$) > MG → $D_1$ ($k_5$). With the exception of conversion of AG to G, the rate constants of the other conversions were higher than the corresponding degradations. The order of activation energy (kcal/mole), for each reaction during moist heating, was: MG → G > G → $D_3$ > Ge → $D_4$ > MG → AG > AG → G > AG → $D_2$, showing that all the conversions and degradations in moist heating had energy barriers similar to dry heating.

By comparison, all the rate constants in moist heating were 10–100 times higher than in dry heating. This result clearly showed that the four isoflavones are more susceptible to moist-heating loss than dry heating. Of the various conversions and degradations, the conversion of malonylgenistin is the most rapid during heating, demonstrating that the stability of malonylgenistin is extremely low when compared to the other three isoflavones.

In conclusion, a kinetic model was developed, based on the concentration change of four isoflavones during dry heating and moist heating. The rate constants for each conversion and degradation were calculated. Moist heating was found to be more susceptible to conversion and degradation of isoflavones than dry heating.

Table 2
Conversion and degradation rate constants of the four isoflavones during moist heating

| Rate equation | | Heating Temperature (°C) | | | | | | Ea[c] |
|---|---|---|---|---|---|---|---|---|
| | | 100 | | 150 | | 200 | | |
| | | $k$ ($h^{-1}$) | $r^2$ | $k$ ($h^{-1}$) | $r^2$ | $k$ ($h^{-1}$) | $r^2$ | |
| MG[a] → AG[a] | $k_1$ | 0.773 ± 0.001[b] | 0.864 | 46.7 ± 0.84 | 0.803 | 76.0 ± 0.96 | 0.944 | 16.6 |
| MG → G[a] | $k_6$ | 1.80 ± 0.003 | 0.664 | 265 ± 5.4 | 0.803 | 318 ± 4. 0 | 0.944 | 18.9 |
| MG → $D_1$[a] | $k_5$ | 0.422 ± 0.007 | 0.664 | 0.012 ± 0.000 | 0.803 | 0.000 ± 0.000 | 0.944 | |
| AG → G | $k_2$ | 0.211 ± 0.011 | 0.953 | 2.54 ± 0.006 | 0.890 | 6.60 ± 0.000 | 0.949 | 12.2 |
| AG → $D_2$ | $k_9$ | –[d] | | 4.40 ± 0.000 | 0.896 | 19.0 ± 0.00 | 0.949 | 12.1 |
| G → $D_3$ | $k_{10}$ | – | | 1.23 ± 0.074 | 0.952 | 11.6 ± 0.53 | 0.977 | 18.6 |
| Ge → $D_4$ | $k_4$ | 0.0743 ± 0.0047 | 0.926 | 5.53 ± 0.29 | 0.972 | 12.6 ± 0.44 | 0.987 | 18.5 |

[a] MG, malonylgenistin; AG, acetylgenistin; G, genistin; Ge, genistein; $D_1$, $D_2$, $D_3$ and $D_4$, degraded products.
[b] Means ± standard deviations.
[c] Activation energy (kcal/mole).
[d] Not detected by HPLC.

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