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Kinetics of daidzin and genistin transformations and water absorption during soybean soaking at different temperatures

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ABSTRACT

The kinetics of the isoflavone transformations (diadzin, diadzein, genistin and genistein) and moisture content in soaked soybeans were studied in the temperature range 30–85 °C. The evolution of the moisture was described by Peleg's model, and a modified first order kinetic equation was used to predict the transformation profiles of different isoflavones in the soaked soybeans. The dependency, on temperature, of the kinetic parameter was modelled by the Arrhenius equation and empirical equations. The measured β-glucosidase activity at different temperatures justifies the experimental profiles of isoflavone conversion.

1. Introduction

Soybeans and the foods made from them are known to have good nutritional and functional qualities, not only for their high protein and oil content, but also because they are a source of phytochemicals. A group of phytochemicals that can be found in soybeans is the isoflavones. These phytochemicals can potentially prevent chronic diseases, such as cancer, osteoporosis and some heart problems and are recommended for alleviation of post-menopause syndrome (Liu, 1997; Messina, Messina, & Setchell, 1994; Snyder & Kwon, 1987). The content of isoflavones in soybeans is affected by the preparation and processing of the beans.

There are 12 isomers of isoflavones in soybeans and soybean products, which are divided into four chemical forms (with three isomers for each form). These forms are 6″-*O*-malonylglucosides, 6″-*O*-acetylglucosides, β-glucosides and aglycones (Liu, 1997). Aglycones are known to be more bioactive than their glucosides and have more functional properties. Two of the aglycones (diadzein and genistein) and their corresponding glucosides (diazin and genistin) have been extensively studied for anti-cancer activity because of their estrogen receptor antagonist and agonist activities (Birt, Hendrich, & Wang, 2001; Chien, Hsieh, Kao, & Chen, 2005).

Soaking is one of the most important steps in the preparation of soybean foods (Toda, Sakamoto, Takayanagi, & Yokotsuka, 2001) as it reduces the energy required for processing and cooking time (Liu, 1997). It has been reported that β-glucosidase is the enzyme responsible for the hydrolysis of isoflavone glucosides into aglycones (Matsuura, Obata, & Fukushima, 1989).

There are a number of recent studies in relation to isoflavones in soybeans, such as the improvement of HPLC methods for determining soybean isoflavones (Careri, Elviri, & Mangia, 2001; Hsieh, Kao, & Chen, 2004), the investigation of isoflavone transformation during soybean processing (Kao, Lu, Hsieh, & Chen, 2004; Kin & Chung, 2007; Lee et al., 2003; Matsuura et al., 1989; Wang & Murphy, 1994) and the study of isoflavone developments during soybean fermentation (Esaki, Onozaki, Kawakishi, & Osawa, 1997; Kim et al., 2006; Lin, Wei, Yu, & Chou, 2006; Pyo, Lee, & Lee, 2005; Romero, Doval, Sturla, & Judis, 2004; Tsangalis, Ashton, McGill, & Shah, 2002). A mathematical approach to the kinetics of isoflavone conversion in well-known mixtures of pure isoflavone solutions under ideal process conditions was studied by Chien et al. (2005). Vaidya, Mathias, Ismail, Hayes, and Corvalan (2007) used kinetic modelling to describe the transformation of malonylgenistin and malonyldaidzin under alkaline conditions at high temperature. However, transformations of this type in real soybean products, and in particular in soaked soybeans at different temperatures, remain unstudied.

In this work, the effect of soaking temperature on water absorption and the transformation of genistin and daidzin into their

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aglycones was investigated. The development of β-glucosidase activity in the soybean was also taken into account. A kinetic approach was used and mathematical, empirical and mechanistic models were proposed in order to: (1) describe appropriately, maintaining statistical robustness, the numerical data obtained experimentally, (2) generate a group of parameters of chemical and statistical significance and (3) formulate consistent relationships of these parameters with temperature.

2. Materials and methods

2.1. Soybean soaking

Ten grams of soybeans were soaked in 50 ml of distilled water at 30 °C, 50 °C, 60 °C and 85 °C for different times. The soybeans were first screened by hand to eliminate broken beans and those with cracked or damaged seed coats. After soaking, the soybeans were filtered using a vacuum pump and the dried beans were then dehulled and ground using a pestle and mortar. Samples were kept at −30 °C in the freezer for later analysis.

2.2. Moisture content

The moisture content was measured using the American Association of Cereal Chemistry method (AACC, 1995); 1.5 g of a representative sample was placed in pre-dried and covered dishes. The covered dishes with the samples were weighed and placed in the oven for 72 h at 103 °C. Dishes were then placed in a desiccator, immediately, and weighed once they reached room temperature. The moisture content was expressed as follows:

$$M(\%) = \left(\frac{W_1 - W_2}{W_1}\right) \times 100 \tag{1}$$

where M denotes the moisture content (%); W_1 denotes the weight before drying; and W_2 denotes the weight after drying.

2.3. Isoflavones extraction

The isoflavones extraction was based on the method of Griffith and Collison (2001). One gram of soaked soybeans was mixed in a 100 ml Duran bottle with 8 ml of acetonitrile, 11.5 ml of distilled water and 500 μl of internal standard (200 μg/ml of fluorescein in methanol). The bottle was shaken for 1 h at 150 rpm and the sample centrifuged for 5 min at 16,249*g*. The supernatant was filtered through a 0.45 mm PVDF filter and put into a vial for HPLC analysis.

2.4. HPLC isoflavones analysis

The HPLC method for isoflavones analysis was adopted from the work of Hsieh et al. (2004). The chromatograph used had a dual pump Varian Pro Star connected to a Phenomenex Gemini 5 μm C_{18}-110A column (250 × 4.6 mm) at 35 °C, and a PDA detector at 277 nm. Solvent A was 0.1% acetic acid in distilled water, solvent B was 0.1% acetic acid in acetonitrile, and the total flow rate was 1.0 ml/min. The gradient system was 92% of A initially, decreased to 90% in 2 min, 88% over 1 min, 78% in 7 min, 77% in 1 min, 65% over 1 min, then to 50% during 1 min, maintained for 5 min, and returned to 92% A in 2 min (the complete cycle lasted 20 min).

For quantification, 25 μg/ml of daidzin, genistin, daidzein and genistein solutions were prepared as working standards. 100, 300, 500, 700 and 1000 μl of each standard were collected and mixed with 125 μl of internal standard (200 μg/ml of fluorescein in methanol). The isoflavone standard curves were prepared using *Varian software* by plotting the concentration ratios between the isoflavone standard and internal standard concentration against the area ratio between the isoflavone standard and the internal standard areas. The coefficients of determination (R^2) ranged from 0.93 to 0.99.

2.5. Crude enzyme extraction

Three grams of ground sample were placed in a 100 ml Duran bottle and mixed with 15 ml of distilled water. The bottles were shaken at 150 rpm and 30 °C for 1 h, and then samples were centrifuged at 12,500*g* and 4 °C for 10 min. Supernatants were filtered through Whatman no. 1 filter paper and kept at −30 °C until used for the enzyme activity assay.

2.6. β-Glucosidase activity assay

β-Glucosidase activity of soaked soybeans was estimated using McCue and Shetty's method (2003). One hundred microliters of 9 mM *p*-nitrophenol-β-D-glucopyranoside were mixed with 800 μl of 200 mM sodium acetate buffer (pH 4.6) in a test tube. The tubes were incubated at 50 °C in a water bath for 5 min before addition of 100 μl of crude enzyme extract. In the blank the extract was replaced with distilled water. The tubes were then incubated for a further 30 min. One thousand microliters of 100 mM sodium carbonate were added to stop the reaction, and then the samples were centrifuged at 16,249*g* for 1 min. The absorbance of *p*-nitrophenol released was measured at 400 nm. The units of enzyme activity were defined (U/mg) as the amount of *p*-nitrophenol (μg) released in 1 min under controlled conditions.

2.7. Numerical methods

Fitting procedures and parametric estimations calculated from the results were carried out by minimisation of the sum of quadratic differences between observed and model/equation predicted values, using the non linear least-squares (quasi-Newton) method provided by the macro solver of the Microsoft excel spreadsheet. Statistica 6.0 software (StatSoft Inc., 2001) was used to evaluate the significance of the estimated parameters by fitting the experimental values to the proposed mathematical models, and the consistency of these equations.

3. Results and discussion

3.1. Effects of soaking temperature on the evolution of moisture content

The behaviour of the soybean water absorption showed a typical exponential increase with time at all assayed temperatures (see Fig. 1). To describe these profiles, the Peleg's equation, an empirical equation commonly used for modelling water absorption in various grains and foods during soaking (Ghannam, 1998; Ghannam & Mckenna, 1997; Peleg, 1988; Sopade and Obekpa, 1990, 1992), was used.

Peleg's model describes the change of moisture content in a solid matrix by the following equation:

$$M = M_0 + \frac{t}{k_1 + k_2 \cdot t} \tag{2}$$

All notation used in this work with units is detailed in Table 1. As the limit of the equation approaches zero and infinity we obtain

$$\lim_{t\to 0} M = M_0 \quad \text{and} \quad \lim_{t\to\infty} M = M_f = M_0 + \frac{1}{k_2} \tag{3}$$

It must be noted that the kinetic parameter k_1 is related to the inverse of the initial rate of water absorption, while k_2 is a constant that defines the equilibrium moisture content (Ghannam & Mckenna, 1997).

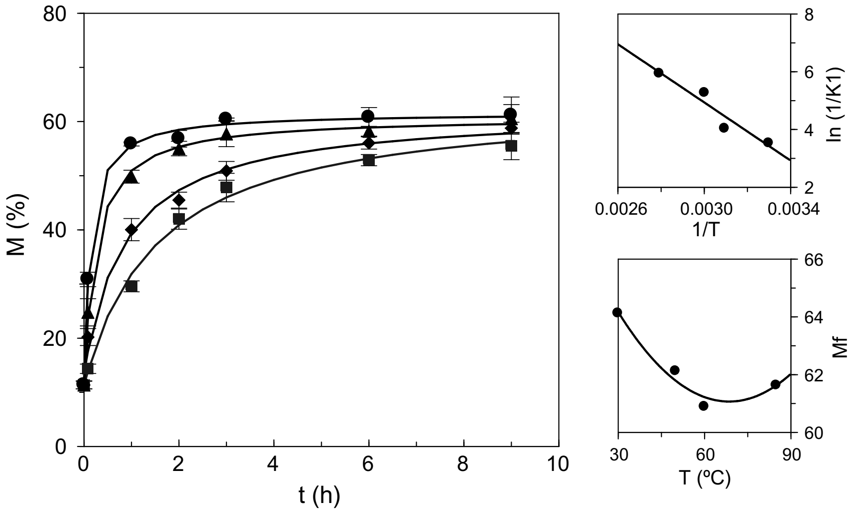

Fig. 1. Left: kinetics of the moisture content of soaked soybeans at different temperatures (■: 30 °C; ◆: 50 °C; ▲: 60 °C; ●: 85 °C) fitted by the Peleg's equation (2). Right: relationships between the kinetic parameters in Eq. (2) with temperature. The error bars are the confidence intervals ($\alpha = 0.05$; $n = 2$).

Table 1
Notation used with units

Peleg's equation (2)	
M	Moisture content,% wet basis (% wb)
M_0	Initial moisture content (% wb)
t	Time (h)
k_1	Peleg's constant 1 (h/(% wb))
k_2	Peleg's constant 2 (% wb^{-1})
M_f	Final or asymptotic moisture content (% wb)
Arrhenius model (4)	
E_a	Activation energy of the reaction (J/mol)
R	Universal gas constant (8.3145 J mol^{-1} K^{-1})
T	Absolute temperature (K)
A_0	Pre-exponential factor or frequency constant (h^{-1})
Modified first order kinetic model for daidzin and daidzein profiles (7) and (8)	
D, De	Daidzin (D) and daidzein (De) concentrations (mg/g; wb)
D_0, De$_0$ Initial	daidzin (D_0) and daidzein (De$_0$) concentrations (mg/g; wb)
D_f, De$_f$	Final daidzin (D_f) and daidzein (De$_f$) concentrations (mg/g; wb)
k_d, k_{de}	Specific rate of daidzin disappearance (k_d) and specific rate of daidzein formation (k_{de}; h^{-1})
t	Time (h)
Modified first order kinetic model for genistin and genistein profiles (9) and (10)	
G, Ge	Genistin (G) and genistein (Ge) concentrations (mg/g; wb)
G_0, Ge$_0$	Initial genistin (G_0) and genistein (Ge$_0$) concentrations (mg/g; wb)
G_f, Ge$_f$	Final genistin (G_f) and genistein (Ge$_f$) concentrations (mg/g; wb)
k_g, k_{ge}	Specific rates of genistin disappearance (k_g) and specific rate of genistein formation (k_{ge}; h^{-1})
t	Time (h)

Fig. 1 (bottom) shows the experimental data and the fitted values according to this model at different temperatures. The statistical analyses of the relevant kinetic parameters are summarized in Table 2. In general, the proposed models are statistically robust (Fisher's F-test and p-values <0.05), and the parametric estimations were significant (Student's t-test $\alpha = 0.05$). The coefficients of linear correlation (r) between predicted and observed values were in all cases >0.99.

The relationship between the specific rate of absorption ($1/k_1$) and temperature was investigated using the Arrhenius equation

$$\ln\left(\frac{1}{k_1}\right) = \ln A_r - \frac{E_a}{R \cdot T}, \quad \text{and} \quad \ln k_w = \ln A_r - \frac{E_a}{R \cdot T} \tag{4}$$

Table 3 shows the activation energy values and the correlation coefficient between observed and predicted data for the model. The Arrhenius equation represents well the variation of the parameters with temperature. This is in line with the result of Gowen, Abu-Ghannam, Frias, and Oliveira (2007), Sopade and Obekpa (1992) and Wang, Kuan, Francis, Ware, and Carman (1990), which found that high soaking temperatures led to complete hydration in a much shorter time. Toda et al. (2001) also reported that the rate of water absorption was faster during the first 5 h and then it gradually decreased. This result suggested that soaking should be carried out only until the soybeans were easily ground with most of the cells rupturing (Lo, Steinkra, Hand, Wilkens, & Hackler, 1968). High temperature (e.g., 40 °C) may be desirable, to reduce soaking time (Gowen et al., 2007; Pan & Tangratanavalee, 2003).

The activation energy value (E_a) was 41.9 kJ/mol (38.6 kJ/mol using %db), very similar to the value (37.2 kJ/mol using %db) obtained by Gowen et al. (2007). Results in Table 3 also demonstrate that the final moisture content parameter (M_f) was temperature-dependent. Pan and Tangratanavalee (2003) suggested that this relationship was caused by the differences in solid losses at different temperatures. M_f is the asymptotic parameter and does not have rate of reaction units.

For this reason, an empirical model was used to explain the relationship between this parameter and temperature. Since only

Table 2
Parametric estimations corresponding to Peleg's kinetic model (2) applied to the moisture content of soaked soybeans at different temperatures

T (°C)	k_1 ± CI	k_2 ± CI	M_f ± CI	F (df$_1$ = 3, df$_2$ = 4; α = 0.05)	p-value	r
30	0.0296 ± 0.0120	0.0189 ± 0.0025	64.12 ± 6.86	1393.44	0.0000	0.997
50	0.0179 ± 0.0098	0.0205 ± 0.0029	62.12 ± 6.21	1155.66	0.0000	0.996
60	0.0052 ± 0.0018	0.0204 ± 0.0012	60.88 ± 1.96	5299.06	0.0000	0.999
85	0.0027 ± 0.0008	0.0199 ± 0.0011	61.62 ± 1.49	6546.68	0.0000	0.999

CI values are confidence intervals ($\alpha = 0.05$), F is the Fisher's test (df$_1$ = degrees of freedom of the model; df$_2$ = degrees of freedom of the error) and r is the correlation coefficient between observed and predicted data.

Table 3
Effect of temperature on the kinetic parameters of the Peleg's model (2)

Peleg's model Mathematical relationships	E_a (kJ/mol)	r
$\ln(1/k_1) = 20.03 - 5034.8/T$	41.85	0.978
$M_f = 0.002T^2 - 0.287T + 70.936$	–	0.990

The Arrhenius equation in linear form (4), and a second order polynomic function (M_f) have been used to fit this parameter. r is the correlation coefficient between observed and predicted data.

four points are available (four temperatures), a second order polynomic function was used (see graphs in Fig. 1, right, and Table 3).

3.2. Isoflavones transformations in soaked soybeans at different temperatures

The transformations of daidzin and genistin into their aglycones (daidzein and genistein) at various temperatures were studied. The kinetics of these transformation were expressed (see Figs. 2 and 3) in terms of the daidzin and genistin disappearance (D and G), and the daidzein and genistein formation (De and Ge). In both cases, this behaviour can be described by a parallel first-order reactions pathway with a kinetic constant of conversion, degradation and transfer to the aqueous phase. The proposed mechanism for the daidzin conversion during soaking is presented in Fig. 4.

As shown in Fig. 2, the production of daidzein was not equimolar to the disappearance of daidzin. The pathway suggests that the unbalance in daidzin concentration is caused by daidzin–daidzein degradation into unknown products (DD and DeD) and by their extraction to the aqueous phase (DEx and DeEx) running in parallel to the daidzein formation. The transformation mechanism should be modified in order to introduce a non-zero final asymptote (see Appendix).

The corresponding integrated equations are as follows:

$$D = (D_0 - D_f)e^{-(k_c + k_{dd} + k_{dex})t} + D_f \tag{5}$$

$$\mathrm{De} = (\mathrm{De}_0 - \mathrm{De}_f)e^{-(k_{ded} + k_{deex} - k_c)t} + \mathrm{De}_f \tag{6}$$

If we assume that the relationship of these kinetic constants to temperature are similar, then

$$k_d = k_c + k_{dd} + k_{dex}$$

and

$$k_{de} = k_{ded} + k_{deex} - k_c$$

where k_d is the rate of disappearance of daidzin and k_{de} the specific rate of daidzein formation. A similar approach has been used by Vaidya et al. (2007) to describe the transformations of malonylglucosides into β-glucosides in an ideal reaction system. According to this:

$$D = (D_0 - D_f)e^{-k_d t} + D_f \tag{7}$$

$$\mathrm{De} = (\mathrm{De}_0 - \mathrm{De}_f)e^{-k_{de} t} + \mathrm{De}_f \tag{8}$$

Similarly, for the transformation of genistin and genistein,

$$G = (G_0 - G_f)e^{-k_g t} + G_f \tag{9}$$

$$\mathrm{Ge} = (\mathrm{Ge}_0 - \mathrm{Ge}_f)e^{-k_{ge} t} + \mathrm{Ge}_f \tag{10}$$

Figs. 2 and 3 (top) and Tables 4 and 5 illustrate the results of the proposed approach. Graphs in Figs. 2 and 3 demonstrate that, during soaking, the concentration of daidzin–genistin decreases accordingly to the daidzein–genistein formation. These results were supported by Wang et al. (1990) who found that daidzein and genistein concentrations greatly increased when the soybeans were presoaked in water. Wang and Murphy (1996) also observed that daidzin and genistin concentrations dropped according to the genistein increment in soymilk and tofu processing. Zhu, Hettiarachchy, Horax, and Chen (2005) found similar results for two soybean varieties during soaking.

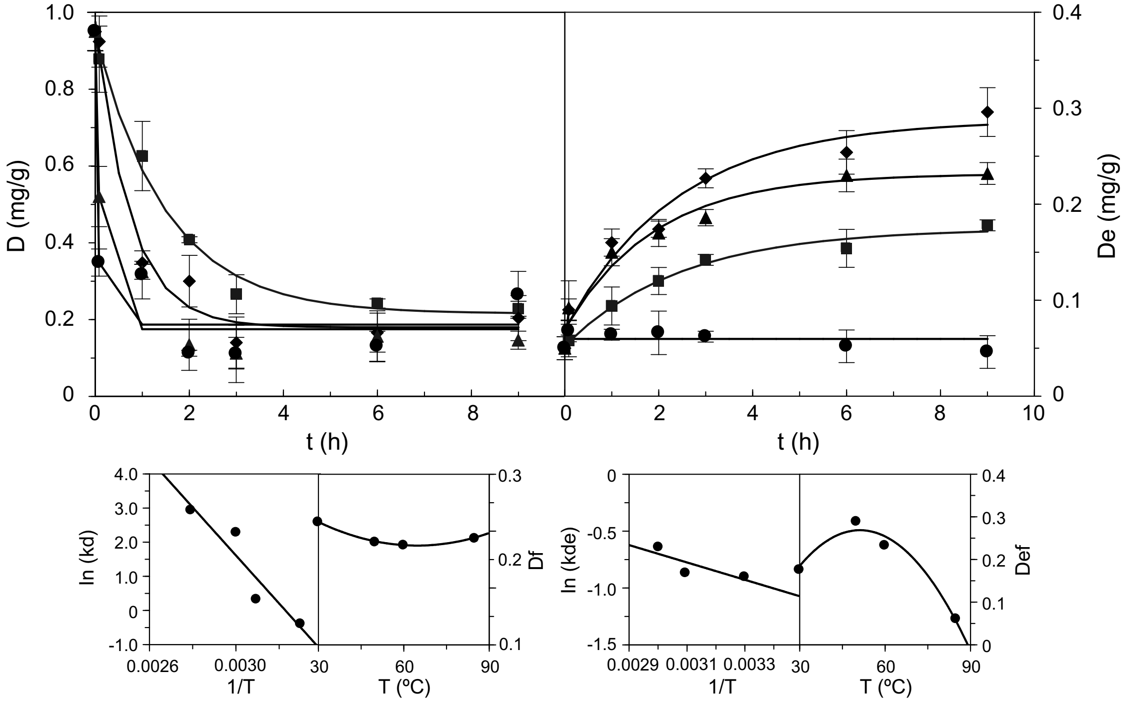

Fig. 2. Daidzin (D) and Daidzein (De) concentrations in soaked soybeans *vs.* time at different temperatures (■: 30 °C; ◆: 50 °C; ▲: 60 °C; ●: 85 °C). The points represent experimental data and the lines the predicted values according to the models shown in Eqs. (7) and (8). At the bottom, the relationship between the kinetic parameters and temperature is shown. The error bars are the confidence intervals ($\alpha = 0.05$; $n = 2$).

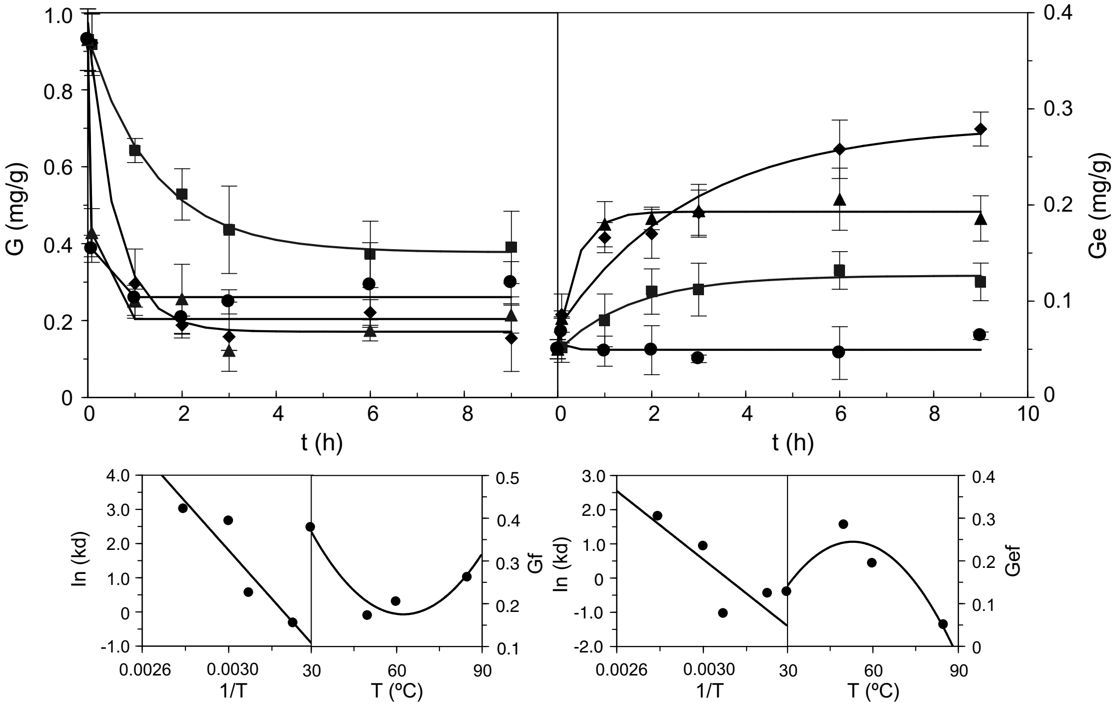

Fig. 3. Genistin (*G*) and Genistein (Ge) concentrations in soaked soybeans *vs.* time at different temperatures (■: 30 °C; ♦: 50 °C; ▲: 60 °C; ●: 85 °C). The points represent experimental data and the lines the values predicted by the models shown in Eqs. (9) and (10). The relationship between the kinetic parameters and temperature is shown at the bottom. The error bars are the confidence intervals ($\alpha = 0.05$; $n = 2$).

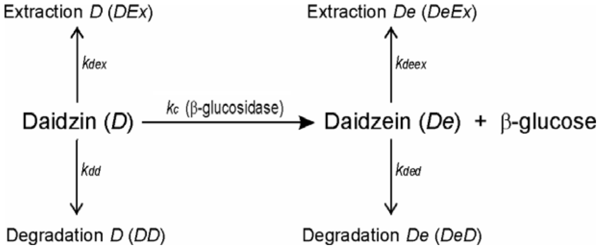

Fig. 4. Reaction mechanism proposed for the transformation between daidzin and daidzein in soaked soybeans. k_c is the kinetic constant (h^{-1}) of conversion of daidzin to daidzein; k_{dd} and k_{ded} are degradation kinetic constants of daidzin and daidzein, respectively (h^{-1}); k_{dex} and k_{deex} are kinetic constants of the transfer of daidzin and daidzein into the aqueous phase (h^{-1}). The error bars are the confidence intervals ($\alpha = 0.05$; $n = 2$).

Table 4
Parametric estimations corresponding to the kinetic model (7) and (8) applied to the disappearance of daidzin (*D*) and formation of daidzein (De) in soaked soybeans at different temperatures

T (°C)	$k_d \pm$ CI	$D_f \pm$ CI	*F* ($df_1 = 3$, $df_2 = 4$; $\alpha = 0.05$)	*p*-value	*r*
30	0.665 ± 0.233	0.215 ± 0.072	315.27	0.0000	0.996
50	1.367 ± 0.812	0.180 ± 0.087	241.39	0.0000	0.992
60	9.693 ± 9.217	0.174 ± 0.106	60.84	0.0009	0.974
85	18.842 ± 18.102	0.186 ± 0.120	42.72	0.0017	0.964
T (°C)	$k_{de} \pm$ CI	$De_f \pm$ CI	*F* ($df_1 = 3$, $df_2 = 4$; $\alpha = 0.05$)	*p*-value	*r*
30	0.404 ± 0.180	0.175 ± 0.019	856.40	0.0000	0.994
50	0.418 ± 0.291	0.288 ± 0.052	274.62	0.0000	0.986
60	0.525 ± 0.399	0.232 ± 0.038	263.03	0.0000	0.981
85	138.521 (NS)	0.060 ± 0.059	83.67	0.0005	0.418

CI values are confidence intervals ($\alpha = 0.05$), *F* is the Fisher's test (df_1 = degrees of freedom of the model; df_2 = degrees of freedom of the error), and *r* is the correlation coefficient between observed and predicted data. NS: non-significant.

Table 5
Parametric estimations corresponding to the kinetic model (9) and (10), applied to the disappearance of genistin (*G*) and formation of genistein (Ge) isoflavones of soaked soybeans at different temperatures

T (°C)	$k_g \pm$ CI	$G_f \pm$ CI	*F* (df_1=3, df_2=4; α=0.05)	*p*-value	*r*
30	0.713 ± 0.143	0.377 ± 0.031	4195.24	0.0000	0.999
50	1.727 ± 0.998	0.171 ± 0.071	302.04	0.0000	0.994
60	14.102 ± 9.145	0.204 ± 0.070	131.47	0.0002	0.986
85	20.107 ± 10.573	0.261 ± 0.046	322.76	0.0000	0.993
T (°C)	$k_{ge} \pm$ CI	$Ge_f \pm$ CI	*F* (df_1=3, df_2=4; α=0.05)	*p*-value	*r*
30	0.631 ± 0.379	0.127 ± 0.014	573.71	0.0000	0.988
50	0.349 ± 0.339	0.283 ± 0.076	168.99	0.0001	0.976
60	2.510 ± 1.915	0.193 ± 0.012	928.62	0.0000	0.994
85	6.000 (NS)	0.049 ± 0.017	49.94	0.0013	0.303

CI values are confidence intervals ($\alpha = 0.05$), *F* is the Fisher's test (df_1 = degrees of freedom of the model; df_2 = degrees of freedom of the error) and *r* is the correlation coefficient between observed and predicted data. NS: non significant.

In general, the proposed models were statistically consistent in all cases. However, two parameters at 85 °C (k_{de} and k_{ge}) were not statistically significant ($\alpha = 0.05$). The correlation between the kinetic coefficients and temperature was established using the Arrhenius equation (k_d, k_{de}, k_g and k_{ge}). The correlation between the final isoflavone concentrations (D_f, De_f, G_f and Ge_f) and temperature was modelled with a second order polynomic equation. These results are depicted in Figs. 2 and 3 (bottom) and in Tables 6 and 7.

These figures show that temperature enhances diadzin–genistin disappearance and diadzein–genistein formation. Very similar conclusions were obtained by Carrao-Panizzi, de Goes-Faroni, and Kikuchi (2004). The representations of the daidzin–genistin final concentrations (D_f and G_f) *vs.* temperature show a minimum value around 50–60 °C. In contrast, the relationship between the

Table 6
Effect of the temperature on the kinetic parameters obtained with the models (7) and (8) and summarized in Table 4

Mathematical relationships	E_a (kJ/mol)	r
Daidzin dissapearance		
$\ln k_d = 22.74 - 7034.2/T$	58.56	0.939
$D_f = 3 \times 10^{-5}T^2 - 0.0045T + 0.319$	–	0.999
Daidzein formation		
$\ln k_{de} = 35.07 - 11300.2/T$	93.95	0.826
$De_f = -2 \times 10^{-4}T^2 + 0.019T - 0.230$	–	0.983

The Arrhenius equation in linear form (with constants k_d and k_{de}) and a second order polynomic function (D_f and De_f) were used. r is the correlation coefficient between observed and predicted data.

Table 7
Effect of temperature on the kinetic parameters obtained with models (9) and (10) and summarized in Table 6

Mathematical relationships	E_a (kJ/mol)	r
Genistin dissapearance		
$\ln k_g = 22.93 - 7043.2/T$	58.56	0.924
$G_f = 2 \times 10^{-4}T^2 - 0.023T + 0.900$	-	0.960
Genistein formation		
$\ln k_{ge} = 15.54 - 5000.1/T$	41.57	0.814
$G_{ef} = -2 \times 10^{-4}T^2 + 0.021T - 0.317$	-	0.937

The Arrhenius equation in linear form (with constants k_g and k_{ge}) and a second order polynomic function (G_f and G_{ef}) were used. r is the correlation coefficient between observed and predicted data.

daidzein–genistein final concentrations (De_f and Ge_f) and temperatures shows a maximum value at 50 °C.

3.3. *β-glucosidase activity in soaked soybeans*

In Fig. 5, the evolution of the β-glucosidase activity during soaking is shown at different temperatures. The kinetic trends of this enzymatic activity give evidence that hydrolysis of daidzin and genistin during soaking contributed to an increase in the contents of their aglycones (Matsuura & Obata, 1993). At 50 °C and 60 °C the maximum activity is observed after 1 h. However, it takes up to 6 h to reach similar levels at 30 °C, and soaking at 85 °C does not develop this activity. Matsuura et al. (1989) and Matsuura and Obata (1993) reported inactivation of β-glucosidase at 60 °C with an optimum temperature of 45 °C. Losses of activity could be due to enzyme inactivation, elution into soaking water or a combination of both. Toda et al. (2001) also observed that soaking increased the β-glucosidase and isoflavones lost in the aqueous phase.

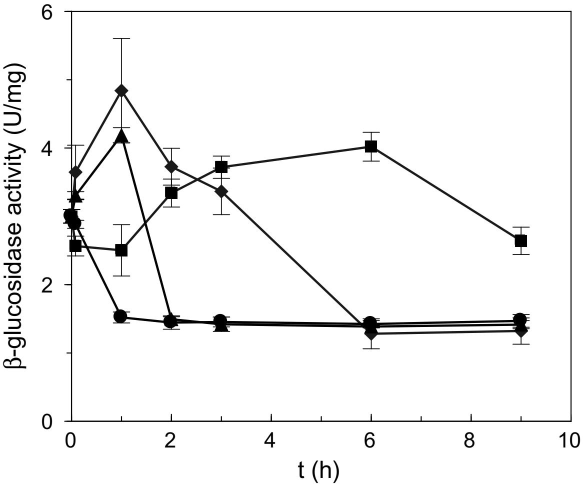

Fig. 5. β-Glucosidase activity in soaked soybeans at different temperatures (■: 30 °C; ♦: 50 °C; ▲: 60 °C; ●: 85 °C). The error bars are the confidence intervals ($\alpha = 0.05$; $n = 2$).

4. Conclusions

The proposed mathematical models provide a statistically consistent description of the evolution of the moisture content and the transformations of isoflavones in soybeans during soaking. These equations can also be used to predict the amount of water absorbed and the dynamics of isoflavone conversions at different temperatures. These mathematical tools could be used to establish a processing strategy that could help in maximising the functionality of soybean processed products.

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Appendix. . Reaction mechanism proposed

According to the pathway in Fig. 4, a mass balance would lead to the differential equations that represent the changes of daidzin with time

$$D \overset{k_{dex}}{\rightarrow} \mathrm{DEx} \ : \ \left.\frac{dD}{dt}\right|_1 = -k_{dex}(D - \mathrm{DEx})$$

$$D \overset{k_{dd}}{\rightarrow} \mathrm{DD} \ : \ \left.\frac{dD}{dt}\right|_2 = -k_{dd}(D - \mathrm{DD})$$

$$D \overset{k_c}{\rightarrow} \mathrm{De} \ : \ \left.\frac{dD}{dt}\right|_3 = -k_c(D - \mathrm{De})$$

Though it is not possible to know, with accuracy, the DEx, DD and De concentrations, it is assumed that they are approximately equal to D_f or final concentration of daidzin ($D_f = \mathrm{De} = \mathrm{DD} = \mathrm{DEx}$). This parameter defines the non-zero final asymptote obtained experimentally (Fig. 2). The total mass balance of the three parallel mechanisms gives

$$\frac{dD}{dt} = \left.\frac{dD}{dt}\right|_1 + \left.\frac{dD}{dt}\right|_2 + \left.\frac{dD}{dt}\right|_3 = -k_{dex} \cdot (D - D_f) - k_{dd} \cdot (D - D_f) - k_c \cdot (D - D_f)$$

$$\frac{dD}{dt} = -(k_c + k_{dd} + k_{dex}) \cdot (D - D_f) \tag{A1}$$

Identical postulates can be used for the daidzein (De) balance to obtain

$$\frac{d\mathrm{De}}{dt} = -(k_{ded} + k_{deex} - k_c)(\mathrm{De} - \mathrm{De}_f) \tag{A2}$$

Separating variables and integrating (A1) between time *0* and *t* gives

$$\int_{D_0}^{D} \frac{dD}{D - D_f} = -(k_c + k_{dd} + k_{dex}) \int_0^t dt \Rightarrow \ln(D - D_f)_{D_0}^{D} = -(k_c + k_{dd} + k_{dex}) \cdot t$$

$$\frac{D - D_f}{D_0 - D_f} = exp[-(k_c + k_{dd} + k_{dex}) \cdot t] \Rightarrow D = (D_0 - D_f) \cdot e^{-(k_c + k_{dd} + k_{dex}) \cdot t} + D_f \tag{A3}$$

Similarly, for the case of the diadzein and using (A2) we would obtain

$$\mathrm{De} = (\mathrm{De}_0 - \mathrm{De}_f) \cdot e^{-(k_{ded} + k_{deex} - k_c)t} + \mathrm{De}_f \tag{A4}$$

The limits of the function as time approaches zero and infinite are

$$\lim_{t\to 0} D = D_0;\ \lim_{t\to 0} \mathrm{De} = \mathrm{De}_0;\ \lim_{t\to\infty} D = D_\mathrm{f}\ \text{and}\ \lim_{t\to\infty} \mathrm{De} = \mathrm{De}_\mathrm{f}$$

A similar mechanism could be drawn for the genistin conversion (not shown).

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