Modeling the stability of green tea catechins EGCG and ECG during the biscuit making process

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ABSTRACT
Tea catechins undergo degradations and epimerization in aqueous systems during thermal processing. It is known that thermal degradation and epimerization of green tea catechins follows pseudo first-order reaction kinetics in aqueous systems. The stability of green tea catechins is also a function of the effects of pH, oxygen concentration, etc. The biscuit system is a complex matrix having both solid and aqueous phases, with sugar, fat, leavening agents and other ingredients added to it. Also the temperature, moisture and water activity profiles during biscuit baking are dynamic rather than static. Not only does this make the modeling of such a system important and necessary, but also a great challenge. This study was to develop mathematical models to predict the stability of green tea catechins during biscuit baking. Degradation and epimerization reactions of EGCG and ECG were taken into consideration for this purpose. The temperature and moisture profiles that were measured at different regions in the biscuit during the baking process were utilized in developing the models. Mathematical models for the simultaneous degradation and epimerization of these catechins were successfully developed, based on pseudo first-order kinetics for each of the reactions. The models also accounted for the dynamic changes occurring in the temperature and moisture profiles of the biscuit during baking. The activation energies obtained previously in aqueous and bread systems remain unchanged in the biscuit system. It was found that degradation and epimerization of catechins followed pseudo first-order kinetics in the biscuit matrix and the rate constant $k_r$ complied with Arrhenius equation.

Keywords: Biscuit; modeling; tea catechins; epimerization; reaction kinetics

INTRODUCTION
Tea antioxidants have drawn increased attention in recent years because of their potential health benefits, not only as an antioxidant agent but also as anti-arteriosclerotic, anti-carcinogenic, and antimicrobial agents. They may contribute to reducing risks of chronic diseases and cancer, promoting oral health, and prolonging shelf life of food products without damage to their organoleptic or nutritional qualities. Four major tea catechins have been identified as (-)-epigallocatechin (EGC), (-)-epigallocatechin gallate (EGCG), (-)-epicatechin (EC) and (-)-epicatechin gallate (ECG). Their corresponding epimers are (-)-gallocatechin (GC), (-)-gallocatechin gallate (GCG), (+)-catechin (C) and (-)-catechin gallate (CG), respectively.

Tea catechins undergo degradations and epimerization in aqueous systems during thermal processing. It is known that thermal degradation and epimerization of green tea catechins follow pseudo first-order reaction kinetics in aqueous and bread systems [1, 2]. The stability of green tea catechins is also a function of the effects of pH, oxygen concentration, etc. The biscuit system is a complex matrix having both solid and aqueous phases, with sugar, fat, leavening agents and other ingredients added to it. Also the temperature, moisture and water activity profiles during biscuit baking are dynamic rather than static. Not only does this make the modeling of such a system important and necessary, but also a great challenge.

This study was to develop mathematical models to predict the stability of green tea catechins (EGCG and ECG, and their epimers) during biscuit baking. Degradation and epimerization reactions of EGCG and ECG were taken into consideration for this purpose. The temperature and moisture profiles that were measured at different regions in the biscuit during the baking process were utilized in developing the models. The developed mathematical models can provide a guideline for manufacturers to select the correct amount of GTE powder in the formulation for a desired concentration in the final product.
MATERIALS & METHODS

The materials used for biscuit sample preparation and HPLC analysis of tea catechins were as described previously [3]. Biscuits were prepared with addition of 500 mg green tea extract (GTE) powder per 100 g of flour. The baking was done at three different temperatures - 140°C, 160°C, and 180°C. Catechin concentrations were measured at 0, 2, 4, 6, 8, and 10 min of baking. Temperature profiles were measured by type T thermocouples which were placed in the oven, and at three different places in the biscuit dough – top surface, bottom surface, and at the centre of the dough. Samples for moisture content analysis were taken from two different regions of the biscuit – outer top/bottom surfaces (more like crust), and the inner region (more like crumb). Briefly, 2 g of sample were accurately weighed to a pre-dried and cooled dish, and then heated in an oven for 24 hrs at 130°C. At the end of drying, the sample was immediately transferred to a desiccator and weighed after reaching room temperature. All experiments were performed in triplicates. Concentrations of catechins in biscuit samples were expressed as mg/Kg of biscuit dough and presented as mean ± standard deviation.

For this study the sampling times for measuring temperature and moisture were the same, but they were different from those for measuring catechin concentrations. Therefore, the average temperature $T_{av}$ used for rate constant calculation also differed. The equations used for modeling were as follows.

Let $t_i$ denote the $i$th sampling time for the measurement of temperature and moisture content, $t_j$ denote the $j$th sampling time for the measurement of catechins. If $t_{i+1} < t_j$, the time interval is taken as $\Delta t = t_{i+1} - t_i$ and $T_{av}$ can be calculated by:

$$T_{av} = (T_{i+1} + T_i)/2 \quad (1)$$

Let $k_y$, $k_1$ and $k_2$ denote the rate constants of degradation, epimerization from EGCG to GCG and epimerization from GCG to EGCG, respectively. As shown in Wang et al. (2006), the rate constant of degradation ($k_y$) is similar between the pair catechins, i.e. $k_{y,EGCG} \approx k_{y,GCG}$. Changes of catechin concentrations can therefore be described by:

$$y' = -(k_2 + k_y)y + k_2z \quad (2)$$

$$z' = -(k_2 + k_y)z + k_1y \quad (3)$$

$$[y + z]' = -k_2(y' + z') \quad (4)$$

where $y$ is the concentration of EGCG or ECG and $z$ is the concentration of their epimers GCG or CG, respectively. $y'$ and $z'$ are the rate of change of $y$ and $z$, respectively, and $[y + z]'$ is the rate of change of total catechins, i.e. $[y + z]'$. Assuming that the amount of total solids (S) remained constant in the dough during baking while the moisture content ($M_{H2O}$) and temperature profile varied with time, mathematical models for the concentrations of EGCG, ECG, GCG, CG and total catechins [EGCG + GCG] and [ECG + CG] at the sampling time $t_{i+1}$ can be revised to:

$$y_{i+1} = (y_i + y'dt) \times (S + M_{H2O,i-1})/(S + M_{H2O,i}) \quad (5)$$

$$z_{i+1} = (z_i + z'dt) \times (S + M_{H2O,i-1})/(S + M_{H2O,i}) \quad (6)$$

$$y + z_{i+1} = ([y + z]_i + [y + z]dt) \times (S + M_{H2O,i-1})/(S + M_{H2O,i}) \quad (7)$$

where $y'$, $z'$ and $[y + z]'$ are described by Eqs. (2) – (4), respectively. If $t_i < t_j < t_{i+1}$, the time interval is taken as $\Delta t = t_j - t_i$. Temperature $T_j$ is estimated by:

$$T_j = T_i + ([T_{i+1} - T_i]/(t_{i+1} - t_i))\Delta t \quad (8)$$

The moisture content at $t_j$ is estimated by:

$$M_{H2O,j} = M_{H2O,i} + ([M_{H2O,i-1} - M_{H2O,i}]/(t_{i+1} - t_i))\Delta t \quad (9)$$

Thus the average temperature for calculating the rate constants becomes:
The concentrations of individual and total tea catechins at the sampling time $t_j$ can then be modelled by:

$$y_j = \frac{(y_0 + y' \Delta t) \times (S + M_{H2O,1})}{(S + M_{H2O,1} + 1)}$$

$$z_j = \frac{(z_0 + z' \Delta t) \times (S + M_{H2O,1})}{(S + M_{H2O,1} + 1)}$$

$$[y + z]_j = \frac{([y]_t + [z]_t \Delta t) \times (S + M_{H2O,1})}{(S + M_{H2O,1} + 1)}$$

The above models account for not only the simultaneous degradations and epimerization of tea catechins, but also the effects of accompanied changes in the moisture and temperature profiles during baking. Matlab software was used to calculate the reaction kinetic parameters through a non-linear optimization procedure. The Marquardt–Levenberg method was used to minimize the mean squared error (MSE) between the experimental and modeled values, i.e.

$$MSE = \frac{1}{n} \sum_{m=1}^{n} \frac{(C_{m}^{Exp} - C_{m}^{Mod})^2}{n}$$

where $C$ is catechin concentration, $m$ is observation number and $n$ is total number of observations. The superscript “Mod” indicates modeled value and “Exp” indicates experimental value. Root mean squared error (RMSE) between the experimental values and modeled values was taken as a measure of the model quality.

RESULTS & DISCUSSION

![Figure 1. Temperature profiles during baking. (A) oven; (B) biscuit centre; (C) bottom surface; (D) top surface]
The temperature profiles monitored in the baking oven and at different positions in the biscuit are as shown in Figure 1. Due to the nature of the baking oven control system, the oven temperature constantly fluctuated within a range of approximately 20°C. It can be seen that the temperature at the biscuit centre is relatively lower than the top and bottom surfaces even at the end of the 10 min biscuit baking. The average moisture profiles of the biscuit samples at the designated baking temperatures are as shown in Figure 2. As can be seen the moisture content decreased with increasing baking time/temperature. The high $R^2$ values (>0.98) suggested that there was probably an exponential relationship between baking time and the moisture content of dough and biscuit samples.

![Figure 2. Average moisture profiles for biscuit at three baking temperatures](image)

The temperature profiles monitored in the baking oven and at different positions in the biscuit are as shown in Figure 1. Due to the nature of the baking oven control system, the oven temperature constantly fluctuated within a range of approximately 20°C. It can be seen that the temperature at the biscuit centre is relatively lower than the top and bottom surfaces even at the end of the 10 min biscuit baking. The average moisture profiles of the biscuit samples at the designated baking temperatures are as shown in Figure 2. As can be seen the moisture content decreased with increasing baking time/temperature. The high $R^2$ values (>0.98) suggested that there was probably an exponential relationship between baking time and the moisture content of dough and biscuit samples.

![Figure 3. Retention of total catechins (mg/Kg dough): [EGCG+GCG] at (A) 140°C; (B) 160°C; (C) 180°C and [ECG+CG] at (D) 140°C; (E) 160°C; (F) 180°C](image)
It has been shown previously [1, 2] that degradations and epimerization of EGCG and GCG follow pseudo first-order kinetics in aqueous systems and bread matrix respectively, and the rate constant (k) complied with Arrhenius equation. Assuming that the activation energy $E_a$ for degradation of [EGCG+GCG] and [ECG+CG] in the biscuit system is the same as that in the bread and aqueous systems, the concentration of total catechins can be estimated according to the developed models (i.e. Eqs. (7) and (13)). Meanwhile the frequency factor was obtained by applying a non-linear optimisation procedure with constraints to the experimental data under the three designated baking temperatures using Matlab software, based on minimizing the MSE between modeled and experimental values (i.e. Eq. (14)). The retentions of total catechins [EGCG+GCG] and [ECG+CG] in the biscuit are shown in Figure 3. The frequency factors for the degradation of [EGCG+GCG] and [ECG+CG] were $1.00 \times 10^3$ and $2.66 \times 10^4$ (Table 1). Catechin stability decreased with increasing baking temperature. This proves that catechin stability is highly dependent on temperature. The large difference between the modeled and experimental values obtained for [EGCG+GCG] at 2 minutes can be explained by the rapidly changing pH of the biscuit dough during the early stages of baking, which has not been accounted for in this model. Presumably the leavening agents would be getting used up during the first few minutes of baking resulting in drastic pH changes in the biscuit dough during that time. Overall, it can be said that the degradation of catechins followed pseudo first-order kinetics in the biscuit matrix and the rate constant $k$ complied with Arrhenius equation and was similar between pair catechins.

Figures 4 and 5 show the stability of individual catechins ECG and CG, and EGCG and GCG respectively in the biscuit. It can be seen that ECG and EGCG decreased while their epimers CG and GCG increased respectively with baking time. The frequency factors obtained for epimerisation from ECG to CG and the epimerisation from CG to ECG were $1.59 \times 10^{10}$ and $1.59 \times 10^9$, respectively. The good correlation between the modeled and experimental values validates the assumptions that the activation energy of epimerization remained unchanged in the biscuit system and the epimerization of tea catechins follow pseudo first-order kinetics. The low values of the activation energy for the epimerization of ECG to GCG and the epimerization of GCG to EGCG can be explained due to the high rate of degradation during the early stages of baking, presumably due to the combined effects of pH and temperature. The dynamic change of pH during baking will need to be studied to fully understand the kinetics of epimerization of EGCG and GCG.
Table 1. Summary of activation energies and frequency factors for the degradations and epimerization of tea catechins

<table>
<thead>
<tr>
<th>Catechins</th>
<th>Activation energy, $E_a$ (KJ/mol)</th>
<th>Frequency factor, $A$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: degradations of total catechins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[EGCG+GCG]</td>
<td>19.78</td>
<td>$1.00 \times 10^4$</td>
</tr>
<tr>
<td>[ECG+CG]</td>
<td>41.58</td>
<td>$2.66 \times 10^4$</td>
</tr>
<tr>
<td>B: epimerization of catechins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EGCG to GCG</td>
<td>107.59</td>
<td>$1.83 \times 10^{11}$</td>
</tr>
<tr>
<td>GCG to EGCG</td>
<td>74.40</td>
<td>$5.57 \times 10^9$</td>
</tr>
<tr>
<td>ECG to CG</td>
<td>119.25</td>
<td>$1.59 \times 10^7$</td>
</tr>
<tr>
<td>CG to ECG</td>
<td>96.22</td>
<td>$1.59 \times 10^7$</td>
</tr>
</tbody>
</table>

CONCLUSION

Mathematical models for the stability of tea catechins during the biscuit making process were successfully developed. The models accounted not only for the degradation and epimerization of tea catechins but also the dynamic changes in temperature and moisture profiles during the baking. It was found that the degradation and epimerization of tea catechins followed pseudo first-order kinetics. The rate constant complied with Arrhenius equation, and the activation energy remained unchanged from that in the aqueous and bread system. Higher frequency factor was found for the degradation and epimerization of EGCG and GCG as compared to ECG and CG. The developed mathematical models can provide a guideline for manufacturers to select the correct amount of GTE powder in the formulation for a desired concentration in the final product.

REFERENCES