CHAPTER 5

EFFECTS OF PROCESSING ON SULFORAPHANE IN DIETARY FIBER POWDER FROM CABBAGE OUTER LEAVES

5.1 Introduction

Sulforaphane is an anticarcinogenic substance that can be derived from glucoraphanin, one of the glucosinolates, by myrosinase (Shen et al., 2010). It has been reported that sulforaphane showed the highest anticarcinogenic activity among other cabbage outer leaves, it was interesting to study the changes of suforaphane during processing.

Previous research works showed that sulforaphane is sensitive and its thermal susceptibility is much dependent on an experimental system (Shen et al., 2010). Heating at certain temperatures and time has been reported to increase the rate of sulforaphane formation. Matusheski et al. (2004), for example, studied the effect of heating on sulforaphane formation in broccoli florets. Their results showed that heating at 60 °C for either 5 or 10 min led to a significantly increased sulforaphane content. Rungapamestry et al. (2006) also reported that heating at temperatures in the range of 50-60 °C helped accelerate the rate of sulforaphane formation in cabbage. However, in other studies (Jin et al., 1999; Van et al., 2007), heating at 50-60 °C was noted to cause sulforaphane degradation. This information implies that processing steps are significant factors controlling the sulforaphane content in DF powder.

As described in Chapters 3 and 4, the attemps had been made to determine the suitable processing steps for production of high of DF powder from cabbage outer leaves with the highest retention of antioxidants and glucosinolates. However, the conditions suggested might not be suitable for the retention of sulforaphane. Therefore, this study was aimed at studying the effect of hot air drying (40-70 °C) on the evolution of sulforaphane during the production of high DF powder. The information obtained can be used for optimization of industrial process to enhance health benefit of DF powder from cabbage outer leaves.

5.2 Materials and Methods

5.2.1 Sample Preparation

Outer leaves of cabbages (*Brassica oleracea* L. var. capitata), which were discarded, were obtained from a local market; the leaves were kept at 4 °C until the time of experiment. Prior to each experiment, the leaves were washed under running tap water and drained on a screen to get rid of excess water.

From preliminary study, the outer leaves of cabbage were prepared following the method described in Section 3.2.1, Chapter 3. The results showed that blanching was not a suitable pretreatment as sulforaphane is heat sensitive and heat during blanching could destroy myrosinase, endogenous enzyme responsible for the formation of sulforaphane in cabbage.

To determine a suitable preparation, the outer leaves of cabbage were chopped using an electric chopper (Moulinex, DPA141, Ecully, France) at 17,000 rpm for 0.5, 1,1.5 and 2 min to obtain the particle size in the range of 9.0-15.0, 6.0-9.0, 2.0-6.0, 1.7-2.5 mm.

5.2.2 Drying Experiments

Chopped sample (200 g) was spread on a tray as a thin layer; the tray dimensions are 25×40 cm. The load per area was 2 kg/m^2. The prepared sample was dried in a hot air oven (Termaks, TS8000, Bergen, Norway) at 40, 50, 60 and 70 °C until a final moisture content of less than 0.1 g/g dry weight was reached (Larrauri, 1999). During each experiment 3-5 g of the sample was taken out at various intervals to determine its moisture content. The moisture content of the sample was determined using a gravimetric method at 105 °C (AOAC Method 984.25; AOAC, 2000). Drying was conducted at a constant air velocity of 2 m/s and ambient air relative humidity in the range of 55-70%. The temperature of the sample was measured continuously using type-K thermocouples, which were inserted into the bed of cabbage.

5.2.3 Determination of Sulforaphane Content

Measurement of sulforaphane content was conducted using a method described by Liang et al. (2006). Five grams of a sample was extracted two times with 50 mL dichloromethane, which was combined with 2.5 g sodium sulfate anhydrous. The dichloromethane fraction was dehydrated using a rotary evaporator (Resona Technics, Laborota S-300, Gossau, Switzerland) at 30 °C. The residue was dissolved in 2 mL acetonitrile and was filtered through a 0.45 μm syringe filter. Ten μL of the filtrate was then injected into a Symmetry® C_{18} 5 μL (3.9×150 mm) HPLC column (Waters, Milford, MA) with gradients as follows: 20% acetonitrile and 80% water, then changed linearly over 10 min to 60% acetonitrile and 40% water and maintained at 100% acetonitrile for 2 min. The flow rate was set at 1 mL/min. A UV detector at a wavelength of 254 mm was used for detecting sulforaphane.

5.2.3 Statistical Analysis

The information of statistical analysis was described in Section 3.3, Chapter 3.

5.3 Results and Discussion

5.3.1 Effect of Preparation on Sulforaphane

Table 5.1 shows sulforaphane content in cabbage at different particle sizes. The results showed that decrease in particle size resulted in higher sulforaphane content. This might be because disruption of cell by grinding caused higher release of glucosinolates and myrosinase leading to larger sulforaphane formation. By determining the results grinding cabbage to obtain sample size in range of 1.7-2.5 mm was suggested for sample preparation.

Table 5.1 Sulforaphane content in cabbage samples having different particle size ranges

Particle size (mm)	Sulforaphane content (mg/ 100 g dry weight)
9.0-15.0	0.53 ± 0.11^{a}
6.0-9.0	0.62 ± 0.08^{a}
2.0-6.0	0.93 ± 0.12^{b}
1.7-2.5	1.18 ± 0.09^{c}

Same letters in the same column indicate that values are not significantly different ($p>0.05$).

N/A = not applicable.

5.3.2 Drying Characteristics of Chopped Cabbages

The moisture content of a sample prior to drying was approximately 13.61 ± 0.53 g/g dry weight. Figure 5.1 shows the drying curves of the samples undergoing hot air drying at various temperatures. It was observed that the drying rates at higher temperatures were higher than those at lower temperatures, as expected. Drying at higher temperatures led to higher moisture diffusivity values and larger driving force for heat/mass transfer than at lower temperatures (Kuljarachanan, et al., 2009).

The equilibrium moisture content and time needed to reach the desired moisture content (less than 0.1 g/g dry weight) are shown in Table 5.2. The results showed that drying at 40 °C could not reduce the moisture content of cabbage to the desired value.

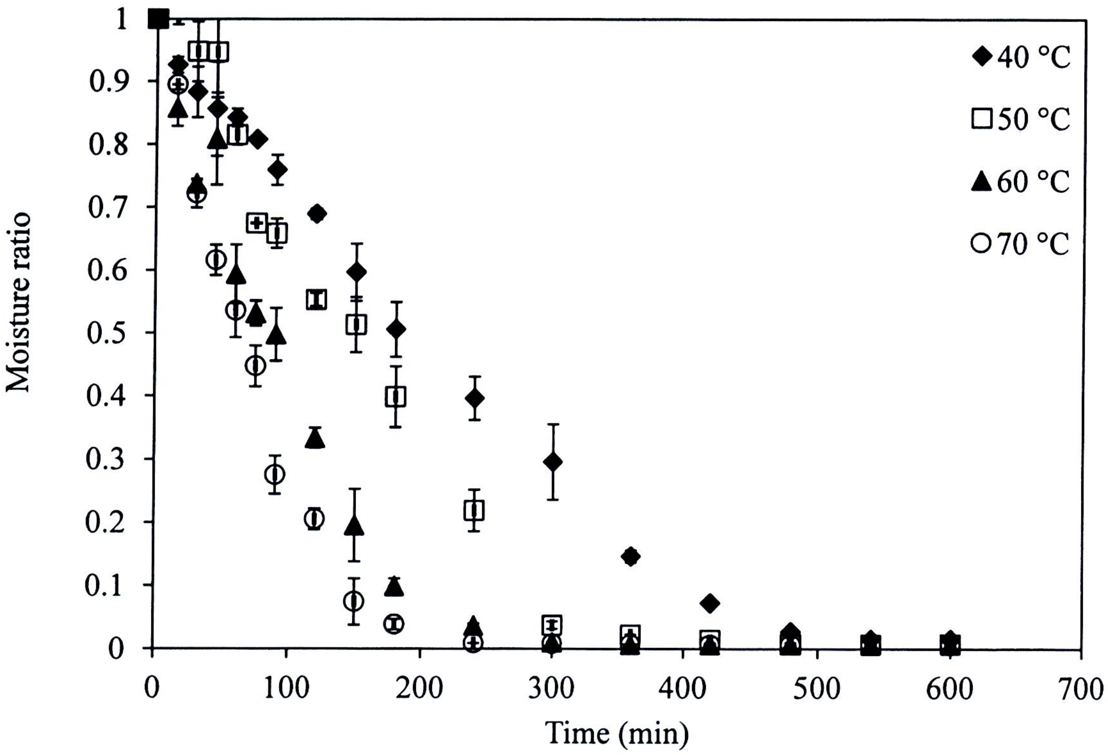

Figure 5.1 Drying curves of cabbage during hot air drying

Table 5.2 Time to dry samples to the final moisture content in the range of 0.089-0.096 g/g dry weight and equilibrium moisture content of samples at different drying temperatures

Temperature (°C)	Drying time (min)	Equilibrium moisture content (g/g dry weight)
40 °C	N/A	0.16 ± 0.02^{b}
50 °C	480	0.09 ± 0.01^{a}
60 °C	420	0.08 ± 0.01^{a}
70 °C	360	0.08 ± 0.01^{a}

Same letters in the same column indicate that values are not significantly different ($p>0.05$).
N/A = not applicable.

5.3.3 Effect of Drying on Sulforaphane Content

Figure 5.2 shows the evolution of sulforaphane in cabbage as well as that of the cabbage temperature during hot air drying at different temperatures. The initial temperature of cabbage was approximately 25.2 ± 0.6 °C. Table 5.3 shows the initial sulforaphane content as well as the highest contents of sulforaphane that could be formed during drying at different temperatures.

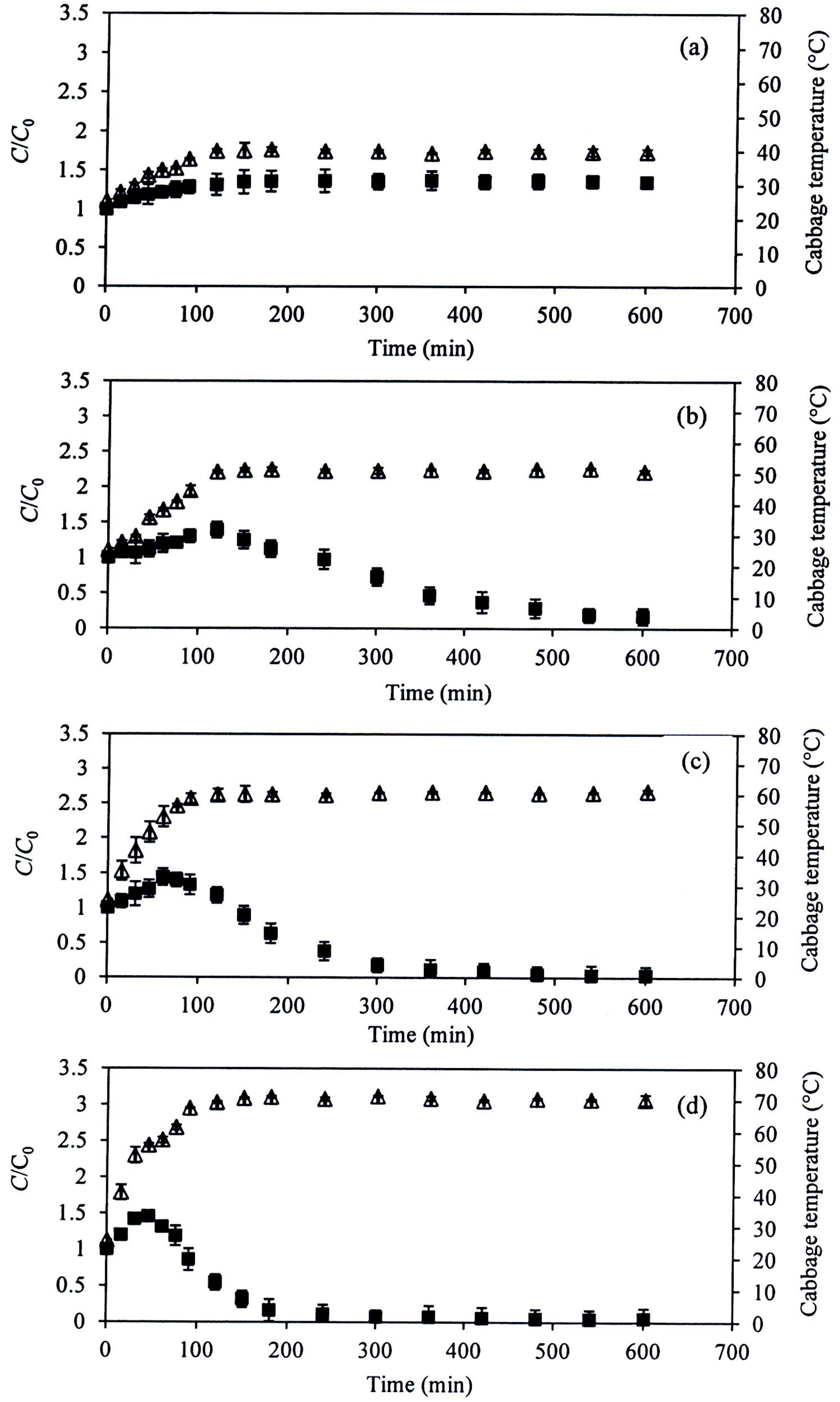

Figure 5.2 Evolution of sulforaphane (■) and cabbage temperature (Δ) during hot air drying at (a) 40 °C, (b) 50 °C, (c) 60 °C and (d) 70 °C. C_0 is sulforaphane content at $t = 0$

Table 5.3 Highest contents of sulforaphane in cabbage during drying at different temperatures; the specified moisture content corresponds to the point of highest sulforaphane content at each temperature

Temperature (°C)	Drying time (min)	Moisture content (g/g dry weight)	Sulforaphane content (mg/100 g dry weight)
Fresh			1.75 ± 0.47^{a}
40	180	7.75 ± 0.25^{b}	2.08 ± 0.23^{b}
50	120	6.50 ± 0.53^{a}	2.39 ± 0.31^{c}
60	60	8.39 ± 0.74^{b}	2.43 ± 0.19^{c}
70	30	9.78 ± 0.66^{b}	2.41 ± 0.11^{c}

Same letters in the same column indicate that values are not significantly different ($p>0.05$).

It has been reported that myrosinase activity is responsible for sulforaphane formation (Rungapamestry et al., 2006) and the mechanism is shown in Figure 5.3. Firstly, glucoraphanin is hydrolyzed by myrosinase. An intermediate is formed before rearranging into sulforaphane. Simultaneously, epithiospecifier protein (ESP), a myrosinase cofactor, can bind the sulfur of the intermediate, resulting in the formation of sulforaphane nitrile, which possesses no anticarcinogenic properties (Matusheski et al., 2004). ESP is nevertheless very heat sensitive in relation to myrosinase; myrosinase is stable up to 50 °C (Yen and Wei, 1993), while ESP is active at 20-40 °C and is damaged at temperatures above 40 °C (Matusheski et al., 2004).

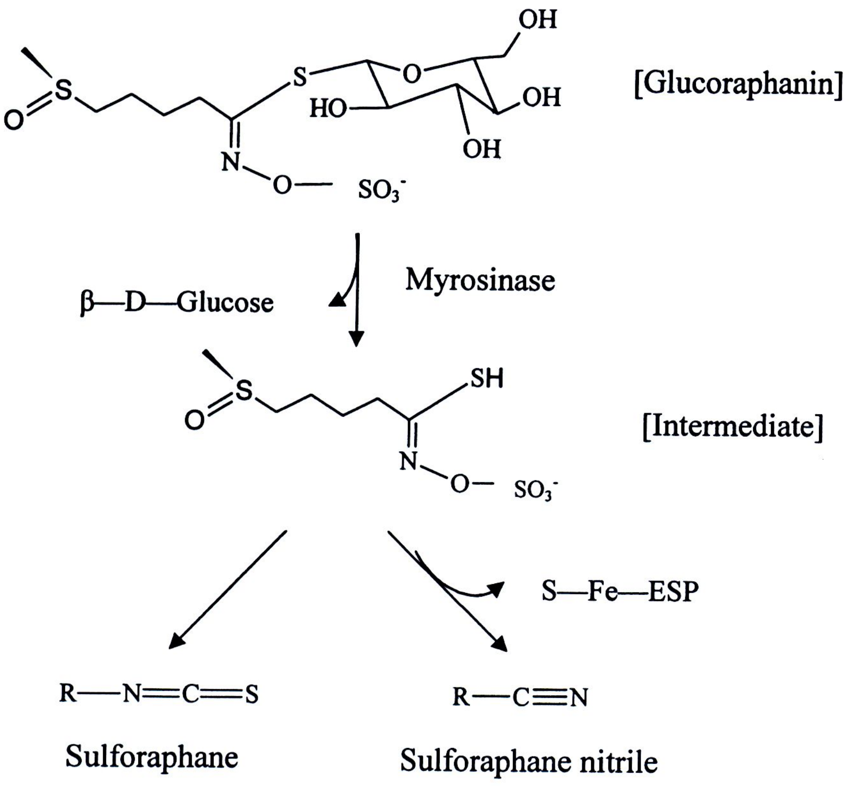

Figure 5.3 The glucoraphanin breakdown pathway (Adapted from Matusheki et al., 2004)

The results showed that the amount of sulforaphane during drying at 40 °C gradually increased and reached a plateau after 180 min (Figure 5.3). This is possibly due to the fact that myrosinase activity was promoted when the cabbage temperature increased from ambient temperature to 40 °C, resulting in an increase in the sulforaphane and suforaphane nitrile (which was not measured in this work) contents. After 180 min of drying, the sulforaphane content remained constant, most probably because there was no longer glucoraphanin available for sulforaphane as well as sulforaphane nitrile formation.

For drying at 50-70 °C, it was observed that sulforaphane first increased and reached the highest values, then gradually decreased. Both formation and degradation rates of sulforaphane were accelerated by the higher drying temperatures. Similar to the phenomena occurring at 40 °C, the formation of sulforaphane and sulforaphane nitrile took place and continued until the cabbage temperature approached 40 °C. At temperatures beyond 40 °C, as mentioned earlier, ESP was damaged and no sulforaphane nitrile was further formed. The remaining substrate, glucoraphanin in this case, would then totally be converted to sulforaphane. This phenomenon gave rise to the higher maximum sulforaphane contents in the cases of cabbage dried at 50-70 °C than that of the cabbage dried at 40 °C.

Figure 5.4 shows the relationship between the sulforaphane content and cabbage temperature. It was observed that sulforaphane diminished rapidly once the cabbage temperature exceeded 50.5-53.5 °C. At higher temperatures, sulforaphane decomposed into thiourea, which is a volatile compound (Jin et al., 1999). The temperature range causing the degradation of sulforaphane observed in this work was close to those reported previously. Jin et al. (1999) reported that sulforaphane in an aqueous solution degraded at 50 °C. Another finding reported that sulforaphane in broccoli juice was destroyed by heating at 60 °C (Van Eylen et al., 2007). The differences between the present and previous results may be from the different experimental systems and internal structures of the samples.

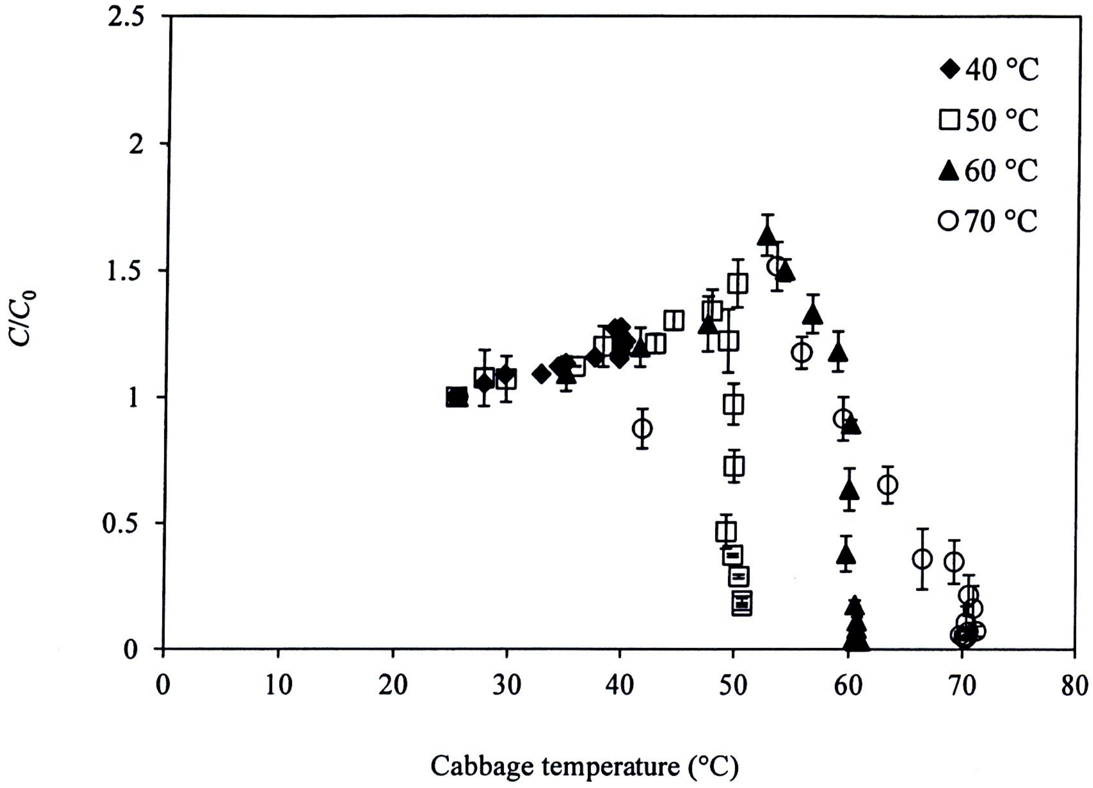

Figure 5.4 Changes of sulforaphane with cabbage temperature during drying. C_0 is sulforaphane content at $t = 0$

The sulforaphane content is also plotted against the cabbage moisture content (Figure 5.5). No correlation between the changes of moisture content and sulforaphane content was observed. The results imply that the major factor controlling the evolution of sulforaphane during drying is the cabbage temperature. The sulforaphane content and the retention of sulforaphane in the final DF powder are shown in Table 5.4. The results showed that approximately 90% loss in sulforaphane content occurred during processing.

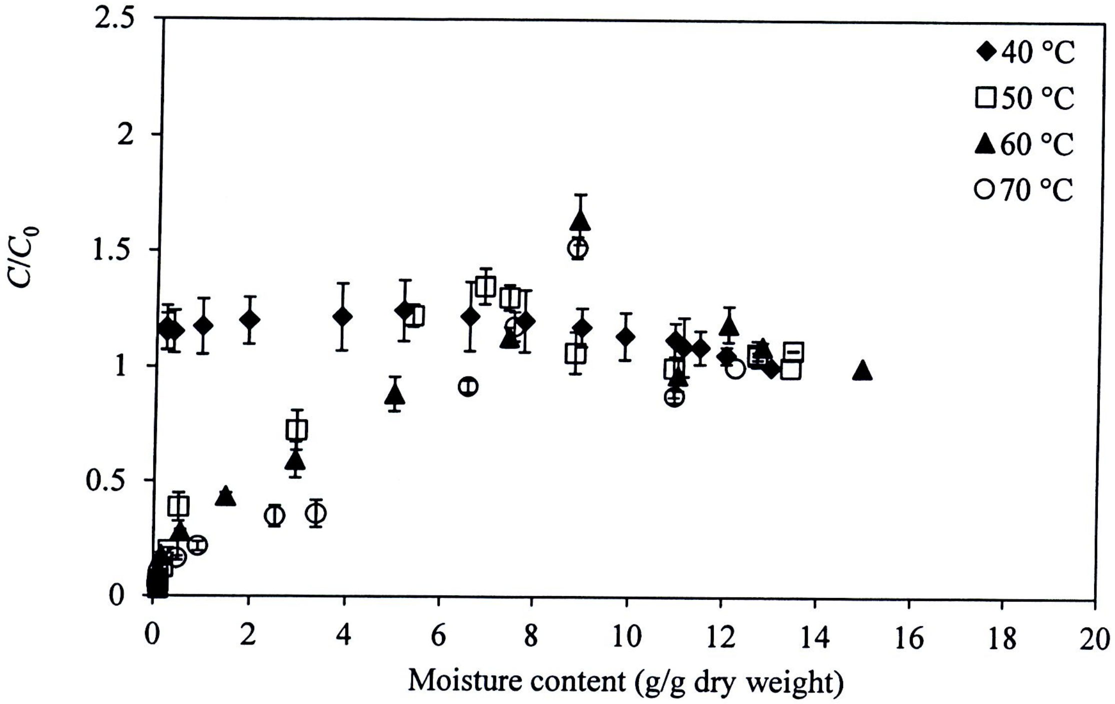

Figure 5.5 Changes of sulforaphane with cabbage moisture content during drying. C_0 is sulforaphane content at $t = 0$

Table 5.4 Sulforaphane content and retention in dietary fiber powder as compared with fresh leaves

Temperature (°C)	Sulforaphane content (mg/100 g dry weight)	Retention* (%)
40	N/A	N/A
50	0.12 ± 0.03^a	6.86 ± 0.98^a
60	0.16 ± 0.03^b	9.14 ± 0.56^b
70	0.11 ± 0.02^a	6.29 ± 0.68^a

Same letters in the same column indicate that values are not significantly different ($p>0.05$). N/A = not applicable. *Retention of sulforaphane = $(C/C_0) \times 100$, where C is the sulforaphane content at any drying time t and C_0 is the initial sulforaphane content.

5.3.4 Modeling of Sulforaphane Evolution

As mentioned earlier, the sulforaphane evolution is noted to be a function of the cabbage temperature during drying. A coupled heat transfer and kinetic model was then proposed to predict the changes of sulforaphane during drying. First, the heat transfer behavior of cabbage during drying was assumed to be described by the following one-dimensional heat conduction equation (Equation 5.1). A simple analytical solution of this equation (assuming that a cabbage particle is a sphere) was then derived; the effect of moisture loss due to evaporation is not explicitly included in the equation but is implicitly and empirically taken into account as shown later in Equation 5.7.

$$\frac{\partial T}{\partial t}=\frac{\alpha}{r^2}\frac{\partial}{\partial r}\left(\frac{1}{r^2}\frac{\partial T}{\partial r}\right) \tag{5.1}$$

Equation 5.1 was solved subject to the following initial condition:

$$T=T_0 \qquad \text{at } t=0 \tag{5.2}$$

and the following boundary conditions:

$$\frac{\partial T}{\partial r}=0 \qquad \text{at } r=0;\ t>0 \tag{5.3}$$

$$-k\frac{\partial T}{\partial r}=h(T_D-T) \qquad \text{at } r=R;\ t>0 \tag{5.4}$$

As mentioned earlier, a cabbage particle was assumed to be a sphere and the following solution can be derived for Equation 1 (Incropera and De Witt, 1990):

$$\frac{T_\mathrm{D}-T}{T_\mathrm{D}-T_0}=\sum_{n=1}^{\infty}\left(\frac{4(\sin\zeta_n-\zeta_n\cos\zeta_n)}{2\zeta_n-\sin(2\zeta_n)}\right)\left(\frac{\sin(\zeta_n r^*)}{\zeta_n r^*}\right)\exp(-\zeta_n^2\mathrm{Fo}) \tag{5.5}$$

where T, T_0 and T_D are the instantaneous and initial cabbage temperatures and drying temperature (°C), respectively; Fo is the Fourier number ($\alpha t/R^2$); α is the cabbage thermal diffusivity (m^2/s); t is the drying time (s); R is the radius of the cabbage particle (1×10^{-3} m); $r*$ is a dimensionless spatial coordinate (r/R); ξ is the positive n^{th} root of the transcendental equation (($1-\xi_n \cot\xi_n$ = Bi where Bi is the Biot number hR/k); h is the heat transfer coefficient (W/m^2K); k is thermal conductivity (W/m K).

In the case where t is large and R is small, terms in series where $n>1$ are negligible and Equation 5.5 could be reduced to:

$$\frac{T_\mathrm{D}-T}{T_\mathrm{D}-T_0}=\left(\frac{4(\sin\zeta_1-\zeta_1\cos\zeta_1)}{2\zeta_1-\sin(2\zeta_1)}\right)\left(\frac{\sin\zeta_1 r^*}{\zeta_1 r^*}\right)\exp(-\zeta_1^2\mathrm{Fo}) \tag{5.6}$$

It was assumed further that the cabbage kept its original shape during drying and that the thermal diffusivity was constant. Equation 5.6 with $r = R$ (indicating the surface temperature of cabbage) can be simplified to:

$$T = T_\mathrm{D}-(T_\mathrm{D}-T_0)c_1(\exp(-c_2 t)) \tag{5.7}$$

where c_1 and c_2 are empirical constants.

The empirical constants in Equation 5.7 could be estimated by fitting the equation to the experimental data using a non-linear regression analysis; the resulting fit is shown as Equation 5.8.

$$T = T_\mathrm{D}-(T_\mathrm{D}-T_0)\exp(-4.17\times10^{-4}t) \tag{5.8}$$

or if t is expressed in min:

$$T = T_\mathrm{D}-(T_\mathrm{D}-T_0)\exp(-0.025t) \tag{5.9}$$

A comparison between the predicted and experimental cabbage temperature evolutions is shown in Fig. 6.6. Excellent agreement with R^2 in the range of 0.95-0.98 was noted in all cases.

To predict the evolution of sulforaphane during drying the following kinetic model was proposed. At the cabbage temperature of less than 40 °C the mechanism as appeared in Equation 5.9 was proposed.

$$\begin{tikzcd} & C_\mathrm{B} \\ C_\mathrm{A} \arrow[ur, "k_1"] \arrow[dr, "k_2"'] & \\ & C_\mathrm{C} \end{tikzcd} \qquad (5.10)$$

Glucoraphanin (C_A) is hydrolyzed by myriosinase to form sulforaphane (C_B) and sulforaphane nitrile (C_C) (See Figure 5.3). The above conversions could be considered as two irreversible first-order reactions in parallel:

$$\frac{dC_\mathrm{A}}{dt} = -(k_1 + k_2)C_\mathrm{A} \qquad (5.11)$$

$$\frac{dC_\mathrm{B}}{dt} = k_1 C_\mathrm{A} \qquad (5.12)$$

Combining Equations 5.11 and 5.12 yields the following expression for C_B:

$$C_\mathrm{B} = C_\mathrm{B0} + \frac{k_1 C_\mathrm{A0}}{(k_1 + k_2)}[1 - \exp(-(k_1 + k_2)t)] \qquad (5.13)$$

Rearranging Equation 5.13 for C_Bt yields:

$$C_{\mathrm{Bt}} = 1 + C_{\mathrm{A}}[\frac{k_1}{k_1 + k_2}][1 - \exp(-(k_1 + k_2)t)] \qquad (5.14)$$

where C_{Bt} is the relative sulforaphane content ($C_{\mathrm{B}}/C_{\mathrm{B0}}$) at time t; C_{B0} is the sulforaphane content at $t = 0$; C_{A} is the relative glucoraphanin content ($C_{\mathrm{A0}}/C_{\mathrm{B0}}$) at $t = 0$; k_1 and k_2 are the rate constants (min^{-1}).

This model could be applied when it is assumed that glucoraphanin is completely converted to the two products. Since the glucoraphanin content was not determined in this study, the glucoraphanin content was estimated based on the recommendation of a previous study, which reported that the molar fractional conversion of glucoraphanin to sulforaphane was about 51% (Matusheski et al., 2006). The highest sulforaphane content during drying at 40 °C (2.08 mg/100 g dry weight) was used for the calculation since both myrosinase and ESP were active, resulting in a complete change of glucoraphanin to sulforaphane and sulforaphane nitrile. The molecular weights of glucoraphanin and sulforaphane are 436 and 177, respectively (Shen et al., 2010). This resulted in the glucoraphanin content of about 23 µmol/100 g dry weight or 10 mg/100 g dry weight. The calculated glucoraphanin content is in agreement with the value reported in a previous study; Kushad et al. (1999) reported that the glucoraphanin content in white cabbage was in the range of 4.36-78.48 mg/100 g dry weight.

The proposed mechanism to describe the formation and degradation of sulforaphane when the cabbage temperature is higher than 40 °C is shown in Equation 5.14. The reaction could be represented by two irreversible consecutive steps, which are the formation of sulforaphane (C_{B}) and thermal degradation of sulforaphane to thiourea (W) (Jin et al., 1999).

$$C_A \xrightarrow{k_3} C_B \xrightarrow{k_4} W \tag{5.15}$$

The equations describing the consecutive model are given as Equations 5.15 and 5.16:

$$\frac{dC_A}{dt} = -k_3 C_A \tag{5.16}$$

$$\frac{dC_B}{dt} = k_3 C_A - k_4 C_B \tag{5.17}$$

These equations were solved by an integrating factor method and the following resulting solution was obtained:

$$C_{Bt} = \exp(-k_4 t) + C_A[\frac{k_3}{k_4 - k_3}][\exp(-k_3 t) - \exp(-k_4 t)] \tag{5.18}$$

where C_{Bt} is the relative sulforaphane content (C_B/C_{B0}) at time t; C_{B0} is the sulforaphane content at $t = 0$; C_A is the relative glucoraphanin content (C_{A0}/C_{B0}) at $t = 0$; k_3 and k_4 are the rate constants (min^{-1}).

As mentioned earlier, there was no correlation between the moisture content and the sulforaphane content. Therefore, only cabbage temperature is a dominant variable influencing the formation and degradation of sulforaphane; the rate constant could thus be expressed with the well-known Arrhenius equation.

$$k_i = A_i \exp(\frac{-B_i}{T}) \tag{5.19}$$

where T is the absolute temperature of cabbage (K) evaluated from Equation 9. For simplicity the rate constants were decoupled from all the kinetic equations and were obtained by fitting Equation 19 directly to the experimental data. The results of the fitting process given as:

$$k_i = A_i \exp(\frac{-B_i}{(T_\mathrm{D} - (T_\mathrm{D} - T_0)\exp(-0.025t)) + 273}) \tag{5.20}$$

where k_i is the rate constant; A_i is the frequency factor (min^{-1}); B_i is the activation energy (kJ/mol) and $i = 1, 2, 3, 4$.

Table 5.5 shows the Arrhenius constants (A_i and B_i) obtained by fitting Equation 20 to the experimental data. The results (Figure 5.7) showed that the proposed model could be adequately used to predict the sulforaphane evolution during drying with R^2 = 0.90-0.97.

Table 5.5 Arrhenius parameters for sulforaphane evolution prediction during drying

i	A	B	R^2
1	85.71×10^{0}	35.04×10^{2}	0.97
2	38.46×10^{-2}	10.13×10^{2}	0.97
3	46.98×10^{4}	58.96×10^{2}	0.90
4	17.37×10^{6}	67.80×10^{2}	0.93

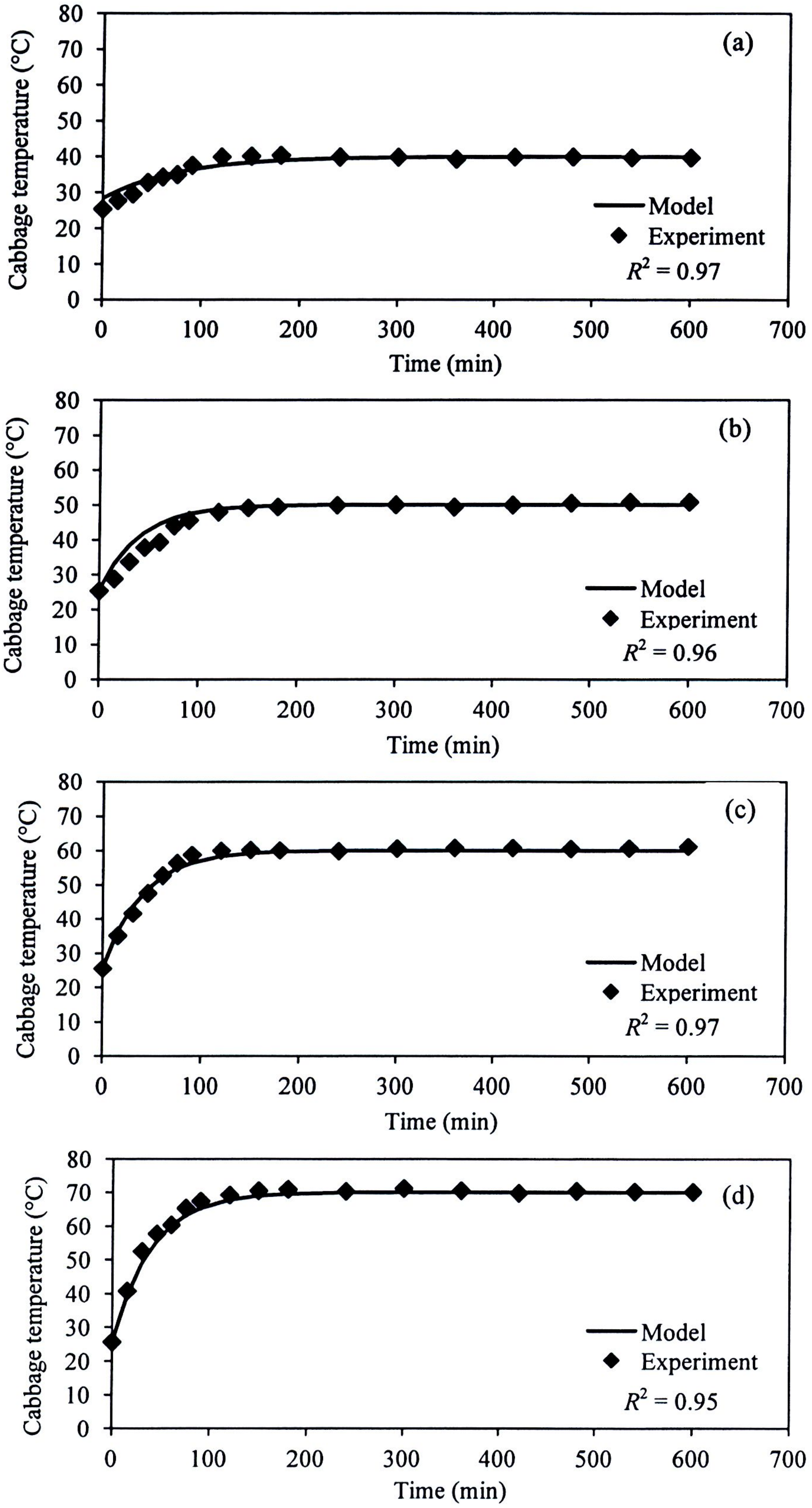

Figure 5.6 Comparison between predicted and experimental cabbage temperature evolutions during drying at (a) 40 °C, (b) 50 °C, (c) 60 °C and (d) 70 °C

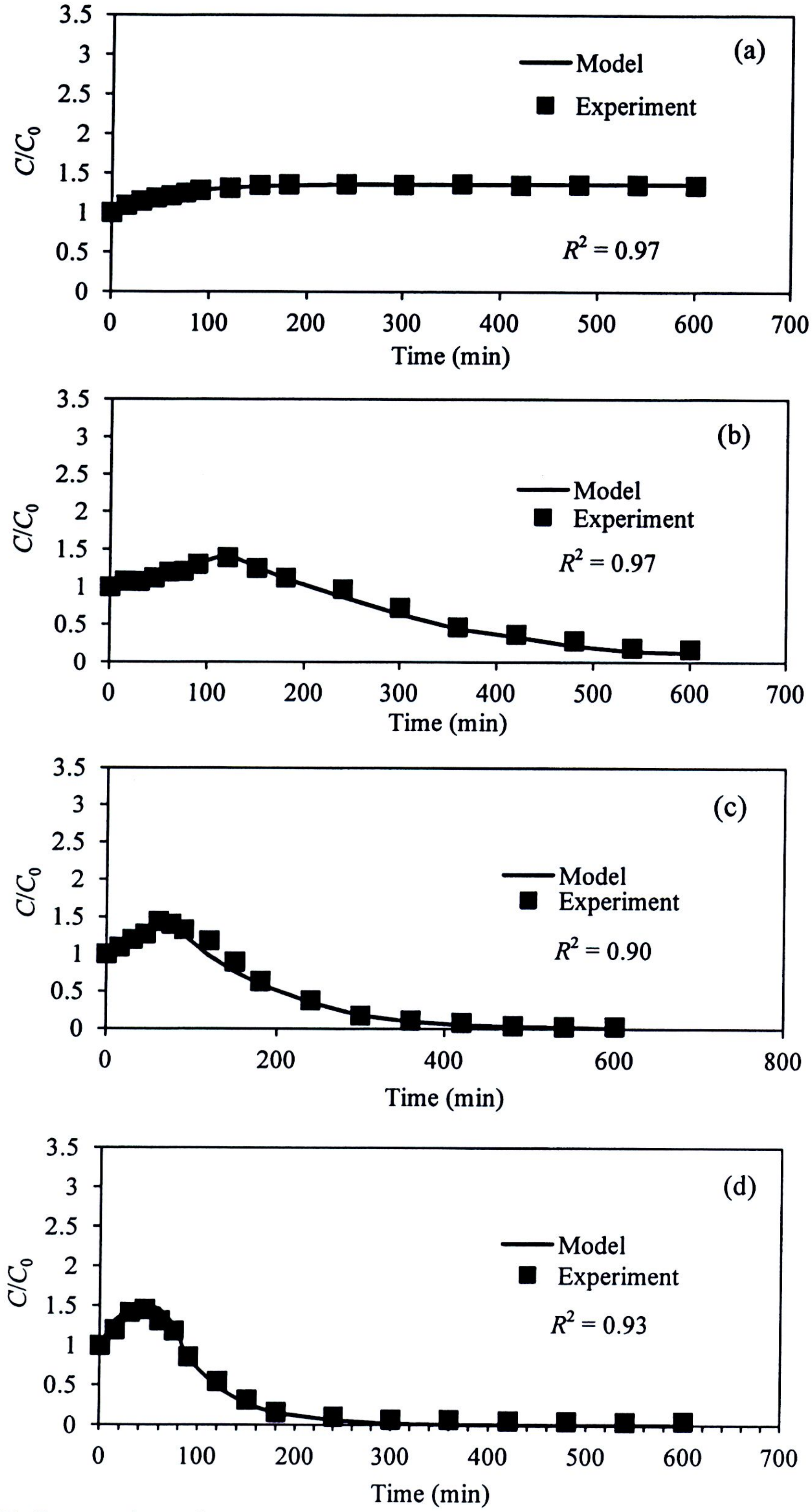

Figure 5.7 Comparison between predicted and experimental sulforaphane evolutions during drying at (a) 40 °C, (b) 50 °C, (C) 60 °C and (d) 70 °C. C_0 is sulforaphane content at $t = 0$