CHAPTER 4

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

4.1 Introduction

Glucosinolates, a group of sulfur-containing plant secondary metabolites, are abundant in *Brassica* vegetables. These compounds are claimed to reduce risk of certain cancers such as lung cancer, colorectal cancer, breast cancer and prostate cancer (Ciska and Pathak, 2004; Higdon et al., 2007).

As shown in Chapter 3, processing of DF powder, i.e., slicing, blanching and drying affected the antioxidant contents and their activities in DF powder. Processing such as blanching and drying has also been reported to cause reduction of glucosinolates in *Brassica* vegetables. Wennberg et al. (2006) studied the effect of blanching in boiling water for 5 min on glucosinolates of white cabbage. They found that the total amount of glucosinolates of cabbage decrease 50-74% because of enzymatic breakdown, thermal breakdown and leaching into blanching water. Mrkic et al. (2010) studied the effect of temperature (50-100 °C) of the air that was used to dry broccoli and reported that glucosinolates content decreased upon drying, especially at higher temperatures. However, the evolution of glucosinolates during drying and the effect of drying method on glucosinolates have not been reported.

To obtain high-quality DF powder from cabbage outer leaves, in this chapter the work was extended to study the effects of processing steps, i.e., preparation, blanching methods, i.e., water and steam blanching as well as drying conditions and methods, i.e., hot air and vacuum drying at 60, 70 and 80 °C, on the evolution of glucosinolates during production of DF powder from cabbage outer leaves.

4.2 Materials and Methods

4.2.1 Sample Preparation

The steps for sample preparation were described in Sector 3.2.1, Chapter 3.

4.2.2 Drying Experiments

Drying conditions and methods was described in Section 3.2.2, Chapter 3.

4.2.3 Determination of Glucosinolates

The determination of the total glucosinolates was carried out following the method of Declercq and Daun (1989) as follows.

4.2.3.1 Sample Extraction

Fresh (1000 mg) or dried cabbage (100 mg) was weighed into a 16×75-mm test tube, which was then placed in a water bath at 95 °C for 15 min. Four mL of boiling water was added. The tube was closed with a screw cap and mixed immediately by a vortex mixer (Scientific Industries, model G-560, Bohemia, NY) for 10 s. The tube was returned to the water bath at 95 °C for 3 min. After cooling with tap water the sample was centrifuged (Hitachi, model Himac CR21, Ibaraki, Japan) at 600× g for 15 min; the supernatant solution was transferred to a different test tube containing 150 μL of 0.5 M barium/lead acetate. The sample was re-extracted by adding 4 mL of hot water (95 °C) into the test tube, which was subsequently placed in a water bath at 95 °C for 3 min and centrifuged at 600× g for 15 min. The extract was combined with the first supernatant and then made up to 10 mL with deionized water.

4.2.3.2 Preparation of Sephadex Pyridine-acetate Column

The isolation of glucosinolates was performed using an ion-exchange column. Twenty five grams of dry DEAE-Sephadex A-25 (Sigma-Aldrich, Steinheim, Germany) was added into a syringe (0.8×4 cm), which was filled subsequently with deionized water. Air bubbles were removed using an ultrasonic generator at 30 kHz for 30 min, the syringe was left overnight at room temperature. Before loading the sample into the syringe 5 mL of 0.5 N NaOH and 10 mL of water were sequentially passed through the syringe respectively to ensure that the pH of the eluate was neutral. DEAE-Sephadex A-25 was converted into an acetate form by adding 5 mL of 0.5 M pyridine acetate solution and then 10 mL of water. An aliquot (3 mL) of the extract was added to the prepared syringe. The syringe was washed twice with 2 mL water, 2 mL 30% (v/v) formic acid and 2 mL water, discarding the eluate each time. Subsequently, the syringe was eluted twice with 4.75 mL of 0.3 M potassium sulfate and adjusted by adding 0.3 M potassium sulfate to 10 mL.

4.2.3.3 Quantification of Glucosinolates

One mL of the sample eluate was transfered to a test tube. Seven mL of 80% (v/v) sulphuric acid was added; this was followed by 1.0 mL of 1% (w/v) thymole solution. The tube was closed with a screw cap, thoroughly mixed by a vortex mixer for 30 s and placed in a water bath at 100 °C for 60 min. The tube was cooled under tap water and mixed again by a vortex mixer for 30 s. The absorbance was then measured using a spectrophotometer (Shimadzu, model UV 21101 PC, Kyoto, Japan) at 505 nm; 0.3 M potassium sulphate was used as blank. The glucosinolates concentration in the sample was calculated from the absorbance of sinigrin standard (Sigma, St. Louis, MO) solution (3 µmol/mL) based on the following equation:

$$C = \frac{A_x}{K} \times \frac{D}{W}$$

where C = total glucosinolates concentration (µmol/mL), A_x = absorbance of the sample, K = absorbance factor (absorbance/concentration of standard)(mL/µmol), D = dilution factor (300), W = mass of the sample taken (g).

4.2.4 Color Measurement

Color of a sample was measured in the Hunter *Lab* color system using a colorimeter (HunterLab, model ColorQuest XE, Reston, VA); D65 illuminant with 10° viewing geometry was employed in all measurements. Three Hunter parameters, i.e., L (lightness), a (redness and greenness) and b (yellowness and blueness), were measured and the total color difference was calculated as follows:

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$$

$$\Delta L = L - L_0,\ \Delta a = a - a_0 \text{ and } \Delta b = b - b_0$$

where L_0, a_0, b_0 are the color values of the fresh leaves. All measurements were performed in triplicate and the data are reported as average values of the three measurements.

4.2.5 Statistical Analysis

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

4.3 Results and Discussion

4.3.1 Effects of Preparation and Blanching Methods on Total Glucosinolates

The content of total glucosinolates in fresh cabbage outer leaves prior to any preparation was 1583.22 ± 69.06 μmol/100 g dry mass. The value obtained is in a similar order to that reported in the literature, which is in the range of 1074 to 1690 μmol/100 g dry mass (Cieślik et al., 2004; Wennberg et al., 2006; Martinez-Villaluenga et al., 2009).

The contents of glucosinolates in the samples undergone different preparation steps are given in Table 4.1. Steam blanching led to no significant losses of glucosinolates, while water blanching caused considerable reduction in total glucosinolates. It has indeed been reported that total glucosinolates in white cabbage, broccoli and cauliflower significantly decreased upon blanching in hot water (Cieślik et al., 2004; Wennberg et al., 2006; Volden et al. 2008); on the other hand, Song and Thornalley (2007) reported that steaming for 20 min presented little effect on total glucosinolates in broccoli, Brussel sprout, cauliflower and green cabbage. This is because glucosinolates is a water-soluble compound and can easily leach into cooking water (Jones et al., 2010).

Slicing prior to blanching was observed to cause a significant decrease in the glucosinolates content. The reason might be that tissue disruption during slicing led to a release of myrosinase, which hydrolyzed glucosinolates to other derivative products (Verkerk et al., 2001). Although this enzyme might be inactivated during blanching, further reduction in glucosinolates took place due to thermal degradation of the compounds. Overall, steam blanching prior to slicing is suggested as the most suitable pretreatment for cabbage outer leaves.

Table 4.1 Effects of preparation methods on retention of total glucosinolates

Sample	Total glucosinolates (μmol/100 g dry mass)	Retention (%)
Fresh	1481.91±85.41^{cd}	
Slicing/water blanching	784.51±52.06^{a}	52.92±0.47^{a}
Slicing/steam blanching	1368.62±62.93^{bc}	92.40±1.82^{b}
Water blanching/slicing	1267.62±38.69^{b}	88.73±5.78^{c}
Steam blanching/slicing	1484.50±60.09^{d}	99.34±1.02^{d}

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

4.3.2 Effect of Drying on Total Glucosinolates Content

The moisture content of the sample prior to drying was approximately 7.55 ± 0.932 g/g dry weight. However, higher moisture content of approximately 10.40 ± 0.184 g/g dry weight was observed for the samples after blanching. Figure 4.1 shows the drying curves of blanched samples undergoing hot air drying and vacuum drying at various drying temperatures. It was observed that drying rate at higher temperature was faster than that at lower temperature. Higher drying temperature provides higher the driving force leading to increase the moisture diffusivity. The result also showed that the rate of moisture reduction of vacuum drying was higher compared with that of hot air drying. This is because lowering the absolute pressure (10 kPa) operating in vacuum drying lead to reducing of boiling point of water resulting in accelerating moisture migration from dried product.

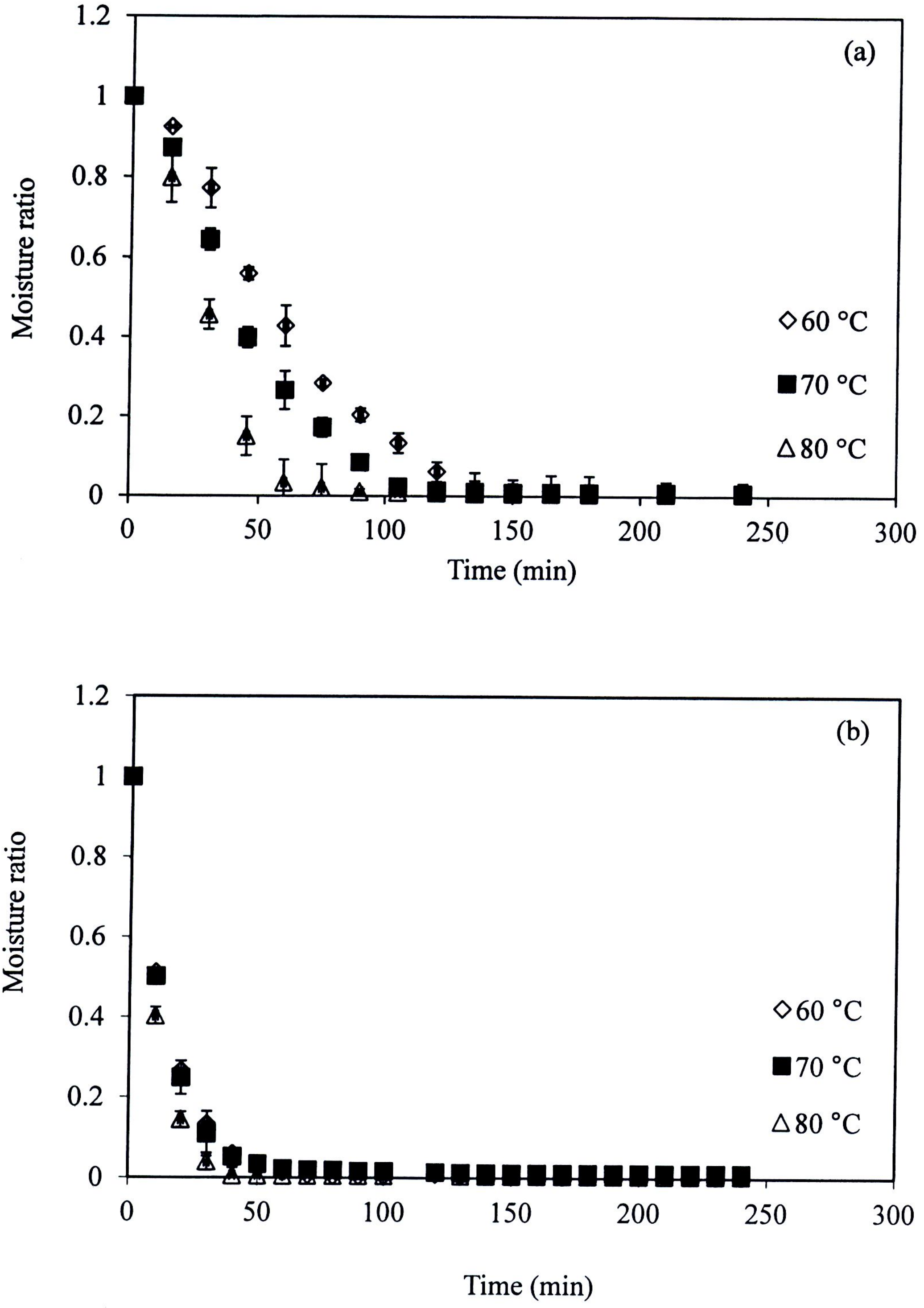

Figure 4.1 Drying kinetics of steam blanched samples undergoing (a) hot air drying and (b) vacuum drying.

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 4.2. It was observed that blanched samples took the shorter drying time than the unblanched ones. This is due to blanching could help softening plant cell resulting in facilitating water removal.

The evolutions of glucosinolates in steam blanched cabbage leaves during hot air and vacuum drying are presented in Figure 4.2. It is seen that glucosinolates content first remained constant and then slowly decreased toward the end of drying. It was also observed that the decrease in the content of glucosinolates occurred when the sample temperature was above 60 °C (Figure 4.3). This might be because indole glucosinolates, which represent around 19% of the total glucosinolates in white cabbage (Verkerk et al., 2001; Oerlemans et al., 2006), degraded during drying. Previous studies have indeed reported that different types of glucosinolates possess different thermal stability (Oerlemans et al., 2006; Dekker et al., 2009). In the case of indole glucosinolates it has been reported that these compounds generally degrade at temperatures lower than 90 °C (Verkerk et al., 2001; Oerlemans et al., 2006). On the other hand, aliphatic glucosinolates, e.g., sinigrin, glucoiberin and gluconapin, identified as the major glucosinolates in white cabbage (more than 75% of the total glucosinolatates) (Verkerk et al., 2001; Martinez-Villaluenga et al., 2009), would degrade at temperatures above 90 °C (Oerlemans et al., 2006).

Table 4.2 Time to dry cabbage outer leaves to approximately 0.1 g/g dry mass as well as their equilibrium moisture contents

Drying	Temperature (°C)	Drying time (min)	Equilibrium moisture content (g/g dry mass)
Hot air	60	180	0.074 ± 0.004
	70	150	0.076 ± 0.014
	80	120	0.050 ± 0.013
Vacuum	60	150	0.051 ± 0.002
	70	120	0.045 ± 0.002
	80	90	0.047 ± 0.009

Table 4.3 Retention of glucosinolates in dietary fiber powder

Drying	Temperature (°C)	Glucosinolates (μmol/100 g dry mass)	Retention* (%)
Hot air	60	1321.58 ± 35.89^a	89.18 ± 2.19^a
	70	1338.60 ± 13.04^a	90.33 ± 1.78^a
	80	1313.42 ± 39.27^a	88.63 ± 1.43^a
Vacuum	60	1405.36 ± 28.54^b	94.87 ± 1.12^b
	70	1410.05 ± 31.67^b	95.19 ± 0.99^b
	80	1380.40 ± 19.06^b	93.15 ± 0.64^b

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

*Comparison was made with the fresh leaves of cabbage

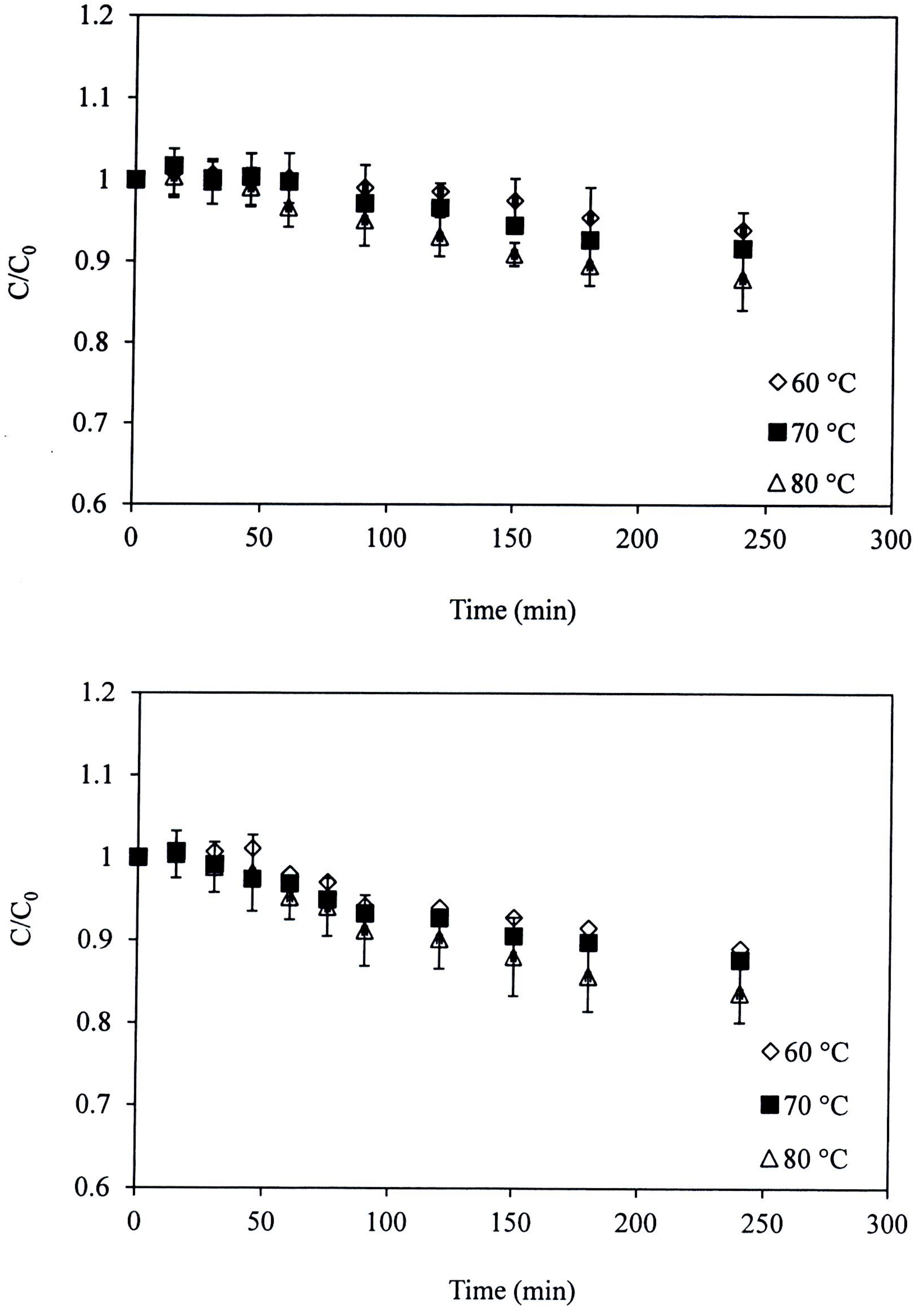

Figure 4.2 Changes of total glucosinolates of steam blanched samples during (a) hot air drying and (b) vacuum drying.

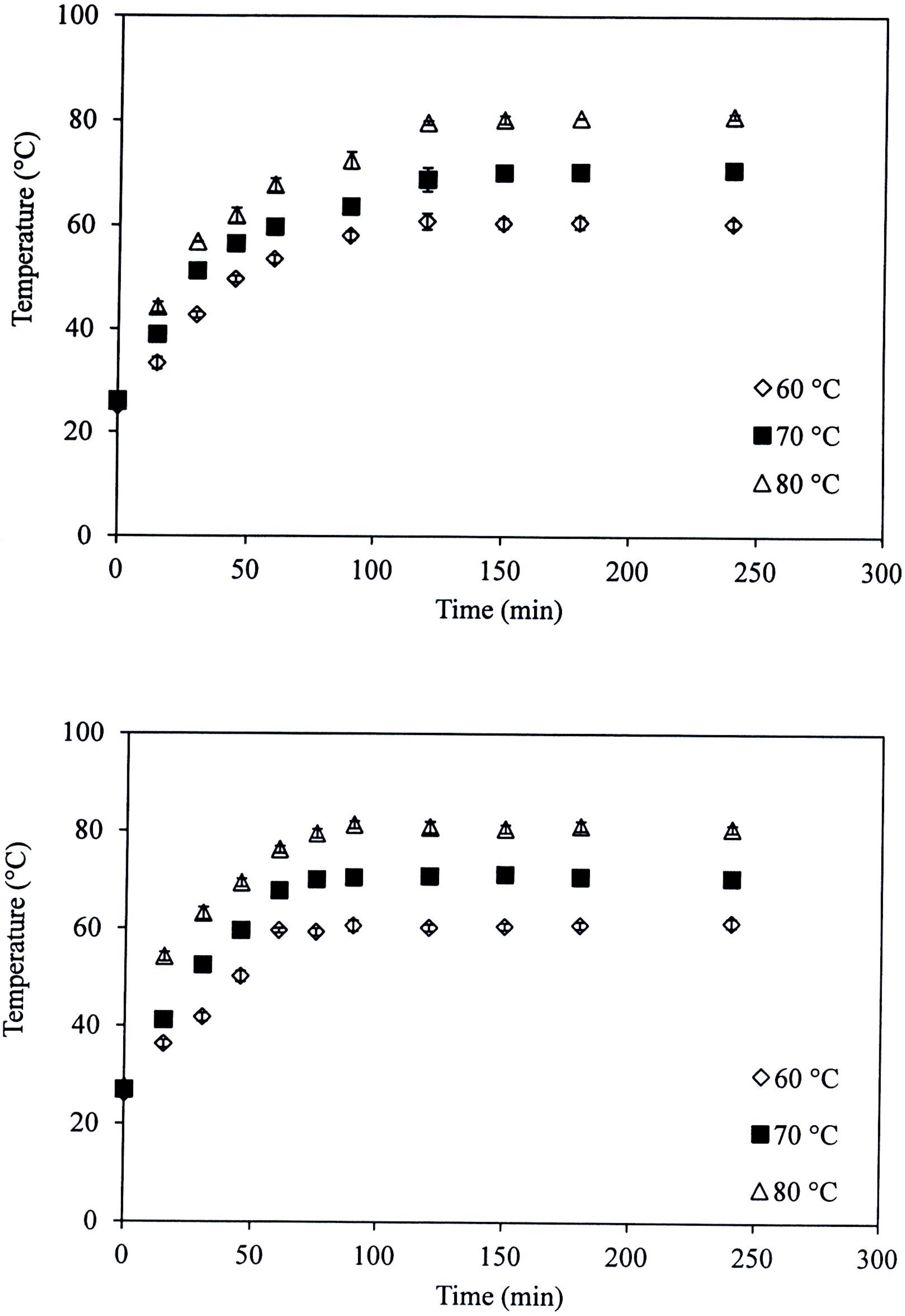

Figure 4.3 Changes of cabbage temperature during (a) hot air drying and (b) vacuum drying

Table 4.3 shows the retention of total glucosinolates in DF powder; comparison was made with the content of glucosinolates in the fresh leaves. The retention of glucosinolates in cabbages undergone hot air and vacuum drying was in the range of 88.63-90.33% and 93.15-95.19%, respectively. High retention of glucosinolates implies that the DF powder prosessed high anticancer properties. It was observed that drying temperature did not have any significant effect on the content of glucosinolates in the DF powder. Vacuum drying provided the DF powder with higher retention of glucosinolates; this is because the drying rates of vacuum drying were higher than those of hot air drying, resulting in the shorter time to reach the desired moisture content, hence lower levels of thermal degradation.

4.3.3 Effect of Drying on Color

Table 4.3 lists the color parameters of both fresh cabbage leaves and DF powder. The lightness and greenness of the DF powder were lower, while the yellowness was slightly higher as compared with the values of the fresh leaves. The dried blanched cabbages were also darker. Similar results were noted by Saencom et al. (2010) who reported that the color of ivy ground was more intense upon blanching. This could be explained by the fact that blanching causes removal of intracellular air, leading to protection of color pigments such as chlorophyll and β-carotene (Dutta et al., 2006). Drying temperature did not significantly affect the color of dried cabbages but the overall results indicated that vacuum drying led to DF powder with better lightness and green color.

Table 4.4 Color of DF powder

Drying	Temperature (°C)	*L*	*a*	*b*	ΔE
Fresh		40.58 ± 0.51^{b}	-7.49 ± 0.13^{b}	16.52 ± 0.23^{a}	
Hot air	60	31.52 ± 2.40^{a}	-6.08 ± 0.28^{a}	19.04 ± 0.52^{b}	10.18 ± 2.16^{a}
	70	32.42 ± 4.39^{a}	-6.41 ± 0.67^{a}	19.15 ± 3.79^{b}	9.40 ± 2.49^{a}
	80	32.47 ± 3.64^{a}	-6.53 ± 0.38^{a}	18.14 ± 0.71^{b}	9.25 ± 3.13^{a}
Vacuum	60	32.01 ± 2.51^{a}	-6.88 ± 0.50^{a}	17.33 ± 0.16^{ab}	9.15 ± 4.35^{a}
	70	32.28 ± 1.83^{a}	-6.92 ± 0.22^{a}	18.32 ± 0.40^{b}	8.86 ± 4.11^{a}
	80	34.65 ± 0.42^{a}	-6.76 ± 0.50^{a}	18.19 ± 0.15^{b}	7.11 ± 3.39^{a}

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

Overall, the results showed that cabbage outer leaves should be prepared into functional DF powder by steam blanching prior to slicing in combination with vacuum drying at 80 °C. This condition was also suggested to produce DF powder with high antioxidants as mentioned in Chapter 3. Therefore, to obtain DF powder with high antioxidants and glucosinolates steam blanching the outer cabbage leaves prior to slicing in combination with vacuum drying at 80 °C was recommended.