CHAPTER 6

MICROWAVE-ASSISTED EXTRACTION OF SULFORAPHANE IN WHITE CABBAGE

6.1 Introduction

Sulforaphane is of interest for the pharmaceutical industry since it possesses the high anticarcenogenic activity. As sulforaphane possesses beneficial health effect, it would be useful to extract the compound from plant cells.

Extraction method commonly used to extract sulforaphane component is solvent extraction (Liang et al., 2006). This method involves mixing of substrate matrix with solvent for a predetermined extraction time. However, it required large amount of solvent and long extraction time.

Recently, microwave technology has been applied to extract organic compounds from food matrix. This extraction method uses microwave energy to heat solvents and samples to increase the mass transfer rate of the solutes from the sample matrix into solvent (Duvernay et al., 2005). Microwave-assisted extraction (MAE) has been reported to largely reduce the extraction time and increase yields of extracted compound (Dai et al., 2010). The extraction process can be influenced by several parameters such as type of solvent, temperature ratio of sample and solvent and food characteristics.

Dichloromethane is well-known as a good solvent for extracting sulforaphane (Han and Row, 2011). However, its toxicity and its harmful character to the environment can

generate a severe regulation in the future (Letellier et al., 1999). Therefore, it is important to test other less toxic solvents. Water is safe and environmentally friendly solvent. Water is also indicated as polar solvent. It has been reported that polar molecules can absorb microwave energy strongly because it has a permanent dipole moment which can be affected by microwave.

Many studies have reported the use of microwave for extracting compounds from plant cells such as polyphenol and caffeine from green tea leaves, piperine from black pepper and phenolic compounds from grape seed (Hong et al., 2001; Raman and Gaikar, 2002; Pan et al. 2003). However, the use of microwave to assist the extraction of sulforaphane has not been reported. Thus, the feasibility of using MAE of sulforaphane from white cabbage was studied.

6.2 Materials and Methods

6.2.1 Preparation of Semi-dried Cabbage Sample

The preparation procedures followed those suggested by Tanongkankit et al. (2011). White cabbages (*Brassica oleracea* L. var. capitata) were obtained from a local supermarket in Pullman and were washed under tap water for 1 min. A cabbage head was cut into 2 portions and the core was removed. Cabbage leaves were then chopped using an electric chopper (Oster, 6878, Tlalnepantla, Mexico) to obtain a sample with the size in the range of 1.7-2.5 mm.

A part of the chopped sample (200 g) was spread on a tray as a thin layer and dried in a hot air dryer (Armfield Limited, UOP8, Ringwood, UK) at 60 °C until a final moisture content of less than 0.1 g/g dry mass was reached (Larrauri, 1999); the drying

temperature was as suggested by Tanongkankit et al. (2011) as the temperature that leads to the highest enhancement of sulforaphane content in cabbages. During drying 3–5 g of the sample was taken out at various time intervals to determine its moisture content following AOAC Method 984.25 (AOAC, 2000); the sulforaphane content was also determined. Drying was conducted at a constant air velocity of 1.5 m/s. The temperature of the sample during drying was measured continuously using type-T thermocouples with an accuracy of ± 0.5 °C.

6.2.3 Extraction Procedures

Cabbage samples were prepared as described in Section 6.2.1. After chopping into the desired particle size range, the chopped samples were then divided into two portions. The first portion was subjected directly to extraction process. Another portion of chopped cabbage was subjected to hot air drying for 2 h to obtain the semi-dried sample before subjecting to extraction. The prepared samples were subsequently extracted by either conventional extraction method or MAE method. The extraction procedures are as follows:

6.2.3.1 Conventional Extraction

Extraction of sulforaphane was conducted using the method described by Liang et al. (2006) with some modifications. Five grams of a sample was stirred with 50 mL dichloromethane which was combined with 2.5 g sodium sulfate anhydrous for 15 min. The extract was filtered through Whatman No. 1 filter paper. The sample was re-extracted by adding 50 mL dichloromethane for 15 min and filtered through Whatman No. 1 filter paper. The both extracts were mixed together. The solvent fraction was then dehydrated using a rotary evaporator at 30 °C.

6.2.3.2 MAE

A sample (5 g) was placed in a 100-mL Erlenmeyer flask, which was then filled with 50 mL of a selected solvent. The extraction was carried out in a domestic microwave oven (Panasonic, NN-SD967S, Shanghai, China) at the set powers of 130, 260 and 390 W for either 1, 2, 3, 4 or 5 min. The extract was dehydrated using the rotary evaporator at 30 °C until it was completely dried. The temperature of the sample and solvent mixture was periodically measured by a digital thermocouple (type T, Omega, RH511, Stamford, CT) with an accuracy of ± 0.5°C. At each measuring time the flask of the mixture was rapidly taken out from the oven. The temperature of the mixture was immediately measured by inserting the temperature probe into the flask. That particular experiment was then discarded and a new experiment was conducted until the next sampling had been reached. The whole procedure was repeated until the whole MAE duration was covered. Two types of solvents, i.e., dichloromethane and water, were used for comparative purpose. Each experiment was performed in duplicate.

6.2.4 HPLC Analysis

The residue after dehydration by rotary evaporator was dissolved in 2 mL acetonitrile and was filtered through a 0.2 μm syringe filter. Ten μL of the filtrate was then injected into a C_{18} 5 μL (4.6×250 mm) HPLC column (Varian Microsorb-MV 100-5, Darmstadt, Germany) with gradients as follows: 20% acetonitrile and 80% water, then changed linearly over 10 min to 60% acetonitrile and 40% water, changed to 100% acetonitrile and maintained this concentration 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. Quantification of sulforaphane was carried out based on standard curve of sulforaphane (Sigma-Aldrich, St. Louis, MO).

6.2.4 Absorbed microwave power evaluation

The actual absorbed microwave power was evaluated by monitoring the temperature change of a mixture of a solvent and sample during microwave heating. Five g of a sample was filled with 50 mL of a selected solvent. The mixture was then heated at the set powers of 130 W, 260 W and 390 W for 1 min. The temperature of the mixture was measured both before and after microwave heating for 1 min. The absorbed microwave power was calculated using Equation 6.1:

$$P=\frac{m\times C_p\times \Delta T}{t} \tag{6.1}$$

where P is the absorbed microwave power (W); m is the mass of the mixture (kg); C_p is the heat capacity of the mixture (J/kg.K); t is the time of heating (s); ΔT is the increase of the mean temperature of the mixture (°C). The absorbed microwave powers at different conditions are given in Table 1.

6.2.5 Statistical Analysis

The information of statistical analysis was described in Section 3.4, Chapter 3.

Table 6.1 Absorbed microwave power of mixtures at different set microwave powers

Sample	Solvent	Set microwave power (W)	Absorbed microwave power (W)
Fresh	Dichloromethane	130	16
		260	18
		390	20
Semi-dried	Dichloromethane	130	15
		260	17
		390	19
Fresh	Water	130	38
		260	90
		390	120
Semi-dried	Water	130	36
		260	84
		390	114

6.3 Results and Discussion

6.3.1 Drying Characteristics of Chopped Cabbage

Figure 6.1 shows the drying curve of chopped cabbages at the drying air temperature of 60 °C. The initial moisture content of cabbages was 13.45 ± 0.59 g/g dry mass. The equilibrium moisture content of 0.07 ± 0.02 g/g dry mass was reached after 240 min of drying.

The evolutions of sulforaphane content and cabbage temperature during drying at 60 °C are illustrated in Figure 6.2. The highest sulforaphane content occurred at 120 min of drying. At this point the cabbage temperature was around 51 °C. The results are indeed consistent with those of Tanongkankit et al. (2011) who reported that the maximum sulforaphane content during drying of cabbage outer leaves was observed when the cabbage temperature was in the range of 50.5-53.5 °C. At the maximum point the moisture content of the cabbages was 1.86 g/g dry mass (see Figure 6.1). Therefore, drying was performed for 120 min to prepare a semi-dried sample for subsequent MAE.

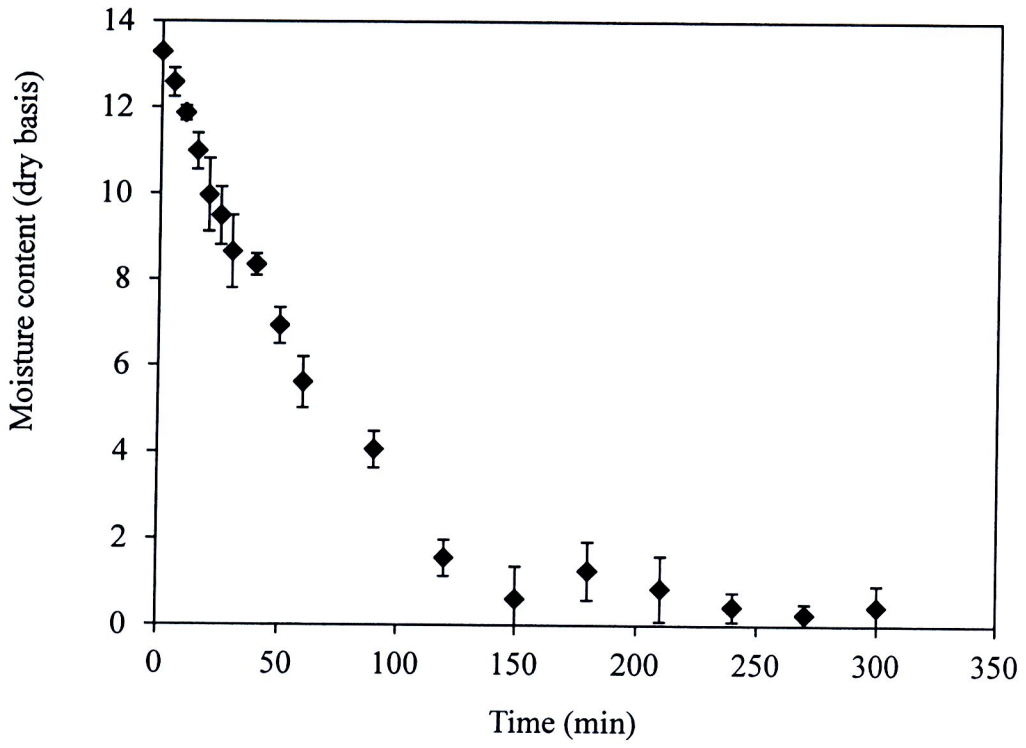

Figure 6.1 Drying curves of cabbage during hot air drying at 60 °C

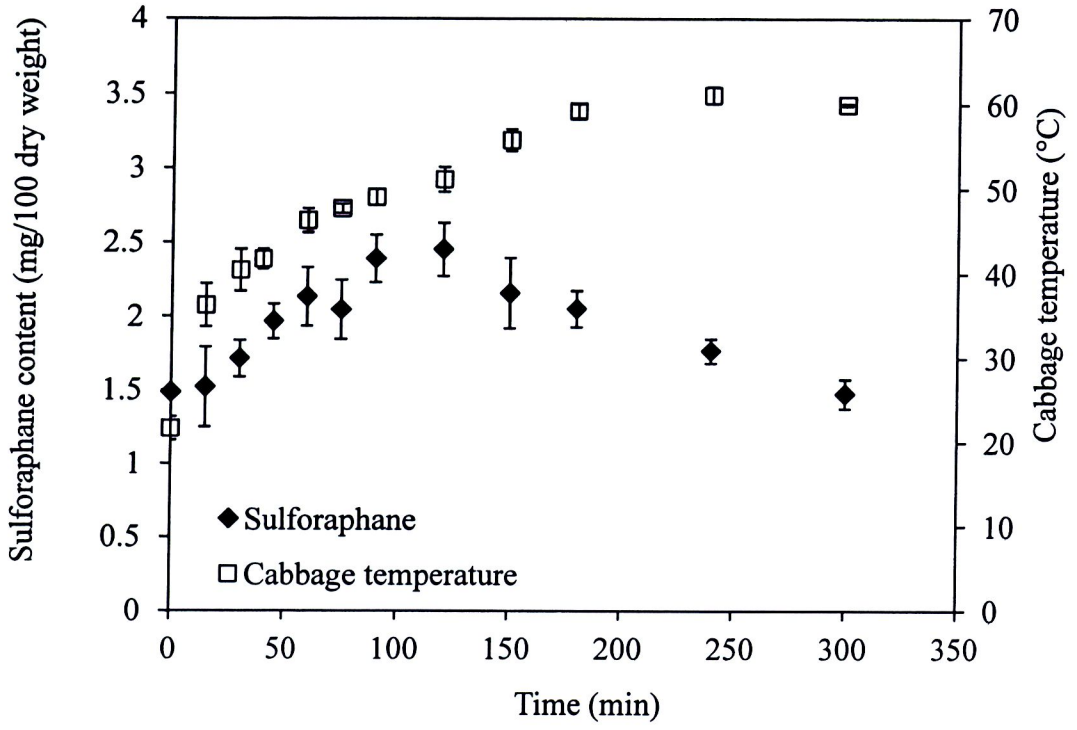

Figure 6.2 Evolution of sulforaphane and cabbage temperature during hot air drying

6.3.2 Effect of Extraction Methods on Sulforaphane Content

Figure 6.3 shows the temperature of the mixtures of cabbages and dichloromethane during MAE at different microwave powers; similar results for the cabbages-water system are shown in Figure 6.4. The initial temperature of the mixture was around 23.5 °C. Once subjecting to microwave irradiation the mixture temperature increased steadily until reaching the boiling point of dichloromethane of 39.8 °C (Eskilsson and Björklund, 2000). The temperature evolution of the solvent suspended either with the fresh or semi-dried cabbages was similar. Higher microwave powers did not lead to significantly higher heating rates. The temperature of the mixture of dichloromethane and fresh cabbages reached the boiling point after 3, 2 and 1.5 min of MAE at the microwave powers of 16, 18 and 20 W, respectively. In the case of the mixture of dichloromethane and semi-dried cabbages, the boiling point was reached at similar

times; the absorbed powers were slightly different, i.e., 15, 17 and 19 W, respectively, however.

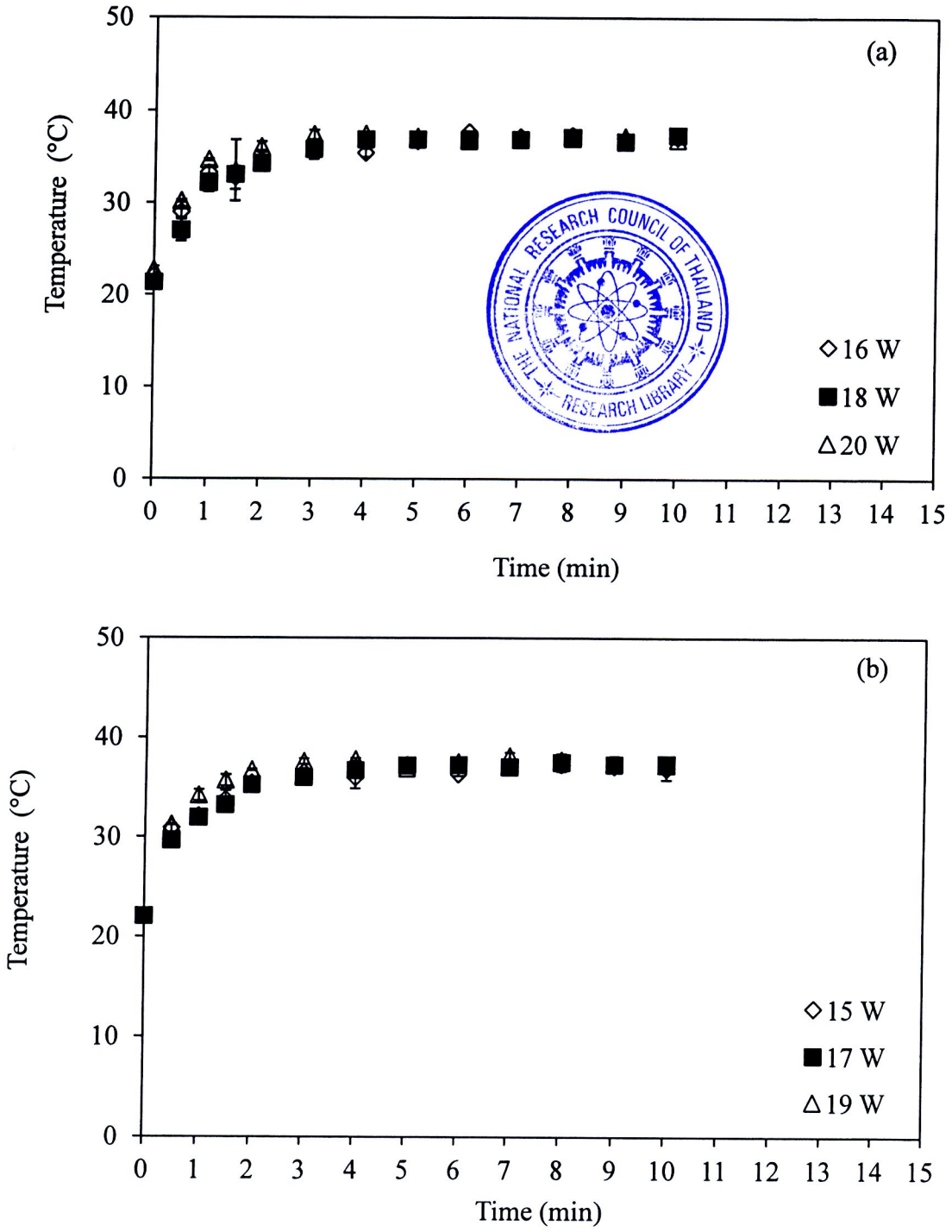

Figure 6.3 Temperature evolution of mixtures of dichloromethane and (a) fresh cabbages and (b) semi-dried cabbages during MAE at different microwave powers

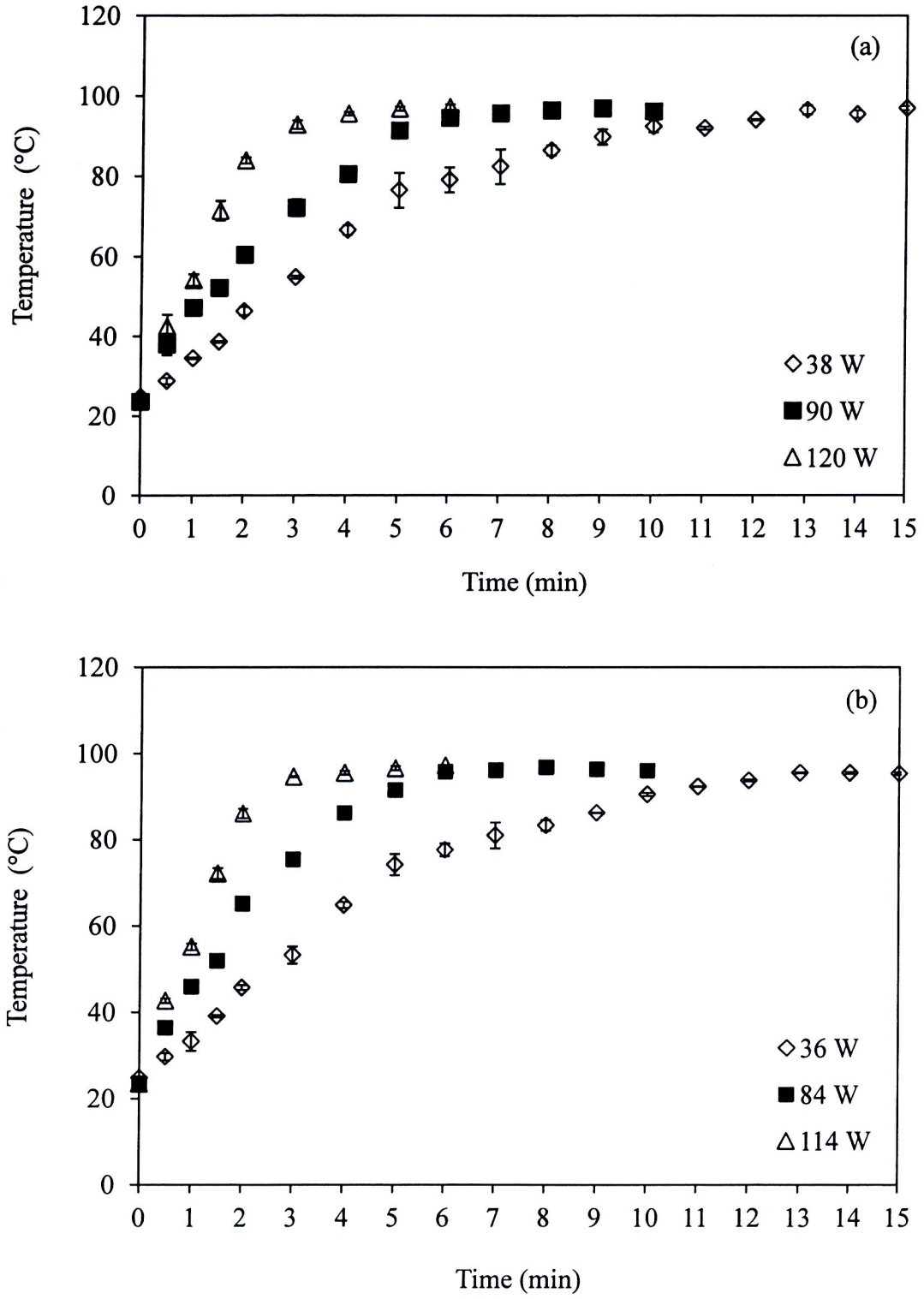

Figure 6.4 Temperature evolution of mixtures of water and (a) fresh cabbages and (b) semi-dried cabbages during MAE at different microwave powers

When using water as a solvent, on the other hand, higher microwave powers significantly accelerated the heating rate (see Figure 6.4). This is because water is a polar solvent and can absorb microwave energy much more efficiently than dichloromethane. Water suspended with either fresh or semi-dried sample reached its boiling point after 12, 6 and 4 min when the microwave powers of 38, 90 and 120 W for fresh sample or 36, 84 and 114 W for semi-dried sample were used, respectively.

6.3.4 Evolution of sulforaphane content when using dichloromethane as a solvent

Figure 6.5 shows the evolution of the extracted sulforaphane contents from fresh and semi-dried cabbages when using dichloromethane as a solvent at different microwave powers; the contents obtained using the conventional extraction are also shown. In the case of the fresh sample the extraction yield rapidly increased before remaining unchanged when the extraction time was beyond 3, 2 and 1.5 min at the microwave powers of 16, 18 and 20 W, respectively. There might be two possible reasons to explain this phenomenon. Firstly, subjecting the fresh sample to microwave heating might activate myrosinase. Generally, this enzyme can change a substrate, which is glucoraphanin in this case, to sulforaphane at a temperature in the range of 25-53.5 °C (Yen and Wei, 1993; Tanongkankit et al., 2011). Increased sample temperature therefore contributed to the formation of sulforaphane. Simultaneously, moisture in the fresh sample when heated up by microwave evaporated and generated pressure, causing rupture of cabbage cells and facilitating the leaching of sulforaphane to the surrounding solvent (Mandal et al., 2007). Once sulforaphane was released from the plant cells, it dissolved and was preserved in dichloromethane as the solvent temperature was always less than or approximately at its boiling point (~ 39.8 °C). Since sulforaphane generally

degraded when the temperature is above 50.5 °C (Tanongkankit et al., 2011), no degradation of sulforaphane was expected during MAE. On the other hand, it was likely that all glucoraphanin was converted to sulforaphane during initial period of extraction. Therefore, extending the extraction duration did not improve the extraction yield and the amount of sulforaphane accumulated in the solvent remained constant.

In the case of the semi-dried sample the initial sulforaphane content was higher than that of the fresh sample due to the partial drying prior to MAE. Similar pattern of the sulforaphane content evolution was noted but the rate of increase in the extraction yield and the level of the increase were much lower than those in the case of the fresh sample. This is probably because there was very small amount of glucoraphanin remained in the semi-dried sample, leading to minimal sulforaphane formation during MAE. Interestingly, no significant differences in terms of the highest extraction yield were noted when using the fresh and semi-dried samples. This is probably because initial glucoraphanin contents in both samples were similar and this substrate could be completely converted into sulforaphane in both cases; glucoraphanin was converted during MAE and drying in the cases of the fresh sample and semi-dried sample, respectively.

The extraction time was determined as the time when the yield of sulforaphane reached its maximum (see Table 6.2). MAE led to significantly reduced extraction time when compared with the conventional method. The results indeed showed that the extraction time was reduced when the microwave power increased.

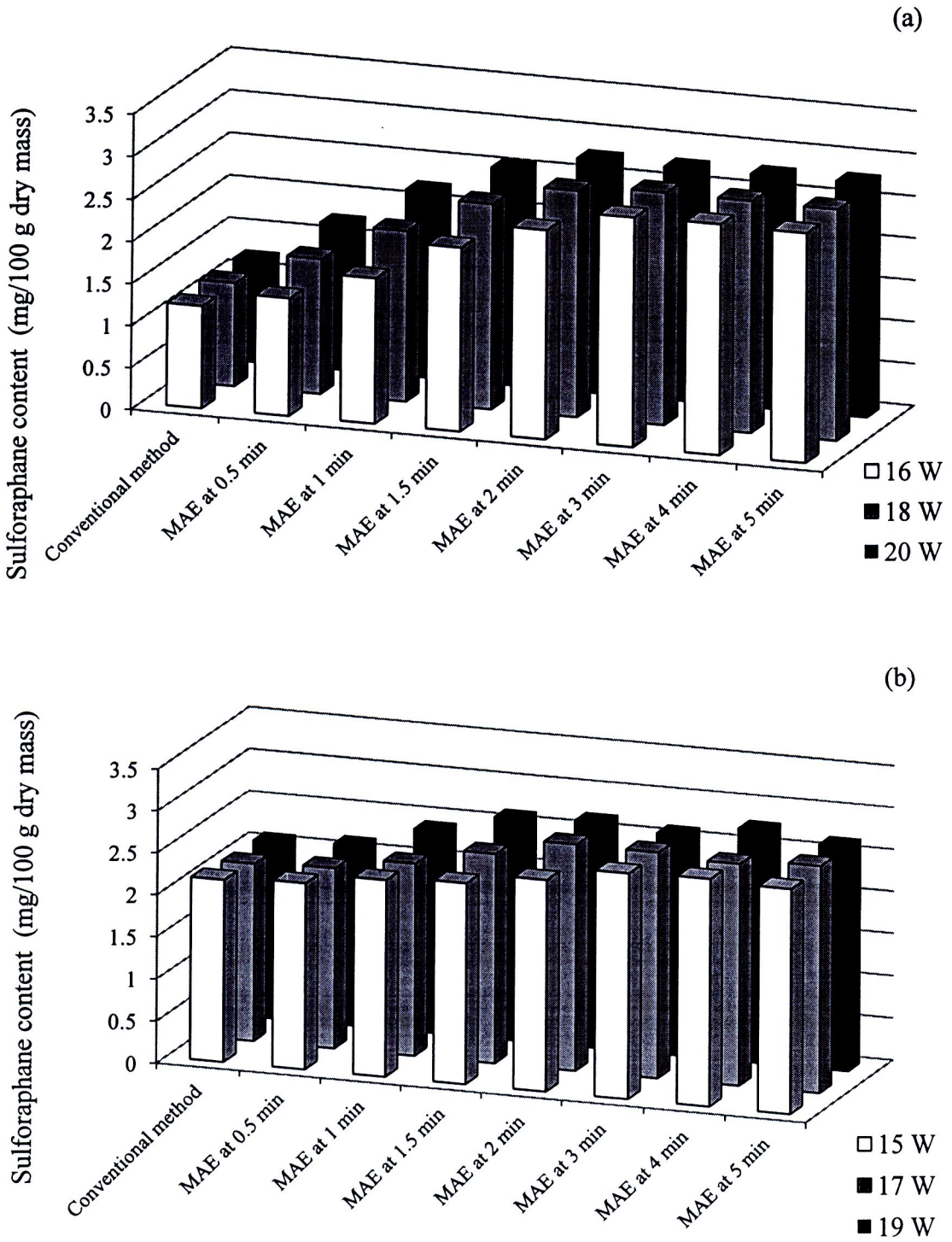

Figure 6.5 Evolution of sulforaphane content from (a) fresh cabbages and (b) semi-dried cabbages during MAE with dichloromethane as a solvent at different microwave powers

Table 6.2 Extraction time when using dichloromethane as a solvent

Sample	Extraction method	Microwave power (W)	Extraction time (min)	Solvent temperature at maximum sulforaphane content (°C)
Fresh	Conventional	-	30	-
	MAE	16	3	37.1±0.3
		18	2	37.8±0.1
		20	1.5	37.5±0.5
Semi-dried	Conventional	-	30	-
	MAE	15	3	37.5±0.4
		17	2	36.8±0.7
		19	1.5	36.7±0.8

6.3.5 Evolution of sulforaphane content when using water as a solvent

A different pattern of the extraction yield evolution was noted when using water as a solvent (Figure 6.6). At each microwave power the extraction yield first increased and reached the highest value before gradually decreased. The maximum extraction yields for both the fresh and semi-dried samples were obtained at the extraction time of 3, 1.5 and 1 min at the microwave powers of 38, 90 and 120 W for the fresh sample and 36, 84 and 114 W for the semi-dried sample, respectively (see Table 6.3).

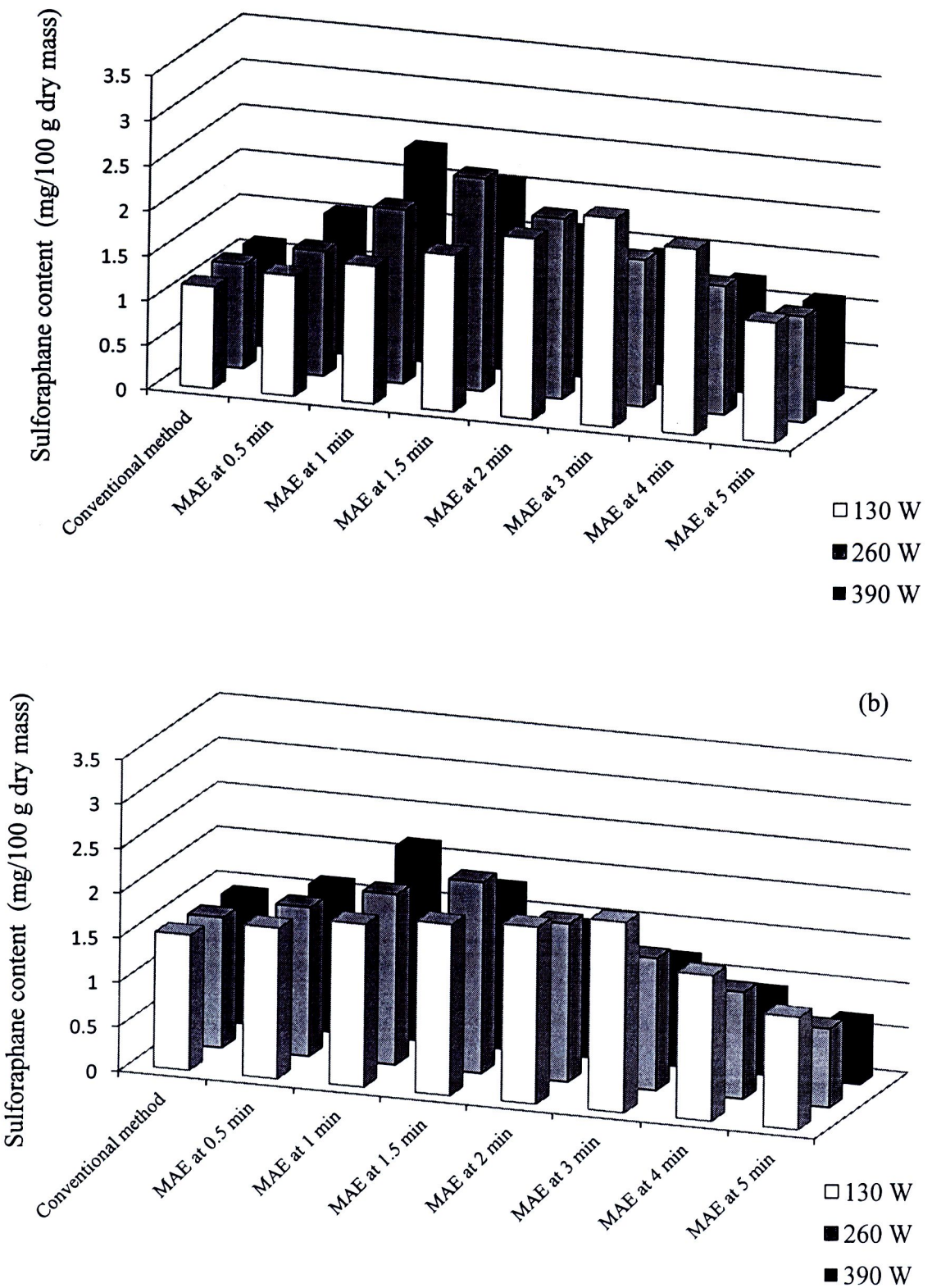

Figure 6.6. Evolution of sulforaphane content from (a) fresh cabbage and (b) semi-dried cabbage during MAE with water as a solvent at different microwave powers

Table 6.3 Extraction time when using water as a solvent

Sample	Extraction method	Microwave power (W)	Extraction time (min)	Solvent temperature at maximum sulforaphane content (°C)
Fresh	Conventional	-	30	-
	MAE	38	3	52.3±1.4
		90	1.5	52.1±0.6
		120	1	54.1±1.1
Semi-dried	Conventional	-	30	-
	MAE	36	3	54.6±2.0
		84	1.5	55.0±0.7
		114	1	54.3±1.4

An increase in the yield of sulforaphane might also be explained by the two reasons mentioned earlier. However, after the extraction time of 3, 1.5 and 1 min at 130, 260 and 390 W, respectively, the temperature of water was higher than 55.0 °C (see Figure 6.4). At this point, myrosinase might be inactivated, no further formation of sulforaphane therefore took place; previous work has reported that myrosinase is damaged at temperatures over 50 °C (Yen and Wei, 1993). In addition, decomposition of sulforaphane might occur, resulting in the decreased yield of sulforaphane.

6.3.6 Enhancement effect of MAE

The extraction yield and enhancement factor, which is defined as the ratio of the sulforaphane content extractable by MAE to that by the conventional method, at

different conditions are presented in Table 6.4. When using the conventional extraction, dichloromethane gave higher extraction yields than water. Similar results were reported by Han and Row (2011) who noted that using dichloromethane as a solvent resulted in the highest extraction yield of sulforaphane from broccoli when compared with acetonitrile and water. Sulforaphane possesses weak polarity, making it easy to be extracted by dichloromethane, which is a non-polar organic solvent (Han and Row, 2011).

The extraction yield significantly increased when applying MAE; the maximum extraction yield could be reached in a much shorter extraction time. Enhancement factors of approximately 2-2.3 and 1.2-1.4 were possible in the cases of MAE of fresh and semi-dried samples, respectively. However, there were no significant differences in the enhancement factor when considering the same sample type extracted by either dichloromethane or water. No significant differences in the extraction yield were also noted at different microwave powers.

Considering in terms of the effect of the sample type, extraction yield from the semi-dried sample was higher than that from the fresh sample in the case of conventional method. However, the results showed that similar extraction yield could be obtained using either fresh or semi-dried sample in the case of MAE; the results were the same both in the cases of using dichloromethane or water as the solvent. This implies that there is no need to prepare a sample into a semi-dried sample prior to MAE. Sulforaphane in a fresh sample could be spontaneously formed during MAE and reaches a similar level to that extractable from a semi-dried sample.

Table 6.4 Maximum extractable sulforaphane contents from fresh and semi-dried cabbages

Sample	Solvent	Extraction method	Microwave power (W)	Extraction time (min)	Extraction yield (mg/ 100 g dry mass)	Enhancement factor
Fresh	Dichloromethane	Conventional	-	30	1.2 ± 0.3^{b}	
		MAE	130	3	2.8 ± 0.5^{d}	2.3 ± 0.1^{cd}
			260	2	2.8 ± 1.0^{d}	2.2 ± 0.1^{cd}
			390	1.5	2.8 ± 1.1^{d}	2.3 ± 0.1^{cd}
Semi-dried	Dichloromethane	Conventional	-	30	2.2 ± 0.4^{c}	
		MAE	130	3	2.6 ± 0.6^{d}	1.2 ± 0.1^{a}
			260	2	2.6 ± 0.8^{d}	1.3 ± 0.1^{ab}
			390	1.5	2.6 ± 0.4^{d}	1.3 ± 0.1^{ab}
Fresh	Water	Conventional	-	30	1.1 ± 0.4^{a}	
		MAE	130	3	2.4 ± 1.1^{c}	2.1 ± 0.1^{c}
			260	1.5	2.4 ± 1.2^{c}	2.2 ± 0.1^{c}
			390	1	2.4 ± 0.8^{c}	2.1 ± 0.1^{c}
Semi-dried	Water	Conventional	-	30	1.6 ± 0.6^{b}	
		MAE	130	3	2.1 ± 0.9^{c}	1.4 ± 0.1^{b}
			260	1.5	2.1 ± 1.2^{c}	1.4 ± 0.1^{b}
			390	1	2.2 ± 1.3^{c}	1.4 ± 0.1^{b}

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