CHAPTER 3

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

3.1 Introduction

Fruits and vegetables are good sources of DF and bioactive compounds. They can be processed into many products to extend the shelf life. During processing, there are many steps involved which many lead to significant losses of antioxidants. For example, slicing leading to damage of plant cells caused higher losses of bioactive compounds. It has been also reported that water blanching exhibited the reduction of various phytochemicals content because of thermal breakdown and leaching of those compounds into blanched water (Podsędek, 2007; Volden et al., 2008). Amine and Lee (2005) reported that the antioxidant activities and total phenolic contents of red cabbage and white cabbage significantly decreased during water blanching at 98 °C. Steam blanching has been reported to be an alternative for minimizing the loss of phytochemicals into blanched water (Roy et al., 2009). Volden et al. (2008) reported that water blanching of red cabbage at 95 °C for 3 min caused significant losses of total phenols, whereas steaming caused no losses of total phenols.

Decline in antioxidants and their activities during drying has also been reported (Porkony et al., 2001; Ball, 2006). Hot air drying is the most common method for drying process. This drying technique has been reported to cause much quality degradation of a dried product, especially in terms of level of the bioactive compounds and activity. The main losses of antioxidants during hot air drying occur due to heat sensitivity and enzymatic oxidation (Katsube et al., 2008). However, many recent studies have shown that vacuum drying could provide better retention of antioxidant than hot air drying due to operating in low pressure under low oxygen environment (Suvanakuta et al., 2005; Kongsoontornkijkul et al., 2006; Gong et al., 2007).

Outer leaves of white cabbage are by-products that can be obtained during industrial preparation or selling in a consumer market. Recently, it has been reported that this by-product can be used as a raw material to produce antioxidant DF (Jongaroontaparangsee et al., 2007; Nilnakara et al., 2009). The suggested processing steps starts from slicing the leaves into size of 0.5 × 5 cm and then hot water blanching prior to hot air drying at 80 °C. However, the data on effects of pretreatment and drying methods on the evolution of antioxidants in cabbage outer leaves during processing had not yet been determined.

To improve processes to produce high antioxidant DF, the effects of various processing steps, i.e., sample preparation, which included sample slicing and blanching using either hot water or steam, as well as drying conditions and methods, i.e., hot air drying and vacuum drying at 60, 70 and 80 °C on the evolutions of antioxidants in cabbage outer leaves during preparation and drying were determined.

3.2 Materials and Methods

3.2.1 Sample Preparation

The outer leaves of cabbages (*Brassica oleracea* L. var. capitata), which were discarded, were obtained from a local market; the samples 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.

Figure 3.1 shows all possible steps for sample preparation. The blanching conditions were predetermined based on peroxidase inactivation. Blanching was performed either as hot-water blanching or steam blanching. In the former case the whole leaves of cabbage were blanched in hot water at 95 ± 2 °C for 2 min. The ratio of cabbage leaves to water was 1:7. Steam blanching, on the other hand, was carried out in a closed water bath (Heto, model AT 110, Allerod, Denmark). A single layer of whole cabbage leaves was steamed on a perforated tray suspended over boiling water for 1 min. After either hot-water or steam blanching the leaves were immediately cooled in cold water (4 °C). The blanched leaves were then sliced into smaller pieces with the dimensions of 5 × 0.5 cm (length × width).

Another set of experiments were conducted by slicing cabbage leaves into 0.5×5 cm slices prior to blanching; blanching was then carried out as described above. The prepared fresh whole leaves or fresh slices were subjected to drying within 30 min after preparation.

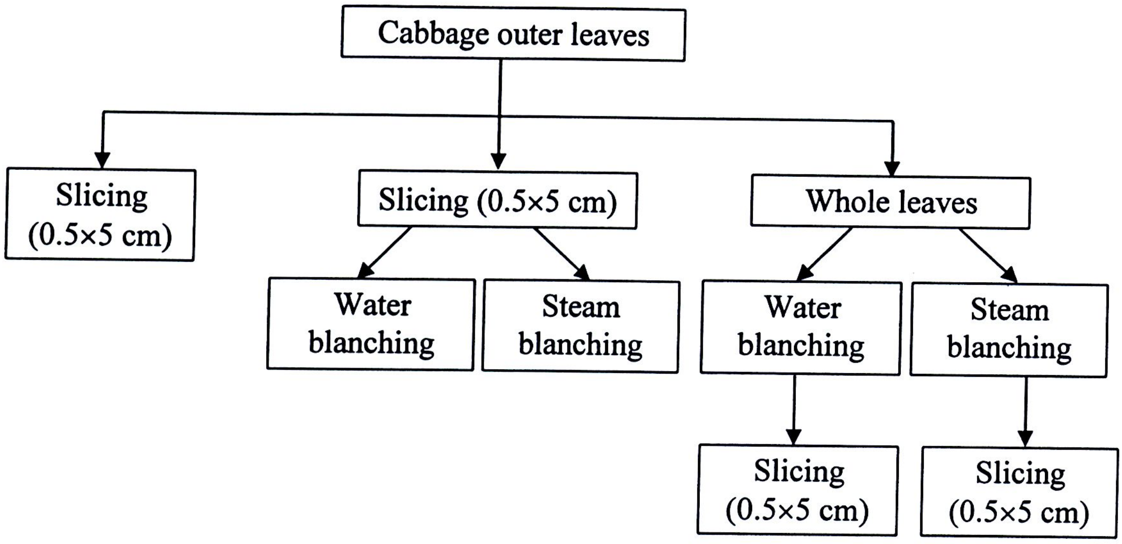


Figure 3.1 Sample preparation steps

3.2.2 Drying Experiments

The cabbages, which were prepared by the best condition obtained from 3.2.2, were subject to either hot air drying or vacuum drying. For a hot air drying experiment approximately 200 g of prepared cabbage sample was spread as a single layer on a tray. An experiment was performed using a laboratory-scale hot air oven (Termaks, TS8000, Bergen, Norway) either at 60, 70 or 80 °C. Samples (3-5 g) were taken at various time intervals to determine the moisture content following the gravimetric method at 105 °C (AOAC Method 984.25; AOAC, 2000).

For a vacuum drying experiment approximately 30 g of prepared cabbage sample was placed as a single layer on a sample holder in a vacuum dryer similar to that used by Devahastin et al. (2004). Experiments were performed at an absolute pressure of 10 kPa and temperatures of 60, 70 and 80 °C.

All samples were dried until a final moisture content of less than 10% (d.b.) was reached (Larrauri, 1999). The sample temperature was measured continuously using a type K thermocouple inserted into the thin layer of the sample. Dried sample was ground into fine powder with the particle size in the range of 150-450 μm (Larrauri, 1999).

3.2.3 Proximate Analysis

Proximate compositions including moisture content, crude protein content, crude fiber, ash and nitrogen free extract were first analyzed. The moisture content was determined by a gravimetric method at 105 °C. Crude protein content was determined by estimating the nitrogen content using the Kjedahl method. Ash content was determined by incineration at 550 °C. Crude fat was determined by the Soxhlet method and the crude fiber was assayed by acid digestion and alkali digestion. Both fresh and dried samples were analyzed for the above compositions in duplicate, in accordance with the AOAC standards (1995). Carbohydrate is defined as the residue excluding protein, lipid, crude fiber and ash (=100 - proteins - lipid - crude fiber - ash).

3.2.4 Determination of DF Compositions

An enzymatic-gravimetric procedure (AOAC Method 991.43; AOAC, 2000) was used for the determination of the DF compositions. Dried powdery sample was first gelatinized with heat-stable α-amylase (Sigma-Aldrich, Steinheim, Germany). After gelatinization the sample was digested with protease and amyloglucosidase (Sigma-Aldrich, Steinheim, Germany) to remove protein and starch in the sample. Subsequently, insoluble dietary fiber (IDF) was filtered and washed with warm distilled water. The filtrate and washed water were combined and added with 4 volumes of 95%

(v/v) ethanol at 60 °C to precipitate soluble dietary fiber (SDF). The residue was weighed after drying at 105 °C in a hot air oven (Memmert, model 800, Schwabach, Germany) until the constant mass was obtained. Ashless filter paper was used as blank. Determination of residual protein (as Kjeldahl N×6.25) and ash contents was also carried out for corresponding correction. Total dietary fiber content was calculated as a sum of SDF and IDF. The percentage of SDF and IDF was calculated by:

$$\%\mathrm{SDF}, \%\mathrm{IDF} = \frac{\left((\mathrm{R}-\mathrm{P}-\mathrm{A})-\mathrm{B}\right)\times 100}{\mathrm{S}}$$

where R, P, A, B and S are mass of residue (g), protein (g), ash (g), blank (g) and sample (g), respectively.

3.2.5 Determination of Total Phenolic Content (TPC)

The total phenolic content was determined using Folin-Ciocalteu reagent (Yu et al., 2005). Five grams of each sample was shaken in a shaker (New Brunswick Scientific, model Innova 4230, Edison, NJ) with 50 mL of acetone-water solution (1:1, v/v) at 120 rpm for 15 h at ambient temperature (~30 °C). The acetone extract was filtrated and kept in dark at room temperature until further analysis. Fifty µL of the sample extract was diluted with 3 mL of distilled water; 250 µL of Folin-Ciocalteu reagent and 0.75 mL of 20 g/mL sodium carbonate solution were then added. The absorbance was measured at 765 nm using a UV-vis scanning spectrophotometer (Shimadzu, model UV 21101 PC, Kyoto, Japan) after 2 h. Estimation of TPC was carried out using gallic acid (Sigma Aldrich, St. Louis, MO) as a standard. The result was expressed as mg gallic acid equivalent per one hundred g of sample (dry basis).

3.2.6 Determination of Vitamin C

The vitamin C analysis method was a modification of that suggested by AOAC (2000). Three grams of fresh sample or one gram of dried sample was homogenized in a blender (Waring, model SS110, Torrington, CT) with 50 mL of 3% metaphosphoric acid. The slurry was adjusted to 100 mL with 3% metaphosphoric acid and filtered through 0.45 μm nylon filter. Ten μL of filtrate was then injected into Atlantis™ dC_{18} 5 μL (4.6×150 mm) HPLC column (Waters, Milford, MA). The HPLC system consists of a pump and controller (Waters, moldel 600, Milford, MA), a tunable absorbance detector (Waters, model 486, Milford, MA) and an autosampler (Waters, model 717 plus, Milford, MA). The mobile phase was 0.2 M KH_2PO_4 (pH 2.4) at a flow rate of 0.5 mL/min. A UV detector at a wavelength of 254 mm was used for detecting vitamin C. The mobile phase was degassed using an ultrasonic generator. Quantification of vitamin C was carried out based on a vitamin C standard curve.

3.2.7 Determination of β-carotene

Analysis of the total amount of β-carotene was performed following a modification of the method described by Kurilich et al. (1999). Fresh cabbage (1000 mg) or dried cabbage (600 mg) was ground for 1 min using a stainless steel pulverizer (Waring, model SS110, Torrington, CT). The ground sample was then placed in a test tube filled with 10 mL of ethanol and 0.1 g of butylated hydroxytoluene before heating in water bath at 50 °C for 15 min. Subsequently, 180 μL of 80% KOH was added to saponify the heated sample at 70 °C for 30 min; the sample was cooled down immediately to 0 °C. De-ionized water and 2.5 mL of hexane:toluene mixture (10:8) was then added. The tube was vortexed for 20 s and centrifuged at 2100 rpm for 5 min. The upper layer was removed to a different tube. The sample was re-extracted twice and combined with the first extract. Aliquot of the extract was concentrated using a rotary evaporator (Resona Technics, LaboRota S-300, Gossau, Switzerland) at 50 °C and subsequently reconstituted with 3 mL of acetone. The extract was filtered through a 0.2 μm filter before being injected into Symmetry® C_{18} 5 μL (3.9 ×150 mm) HPLC column (Waters, Milford, MA). The mobile phase was methanol and acetronitrile (90:10) and its flow rate was set at 1.5 mL/min. A UV spectrophotometer, operated at a wavelength of 450 nm, was used for detecting β-carotene. Quantification of β-carotene was carried out based on a β-carotene (Fluka, Buchs, Switzerland) standard curve.

3.2.8 Determination of α-tocopherol

α-tocopherol in each sample was extracted and analyzed following the same procedure as that for β-carotene (Kurilich et al., 1999). A UV-vis scanning spectrophotometer (Shimadzu, model UV 21101 PC, Kyoto, Japan) at a wavelength of 290 nm was used for determination of the α-tocopherol content. Quantification of α-tocopherol was carried out based on an α-tocopherol (Sigma Aldrich, St. Louis, MO) standard curve.

3.2.9 Total Antioxidant Activity

3.2.9.1 Preparation of Extract for Antioxidant Activity Determination

Ten grams of each homogenized sample was shaken with 90% ethanol in a shaker (New Brunswick Scientific, model Innova 4230, Edison, NJ) at 120 rpm for 6 h and then filtered through Whatman No.1 filter paper. The sample extract was then concentrated in a rotary evaporator (Resona Technics, LaboRota S-300, Gossau, Switzerland) at 50 °C for 5 min and then made up to 5 mL by 90% ethanol. The obtained extract was stored in amber colored air-tight container at -20 °C until subsequent analysis.

3.2.9.2 Free Radical Scavenging Activity (DPPH assay)

This assay is based on the scavenging ability of 2,2-diphenyl-2-picryl-hydrazyl (DPPH·) radicals by the radical scavenging constituents in the samples. Free radical scavenging activity of a sample was determined using the method described by Turkmen et al. (2005) with some modifications. An aliquot of 1.5 mL of 0.2 mM DPPH radical in methanol was added to a test tube with 0.5 mL of the prepared sample extract. The reaction mixture was vortex-mixed for 30 s and left to stand at room temperature in dark for 30 min. The absorbance was measured at 517 nm using a UV-vis scanning spectrophotometer (Shimadzu, model UV 21101 PC, Kyoto, Japan). Pure ethanol was used to calibrate the spectrophotometer. The absorbance of aliquot of 1.5 mL of 0.2 mM DPPH radical in methanol as control was determined. The total antioxidant activity (TAA) was expressed as the percentage of inhibition of DPPH radical and was determined by the following equation:

$$\%\mathrm{TAA} = \frac{\mathrm{Abs}_{\mathrm{control}} - \mathrm{Abs}_{\mathrm{sample}}}{\mathrm{Abs}_{\mathrm{control}}} \times 100$$

where TAA is the total antioxidant activity and Abs is the absorbance.

3.2.9.3 β-carotene Bleaching Assay

Antioxidant activity of each sample was measured according to the method described by Amin et al. (2006). The measured antioxidant activity is based on the ability to decrease the oxidative bleaching on β-carotene in β-carotene/linoleic acid emulsion. Two mg of β-carotene powder (AR Grade, Fluka) was dissolved in chloroform (20 mL). An aliquot (5 mL) of the solution was added with linoleic acid (20 mL) and Tween 40 (200 mL). Chloroform was removed using a stream of nitrogen gas; the mixture was made up to 100 mL with oxygenated water. An aliquot of the β-carotene-linoleic acid emulsion was mixed with 40 µL of the sample extract and placed in water bath at 50 °C for 120 min. The absorbance of the oxidized emulsion was measured using a UV-vis scanning spectrophotometer (Shimadzu, model UV 21101 PC, Kyoto, Japan) at 470 nm. Distilled water was used as control instead of the sample extract. All samples were assayed in triplicate. The total antioxidant activity (TAA) was then calculated using the following equation:

$$\%\mathrm{TAA} = \left[1 - \frac{A_s^0 - A_s^{120}}{A_c^0 - A_c^{120}}\right] \times 100$$

where TAA is the total antioxidant activity. A_s^0 and A_s^{120} are the absorbance of the sample at $t = 0$ min and $t = 120$ min, respectively. A_c^0 and A_c^{120} are the absorbance of the sample at $t = 0$ min and $t = 120$ min, respectively.

3.2.10 Statistical Analysis

The experiments were designed in complete random. The data were analyzed and presented as mean values with standard deviations. Differences between mean values were established using Duncan's multiple range tests. Values were considered at a confidence level of 95%. All statistical analyses were performed using SPSS® program (version 17). All experiments were performed in duplicate unless specified otherwise.

3.3 Results and Discussion

3.3.1 Effects of Preparation and Blanching Methods on Proximate and DF Compositions

Table 3.1 shows the proximate compositions of fresh and blanched cabbage outer leaves. The results illustrated that cabbage outer leaves contained lower amounts of protein, lipid, ash and carbohydrates, implying that cabbage outer leaves possess low caloric value and is good for health. Nevertheless, it was found that the contents of protein and lipid increased, while the content of ash decreased after water blanching; steam blanching, however, did not lead to any significant changes of the chemical compositions. This may be due to the losses of such substances as minerals, vitamins and sugars to the blanching water, resulting in the changes of the total solids, which resulted in an increase in the contents of protein and lipid on a dry basis (Wennberg et al., 2006; Nilnakara et al., 2009).

The amounts of IDF, SDF and TDF in fresh and blanched samples as well as the ratio of IDF to SDF are given in Table 3.2. The results showed that cabbage outer leaves contained high amount of DF and the ratio of SDF:IDF was 1:4.6. The values obtained here were in agreement with those reported by Jongaroontaprangsee et al. (2007). It is also seen that the preparation and blanching methods did not have any significant effect on IDF, SDF and TDF. This finding is consistent with the results of Margarita and Nyman (2003) who reported that the DF compositions in carrot, green pea and Brussels sprout were unaffected by both water blanching and boiling.

Table 3.1 Proximate compositions of cabbage outer leaves

Sample	Composition (g/100 dry basis)			
	Protein	Lipid	Ash	Carbohydrates
Fresh	18.43 ± 0.63^a	1.02 ± 0.11^a	9.02 ± 0.90^b	30.64 ± 0.99^a
Slicing/water blanching	22.06 ± 0.52^b	1.54 ± 0.13^b	5.56 ± 0.58^a	32.62 ± 0.75^a
Slicing/steam blanching	19.16 ± 0.38^a	1.27 ± 0.26^a	8.25 ± 0.39^b	33.11 ± 1.21^a
Water blanching/slicing	21.02 ± 0.49^b	1.42 ± 0.28^b	6.29 ± 0.72^a	32.33 ± 0.82^a
Steam blanching/slicing	18.64 ± 0.66^a	1.18 ± 0.47^a	7.93 ± 0.48^b	34.43 ± 0.64^a

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

Table 3.2 Dietary fiber contents (g/100 dry basis) of cabbage outer leaves

Sample	IDF	SDF	TDF	SDF:IDF
Fresh	33.54 ± 1.44^a	7.35 ± 0.81^a	40.89 ± 2.25^a	1:4.56
Slicing/water blanching	31.31 ± 0.86^a	7.91 ± 0.96^a	39.22 ± 1.13^a	1:3.95
Slicing/steam blanching	32.07 ± 1.02^a	7.14 ± 0.58^a	39.21 ± 1.79^a	1:4.49
Water blanching/slicing	31.64 ± 0.67^a	7.70 ± 0.74^a	39.94 ± 1.86^a	1:4.11
Steam blanching/slicing	32.91 ± 1.23^a	7.31 ± 0.67^a	40.22 ± 2.02^a	1:4.50

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

3.3.2 Effects of Preparation and Blanching Methods on Antioxidants

Table 3.3 shows the amounts of antioxidants of interest in fresh cabbage outer leaves. The amount of phenolic compounds, vitamin C, β-carotene and α-tocopherol were found to be higher than those reported in previous studies (Kurilich et al., 1999; Podsędek, 2005; Singh et al., 2006). The higher amounts of antioxidants might be due to the use of different parts of cabbage; outer leaves of cabbage were used in this study, whereas inner leaves (typical edible part) were used in other studies (Kurilich et al., 1999; Podsędek, 2005; Singh et al., 2006). Kalt (2005) indeed reported that cabbage outer leaves had darker green color than inner leaves due to more exposure to sunlight of the former; this led to larger amounts of color compounds such as chlorophyll and β-carotene in the outer leaves. Vrchovská et al. (2006) also found that external leaves of tronchuda cabbage exhibited higher amounts of phenolic compounds and ascorbic acid. These findings indicated that outer part of cabbage leaves have more potential to be produced into antioxidant DF.

Table 3.3 Antioxidants in fresh cabbage outer leaves

Antioxidant	Content	Unit
Total phenolics	739.24±37.25	mg GAE/100 dry weight
Vitamin C	639.55±21.21	mg/100 g dry weight
β-carotene	9.44±0.13	mg/100 g dry weight
α-tocopherol	5.45±0.09	mg/100 g dry weight
Glucosinolates	1583.22 ± 69.06	μmol/100 g dry basis

Table 3.4 presents the total phenolic contents (TPC) of the samples after different preparation steps. The results showed that steam blanching led to better retention of the TPC than water blanching. This is due to the shorter blanching time; loss of water-soluble constituents with the blanched medium was also naturally less in the case of steam blanching. The results were in agreement with those of Volden et al. (2008) who reported that steaming resulted in no change of the TPC in cauliflower, whereas water blanching caused a 21% reduction in the TPC. It was also observed that slicing prior to blanching caused higher loss of the TPC. Slicing might cause a damage to plant tissues, thus allowing exposure of enzymes to their substrates as well as of inner cells to oxygen and heat. Steam blanching prior to slicing, on the other hand, led to the highest retention of TPC. As mentioned earlier, blanching the whole leaves using steam could reduce the loss of TPC. Furthermore, steaming could provide faster heat penetration into plant cells, hence the browning enzymes were inactivated in shorter time. This would then result in less loss of TPC by thermal degradation. In addition, as enzymes using phenolic compounds as substrates were already destroyed during blanching, there was no further loss of TPC due to enzyme activities during slicing. Therefore, the highest retention of TPC obtained was a result of the combination of the phenomena described above.

Table 3.4 Effect of preparation on retention of TPC

Sample	TPC (mg GAE/100 dry weight)	% Retention
Fresh	739.24±37.25^{b}	N/A
Slicing/water blanching	496.92±22.66^{a}	56.49±1.35^{a}
Slicing/steam blanching	652.43±38.55^{b}	89.07±3.24^{c}
Water blanching/slicing	505.06±43.51^{a}	66.76±1.75^{b}
Steam blanching/slicing	719.70±15.85^{b}	95.41±4.84^{c}

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

The concentrations of vitamin C in the samples after water and steam blanching are presented in Table 3.5. It was observed that steam blanching prior to slicing led to the highest retention of vitamin C (95.24%); this was followed by slicing prior to steam blanching (86.38%), water blanching prior to slicing (77.54%) and slicing prior to water blanching (65.71%). Steam blanching led to better retention of vitamin C than water blanching in all cases. Volden et al. (2008) also reported that the retention of vitamin C in red cabbage after water blanching (52.3%) was lower than that after steaming (88.7%). Slicing the leaves prior to blanching also led to higher loss of vitamin C due to the reasons already stated above.

Table 3.5 Effect of preparation on retention of vitamin C

Sample	Vitamin C (mg/100 g dry weight)	% Retention
Fresh	639.55±21.21^{cd}	N/A
Slicing/water blanching	432.89±14.58^{a}	65.71±4.47^{a}
Slicing/steam blanching	565.12±14.73^{c}	86.38±1.13^{b}
Water blanching/slicing	511.84±17.80^{b}	77.54±0.99^{c}
Steam blanching/slicing	628.66±13.64^{c}	95.24±0.19^{d}

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

Tables 3.6 and 3.7 list the β-carotene and α-tocopherol contents in cabbage leaves after preparation. The results showed that preparation steps did not have a significant effect on the retentions of β-carotene and α-tocopherol. These were probably due to the fact that the two antioxidants are lipid-soluble; therefore, losses with the blanched water were not significant (Pokorny et al., 2001; Gliszczyńska-Świglo et al., 2006; Podsędek, 2007). The results also indicated that β-carotene and vitamin E were more heat stable than phenolic compounds and vitamin C. Losses due to thermal degradation were not observed. The results were in agreement with the previous works (Khachik et al., 1992; Gliszczyńska-Świglo et al., 2006). Although slicing might led to the direct exposure of β-carotene or α-tocopherol to oxygen and light as well as the remaining lipoxygenase, this effect was not significant.

Table 3.6 Effect of preparation on retention of β-carotene

Sample	β-carotene (mg/100 g dry weight)	% Retention
Fresh	9.44±0.13[a]	N/A
Slicing/water blanching	9.33±0.39[a]	98.83±2.23[a]
Slicing/steam blanching	9.46±0.47[a]	100.21±0.94[a]
Water blanching/slicing	9.70±0.16[a]	100.27±0.48[a]
Steam blanching/slicing	9.71±0.43[a]	100.35±0.70[a]

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

N/A = not applicable.

Table 3.7 Effect of preparation on retention of α-tocopherol

Sample	α-tocopherol (mg/100 g dry weight)	% Retention
Fresh	5.45±0.09[a]	N/A
Slicing/water blanching	5.41±0.17[a]	99.22±1.56[a]
Slicing/steam blanching	5.36±0.17[a]	98.98±1.79[a]
Water blanching/slicing	5.39±0.11[a]	98.87±0.89[a]
Steam blanching/slicing	5.36±0.05[a]	98.30±1.28[a]

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

N/A = not applicable.

The total antioxidant activity (TAA), as measured by the DPPH radical scavenging activity assay and the β-carotene bleaching assay, is presented in Table 3.8. The results showed that the TAA of all samples decreased significantly after water and steam blanching. Steam blanching resulted in higher retention of TAA than water blanching, as expected. It was also observed that blanching prior to slicing could better retain the TAA of the leaves.

By considering the whole process steam blanching the whole leaves of cabbage and then slicing prior to drying is recommended for the production of high DF from cabbage outer leaves as this combination would lead to the highest retention of phytochemicals as well as the total antioxidant activity of the final product.

Table 3.8 Effect of preparation on total antioxidant activity using DPPH radical scavenging activity assay and β-carotene bleaching assay

Sample	DPPH	β-carotene bleaching assay
Fresh	82.94±1.52^{b}	79.20±2.12^{b}
Slicing/water blanching	76.52±0.46^{a}	74.86±1.44^{a}
Slicing/steam blanching	79.05±0.83^{b}	77.88±0.65^{b}
Water blanching/slicing	76.68±1.18^{a}	75.42±0.81^{ab}
Steam blanching/slicing	80.82±1.25^{b}	78.38±0.56^{b}

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

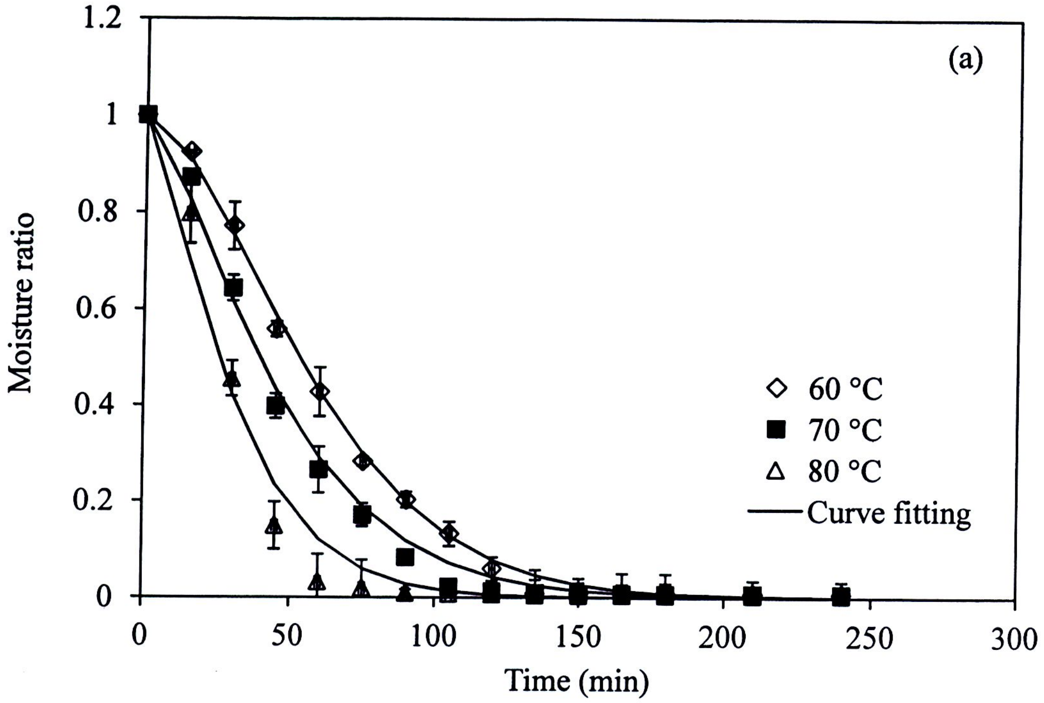
3.3.3 Effects of Drying Conditions and Methods on Antioxidants

After steam blanching and slicing (suitable preparation method suggested in Section 3.2) the cabbage samples were subject to either hot air or vacuum drying. The moisture content of the leaves prior to drying was approximately 7.55 ± 0.93 g/g dry basis. Higher moisture content of approximately 10.40 ± 0.184 g/g dry basis was observed for the samples having undergone steam blanching.

Figure 3.2 shows the drying curves of steam blanched samples undergoing hot air drying and vacuum drying at different drying temperatures. To describe the drying behavior of cabbage during hot air and vacuum drying, moisture ratio (MR) of sample was fitted to Page's equation as follows:

$$MR = \frac{M_{t} - M_{eq}}{M_{i} - M_{eq}} = \exp(-Kt^{N})$$

where MR is the moisture ratio; M_t is the moisture content at time t (kg/kg); M_{eq} is the equilibrium moisture content (kg/kg); M_i is the initial moisture content (kg/kg); K is the drying constant; N is the degree of nonlinearity of the drying curve.



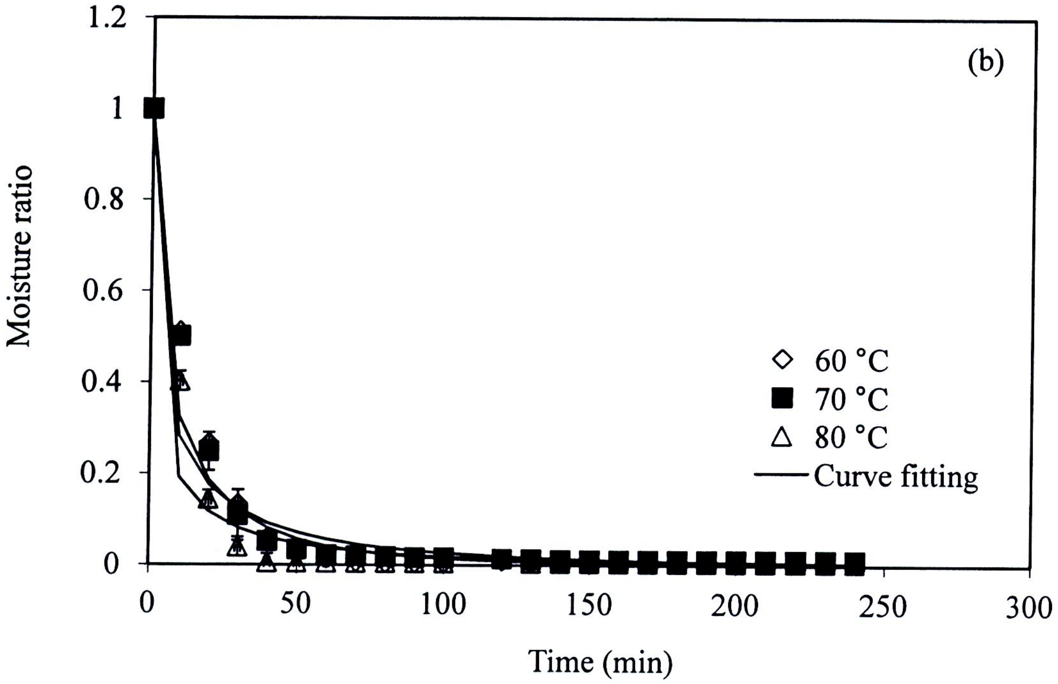


Figure 3.2 Drying kinetics of steam blanched samples undergoing (a) hot air drying and (b) vacuum drying

Table 3.9 shows drying parameters obtained by fitting Page's equation to experimental data. Excellent agreement with R^2 in the range of 0.950–0.998 was found between fitted and experimental values. It was observed that *K* value increased as drying temperature was higher. This was due to drying at higher temperatures results in higher moisture diffusivity values and larger driving force for heat and mass transfer than at lower temperatures. The results also showed that at the same drying temperature *K* values for vacuum drying were higher than hot air drying. This is because lowering the absolute pressure of the drying chamber (10 kPa) led to reduced boiling point of water, resulting in accelerated moisture migration from the samples. For *N* value, no relationship between *N* values and drying temperature was found in this study. Similar results were noted by Methakhup et al. (2005) who reported that there was not clear trend between *N* values and drying temperature. The equilibrium moisture contents and time needed to reach the desired moisture content of less than 0.1 g/g dry basis at various drying conditions are given in Table 3.10.

Table 3.9 Parameters of Page's equation

Drying	Temperature (°C)	Page's parameter		R^2
		K ($\times 10^{-4}$ min^{-1})	N	
Hot air	60	0.12	1.59	0.998
	70	0.48	1.35	0.996
	80	0.79	1.37	0.987
Vacuum	60	29.42	0.58	0.965
	70	43.99	0.46	0.951
	80	67.99	0.38	0.950

Table 3.10 Time to dry cabbage outer leaves to approximately 0.1 g/g dry basis as well as their equilibrium moisture contents

Drying	Temperature (°C)	Drying time (min)	Equilibrium moisture content (g/g dry basis)
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

The evolutions of selected antioxidants during hot air drying and vacuum drying are presented in Figures 3.3 and 3.4. The results showed that contents of all antioxidants decreased gradually during drying. It was also observed that higher drying temperature led to higher losses of antioxidants due to thermal degradation. Kuljarachanan et al. (2008) reported that higher reduction of TPC and vitamin C in lime residues was observed at higher drying temperature. Scalar and Crapiste (2008) also reported that rate of degradation of β-carotene and vitamin C in red pepper was higher when drying at higher temperature.

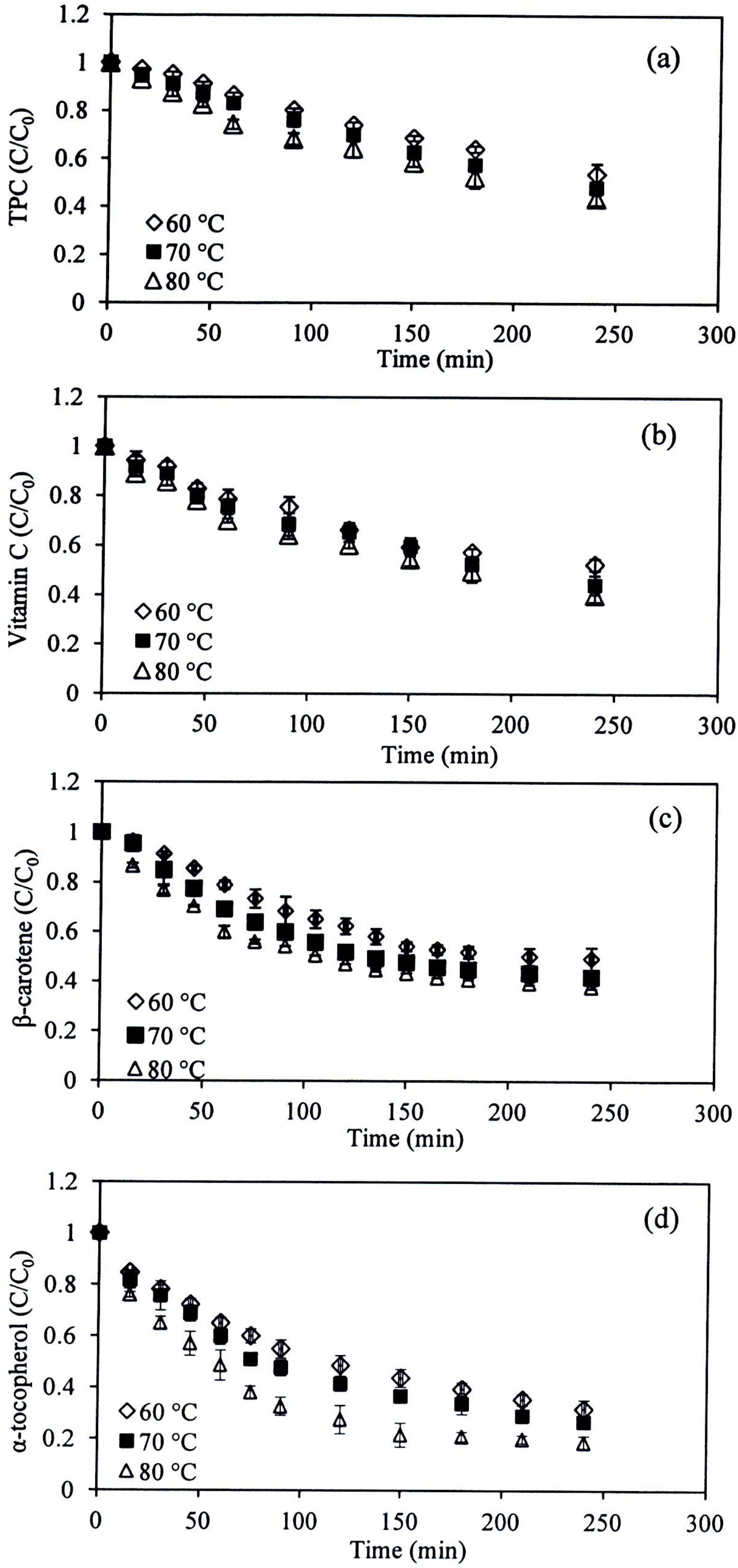


Figure 3.3 Evolution of (a) total phenolic content; (b) vitamin C; (c) β-carotene; (d) α-tocopherol during hot air drying

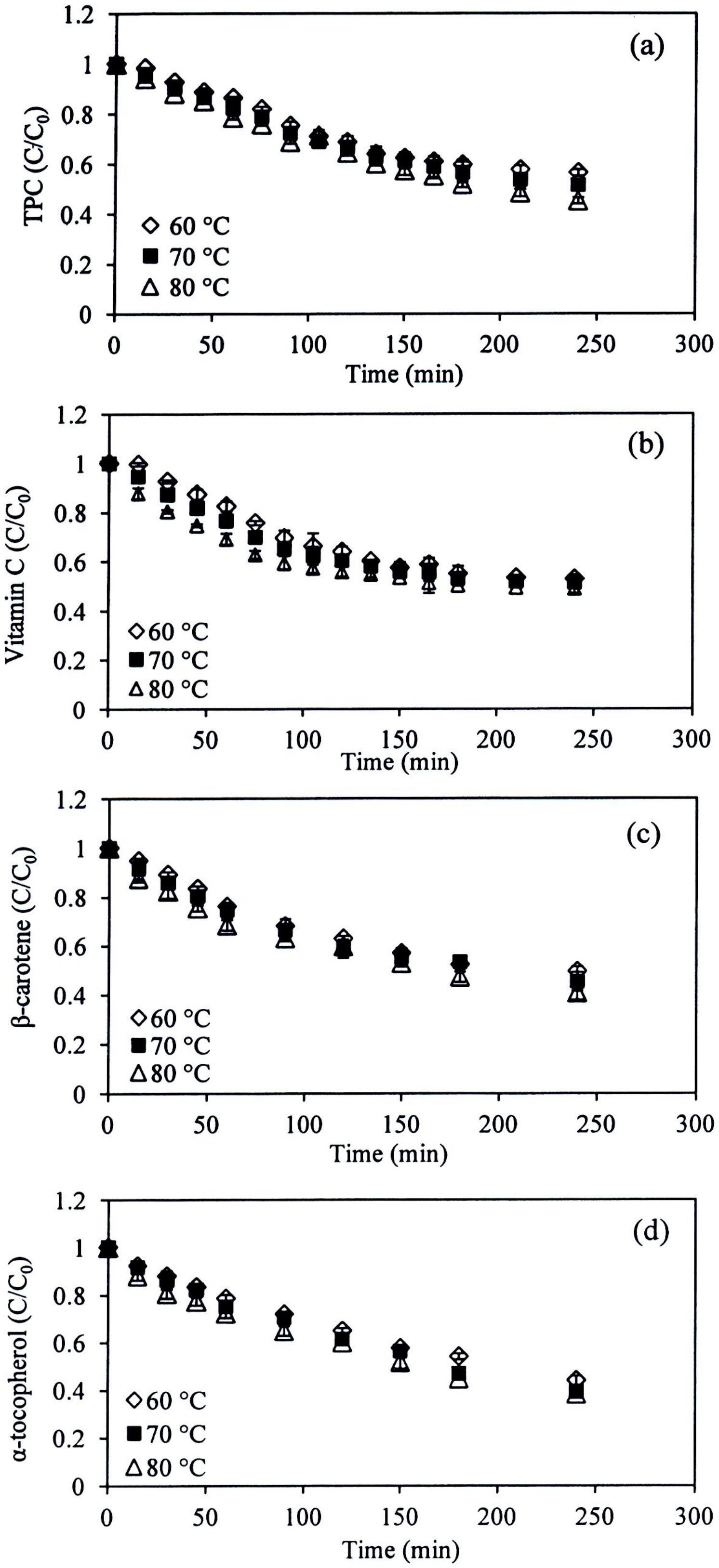


Figure 3.4 Evolution of (a) total phenolic content; (b) vitamin C; (c) β-carotene; (d) α-tocopherol during vacuum drying

Figures 3.5 and 3.6 presents the retention of total antioxidant activity (TAA), as measured by the DPPH radical scavenging assay and β-carotene bleaching assay, during hot air drying and vacuum drying. The results showed that the TAA of all samples decreased gradually during drying for both drying methods. A reduction of TAA was higher at higher drying temperature. It was also observed that the loss of TAA was in accordance with the loss of phenolic compounds. The results imply that phenolic compounds might play an important role on the antioxidant activity of the DF. This finding was consistent with Nilnakara et al. (2009) who reported that phenolic compound was a major contributor to TAA of DF produced from cabbage outer leaves. Table 3.11 summarizes the retention of antioxidants in DF powder. It is seen that vacuum drying provided better retention of each antioxidants and their activities in DF powder than hot air drying. This might be because smaller losses of antioxidants via oxidative reaction occurred during vacuum drying. These results are similar to those reported by Vashisth et al. (2011) who reported that hot air drying caused higher losses of phenolic compounds than vacuum drying due to the oxygen-deficient environment of the latter process. The drying rate of vacuum drying was also noted to be higher than that of hot air drying resulting in the shorter time to reach the desired moisture content, hence lower thermal degradation of phenolic compounds.

No differences in terms of the retention of antioxidants were observed at different drying temperatures. Generally, higher degradation rate was observed at higher drying temperature. However, shorter drying time was required to dry the sample at a higher temperature. Therefore, both drying temperature and time had an effect on the retention of antioxidants. Garau et al. (2007) reported that drying at 30 °C resulted in a decrease of phenolic compounds and antioxidant activity in orange than that at 50, 60 and 70 °C.

This could be because drying at low temperature led to longer time of sample exposure to heat, light and oxygen.

By considering the whole process steam blanching the whole leaves of cabbage and then slicing prior to vacuum drying at 80 °C is recommended for the production of high DF from cabbage outer leaves as this combination would lead to the highest retention of antioxidants as well as the total antioxidant activity of the final product.

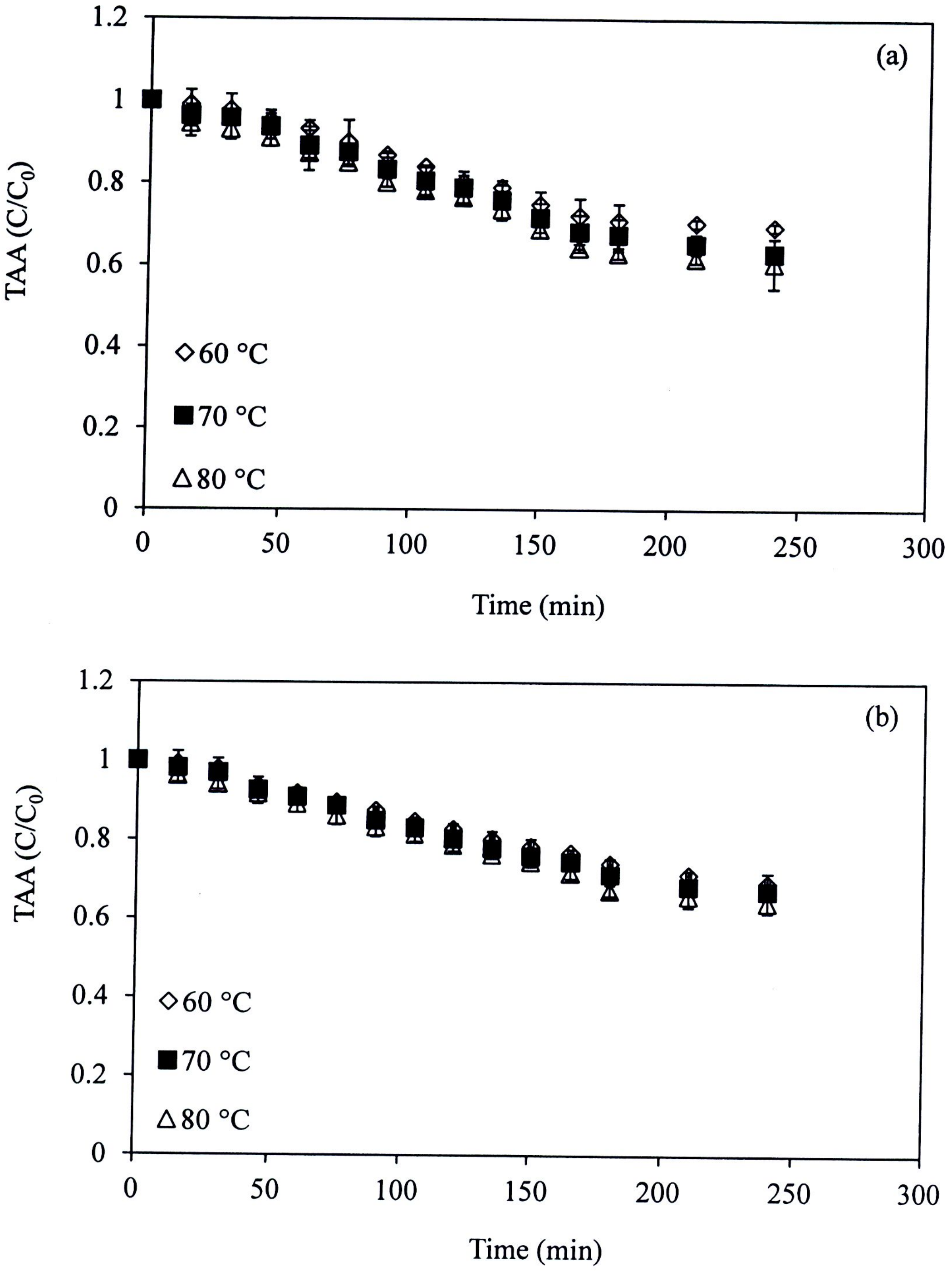
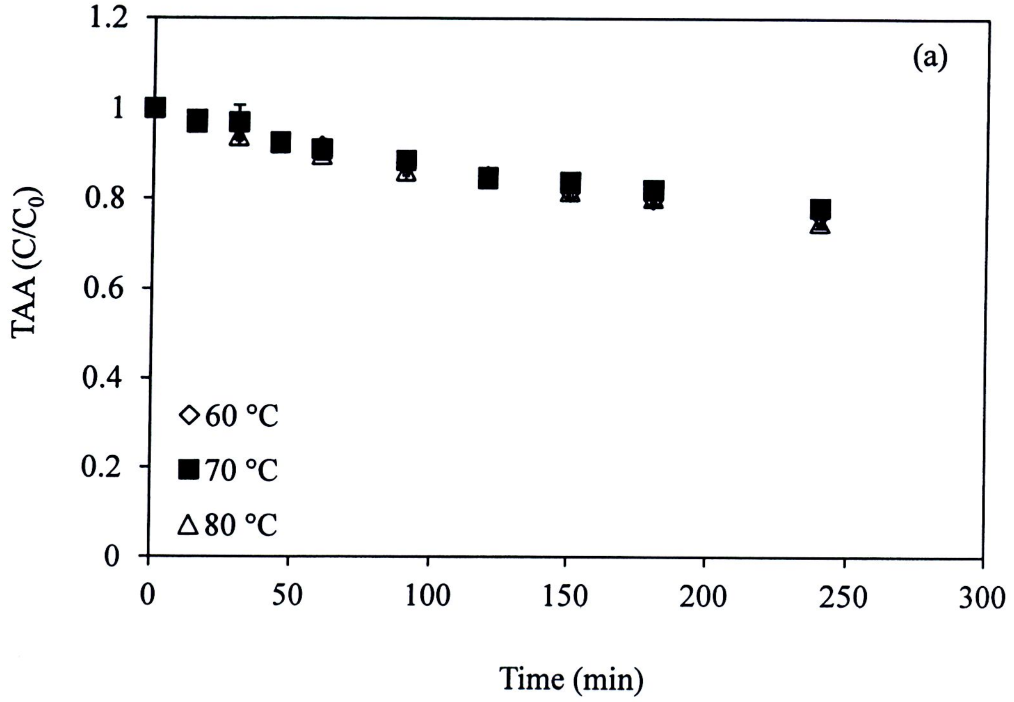


Figure 3.5 Evolution of total antioxidant activity during drying as assessed by (a) DPPH assay and (b) β-carotene bleaching assay during hot air drying



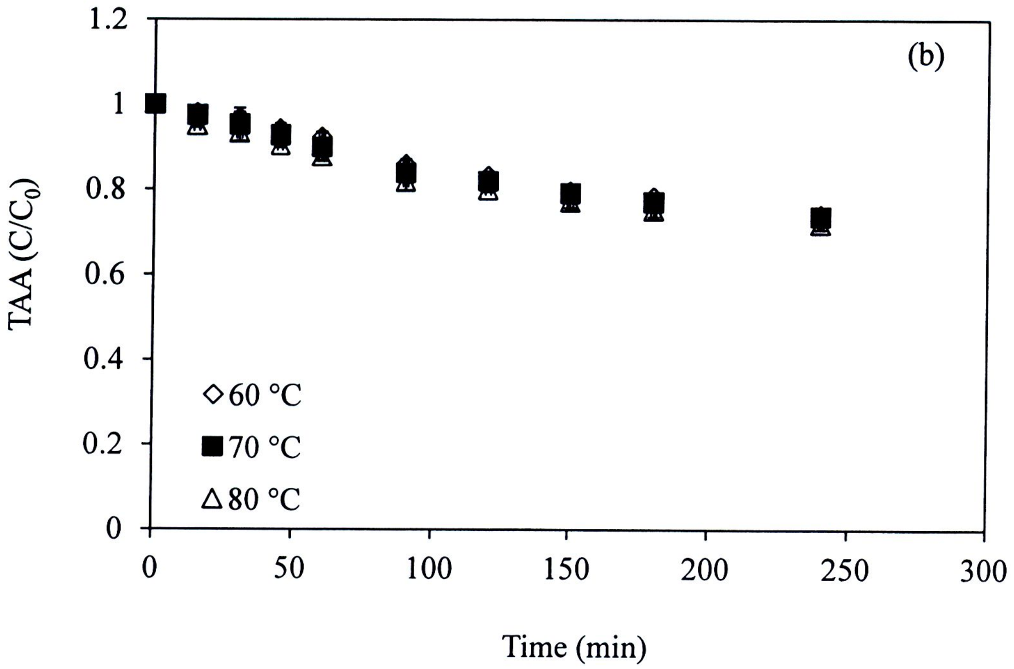


Figure 3.6 Evolution of total antioxidant activity during drying as assessed by (a) DPPH assay and (b) β-carotene bleaching assay during hot air drying

Table 3.11 Retention of antioxidants in DF and their activity. Comparison was made with fresh leaves

Drying method	Temperature (°C)	TPC	Vitamin C	β-carotene	α-tocopherol	TAA (%)	
						DPPH	β-carotene bleaching
Hot air drying	60	57.60 ± 1.13^{b}	54.99 ± 4.41^{b}	51.86 ± 1.34^{b}	44.50 ± 0.81^{b}	74.16 ± 3.53^{cd}	71.00 ± 3.53^{c}
	70	55.31 ± 1.90^{b}	55.50 ± 1.89^{b}	52.14 ± 1.01^{b}	47.12 ± 2.36^{b}	$71.12.\ \pm1.93^{cd}$	69.08 ± 1.93^{bc}
	80	42.87 ± 0.43^{a}	49.33 ± 2.62^{a}	50.57 ± 1.32^{a}	33.50 ± 4.38^{a}	67.98 ± 0.12^{b}	68.28 ± 0.94^{b}
Vacuum drying	60	67.68 ± 1.44^{c}	59.73 ± 1.89^{c}	58.35 ± 1.32^{c}	57.42 ± 2.58^{b}	84.65 ± 2.19^{e}	79.34 ± 2.67^{e}
	70	69.40 ± 0.69^{c}	59.70 ± 0.69^{c}	59.57 ± 1.35^{c}	61.26 ± 4.51^{d}	85.03 ± 1.05^{e}	81.58 ± 3.39^{ef}
	80	68.39 ± 1.04^{c}	63.31 ± 3.53^{c}	62.32 ± 3.20^{c}	60.10 ± 2.65^{d}	86.72 ± 2.73^{ef}	82.94 ± 2.22^{ef}

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