Evolution of antioxidants in dietary fiber powder produced from white cabbage outer leaves: effects of blanching and drying methods

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Abstract White cabbage outer leaves, which are usually discarded both during industrial processing and selling in a consumer market, have proven to be a good source of dietary fiber (DF) as well as antioxidants and can be effectively transformed into functional DF powder. In the past, however, only hot air drying was used to prepare DF powder from cabbage outer leaves although it is well recognized that this drying technique could lead to much quality degradation of a dried product. This work was therefore aimed at studying the evolution of selected important antioxidants, e.g., phenolic compounds and vitamin C, in white cabbage outer leaves during vacuum drying at 60, 70 and 80 °C. The effect of pretreatment viz. steam blanching was also evaluated. The results showed that there were losses of antioxidants during steam blanching; vacuum dried blanched leaves nevertheless contained higher antioxidant contents and activity than dried unblanched leaves. Losses of antioxidants during vacuum drying were also noted to be less than those during hot air drying. Overall, the results showed that there were no differences in the antioxidants retention among all the tested conditions performed in this study. Therefore, the most suitable condition for the production of antioxidant DF powder from cabbage outer leaves is vacuum drying at 80 °C as this condition requires the minimum specific energy consumption.

Keywords *Brassica* vegetables · Dietary fiber · Phenolic compounds · Steaming · Vacuum drying · Vitamin C

Introduction

Use of by-products from fruit and vegetable processing plants is currently of much interest, both from the waste reduction and product value addition points of view. The latter is possible because these by-products contain various nutritional substances, including dietary fiber (DF) and other bioactive compounds.

White cabbages (*Brassica oleracea* var. capitata) are a good source of DF (Wennberg et al. 2006) and various beneficial phytochemicals including phenolics, vitamin C, β -carotene and tocopherols (Kurilich et al. 1999; Singh et al. 2007; Nilnakara et al. 2009; and Tanongkankit et al. 2010). Recently, outer leaves of white cabbages, the residues that are usually discarded during industrial processing and selling in a market, have been reported to have potential to be transformed into functional DF powder. Our previous works have indeed shown that DF powder from outer leaves of white cabbages contains 41–43 % of total dietary fiber (dry basis) (Jongaroontaprangsee et al. 2007; Tanongkankit et al. 2010); the DF powder exhibits high water holding and swelling capacities (Jongaroontaprangsee et al. 2007), making it suitable for food formulation. The DF powder from white cabbage outer leaves was also noted to contain high contents of phenolics, and vitamin C (Nilnakara et al. 2009; Tanongkankit et al. 2010) and small amounts of β -carotene and α -tocopherol (Tanongkankit et al. 2010). In addition, the powder was noted to contain significant amounts of glucosinolates and sulforaphane, which are anticarcinogenic substances (Tanongkankit et al. 2011a, b).

A typical process for the production of DF powder starts from washing a raw material and then cutting it into small pieces, which are then subject to a drying process (Larrauri 1999; Lario et al. 2004). Pretreatment, e.g., blanching and/or

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soaking in chemical solutions, may be required to improve the powder quality, both in terms of the functional properties and color (Chantaro et al. 2008; Wuttipalakorn et al. 2009).

Drying is also an important step to produce DF powder. Although hot air drying is most commonly applied to produce DF powder, it is well recognized that this drying technique leads to much quality degradation, especially in terms of the functional properties of a product. Tanongkankit et al. (2010), for example, studied the effect of hot air drying at various temperatures (60–80 °C) on the quality of DF powder produced from cabbage outer leaves and noted that the contents of phenolics, vitamin C, β -carotene, α -tocopherol as well as the total antioxidant activity of the powder decreased quite significantly upon hot air drying. This is probably due to the lengthy required drying time and the oxygen-rich nature of the process (Kumar and Sagar 2012; Verma et al. 2013). For this reason vacuum drying could be an alternative for producing high-quality DF powder. Vacuum drying not only decreases the oxygen concentration in the drying chamber but also helps lower the absolute pressure of the drying chamber. Lowering the absolute pressure leads to reduced boiling point of water, resulting in accelerated moisture migration from a sample.

The present study was aimed at studying the evolution of various antioxidants in white cabbage outer leaves during vacuum drying at 60–80 °C. The effect of steam blanching, which is a recommended pretreatment step by Tanongkankit et al. (2010), prior to further processing and vacuum drying was also evaluated.

Materials and methods

Chemicals

Gallic acid standard and 2,2-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma Aldrich (St. Louis, MO). Folin-Ciocalteu reagent and metaphosphoric acid were purchased from Carlo Erba (Milan, Italy). L-ascorbic acid standard was purchased from Riedel-de Haën (Seelze, Germany). Linoleic acid and β -carotene standard were purchased from Fluka (Buchs, Switzerland). All chemicals were of analytical grade. Methanol, acetone, chloroform and acetonitrile (HPLC grade) were purchased from Lab-Scan Analytical Sciences (Bangkok, Thailand).

Sample preparation

The outer leaves of white cabbages (*Brassica oleracea* var. capitata), which were discarded, were obtained from a specific seller at Pakklong Talad Market, Bangkok; the fresh cabbages were grown in Omkoi, Chiang Mai Province. The leaves were kept at 4 °C until the time of an experiment. Prior to each experiment the leaves were washed under running tap

water and drained on a screen to get rid of excess water. The moisture content of a fresh sample was approximately 7.55 ± 0.93 g/g dry basis.

Steam blanching was carried out in a closed water bath (Heto, model AT 110, Allerød, Denmark). A single layer of the whole leaves was steamed on a perforated tray suspended over boiling water for 1 min (Tanongkankit et al. 2010). After 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 0.5×5 cm. Fresh sliced cabbages were used as a control sample.

Vacuum drying

The vacuum drying system used in this study consists of a stainless steel drying chamber, with the inner dimensions of 45×45×45 cm, and a liquid ring vacuum pump (Nash, model ET32030, Trumbull, CT) rated at 2 hp (or 1.49 kW). An electric heater, rated at 1.5 kW, which was controlled by a PID controller (Omron, model E5CN, Tokyo, Japan) was installed in the drying chamber to control the drying temperature. Two variable-speed electric fans were used to disperse heat throughout the drying chamber. A sample holder was made of a stainless steel screen with the dimensions of 16.5×16.5 cm. The change of the mass of the sample was detected continuously (at 60 s intervals) using a load cell (Minebea, model UCG-3 kg, Nagano, Japan). The drying experiments were conducted at 60–80 °C, which is a typical temperature range for drying of vegetables (Soponronnairit et al. 1998). The vacuum pressure (10 kPa) selected in this study also falls in the typical range for vacuum drying of food products (Arevalo-Pinedo and Murr 2007).

Approximately 30 g of a sample was placed as a single layer on the sample holder. Drying was continued until the mass of the sample reached an equilibrium value. The drying kinetics were determined to find the time needed to dry the sample to the desired final moisture content of less than 10 % dry basis (Larrauri 1999). Moisture content of the sample was checked using a gravimetric method at 105 °C. (AOAC Method 984.25; AOAC 2000). The dried sample was ground into fine powder with the particle size in the range of 150–450 μ m (Larrauri 1999).

Determination of total phenolics content (TPC)

The total phenolics content of a sample was determined using Folin-Ciocalteu reagent (Yu et al. 2005). Five g of a sample was first shaken with 50 mL of acetone-water solution (1:1, v/v) via the use of a shaker (New Brunswick Scientific, model Innova 4230, Edison, NJ) at 120 rpm for 15 h at ambient temperature (~30 °C). The acetone extract was then filtered through Whatman No. 1 filter paper and kept in dark at

ambient temperature until further analysis. The sample extract (50 μ L) was diluted with 3 mL of distilled water; 250 μ L of Folin-Ciocalteu reagent and 0.75 mL of 20 g/mL sodium carbonate solution were 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 as a standard. The results are expressed as mg gallic acid equivalent per one hundred g of sample (dry basis).

Determination of vitamin C

The vitamin C analysis method was a modification of that suggested by Vikram et al. (2005). Three g of a fresh sample or 1 g of a dried sample was first 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 μ m nylon filter. The filtrate (10 μ L) was then injected into AtlantisTM C₁₈ 5 μ L (4.6 $\times$ 150 mm) HPLC column (Waters, Milford, MA). The HPLC system consists of a pump and controller (Waters, model 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 0.8 % metaphosphoric acid at a flow rate of 0.5 mL/min. A UV detector at a wavelength of 254 nm was used for the detection of vitamin C. The mobile phase was degassed using an ultrasonic generator. Quantification of vitamin C was carried out based on a vitamin C (L-ascorbic acid) standard curve.

Total antioxidant activity evaluation

As the total antioxidant activity depends on the measurement method, a single testing method is not sufficient to investigate the total antioxidant activity (Kulisic et al. 2004; Leusink et al. 2010). Two different methods, namely, DPPH assay and β -carotene bleaching assay, were used for determining the total antioxidant activity in this study.

Preparation of extract for antioxidant activity determination

Ten g of a homogenized sample was shaken with 90 % (v/v) ethanol in the shaker 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 (Buchi, model R-215, Flawil, Switzerland) at 50 $^{\circ}$ C for 5 min and then made up to 5 mL by 90 % (v/v) ethanol. The obtained extract was stored in an amber colored air-tight container at -20° C until subsequent analysis.

Fig. 1 Drying curves of (a) fresh and (b) blanched samples during drying. *Data from Tanongkankit et al. (2010)

DPPH assay

The total antioxidant activity was assessed in terms of the hydrogen denoting or radical scavenging ability. Free radical scavenging activity of a sample was first determined using the 2,2-diphenyl-2-picrylhydrazyl (DPPH) assay (Turkmen et al. (2005) with some modifications). An aliquot of 1.5 mL of

Table 1 Equilibrium moisture contents (g/g dry basis) of cabbage outer leaves and drying time to reach the final moisture content of less than 0.1 g/g dry basis

Sample	Temperature ($^{\circ}$ C)	Equilibrium moisture content (g/g dry basis)	Drying time (min)
Fresh	60	0.065 ± 0.08	180
	70	0.061 ± 0.01	150
	80	0.044 ± 0.005	120
Blanched vacuum dried	60	0.051 ± 0.002	150
	70	0.045 ± 0.002	120
	80	0.047 ± 0.009	90
Blanched hot air dried	80 ^a	0.050 ± 0.013	120

^a Data from Tanongkankit et al. (2010)

Table 2 Antioxidant profiles and activities of fresh and blanched cabbage outer leaves

Sample	TPC (mg GAE/100 dry basis)	Vitamin C (mg/100 g dry basis)	TAA (%)	
			DPPH	β-carotene bleaching
Fresh	711.55±29.82	619.85±19.53 ^a	80.32±1.95 ^a	78.26±2.21 ^a
Blanched	710.05±24.24 ^a	626.60±24.24 ^a	79.49±1.48 ^a	78.43±2.21 ^a

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

0.2 mM DPPH radicals 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 ambient temperature ($\sim 30^\circ\text{C}$) in dark for 30 min. The absorbance was measured at 517 nm using the UV–vis scanning spectrophotometer. Pure ethanol was used as a control sample to calibrate the spectrophotometer. The total antioxidant activity (TAA) is expressed as the percentage of inhibition of DPPH radicals and was determined by the following equation:

$$\%TAA = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100 \quad (1)$$

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

Fig. 2 Changes in (a) total phenolics and (b) vitamin C. *Data from Tanongkankit et al. (2010)

β-carotene bleaching assay

The assay was that described by Amin et al. (2006). The measured antioxidant activity is based on the ability of a sample to decrease the oxidative bleaching of β-carotene in an emulsion of β-carotene and linoleic acid. Two mg of β-carotene powder was dissolved in 20 mL of chloroform. Twenty mL of linoleic acid and 200 mL of Tween 40 were then added to an aliquot (5 mL) of the solution. Chloroform was subsequently 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 a water bath at 50 °C for 120 min. The absorbance of the oxidized emulsion

Fig. 3 Changes in total antioxidant activity during drying as assessed by (a) DPPH radical scavenging activity assay and (b) β-carotene bleaching assay. TAA is the total antioxidant activity at any drying time t and TAA_0 is the initial total antioxidant activity. *Data from Tanongkankit et al. (2010)

Table 3 Correlations among TPC, vitamin C and their TAA

Correlation between	Relationship	R^2
TPC and TAA (via DPPH)	$Y \text{ (DPPH)} = 1.87(\text{TPC}) - 0.86$	0.92
Vitamin C and TAA (via DPPH)	$Y \text{ (DPPH)} = 1.97(\text{Vitamin C}) - 1$	0.91
TPC and TAA (via β -carotene bleaching)	$Y \text{ (}\beta\text{-carotene bleaching)} = 1.67(\text{TPC}) - 0.65$	0.95
Vitamin C and TAA (via β -carotene bleaching)	$Y \text{ (}\beta\text{-carotene bleaching)} = 1.78(\text{Vitamin C}) - 0.80$	0.89

was measured using the UV–vis scanning spectrophotometer at 470 nm. Distilled water was used as a control sample. The total antioxidant activity (TAA) was calculated using the following equation:

$$\%TAA = \left[1 - \frac{A_s^0 - A_s^{120}}{A_c^0 - A_c^{120}} \right] \times 100 \quad (2)$$

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 control at $t=0$ min and $t=120$ min, respectively. All samples were assayed in triplicate.

Specific energy consumption (SEC)

Specific energy consumption (SEC, MJ/kg water evaporated), which is the energy needed to evaporate a unit mass of water, was determined to help select the best drying condition. The energy consumed at different conditions was calculated using the following equation:

$$SEC = \frac{E_p \text{ (or } E_h)}{W} \quad (3)$$

where E_p and E_h are the amounts of energy (MJ) consumed by the vacuum pump and electric heater of the dryer, respectively. W is the mass of evaporated water (kg).

Statistical analysis

The experiments were designed in complete random. Two factors, pretreatment method (2 levels) and drying temperature (3 levels), were studied to determine their influences on the contents of antioxidants and their activity. The data were subjected to the analysis of variance (ANOVA) and are presented as mean values with standard deviations. Differences between mean values were established using Duncan's multiple range tests at a confidence level of 95 %. All statistical analyses were performed using SPSS® software (version 17, SPSS Inc., Chicago, IL). All experiments were performed in duplicate unless when specified otherwise.

Results and discussion

Drying kinetics of cabbage outer leaves

The sample moisture content of approximately 10.40 ± 0.184 g/g dry basis was observed after steam blanching. Figure 1 shows the drying curves of fresh and steam blanched samples undergoing vacuum drying at various temperatures; for comparison the drying curve of a blanched sample undergoing hot air drying at 80 °C is also shown. As expected, the drying rate increased with the drying temperature. At the same drying temperature (80 °C) vacuum drying exhibited a higher drying rate than hot air drying as expected. The equilibrium

Table 4 Retention of antioxidants in dietary fiber powder and their activity

Sample	Temperature (°C)	TPC	Vitamin C	TAA (%)	
				DPPH	β -carotene bleaching
Fresh vacuum dried	60	60.56±2.49 ^a	54.61±2.13 ^a	77.66±3.12 ^a	74.79±1.63 ^a
	70	62.00±1.16 ^a	54.80±2.12 ^a	81.08±1.49 ^a	75.52±4.82 ^a
	80	60.01±2.30 ^a	56.00±2.94 ^{ab}	80.72±1.08 ^a	74.97±1.10 ^a
Blanched vacuum dried	60	67.68±1.44 ^b	59.73±1.89 ^b	84.65±2.19 ^b	79.34±2.67 ^{ab}
	70	69.40±0.69 ^b	59.70±0.69 ^b	85.03±1.05 ^b	81.58±3.39 ^b
	80	68.39±1.04 ^b	63.31±3.53 ^b	86.72±2.73 ^b	82.94±2.22 ^b
Blanched hot air dried	80*	42.87±0.43*	49.33±2.62*	67.98±0.12*	68.28±0.94*

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

Comparison was made with fresh leaves

*Data from Tanongkankit et al. (2010)

Table 5 Analysis of variance for the effect of drying on retention of antioxidants in dietary fiber powder and their activity

	Source	Sum of Squares	df	Mean Square	F	Sig.
TPC	Between Groups	1000.157	6	166.693	54.029	0.000
	Within Groups	21.597	7	3.085		
	Total	1021.754	13			
Vitamin C	Between Groups	249.220	6	41.537	7.016	0.011
	Within Groups	41.442	7	5.920		
	Total	290.662	13			
DPPH	Between Groups	483.232	6	80.539	21.458	0.000
	Within Groups	26.273	7	3.753		
	Total	509.505	13			
β -carotene Bleaching	Between Groups	323.840	6	53.973	6.141	0.015
	Within Groups	61.524	7	8.789		
	Total	385.364	13			

moisture content (EMC) and time needed to dry the samples to the desired moisture content of less than 0.1 g/g dry basis are listed in Table 1. EMC of the samples was in the range of 0.044–0.065 g/g dry basis. Blanched samples required shorter drying time than unblanched samples since blanching helped soften the sample cell structure, resulting in enhanced water removal.

Effect of drying on antioxidants

The amounts of antioxidants in fresh and blanched cabbage outer leaves are listed in Table 2. The contents of antioxidants of interest, i.e., total phenolics content and vitamin C as well as their total antioxidant activity were monitored during drying (Fig. 2). All data were normalized with the properties of the raw materials prior to processing to eliminate the effect of different raw materials; the results are therefore presented in terms of C/C_0 , where C is the antioxidant content at any drying time and C_0 is the initial antioxidant content of each batch of cabbages. Comparing with the drying curves (Fig. 1), the exponential decay relationship between the antioxidant contents and drying time was observed (Fig. 2). The extents of antioxidant losses depended significantly on the drying rate.

The results indicated that all antioxidants degraded upon drying. It was also observed that higher drying temperatures led to higher losses of antioxidants, due most probably to the larger extent of thermal degradation. Kuljarachanan et al. (2009) reported a similar trend of the higher losses of phenolics and vitamin C in lime residues during higher-temperature drying. Di Scala and Crapiste (2008) also observed higher degradation rates of vitamin C in red pepper during drying at higher drying temperatures.

During drying the losses of antioxidants in the unblanched samples were more extensive than those in the blanched samples. This might be because blanching could help inactivate polyphenoloxidase (PPO) and peroxidase (POD) as well as ascorbate oxidase (Maccarone et al. 1993), leading to negligible enzymatic degradation of phenolic compounds and vitamin C. Less availability of oxygen also led to the lower levels of oxidative reactions. Therefore, the antioxidants were more preserved during vacuum drying.

Figure 3 illustrates the changes in the total antioxidant activity (TAA), as measured by the DPPH radical scavenging assay and β -carotene bleaching assay, of unblanched and blanched samples during vacuum drying. The results showed that TAA of all samples continuously decreased during drying. The relationships between the antioxidants and TAA were

Table 6 Specific energy consumption of vacuum pump (SEC_p) and electric heater (SEC_h) at various conditions

Sample	Temperature (°C)	Drying time (min)	E_p (MJ)	E_h (MJ)	Amount of evaporated water (kg) ^a	SEC_p (MJ/kg)	SEC_h (MJ/kg)
Fresh	60	180	16.09	16.20	0.023	699.57	704.35
	70	150	13.43	13.50	0.025	537.20	540.00
	80	120	10.73	10.80	0.026	412.69	415.38
Blanched	60	150	13.43	13.50	0.025	537.20	540.00
	70	120	10.73	10.80	0.026	412.69	415.38
	80	90	8.06	8.10	0.026	310.00	311.54

^a The amount of evaporated water = Initial mass of sample – Final mass of sample

determined (Table 3); linear relationships were noted with high correlation coefficients, especially in the case of TPC and TAA, indicating that TPC plays an important role on the antioxidant activity of cabbage DF powder. Similar results was noted by Kaur et al. (2007) who reported that TAA, as measured by the DPPH assay, of broccoli showed a higher correlation with phenolics than with vitamin C, β -carotene and α -tocopherol.

Table 4 summarizes the retention of all antioxidants in the DF powder. For comparison the data of Tanongkankit et al. (2010) who steam blanched the whole leaves of cabbages prior to slicing and hot air drying at 80 °C are also listed. It is important to note that the fresh cabbage leaves used in the present work were of the same variety although they were not exactly the same harvest to that of the previous work by Tanongkankit et al. (2010). Nevertheless, we were always aware of the possible errors from different sample lots. Therefore, all data were normalized with the properties of the fresh leaves prior to processing to eliminate the effect of different raw materials; the data were compared again in terms of C/C_0 to eliminate the differences in the initial amounts of antioxidants. It is seen that vacuum drying provided better retention of each antioxidant and its activity than hot air drying. As mentioned earlier, smaller losses of antioxidants via oxidative reactions might have occurred during vacuum drying. 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.

Table 5 shows the analysis of variance for the effect of drying on the retention of antioxidants in DF powder and their activity. No differences in terms of the retention of antioxidants were observed at different drying temperatures during vacuum drying. Generally, higher degradation rates should be expected at higher drying temperatures. However, shorter drying time is required to dry a sample at a higher temperature. Therefore, both the drying temperature and time had an effect on the retention of antioxidants. Garau et al. (2007), for example, also reported that drying at 30 °C resulted in a more extensive reduction in the phenolic compounds and antioxidant activity in orange than at 50, 60 and 70 °C.

Specific energy consumption

Table 6 summarizes the SEC of the drying process at different drying temperatures. It can be seen that the energy consumption decreased with the drying temperature. This is due to an increase in the temperature gradient within the sample, resulting in accelerated evaporation rate, which led to reduced drying time and hence required energy consumption. The analysis showed that there were no differences in the antioxidants retention among all the tested conditions. Note that the seemingly excessively large SEC was due to the fact that only

a very small amount of sample was dried in each experiment. The dryer was indeed capable of drying much more sample; the dryer should indeed be filled with maximum possible loading to reduce the SEC.

The most suitable condition for the production of antioxidant DF powder from cabbage outer leaves is vacuum drying at 80 °C as this condition requires the minimum specific energy consumption.

Conclusion

The effects of steam blanching and subsequent vacuum drying of outer leaves of cabbages on the antioxidants and their activity in the obtained DF powder were investigated. The DF powder produced by vacuum drying contained higher amounts of antioxidants and antioxidant activity than the powder produced by hot air drying. By considering the whole production process steam blanching prior to slicing and vacuum drying at 80 °C is a recommended process for the production of antioxidant DF powder as it provided the highest retention of antioxidants. Studies on physical and functional properties of the DF powder and its application as a food ingredient are suggested as a future work.

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