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Research Article

Evaluation of Phytochemical Content and the Antioxidant and Antiproliferative Potentials of Leaf Layers of Cabbage Subjected to Hot Air and Freeze-Drying

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Cabbage (*Brassica oleracea* var. capitata f. alba), a cruciferous vegetable, is one of the most widely consumed vegetables worldwide. However, research on whether there are differences in its contents of phytochemicals and biological activities according to the drying method for each portion of the vegetable remains insufficient. The present study investigated the contents of representative polyphenols and isothiocyanates, the antioxidant capacity, and the antiproliferative effects among six leaf layers of cabbage subjected to hot air and freeze-drying. High-performance liquid chromatography analysis showed that most phenolic and flavonoid bioactive compounds were significantly accumulated in the outer cabbage leaf layer (P1), whereas isothiocyanates were most abundant in the leaf layer close to the core of the head (P5). The contents of isothiocyanates, gallic acid, epicatechin, p-coumaric acid, sinapic acid, and myricetin were significantly higher in the hot air-dried sample than in the freeze-dried sample, whereas the contents of catechin hydrate, chlorogenic acid, 4-hydroxybenzoic acid, and rutin hydrate were significantly higher in the freeze-dried sample. Compared to other leaf layers, P1 exhibited high antiproliferative efficacy against pancreatic, breast, and gastric cancer cells. P1 also showed excellent DPPH·(EC $_{50}$–4.208 ± 0.033 and 4.611 ± 0.053 mg/mL for hot air and freeze-dried samples, respectively) and ABTS· (2.422 ± 0.068 and 2.224 ± 0.070 mg/mL for hot air and freeze-dried samples, respectively) radical-scavenging effects. These results indicate that the contents of polyphenols and isothiocyanates in cabbage may vary depending on the leaf layer and the drying method. Our findings provide insight for applying appropriate food drying methods that can be used to produce cabbage leaf-based products with enhanced bioactivity.

1. Introduction

Cabbage (*Brassica oleracea* var. capitata) is a popular vegetable in many countries, which has been reported with high nutritional value and distinct health benefits [1]. It contains abundant polyphenols, flavonoids, anthocyanins, glucosinolates, fiber, and vitamins [1]. Among these, phenolic acids and flavonoids possess important health benefits, including anticancer and antioxidant activities [2]. In addition to phenolic acids and flavonoids, structurally and functionally diverse glucosinolates and isothiocyanates are abundant in cabbage [3]. Several epidemiological investigations have demonstrated an inverse correlation between the consumption of cabbage and the risk for several diseases, including cardiovascular diseases, cancer, and hepatic steatosis [4, 5].

The moisture content of cabbage affects its shelf life and quality [6]. Moreover, the high perishability of cruciferous vegetables, including cabbage and broccoli, mandates the need for suitable processing methods to improve the shelf life and quality of the products [7]. At the individual household and industrial levels, heat application is commonly used as a food processing method to improve the shelf life of many cruciferous vegetables [8]. Drying using hot air leads to dehydration of the raw food material, by which microbial spoilage is reduced [9]. Furthermore, it prevents moisture-mediated deterioration of reactions and facilitates food transportation due to the reduction in water mass [6]. However, it alters the levels of major bioactive constituents, natural food color, and food structures [10]. Freeze-drying is another food-processing method for perishable food items [11]. It removes water from food through sublimation of ice and provides high-quality products by maintaining the inherent physical and biological properties. However, it is costly and energy inefficient [11].

Several researchers have demonstrated the effects of different drying methods on the physicochemical properties of cabbage. Recently, the effects of freeze drying, hot air drying, microwave vacuum drying, vacuum drying, and combined drying methods on the nutritive value and physicochemical characteristics of portions of cabbage were analyzed [6]. In another investigation, the volatile and physical properties of hot air-dried and freeze-dried cabbage pieces were evaluated [10]. The effects of belching, steam, and hot air drying on fiber content and the effects of freeze drying, sun drying, oven drying, and solar cabinet drying on antioxidant properties and phenolic compounds in cabbage leaf have also been investigated [12]. In another recent investigation, the amounts of different primary metabolites (soluble sugar, protein, and mineral) and secondary metabolites (carotenoid, vitamin C, anthocyanin, flavonoid, and total phenolic compound) in cabbage leaf layers were investigated [13]. However, comprehensive studies on hot air- and freeze-dried cabbage leaves, including studies of the contents of various polyphenols and isothiocyanates, antioxidant activity, and potential antiproliferative effects on various types of cancer cell lines, are lacking.

The main components contained in six parts of the cabbage leaf layers (P1–P6), including 11 phenolic compounds and 3 isothiocyanates, subjected to hot air and freeze-drying, were analyzed by high-performance liquid chromatography (HPLC). The antioxidant and cancer cell proliferation inhibitory effects of each part were also evaluated. Our findings provide insight for applying appropriate food drying methods that can be used to produce cabbage leaf-based products with enhanced bioactivity.

2. Materials and Methods

2.1. Chemicals. The standards for HPLC analysis, i.e., ascorbic acid, gallic acid, caffeic acid, p-coumaric acid, chlorogenic acid, 4-hydroxybenzoic acid, sinapic acid, catechin hydrate, rutin hydrate, myricetin, quercetin, epicatechin, L-sulforaphane, erucin, phenyl isothiocyanate, and HPLC-grade solvents such as methanol, water, and acetic acid, were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Raw Materials and Sample Preparation. Fresh cabbage was purchased from local markets on Jeju Island, South Korea. After cutting a whole cabbage head into four sides, the outermost leaf of the vivid green leaf layer was named P1, the core was classified as P6, and the leaves directly adjacent to the core were named P5. The remaining layers were divided into three equal parts and designated P2, P3, and P4 in order from the outside, as shown in Figure 1(a). The leaf layers were separated from nine cabbages, as described above, and P1–P6 derived from each cabbage head were mixed with the same parts to obtain sufficient cabbage sample for each part for use in the experiment.

2.3. Color Measurement. The chromaticity of the different cabbage parts was measured using a colorimeter (Chroma Meter CR-400, Konica Minolta, Tokyo, Japan) as described in a previous study [14].

2.4. Drying Experiments. A vacuum freeze-drying machine (LP 20, Ilshinbiobase Co., Ltd., Dongducheon, Korea) was used to process the different cabbage leaf layers. A previously published method was used for drying experiments with slight modifications [11]. The freeze-dried materials were stored in airtight plastic bags in a −80°C deep freezer for 48 h. Next, the different parts of the cabbage were placed in the vacuum freeze-drying machine and dried for 72 h at −80°C. For hot air drying, the different parts of the cabbage were placed on a tray in the drying chamber of a hot air dryer and dried for 72 h at 55°C. The dried materials were stored in airtight plastic bags and kept in a −80°C deep freezer.

2.5. Extraction. A previously published method was used for extractions with slight modifications [15]. Powdered freeze-dried and hot air-dried (10 g of each) cabbage leaf samples were extracted with 80% methanol and dichloromethane. For the extraction with methanol, powdered samples of the different cabbage parts were extracted using 500 mL of 80% methanol with sonication for 45 min at 20°C. Following the extraction, the resulting extracts were evaporated using a rotary evaporator and then freeze dried.

2.6. 2,2-Diphenyl-1-picrylhydrazyl Radical-Scavenging Activity Assay. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity was determined as described previously by Kim et al. [11]. Prior to analysis, 40 μL of each CH_3OH cabbage extract was mixed with 160 μL of freshly prepared DPPH solution (200 μM in ethanol solution) in 96 well plates and incubated at 37°C for 30 min in the dark. The DPPH radical-scavenging activity was measured using the recorded absorbance values at 517 nm. Catechin was used as the positive control. Formula $(A - B)/A \times 100\%$ (where A is the absorbance of the DPPH solution and B is the absorbance of the sample) was used to calculate the percentage radical-scavenging activity of each extract. All experiments were performed in triplicate, and the EC_{50} values for each extract were generated using GraphPad Prism 7.0 software (La Jolla, CA, USA).

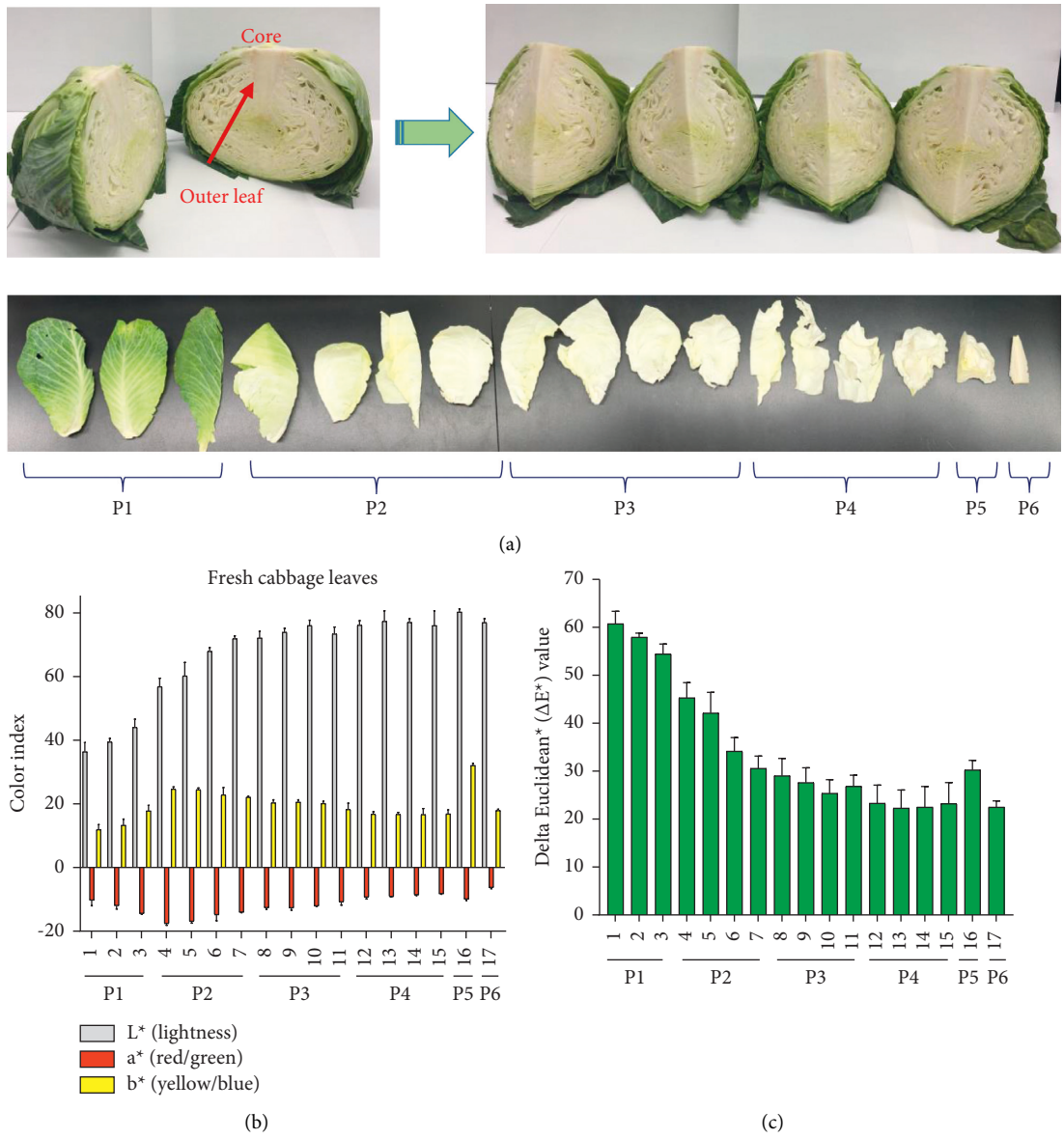


Figure 1: Separation of different cabbage leaf layers and analysis of color index. (a) After cutting into quarters, the leaf layer was peeled off from the outer leaf, and all leaf layers were divided into 6 parts (P1–P6). P1 is the outer layer, and P6 is the core part. (b and c) Color index and total color difference between the different parts of cabbage leaves (ΔE^*). L^*, lightness; a^*, red/green coordinate; b^*, yellow/blue coordinate; ΔE^*, total color difference. The mean and standard deviation were calculated following three independent experiments.

2.7. 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) Radical-Scavenging Activity Assay. The 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical-scavenging assay was conducted as described previously [11]. The assay was started by preparing a fresh ABTS radical solution (7 mM ABTS in 2.45 mM potassium persulfate). The ABTS stock solution was diluted with distilled water to obtain an optical density (OD) of 0.700 ± 0.005 at 734 nm. Then, 900 μL of ABTS solution (diluted) was mixed with 100 μL of each cabbage CH_3OH solvent extract and incubated at room temperature for 2 min. α-tocopherol was used as the positive control. The ABTS radical-scavenging activity was measured using the recorded absorbance values at 734 nm. The percentage radical-scavenging activity of each extract was calculated using the formula $(A - B)/A \times 100\%$, where A is the absorbance of the control group and B is the absorbance of the treatment group. All experiments were performed in triplicate, and GraphPad Prism 7.0 software (La Jolla, CA, USA) was used to calculate the EC_{50} value of each extract.

2.8. Assessment of Total Polyphenol and Flavonoid Contents. The total polyphenol content (TPC) and total flavonoid content (TFC) were determined as described previously by Kim et al. [11]. To estimate the TPC, 125 μL of each extract was mixed with 1.375 mL of water and 500 μL of Folin-Ciocalteu phenol reagent and incubated for 5 min at room

temperature. Following incubation, 1 mL of 10% Na_2CO_3 was added to the reaction mixtures, which were incubated for 30 min in the dark. Then the absorbance at 700 nm was recorded using a micro-plate reader. The TPC was expressed as milligrams of gallic acid equivalent (GAE) per Gram of extract. To estimate TFC, 40 μL of extract was mixed with 80 μL of distilled water and 6 μL of 5% $NaNO_2$. After 5 min of incubation at room temperature, 12 μL of 10% $AlCl_3$ was mixed with the reaction mixture, which was incubated at room temperature for 6 min. Following incubation, 40 μL of 1 N NaOH and 42 μL of DW were added to the reaction mixture, which was mixed well by pipetting. The absorbance at 510 nm was measured using a micro-plate reader. The total flavonoid content was expressed as milligrams of rutin equivalent (RE) per gram of extract.

2.9. HPLC Analysis. A Shim-pack ODS 5 μm column (Shimadzu, Kyoto, Japan) was used for all HPLC separations. All samples were analyzed in triplicate. To quantify the phenolic compounds, a previously published method was used, with slight modifications [15]. The powdered material (100 mg) was extracted with 4 mL of 80% (v/v) methanol with sonication for 60 min. After centrifuging the extracts at 13,000 rpm for 10 min, the supernatant was filtered with a 0.22 μm Acrodisc syringe filter (Merck KGaA, Darmstadt, Germany) and analyzed using an HPLC-ultraviolet detector (HPLC-UVD) system (Shimadzu CBM-20A, Tokyo, Japan). Phenolic compounds were analyzed as previously described [15]. The concentrations of phenolic compounds were determined using a standard curve.

L-sulforaphane content was analyzed as follows. A mass of 1 g of each cabbage sample was mixed with 4 mL of distilled water, vortexed for 1 min, and then kept at room temperature for 10 min. Then, 15 mL of dichloromethane was added and the mixture was vortexed for 1 min and kept at room temperature for 1 h. The resulting extracts were filtered, evaporated, and subjected to HPLC. The mobile phase consisted of solvent A (water) and solvent B (acetonitrile). A binary gradient system was used: 0 min (100% A, 0% B); 10 min (80% A, 20% B); 30 min (40% A, 60% B); 40 min (0% A, 100% B); 45 min (80% A, 20% B), and 50 min (80% A, 20% B). The column was maintained at 40°C. The flow rate was maintained at 0.8 mL/min, the injection volume was 20 μL, and the detection wavelength was 205 nm. The concentrations of L-sulforaphane were determined using a standard curve. For the quantification of erucin and phenyl isothiocyanate, 0.5 g of each cabbage sample was mixed with 10 mL of dichloromethane, vortexed for 1 min, and kept at room temperature for 120 min. Extractions were repeated two times, and the resulting extracts were combined, filtered, evaporated, and subjected to HPLC analysis. The mobile phase consisted of solvent A (water) and solvent B (acetonitrile). The binary gradient setup was 0 min (90% A, 10% B); 38 min (10% A, 90% B); 38.1 min (90% A, 10% B); and 40 min (90% A, 10% B). The column was maintained at 40°C. The flow rate was maintained at 1.0 mL/min, and the injection volume was 20 μL. The detection wavelength was 205 nm. The concentrations of erucin or phenyl isothiocyanate were determined using a standard curve.

2.10. Cell Lines and Cell Culture. Human pancreatic carcinoma MIA PaCa-2 cells, human breast adenocarcinoma MCF7 cells, and human gastric adenocarcinoma AGS cell lines were purchased from the American Type Culture Collection (ATCC) and maintained according to their recommended culture conditions [11]. Human hepatoma HepG2 cells obtained from the Korean Cell Line Bank (KCLB) were cultured in a RPMI1640 medium (Roswell Park Memorial Institute) supplemented with 10% FBS (v/v), 100 μg/mL of penicillin, and 100 μg/mL of streptomycin at 37°C in a 5% CO_2 atmosphere.

2.11. Cell Viability Assays. The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) cell viability assay was conducted as described previously [11]. Prior to cell viability assays, MIA PaCa-2, MCF7, AGS, and HepG2 cells (4000 cells/well) were exposed to different concentrations (0, 100, 200, 400, 800, or 1600 μg/mL) of methanol cabbage extracts in 96 well-plates and incubated for 48 h. All experiments were performed in triplicate. GraphPad Prism 7.0 software (La Jolla, CA, USA) was used to calculate the IC_{50} value of each extract.

2.12. Statistical Analysis. All experiments were performed in triplicate. GraphPad Prism 7.0 software (La Jolla, CA, USA) was used for statistical analysis. Data were presented as mean ± standard deviation (SD). The level of $p < 0.05$ was considered statistically significant. Statistical analysis of TPC, TFC, and phenolic content was performed using one-way analysis of variance (ANOVA) with Tukey's multiple comparison test (95% level of significance) using GraphPad Prism 7.0. Pearson's correlation was performed using the "correlation" tool in GraphPad Prism 7.0 software.

3. Results and Discussion

3.1. Color Index of the Different Parts of Cabbage Leaves. The color index of the different parts of cabbage is shown in Figure 1(b). The letter L indicates the lightness, which showed variation from 36.35 ± 2.94 (P1) to 80.26 ± 0.99 (P5). The red/green coordinate (a^*) showed variation from −17.64 ± 0.48 (P2) to −6.28 ± 0.37 (P6). The yellow/blue (b^*) coordinate indicated variation from 11.94 ± 1.52 (P1) to 32.09 ± 0.64 (P5). The total color difference between the different parts of cabbage (ΔE^*) showed that P1 had the highest color difference among the parts of cabbage (60.73 ± 2.63 to 54.46 ± 2.07) (Figure 1(c)). Compared to P1, parts P2, P3, and P4 showed smaller ΔE^* values (P2 (45.32 ± 3.15 to 30.58 ± 2.56); P3 (29.04 ± 3.64 to 26.84 ± 2.30), and P4 (23.32 ± 3.76 to 22.31 ± 3.75)). P5 showed a larger ΔE^* (30.27 ± 1.97). Compared to P5, P6 had a smaller ΔE^* (22.48 ± 1.34). Collectively, these data indicate that the separated raw cabbage leaf layers have different color indices.

Table 1: Total polyphenol content (TPC) and total flavonoid content (TFC) of different cabbage leaf layers (mg/g dry weight powder extract).

Method	Layer	TPC (mg GAE[1])/g dry weight)	TFC (mg RE[2])/g dry weight)
Hot air-dried samples	H-P1	1.499 ± 0.107^{a}	0.293 ± 0.051^{b}
	H-P2	0.729 ± 0.026^{c}	0.100 ± 0.012^{c}
	H-P3	0.729 ± 0.037^{c}	0.071 ± 0.006^{de}
	H-P4	0.771 ± 0.002^{c}	0.068 ± 0.005^{e}
	H-P5	0.940 ± 0.021^{b}	0.082 ± 0.007^{d}
	H-P6	0.619 ± 0.008^{d}	0.043 ± 0.005^{f}
Freeze-dried samples	F-P1	1.337 ± 0.108^{a}	0.354 ± 0.025^{a}
	F-P2	0.521 ± 0.033^{e}	0.101 ± 0.015^{c}
	F-P3	0.486 ± 0.040^{e}	0.083 ± 0.011^{d}
	F-P4	0.517 ± 0.049^{e}	0.071 ± 0.012^{de}
	F-P5	0.638 ± 0.060^{d}	0.085 ± 0.013^{d}
	F-P6	0.638 ± 0.060^{d}	0.057 ± 0.006^{ef}

[1]Gallic acid equivalent, [2]rutin equivalent. Mean and standard deviation were obtained from three independent experiments. Letters a–f indicate significant differences ($P < 0.05$). H-P1, hot air-dried part 1; H-P2, hot air-dried part 2; H-P3, hot air-dried part 3; H-P4, hot air-dried part 4; H-P5, hot air-dried part 5; H-P6, hot air-dried part 6; F-P1, freeze-dried part 1; F-P2, freeze-dried part 2; F-P3, freeze-dried part 3; F-P4, freeze-dried part 4; F-P5, freeze-dried part 5; F-P6, freeze-dried part 6.

3.2. TPC and TFC Contents. Phenolics and flavonoids in vegetables have a range of biological properties [16]. The TPC and TFC of the different cabbage leaf layers subjected to heat and freezing are shown in Table 1. As shown in the table, the highest TPC and TFC occurred in the outer cabbage leaves (H-P1 and F-P1) for both heat and freeze-drying. Among the hot air-dried samples, the lowest TPC (0.619 ± 0.008 mg GAE/g dry weight) was found in the core part (H-P6). However, among the freeze-dried samples, layer 3 (F-P3) showed the lowest TPC (0.486 ± 0.040 mg GAE/g dry weight). The lowest TFC occurred in the inner cabbage leaves (0.057 ± 0.006 and 0.043 ± 0.005 mg RE/g dry weight for F-P6 and H-P6, respectively) for both drying methods (Table 1). The TPC values of the cabbage leaf layers were higher when the layers were processed by hot air drying than when processed by freeze-drying. By contrast, the TFC values of the freeze-dried samples were higher than the values of the hot air-dried samples (Table 1). These results indicate that the different cabbage layers subjected to hot air and freeze-drying possess varying levels of phenolics and flavonoids. According to the results of Xu et al. [6], cabbage leaves subjected to freeze-drying contained higher TPC. Freeze-drying results in the development of ice crystals in plant matrix, which could cause rupturing of plant cell walls, possibly allowing better organic solvent access [17]. The correlation between drying methods (hot air-dried and freeze-dried) and TFC in different cabbage leaf layers are shown in Table S3. Specifically, strong positive correlations were observed between H-P1 and H-P3, H-P4 ($r = 0.99$, $P < 0.05$); H-P2 and F-P4, F-P5 ($r = 1.00$, and $r = 0.99$ respectively, $P < 0.05$); H-P4 and F-P5 ($r = 1.00$, $P < 0.05$); however, strong negative correlations were observed between H-P6 and F-P1. ($r = -1.00$, $P < 0.05$). According to table S4, strong positive correlations between drying methods and TPC were observed between H-P1 and H-P2, H-P3 ($r = 1.00$, $P < 0.05$); H-P6 and F-P3, F-P4, F-P5, and F-P6 ($r = 1.00$, $P < 0.05$); F-P3 and F-P4, F-P5, and F-P6 ($r = 1.00$, $P < 0.05$); F-P4 and F-P5 ($r = 1.00$, $P < 0.05$); F-P5 and F-P6 ($r = 1.00$, $P < 0.05$).

3.3. HPLC Analysis of Phenolic Compounds in the Different Parts of Cabbage. Several studies have revealed that phenolic compounds possess potent antioxidant, anticancer, and antiinflammatory activities [16, 18, 19]. In the present investigation, the amounts of 11 different phenolics and flavonoids in different cabbage leaves subjected to hot air or freeze-drying were analyzed (Figure 2 and Table S1). Under both drying methods, most of the compounds were abundant in the outer leaves but had lower abundance in the inside cabbage leaves. However, the amount of quercetin was higher in the innermost leaves of both hot air dried and freeze-dried samples (Figure 2). Myricetin and epicatechin were detected only in the outer leaf layers processed by the hot air drying and freeze-drying methods (Figure 2). The chlorogenic acid content was highest in the outer leaves (548.01 and 517.68 μg/g dry weight for F-P1 and H-P1, respectively) subjected to both processing methods (Figure S2), but it decreased in the inner leaves (P2-6). 4-hydroxybenzoic acid accumulation was highest in the outer leaves (339.65 and 289.51 μg/g DW of F-P1 and H-P1, respectively) but diminished in parts P2, P3, and P4. Compared to P4, P5 had higher amounts of 4-hydroxybenzoic acid. However, this compound was not detected in P6. Accumulations of catechin hydrate and caffeic acid were significantly higher in the outer leaves than in the inner layers. Accumulations of gallic acid, epicatechin, p-coumaric acid, sinapic acid, and myricetin were significantly higher in the different cabbage leaves processed by hot air drying than in the leaves processed by freeze drying. By contrast, catechin, chlorogenic acid, 4-hydroxybenzoic acid, and rutin were higher in the different cabbage leaves processed by freeze-drying than in the leaves processed by hot air drying. According to the HPLC quantification results, the different drying methods resulted in varying degrees of accumulation of phenolic compounds in the cabbage leaf layers.

3.4. HPLC Quantification of Three Major Isothiocyanates in Cabbage. Cabbage is rich in glucosinolates (GLSs) and isothiocyanates (ITCs) [3]. Glucosinolates, precursors of

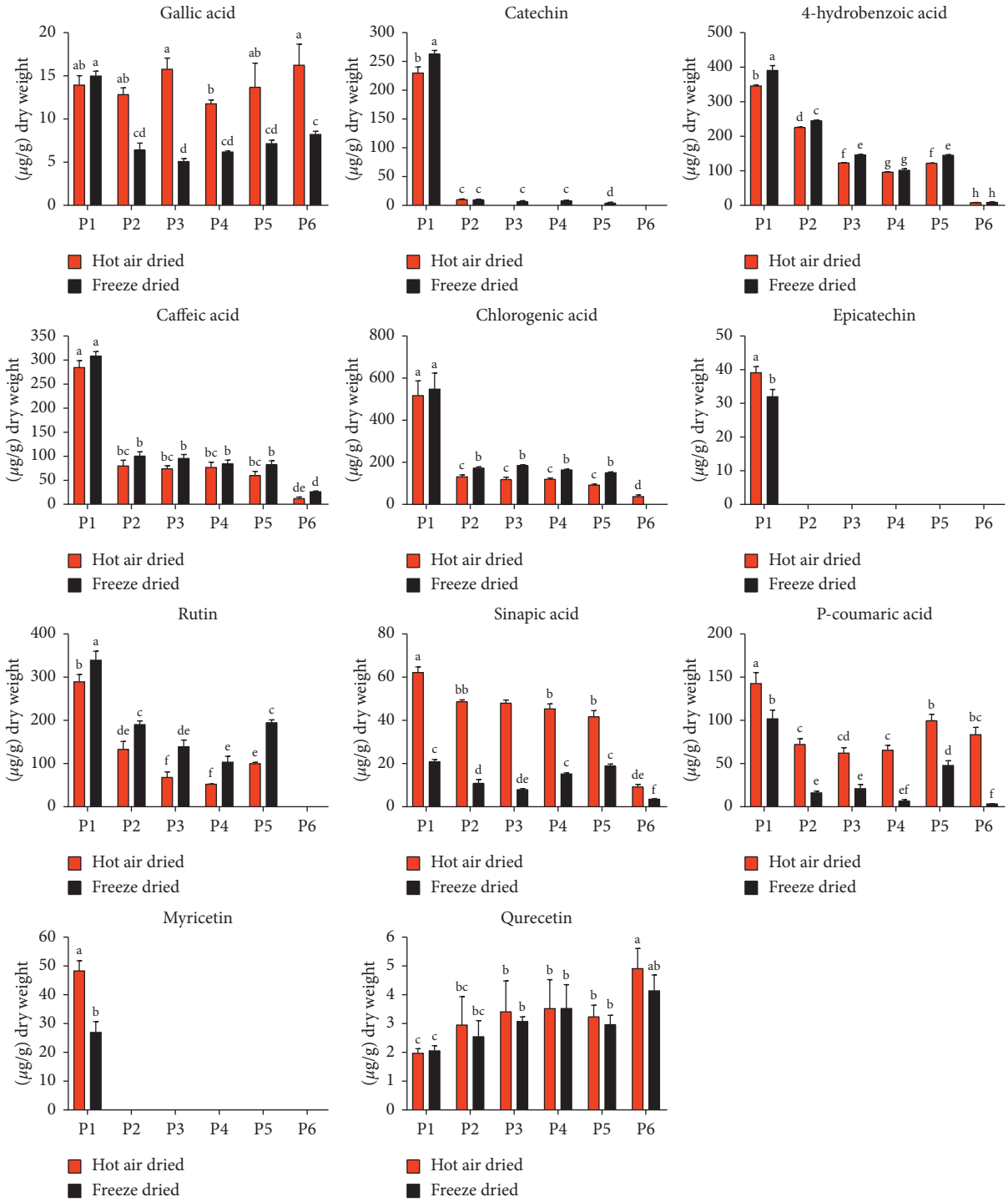


Figure 2: Accumulation of phenolic compounds in different parts of cabbage (μg/g DW). Mean and standard deviation were obtained from three independent experiments. Letters a–h indicate significant differences ($P < 0.05$). P1, leaves part 1; P2, leaves part 2; P3, leaves part 3; P4, leaves part 4; P5, leaves part 5, P6, leaves part 6.

isothiocyanates, are hydrolyzed into isothiocyanates via the enzyme myrosinase, which is discharged when the plant part including the GLS fraction is broken due to chewing, heating, or insect attack [20, 21]. Once the GSLs are ingested, the absorption of a small portion of intact GSLs can occur in the stomach, although most transit to the colon where they are hydrolyzed by bacterial myrosinase activity, and the generated ITCs are absorbed by the lining of the small intestine [22]. ITCs are highly reactive, have potent inhibitory effects on mitosis, and stimulate apoptosis in human tumor cells [23]. Erucin, an ITC obtained from the enzymatic hydrolysis of glucoerucin, has anti-inflammatory, antioxidant, and anticancer effects [24–26]. Phenyl isothiocyanate (PEITC), which is biosynthesized from

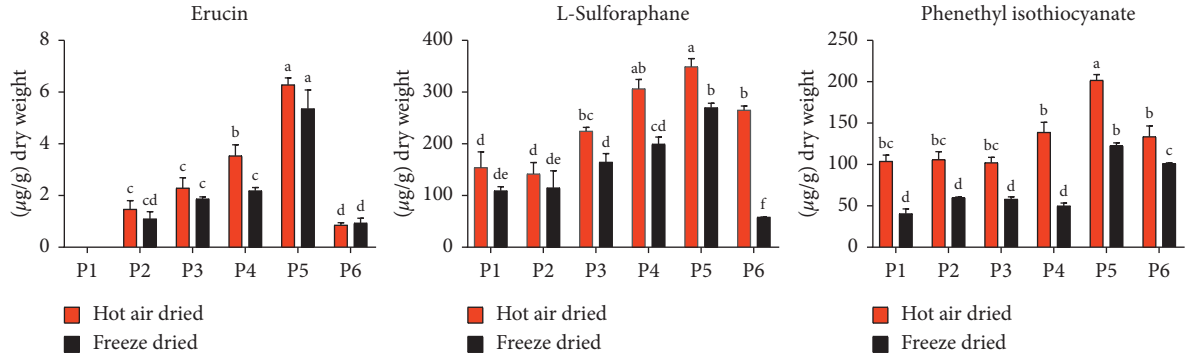


FIGURE 3: Accumulation of isothiocyanate compounds in different parts of cabbage (μg/g DW). Mean and standard deviation obtained from three independent experiments. a–f indicate significant differences ($P < 0.05$). P1, leaves part 1; P2, leaves part 2; P3, leaves part 3; P4, leaves part 4; P5, leaves part 5, P6, leaves part 6.

gluconasturtiin by the action of the enzyme myrosinase [27], has been studied for its potential antiproliferative effects in prostate [28], breast [29], and pancreatic cancer cells [30]. L-sulforaphane, obtained from enzymatic hydrolysis of glucoraphanin by the enzyme myrosinase [20], has also been reported to have anti-inflammatory, antioxidant, and anticancer effects [31]. Here, considering the importance of ITCs as emerging antioxidant and anticancer agents, we quantified the amounts of these three representative ITCs (L-sulforaphane, PEITC, and erucin) in the different cabbage layers processed by hot air drying or freeze-drying (Figure 3 and Table S2). Among the three ITCs, the most abundant in cabbage was L-sulforaphane, followed by PEITC and erucin, respectively. This result is somewhat similar to the result of a previous study that indicated that cabbage has a higher content of sulforaphane and its precursor, glucoraphanin, than other cruciferous vegetables [32]. Under both drying methods, erucin was not detected in P1, but its level increased from P2 to P4, peaked in P5, and decreased in P6. Similarly, the amounts of L-sulforaphane and PEITC reached their maximum in the P5 part adjacent to the cabbage core, increasing toward the inner core rather than the outer leaf. The temperature during food processing affects the L-sulforaphane content [33]. In our study, the contents of L-sulforaphane were significantly higher in the hot air-dried samples than in the freeze-dried samples. Our results are similar to the results of previous studies that have indicated a significant increase in the sulforaphane content of broccoli subjected to heating [34]. The study of Matusheski et al. [35] also reported that preheating broccoli florets and sprouts to 60°C significantly increased the sulforaphane in vegetable tissue extracts following crushing. In addition, we observed that the contents of two types of isothiocyanates (erucin and PEITC) also increased in the hot air-dried samples (Figure 3). These results indicate that the hot air drying method is more advantageous than the freeze-drying method for increasing isothiocyanate accumulation.

3.5. Antioxidant Activities. Several studies have investigated the antioxidant activity of cabbage [36, 37]. However, studies assessing the antioxidant activities of different cabbage layers are limited. To obtain a comprehensive profile of the antioxidant activities of different cabbage leaf layers, we performed ABTS· and DPPH· scavenging assays for leaf layer extracts following extraction with methanol. As shown in Figure 4, the extracts from outer leaves (P1) had significantly higher radical-scavenging activities than the other parts according to the results of both antioxidant assessments. The highest antioxidant activities was detected at H-P1, with an EC_{50} value of 4.208 mg/mL for the DPPH· radical-scavenging assay, and F-P1 with an EC_{50} value of 2.224 mg/mL ABTS radical-scavenging activity (Table 2 and Figure 4). This result is consistent with the total polyphenol and flavonoid contents and the accumulation of phenolic compounds, for which the highest accumulation in the outer leaves of cabbage suggests that a positive relationship exists between total phenolic and flavonoid contents and accumulation of phenolic compounds and antioxidant activity in the different parts of cabbage. According to the results of the DPPH radical-scavenging assay as well, it is evident that the outer leaf layers possess higher radical-scavenging effects (Table 2 and Figure 4). Among the hot-air dried samples, H-P2, H-P3, and H-P4 showed moderate radical-scavenging potentials as assessed by both assays (Table 2 and Figure 4). Same radical-scavenging pattern was observed for freeze-dried samples (Table 2 and Figure 4). Similar results have been reported for Chinese cabbage (*Brassica rapa* L. ssp. *Pekinensis*), which shows the highest levels of both polyphenolics and antioxidant capacities in outer leaves [38]. Furthermore, the DPPH radical-scavenging activities in most parts of cabbage were higher in the samples processed by hot air drying than those processed by freeze-drying. Correlation analysis between drying methods (hot air-dried and freeze-dried) and DPPH radical-scavenging activity of different cabbage leaf layers showed strong positive correlations ($r > 0.9$) (Table S5 and Table S6). Interestingly, the highest dose (200 μM) of the positive control, α-tocopherol and catechin, had a lower radical-scavenging effect compared to the highest dose of P1 used in the experiment, regardless of the drying method (Figure 4), indicating that the outer leaves had very good antioxidant activity. This may be due to the accumulation of higher amounts of catechin, 4-hydroxybenzoic acid, caffeic acid, chlorogenic acid, epicatechin, rutin, p-coumaric acid, and myricetin in the outer leaf layers (Figure 2).

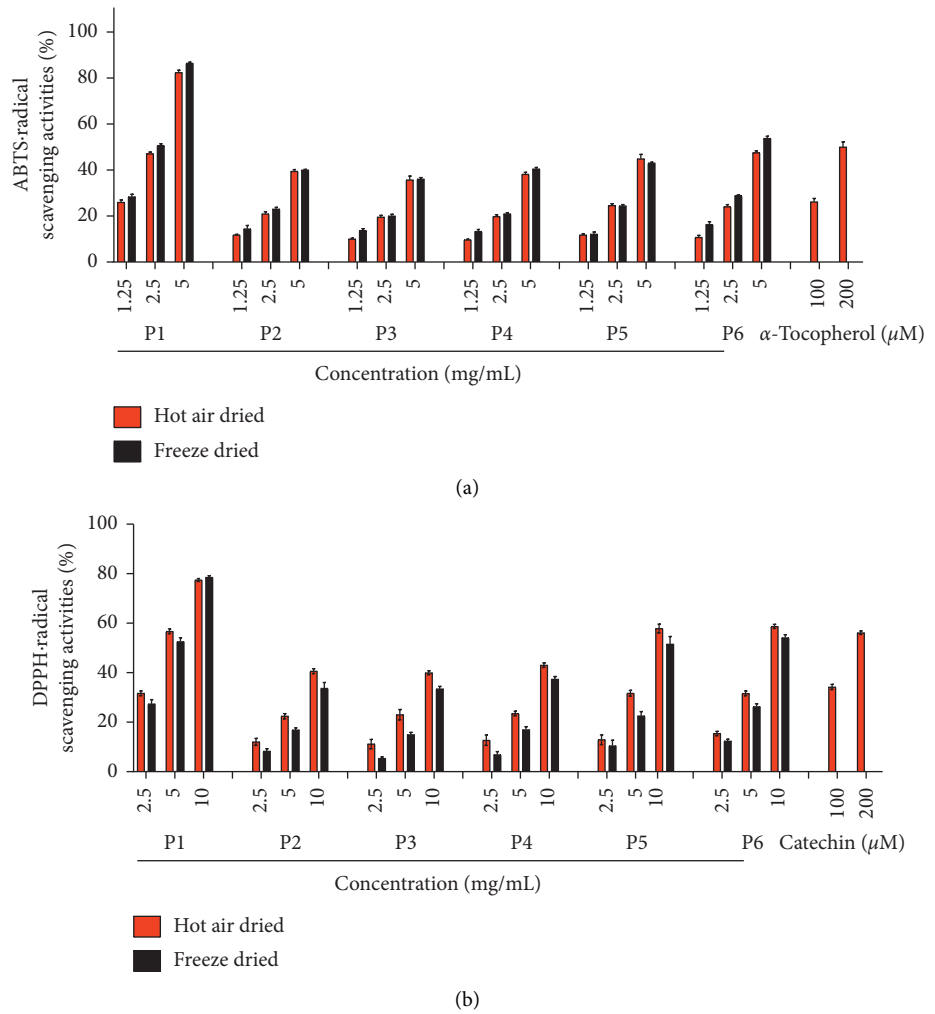


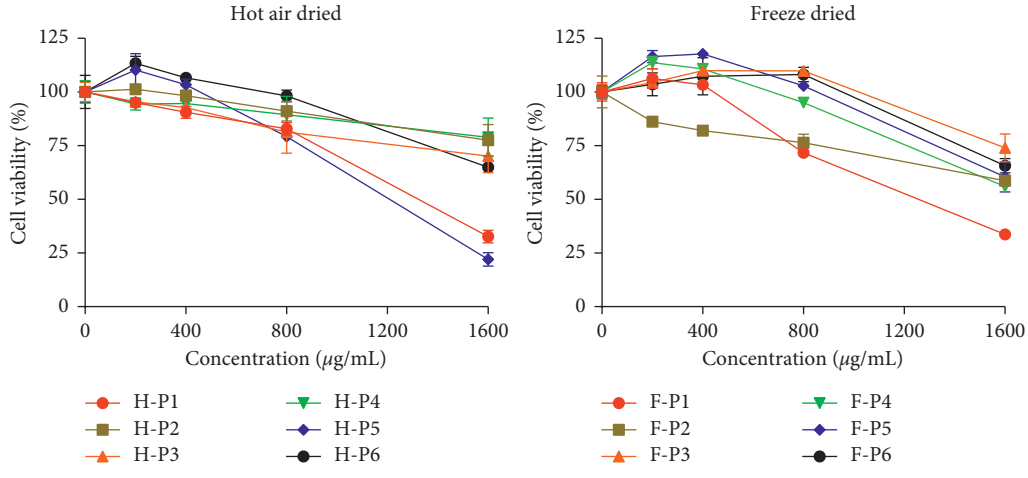
Figure 4: DPPH (a) and ABTS (b) free radical-scavenging potentials of CH_3OH cabbage extracts. The mean and standard deviation are obtained from three independent experiments. Letters a–g indicate significant differences ($P < 0.05$). P1, leaves part 1; P2, leaves part 2; P3, leaves part 3; P4, leaves part 4; P5, leaves part 5, P6, leaves part 6.

Table 2: EC_{50} (mg/mL) values obtained from various antioxidant assays of cabbage extracts.

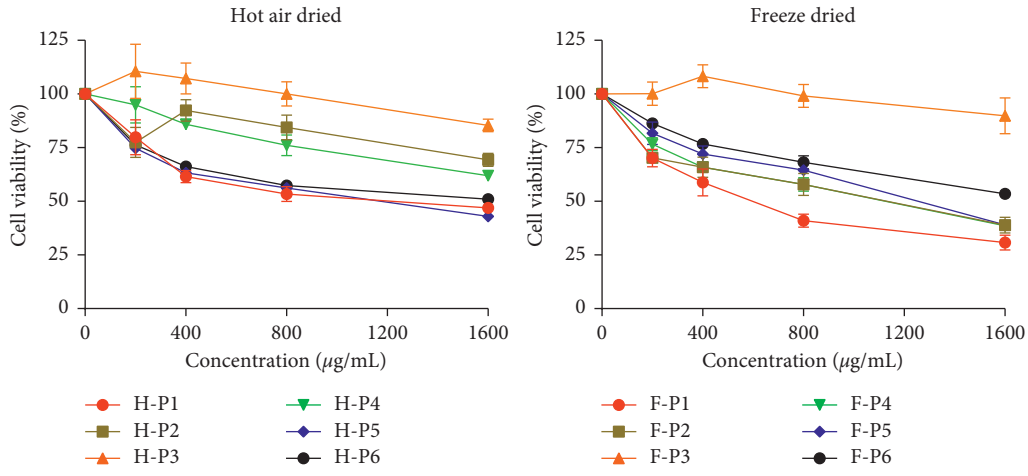
Method	Samples	DPPH·radical-scavenging activity assay	ABTS·radical-scavenging activity assay
Hot air-dried samples	H-P1	4.208 ± 0.033^{a}	2.422 ± 0.068^{a}
	H-P2	13.84 ± 0.353^{d}	7.239 ± 0.167^{e}
	H-P3	14.03 ± 0.495^{d}	8.287 ± 0.237^{f}
	H-P4	12.69 ± 0.355^{e}	7.311 ± 0.111^{e}
	H-P5	8.166 ± 0.118^{b}	5.849 ± 0.107^{d}
	H-P6	8.035 ± 0.094^{b}	5.363 ± 0.061^{c}
Freeze-dried samples	F-P1	4.611 ± 0.053^{a}	2.224 ± 0.070^{a}
	F-P2	17.07 ± 0.712^{g}	7.529 ± 0.261^{e}
	F-P3	15.58 v 0.286^{f}	9.189 ± 0.490^{g}
	F-P4	14.02 ± 0.306^{d}	7.14 ± 0.277^{e}
	F-P5	9.774 ± 0.266^{c}	6.295 ± 0.073^{d}
	F-P6	9.134 ± 0.139^{c}	4.578 ± 0.095^{b}

Mean and standard deviation obtained from three independent experiments. Letters a–g indicate significant differences ($P < 0.05$). H-P1, hot air-dried part 1; H-P2, hot air-dried part 2; H-P3, hot air-dried part 3; H-P4, hot air-dried part 4; H-P5, hot air-dried part 5; H-P6, hot air-dried part 6; F-P1, freeze-dried part 1; F-P2, freeze-dried part 2; F-P3, freeze-dried part 3; F-P4, freeze-dried part 4; F-P5, freeze-dried part 5; F-P6, freeze-dried part 6.

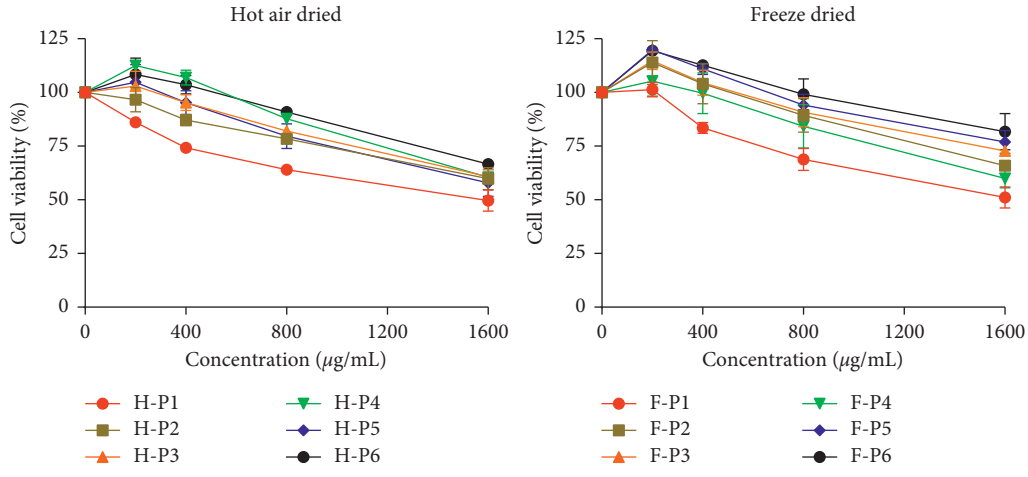
3.6. Antiproliferative Activities. Extracts of several vegetables have shown anticancer effects *in vitro* and *in vivo* [39, 40]. In the present study, antiproliferative effects of methanol extracts of cabbage leaf layers under the two drying methods were evaluated in human pancreatic carcinoma MIA PaCa-2 cells, human breast adenocarcinoma MCF7 cells, human



(a)



(b)



(c)

Figure 5: Continued.

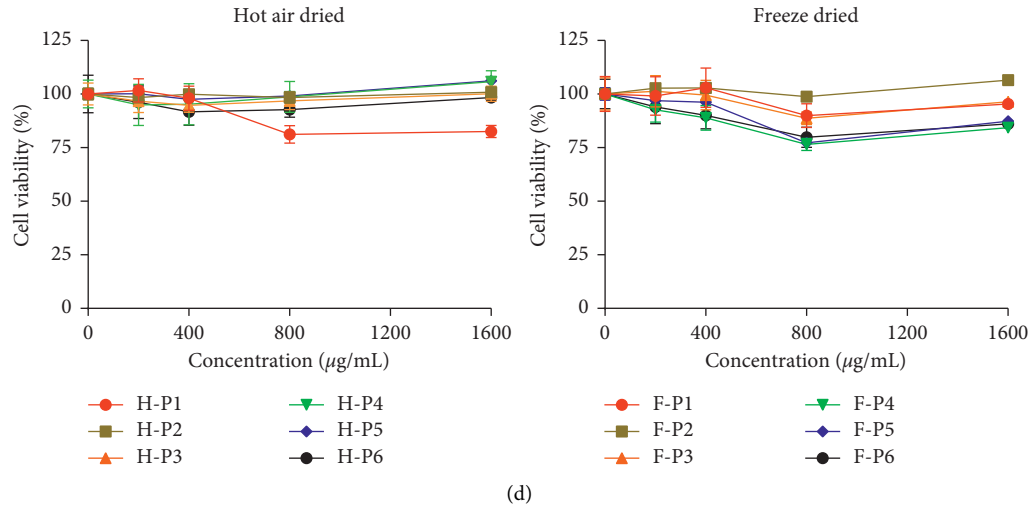


Figure 5: Antiproliferative effects of methanol extracts of cabbage leaf layers as assessed by the MTT assay following 48 h of exposure to the MIA PaCa-2 cell line, (a) MCF7 cell line, (b) AGS cell line, (c) and HepG2 cell line (d). The mean and standard deviation were obtained from three independent experiments. H-P1, hot air-dried part 1; H-P2, hot air-dried part 2; H-P3, hot air-dried part 3; H-P4, hot air-dried part 4; H-P5, hot air-dried part 5; H-P6, hot air-dried part 6; F-P1, freeze-dried part 1; F-P2, freeze-dried part 2; F-P3, freeze-dried part 3; F-P4, freeze-dried part 4; F-P5, freeze-dried part 5; F-P6, freeze-dried part 6.

Table 3: IC_{50} (μg/mL) values of methanol extract cabbage leaf layers.

Method	Samples	MIA PACA-2	MCF7	AGS	HepG2
Hot air-dried samples	H-P1	$1276^{\dagger} \pm 39.97^{a}$	1091 ± 104.1^{b}	1564 ± 93.98^{a}	NM
	H-P2	3097 ± 58.2^{d}	NM	2227 ± 93.7^{cd}	NM
	H-P3	3363 ± 72^{d}	NM	2008 ± 45.4^{bc}	NM
	H-P4	NM	2207 ± 94.6^{e}	1843 ± 95.6^{b}	NM
	H-P5	1148 ± 57^{a}	1064 ± 55.34^{b}	1857 ± 73.7^{b}	NM
	H-P6	1802 ± 218^{b}	1410 ± 117.7^{d}	2043 ± 100.4^{bc}	NM
Freeze-dried samples	F-P1	1214 ± 59.81^{a}	568.3 ± 28.43^{a}	1567 ± 62.3^{a}	NM
	F-P2	2554 ± 77^{c}	991.5 ± 83.39^{b}	2027 ± 113.6^{bc}	NM
	F-P3	NM	NM	2305 ± 114.6^{d}	NM
	F-P4	1689 ± 94^{b}	988.7 ± 47.31^{b}	1900 ± 93.8^{b}	NM
	F-P5	NM	1161 ± 71.24^{b}	2287 ± 97.4^{cd}	NM
	F-P6	NM	1838 ± 112.8^{c}	2074 ± 94^{bc}	NM

IC_{50} values (μg/mL) of methanol extracts of cabbage leaf layers as assessed by the MTT assay following 48 h of exposure to the MIA PaCa-2, MCF7, AGS, and HepG2 cell line. †Mean and standard deviation obtained from three independent experiments. NM, not measurable. Letters a–g indicate significant differences ($P < 0.05$). H-P1, hot air-dried part 1; H-P2, hot air-dried part 2; H-P3, hot air-dried part 3; H-P4, hot air-dried part 4; H-P5, hot air-dried part 5; H-P6, hot air-dried part 6; F-P1, freeze-dried part 1; F-P2, freeze-dried part 2; F-P3, freeze-dried part 3; F-P4, freeze-dried part 4; F-P5, freeze-dried part 5; F-P6, freeze-dried part 6.

gastric adenocarcinoma AGS cells, and human hepatoma HepG2 cells. As shown in Figure 5, we found that proliferation of MIA PaCa-2, MCF7, and AGS was inhibited in a dose-dependent manner by the cabbage extracts, whereas little antiproliferative effect was observed in HepG2 cells. According to Tables S7–S9, strong positive correlations were observed for AGS cells, whereas less strong positive correlations were observed for MIA PaCa-2 and MCF7 cells. The methanol extract of outer leaf layer (P1) processed by both methods showed higher antiproliferative in most cancer cell lines tested, except in HepG2 cells (Figure 5 and Table 3). Among the phenolic compounds analyzed by HPLC, chlorogenic acid had the highest content in P1, followed by 4-hydrobenzoic acid, rutin, caffeic acid, catechin, and p-coumaric acid (Figure 2). Many studies have shown that phenolic compounds are effective for treating cancer. Chlorogenic acid and 4-hydroxybenzoic acid, rutin, and caffeic acid, catechin, and p-coumaric acid exerted anticancer effects in a various cancer cell lines by inducing apoptosis [41–46]. Altogether, the antiproliferative effects of methanol extracts of cabbage leaf layers are consistent with the accumulation of phenolic, polyphenol, and flavonoid compounds in different parts of cabbage leaves, suggesting that a positive relationship exists between antiproliferative activities and these compounds in cabbage.

4. Conclusion

We compared the contents of bioactive compounds (11 polyphenols and 3 isothiocyanates) and analyzed the antioxidant and antiproliferative activities in different parts of cabbage processed by hot air drying and freeze drying. The

different drying methods led to different accumulations of polyphenols and isothiocyanate compounds. The contents of isothiocyanate, gallic acid, epicatechin, p-coumaric acid, sinapic acid, and myricetin were higher in the hot air-dried sample, whereas catechin, chlorogenic acid, 4-hydroxybenzoic acid, and rutin were significantly higher in the freeze-dried sample. Most of the phenolic compounds were concentrated in the outer leaves (P1), consistent with the greatest antioxidant and antiproliferative effects of cabbage extracted with methanol. However, isothiocyanate compounds were concentrated in the innermost layer leaves (P5) adjacent to the cabbage core. Our results indicate that cabbage has potential as a dietary supplement for promoting health. These results may attract the attention of consumers to the leaves of cabbage, which have the highest antioxidant potency and antiproliferative properties, but are usually discarded.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no onflicts of interest or personal relationships that could have appeared to influence the work reported in this paper.

Authors' Contributions

Somi Kim Cho conceived the study and revised the paper. Do Manh Cuong and Meran Keshawa Ediriweerab wrote the paper. The experiments were performed by Do Manh Cuong and Hee Young Kim. All authors participated equally in reviewing and finalizing the manuscript. Do Manh Cuong and Hee Young Kim contributed equally to this work.

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Supplementary Materials

Table S1: accumulation of phenolic compounds in different parts of cabbage (μg/g DW). Table S2: accumulation of isothiocyanates in different parts of cabbage (μg/g DW). Table S3: correlation analysis between drying methods (hot air-dried and freeze-dried) and TFC in different cabbage leaf layers. Table S4: correlation analysis between drying methods (hot air-dried and freeze-dried) and TPC in different cabbage leaf layers. Table S5: correlation analysis between drying methods (hot air-dried and freeze-dried) and DPPH radical-scavenging activity of different cabbage leaf layers. Table S6: correlation analysis between drying methods (hot air-dried and freeze-dried) and ABTS radical-scavenging activity of different cabbage leaf layers. Table S7: correlation analysis between drying methods (hot air-dried and freeze-dried) and antiproliferative activity in MIAPaCa-2 cells. Table S8: correlation analysis between drying methods (hot air-dried and freeze-dried) and antiproliferative activity in MCF-7 cells. Table S9: correlation analysis between drying methods (hot air-dried and freeze-dried) and antiproliferative activity in AGS cells. Figure S1: cultured MIA PaCa-2, (a) MCF-7, (b) AGS, (c) and HepG2 (d) cells. Figure S2: HPLC-UVD chromatograms of freeze-dried (a) and hot air-dried (b) cabbage leaf layers (P1) and standard compounds (c). (*Supplementary Materials*)

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