Food Sci. Biotechnol. 26(1): 221-228 (2017)
DOI 10.1007/s10068-017-0030-5

Food Science
and
Biotechnology

Effects of freeze-drying on antioxidant and anticholinesterase activities in various cultivars of kiwifruit (*Actinidia* spp.)

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Received July 14, 2016
Revised December 13, 2016
Accepted December 21, 2016
Published online February 28, 2017

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pISSN 1226-7708
eISSN 2092-6456

Abstract Oxidative stress contributes to neurodegenerative disorders such as Alzheimer's disease. Phenolic antioxidants can efficiently reduce oxidative stress. In this study, we evaluated the effects of the freeze-drying process on phenolics, antioxidants, and cholinesterase inhibition in five cultivars of kiwifruits grown in Korea, *Actinidia chinensis* cv. Hort16A, cv. Happygold, and cv. Haegeum; *A. deliciosa* cv. Hayward; and *A. eriantha* cv. Bidan, by comparing them with their fresh counterparts. Among the five cultivars of both fresh and freeze-dried kiwifruits tested in this study, cv. Bidan had the highest levels of total phenolics, total flavonoids, and antioxidants, and cv. Hayward had the lowest. Freeze-dried kiwifruits inhibited acetylcholinesterase and butyrylcholinesterase that catalyze the breakdown of acetylcholine (neurotransmitter). On sensory evaluation, cv. Happygold had the highest overall preference scores among the freeze-dried kiwifruits. The results suggest that freeze-dried kiwifruit could serve as a good source of antioxidants and cholinesterase inhibitors.

Keywords: acetylcholinesterase, butyrylcholinesterase, golden kiwifruit, green kiwifruit, white kiwifruit

Introduction

As one of the most popular fruits in the world, kiwifruits of the *Actinidia* species are known as a good source of vitamin C (1,2). Kiwifruits are also reported to contain flavonoids, phenolic acid, minerals, dietary fiber, vitamin E, and carotenoids (3,4). Kiwifruits are an economically important fruit crop in New Zealand, Italy, France, and Chile (5). Kiwifruit seedlings were introduced in Korea in 1977, and kiwifruits emerged in the Korean market in 1981 (6). Many kiwifruit cultivars, such as Hayward, Bruno, Abbott, and Monty, are grown in southern coastal areas and in Jeju Province, Korea (7). Domestically bred kiwifruit varieties including Haegeum, Bidan, Halla Gold, and Jecy Gold have been cultivated in Korea.

Reactive oxygen species (ROS) are well recognized for being both deleterious and beneficial. ROS within cells act as secondary messengers in intracellular signaling cascades and have anti-tumorigenic activity (8). However, ROS can also induce cellular senescence and apoptosis (8). Oxidative stress caused by ROS overproduction can lead to neurodegenerative diseases, such as Alzheimer's disease, through the oxidation of protein and lipids inside brain cells (9). Antioxidants can scavenge ROS. Many phenolic compounds, such as phenolic acids and flavonoids, exert antioxidant properties and protect cells and their components (10), and some of them have been identified in a variety of kiwifruits (4,11). It has been reported that kiwifruits grown in Korea carry biological benefits such as neuroprotection and antioxidant effects (12-14). Flavonoids and phenolic acids are reported to inhibit acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), which break down the neurotransmitter acetylcholine into choline and acetate in cholinergic neurons (15,16). Inhibition of AChE and BChE significantly correlates with the total phenolics and total flavonoids, respectively, of kiwifruits (1).

Drying as a food preservation process results in physical and biological changes to foodstuffs (17). Freeze-drying is a well-known drying process used to manufacture high-quality dehydrated foods and preserve heat-sensitive bioactive components (17). Consumers currently pay great attention to the health-improving properties of fruits, and demand for processed fruit products, such as juices and snacks, has gradually increased (18). Freeze-drying, however, decreases the total phenolic and flavonoid content and the antioxidant capacity of golden kiwifruits, along with a reduction in vitamin C (19). Limited information is available on the influence of freeze-drying on biological effects, including antioxidant capacity and cholinesterase inhibition,

of kiwifruits commercially bred in Korea.

In this study, we freeze-dried five different cultivars of kiwifruits grown in Korea: *Actinidia chinensis* cv. Hort16A, *A. chinensis* cv. Happygold, *A. chinensis* cv. Haegeum, *A. deliciosa* cv. Hayward, and *A. eriantha* cv. Bidan. We then used fresh and freeze-dried kiwifruits to comparatively evaluate the total phenolic and flavonoid contents and antioxidant capacity. We also looked for AChE and BChE inhibition to evaluate anticholinesterase activity and find potential anti-neurodegenerative effects in freeze-dried kiwifruits.

Materials and Methods

Chemicals We purchased ascorbic acid, ABTS, catechin, DPPH, 2,2'-azobis-(2-methylpropionamidine) dihydrochloride (AAPH), Folin-Ciocalteu's phenol reagent, gallic acid, AChE, BChE, acetylcholine iodide (ATCI), butyrylthiocholine chloride (BTCC), 9-amino-1,2,3,4-tetrahydroacridine hydrochloride hydrate (tacrine), 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), and phosphate buffered saline (PBS) from Sigma-Aldrich, Inc. (St. Louis, MO, USA). Dulbecco's phosphate buffered saline (DPBS) was purchased from Welgene Inc. (Daegu, Korea). All other reagents were of analytical or HPLC grade.

Kiwifruits We grew five different types of *Actinidia* genotypes: cv. Hort16A, cv. Happygold, cv. Haegeum, cv. Hayward, and cv. Bidan. Kiwifruits were grown and harvested in Gwangyang, Jeonnam, Republic of Korea, in 2014. Among the five kiwifruit cultivars tested in this study, cv. Happygold, cv. Haegeum, and cv. Hort16A are golden kiwifruit (*Actinidia chinensis*), cv. Hayward is a green kiwifruit (*Actinidia deliciosa*), and cv. Bidan is a white kiwifruit (*Actinidia eriantha*) with white hairy skin. Cv. Bidan, cv. Haegeum, and cv. Happygold are domestically bred kiwifruit varieties, whereas cv. Hort16A and cv. Hayward originated in New Zealand. The harvested kiwifruits were stored at 4°C before being freeze-dried and extracted. We peeled the kiwifruits and sliced them to a thickness of 0.5 cm. The sliced kiwifruits were freeze-dried at −40 to 70°C using a freeze-dryer (Lyoph-Pride Series; ilShinBiobase, Yangju, Korea).

Determination of water content We measured the water content using the drying method (20) and used the measured water content to determine the solid content when comparing total phenolic and flavonoid contents as well as antioxidant capacity between fresh and freeze-dried kiwifruits. The five kiwifruit cultivars in fresh and freeze-dried forms showed the water content of 87.1 and 9.2% in cv. Bidan, 84.0 and 8.0% in cv. Haegeum, 84.1 and 10.6% in cv. Happygold, 83.2 and 14.1% in cv. Hayward, and 84.3 and 9.1% in cv. Hort16A, respectively (data not shown).

Extraction The flesh of fresh or freeze-dried kiwifruit was mixed with 100 mL of absolute methanol and homogenized using a Polytron homogenizer (PT 10/35; Kinematica, Kriens-Luzern, Switzerland) at 15,000 rpm for 2 min. We filtered the homogenized mixture through Whatman #2 filter paper (Whatman International Limited, Kent, England) using an aspirator. Filter cakes were re-extracted using the procedure above with 100 mL of 80% (v/v) aqueous methanol instead of absolute methanol. We then combined the two filtrates and evaporated them until dry under reduced pressure using a rotary evaporator (Eyela, Tokyo, Japan) in a water bath at 37°C. The concentrated extracts were dissolved in 50 mL of absolute methanol and brought to a final volume of 100 mL with deionized water. We stored the fresh and freeze-dried kiwifruit extracts at −20°C prior to use. The extraction of each kiwifruit was performed in triplicate.

Determination of total phenolic content We measured the total phenolic content via a colorimetric method using the Folin-Ciocalteu's phenol reagent (21). We mixed properly diluted extracts (0.2 mL) with 2.6 mL of deionized water and added an aliquot (0.2 mL) of Folin-Ciocalteu's phenol reagent to the mixture. After 6 min, we added 2.0 mL of 7% (w/v) Na_2CO_3 solution to the reaction mixture. At 90 min, we measured the absorbance at 750 nm. A gallic acid standard was used to build a calibration curve with concentrations of 10, 30, 60, and 100 mg/L. The content of total phenolics was expressed as mg gallic acid equivalents (GAE)/g of dried kiwifruit. Each extract was analyzed in triplicate.

Determination of total flavonoid content We measured the total flavonoid content using a modified version of the method described by Kim *et al.* (22). Briefly, a mixture of 500 μL of properly diluted extract and 3.2 mL of distilled water was added to 150 μL of 5% (w/v) $NaNO_2$. After 5 min, we added 150 μL of 10% (w/v) $AlCl_3$. After 6 min, we added 1 mL of 1 M NaOH and then immediately measured the absorbance of the mixture at 510 nm versus a deionized water blank. A calibration curve was built using a catechin standard solution at 10, 30, 60, and 100 mg/L. The total flavonoid content of each kiwifruit was expressed as mg catechin equivalents (CE)/g of dried kiwifruit. Each extract was analyzed in triplicate.

ABTS radical scavenging capacity We measured the antioxidant capacity of kiwifruit extracts using blue/green ABTS radicals (10). We mixed 1 mM of AAPH was mixed with 2.5 mM ABTS in PBS, which we heated in a water bath at 70°C for 30 min to create ABTS radicals. The solution of ABTS radicals was adjusted to an absorbance of 0.650± 0.020 at 734 nm. The reaction between the ABTS radical solution (980 μL) and the appropriately diluted extracts (20 μL) was conducted at 37°C for 10 min. Absorbance at 734 nm was measured immediately. We used a vitamin C standard to build a calibration curve with concentrations of 10, 30, 60, and 100 mg/L. The antioxidant capacity of kiwifruit was expressed as mg vitamin C equivalents (VCE)/g of dried kiwifruit. Each extract was analyzed in triplicate.

DPPH radical scavenging capacity We used a method described by Brand-Williams *et al.* (23) to measure DPPH radical scavenging

capacity. We set the absorbance of fresh deep-purple DPPH radicals in 80% (v/v) aqueous methanol to 0.650±0.020 at 517 nm. The reaction between the DPPH radical solution (2.95 mL) and the appropriately diluted extracts (50 μL) took place at 23°C for 30 min. The reduction of absorbance at 517 nm was measured immediately. We used a vitamin C standard to build a calibration curve with concentrations of 10, 30, 60, and 100 mg/L. The antioxidant capacity of kiwifruit was expressed as mg VCE/g of dried kiwifruit. Each extract was analyzed in triplicate.

Oxygen radical absorbance capacity We performed the ORAC assay as described by Huang *et al.* (24). Appropriately diluted extracts (25 μL) and 150 μL of 81.6 nM fluorescein solution were added to a 96-well plate and incubated at 37°C for 10 min with 3 min of shaking. We added 25 μL of 153 mM AAPH solution and the detected the fluorescence every minute for 90 min using a microplate reader (Infinite M200; Tecan Austria GmbH, Grödig, Austria) with 485 nm excitation and 520 nm emission wavelengths. Antioxidant capacity of kiwifruit was expressed as mg VCE/g of dried kiwifruit. Each extract was analyzed in triplicate.

Anticholinesterase activity We determined anticholinesterase activity using AChE and BChE assays performed in a 96-well plate format. We used ATCI and BTCC substrates for the AChE and BChE inhibitory assays, respectively, and DTNB as the color developing reagent. For the AChE inhibition assay, 20 μL of each concentration (15.6 to 1,000 μg/mL) of kiwifruit extracts and 20 μL AChE (0.2 U/mL) were added to 150 μL DPBS. After incubation for 5 min at 37°C, 30 μL DTNB (10 mM) and 20 μL ATCI substrate (15 mM) were added. The BChE inhibition assay was performed using a similar protocol, except that instead of AChE and ATCI, we used 0.2 U/mL BChE and 10 mM BTCC. After 5 min at 37°C, we measured absorbance at 415 nm using a microplate reader (Infinite M200; Tecan Austria GmbH). Tacrine was used as a positive control. We made a tacrine standard curve relating various concentrations of tacrine to cholinesterase inhibition (%) for our quantitative evaluation of cholinesterase inhibitory activity in freeze-dried kiwifruits. We expressed the inhibitory activities of AChE and BChE under treatment in the freeze-dried kiwifruits as nM of tacrine equivalents. Measurements of AChE and BChE inhibitory activity were done in triplicate.

Sensory evaluation We used 31 panelists to perform the sensory evaluation of the freeze-dried kiwifruits. We asked the panelists to assess the color, texture, flavor, sweetness, bitterness, sourness, and their overall preference for the freeze-dried kiwifruits presented in a random order. Characteristics of the freeze-dried kiwifruits were scored on a 9-point line scale (1; not undesirable, 9; preferable).

Statistical analysis All experiments were performed in triplicate. Statistical tests were performed using a dedicated software package (SAS 9.4, SAS Institute, Inc., Cary, NC, USA). Significant differences were verified using Duncan's multiple range test with a 95% confidence level.

Results and Discussion

Total phenolic and total flavonoid contents of kiwifruits The levels of total phenolics in fresh and freeze-dried kiwifruits are presented in Fig. 1A. The total phenolic content (mg GAE/g dry weight) among the various fresh kiwifruits was as follows in descending order: cv. Bidan (28.1±0.2)>cv. Happygold (9.0±0.1)>cv. Haegeum (7.7±0.2)>cv. Hort16A (6.9±0.1)>cv. Hayward (5.0±0.0), whereas the total phenolic content of freeze-dried kiwifruits was as follows in descending order: cv. Bidan (22.4±0.0)>cv. Haegeum (6.0±0.0)>cv. Happygold (5.6±0.1)>cv. Hort16A (5.2±0.1)>cv. Hayward (4.5±0.1). Fresh kiwifruits had significantly (p<0.05) higher total phenolic content than their freeze-dried counterparts. The freeze-dried kiwifruits contained 61.9–90.7% of the total phenolics present in fresh kiwifruits. The total phenolic levels of the domestically developed cultivars (cv. Bidan, cv. Happygold, and cv. Haegeum) were significantly (p<0.05) higher than those of the exotic cultivars (cv. Hort16A and cv. Hayward). Golden kiwifruits (*A. chinensis*) grown in Turkey had a total phenolic content of 16.67 mg GAE/g of lyophilized aqueous extract (3). Similar to the results in our study, a previous study found that *A. eriantha* contained a higher total phenolic content than *A. chinensis* and *A. deliciosa* (25). With similar soluble solid content harvested in 2007, *A. eriantha* cv. Bidan showed significantly (p<0.05) higher total phenolic content than *A. deliciosa* cv. Hayward (26).

The contents of total flavonoids in the fresh and freeze-dried kiwifruits are presented in Fig. 1B. The total flavonoid contents (mg CE/g dry weight) among the fresh kiwifruits were as follows in descending order: cv. Bidan (3.1±0.4)>cv. Happygold (1.7±0.1)>cv. Haegeum (1.2±0.0)>cv. Hort16A (1.1±0.0)>cv. Hayward (0.7±0.0), whereas the freeze-dried kiwifruits had total flavonoid contents as follows in descending order: cv. Bidan (1.4±0.1)>cv. Haegeum (1.1±0.1)>cv. Happygold (1.0±0.0)≈cv. Hort16A (1.0±0.0)>cv. Hayward (0.7±0.0). As with total phenolic content, fresh kiwifruits contained higher total flavonoid content than their freeze-dried counterparts. Compared with fresh kiwifruits, lower amounts of total phenolics and flavonoids in their freeze-dried kiwifruits might result from decrease and/or loss of some phenolic compounds such as phenolic acids and flavonoids via tissue damage during sample preparation and lyophilization. Freeze-dried kiwifruits had 45.2–95.6% of the total flavonoid content of fresh kiwifruits. As in our results, the total flavonoid content of fresh *A. eriantha* cv. Bidan harvested in 2007 was significantly (p<0.05) higher than that of fresh *A. deliciosa* cv. Hayward (26).

Antioxidant capacity of kiwifruits Phenolic compounds are known as antioxidants, which neutralize free radicals, inhibit radical-producing enzymes, and scavenge ROS (10,27,28). ROS in a human

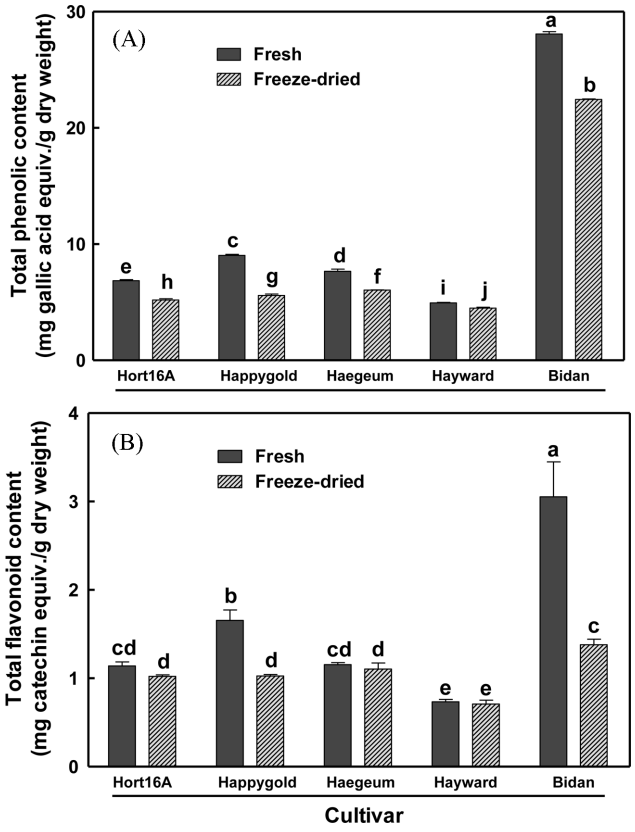

Fig. 1. Contents of total phenolics (A) and total flavonoids (B) in fresh and freeze-dried kiwifruits. Data are expressed as mean±standard deviation (bars) of three replications. Different letters on the bars indicate significant differences by Duncan's multiple range test (p<0.05).

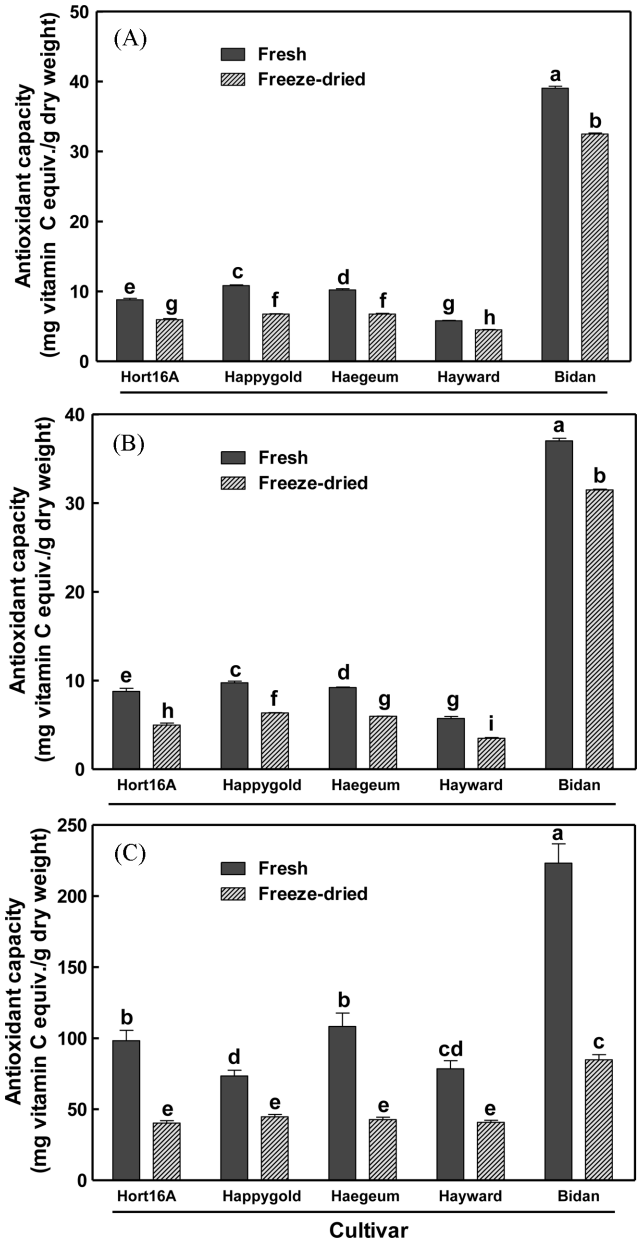

Fig. 2. Antioxidant capacity of fresh and freeze-dried kiwifruits using the ABTS (A), DPPH (B), and ORAC (C) assays. Data are expressed as mean±standard deviation (bars) of three replications. Different letters on the bars indicate significant differences by Duncan's multiple range test (p<0.05).

body cause oxidative destruction of various cellular components, leading to cellular senescence and age-related neurodegeneration. Therefore, it is important to quantitatively measure antioxidants that are responsible for quenching oxidants such as ROS. In this study, we used three different measurements of antioxidants, the ABTS, DPPH, and ORAC assays, to comparatively assess the antioxidant capacity of various kiwifruits (Fig. 2).

The antioxidant capacity of fresh and freeze-dried kiwifruits measured using the ABTS assay is presented in Fig. 2A. The antioxidant capacity (mg VCE/g dry weight) among the fresh kiwifruits was as follows in descending order: cv. Bidan (39.0±0.3)>cv. Happygold (10.8±0.1)>cv. Haegeum (10.2±0.2)>cv. Hort16A (8.8±0.2) >cv. Hayward (5.8±0.1), whereas freeze-dried kiwifruits had antioxidant capacity as follows in descending order: cv. Bidan (32.5±0.1)>cv. Haegeum (6.8±0.1)≈cv. Happygold (6.8±0.1)>cv. Hort16A (6.0±0.1) >cv. Hayward (4.5±0.0). Freeze-dried kiwifruits had 62.4–83.3% of the antioxidant capacity of fresh kiwifruits measured using the ABTS assay.

The antioxidant capacity of fresh and freeze-dried kiwifruits as measured using the DPPH assay is presented in Fig. 2B. The antioxidant capacity among the fresh kiwifruits was as follows in descending order: cv. Bidan (37.0±0.3)>cv. Happygold (9.7±0.2)>cv. Haegeum (9.2±0.1)>cv. Hort16A (8.8±0.4)>cv. Hayward (5.7±0.2), whereas the freeze-dried kiwifruits contained antioxidants as follows in descending order: cv. Bidan (31.5±0.1)>cv. Happygold (6.3±0.0)>cv. Haegeum (6.0±0.0)>cv. Hort16A (5.0±0.2)>cv. Hayward (3.5±0.1). Freeze-dried kiwifruits thus have 56.7–85.0% of the antioxidant capacity of fresh kiwifruits measured using the DPPH assay.

The antioxidant capacity of fresh and freeze-dried kiwifruits as measured using the ORAC assay is presented in Fig. 2C. Fresh kiwifruits had antioxidant capacity as follows in descending order: cv. Bidan (223.1±13.7)>cv. Haegeum (108.2±9.6)>cv. Hort16A (98.3±7.2)

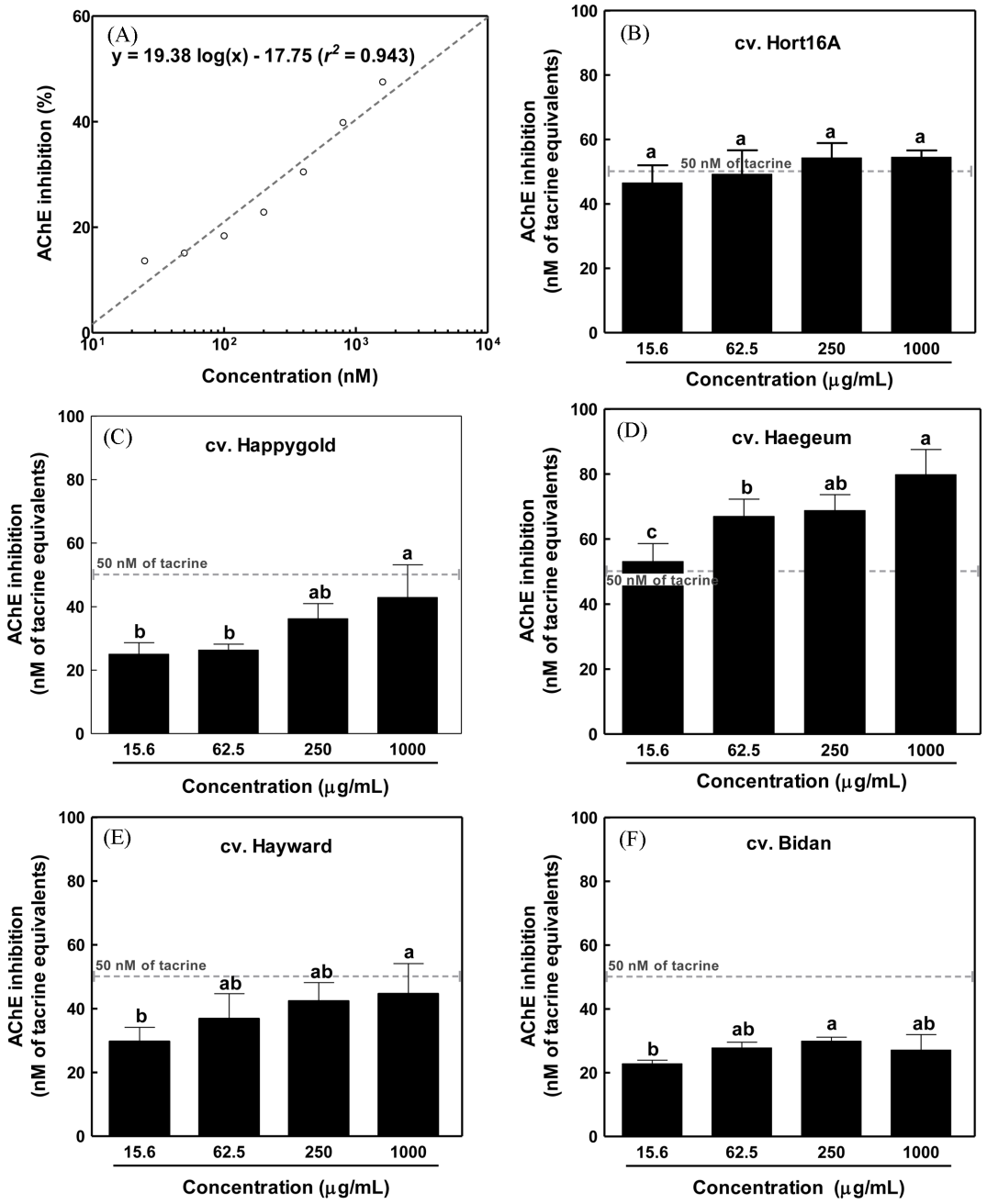

Fig. 3. Effects of freeze-dried kiwifruits on acetylcholinesterase (AChE) activity. Tracrine standard cruve (A); cv. Hort16A (B); cv. Happygold (C); cv. Haegeum (D); cv. Hayward (E); cv. Bidan (F). Data are expressed as mean±standard deviation (bars) of three replications. Different letters on the bars indicate significant differences by Duncan's multiple range test (p<0.05).

>cv. Hayward (78.5±5.7)>cv. Happygold (73.5±4.0), whereas freeze-dried kiwifruits showed antioxidant capacity as follows in descending order: cv. Bidan (84.9±3.6)>cv. Happygold (44.7±1.8)>cv. Haegeum (42.8±1.7)>cv. Hayward (40.8±1.5)>cv. Hort16A (40.3±1.7). In the ORAC assay, freeze-dried kiwifruits had 38.0–60.8% of the antioxidant capacity of fresh kiwifruits.

In all three different antioxidant measurements used in this study, fresh kiwifruits had significantly (p<0.05) higher antioxidant levels than freeze-dried kiwifruits (Fig. 2). As with the levels of total phenolics and flavonoids, the varieties developed in Korea, cv. Bidan, cv. Haegeum, and cv. Happygold, had higher antioxidants than the varieties from New Zealand, cv. Hort16A and cv. Hayward. In previous studies, the white kiwifruit, cv. Bidan, showed higher antioxidant capacity than the golden kiwifruit cv. Haegeum and the green kiwifruit cv. Hayward (13,14). Based on DPPH radical scavenging activity, a previous study also found that the antioxidant capacity of cv. Bidan was higher than that of cv. Hayward (26), and using the ABTS and DPPH assays, it was previously found that cv.

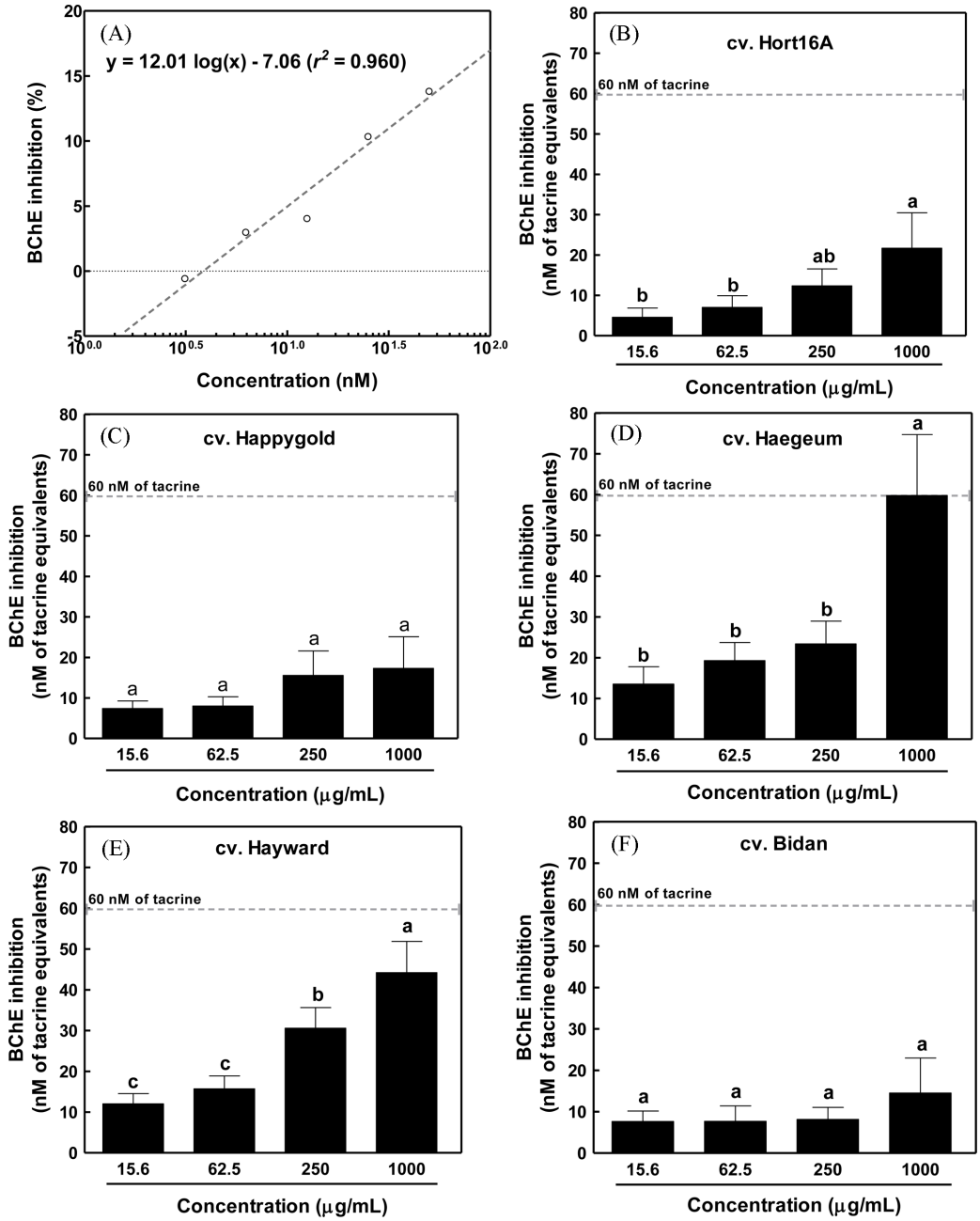

Fig. 4. Effects of the freeze-dried kiwifruits on butyrylcholinesterase (BChE) activity. Tracrine standard cruve (A); cv. Hort16A (B); cv. Happygold (C); cv. Haegeum (D); cv. Hayward (E); cv. Bidan (F). Data are expressed as mean±standard deviation (bars) of three replications. Different letters on the bars indicate significant differences by Duncan's multiple range test (p<0.05).

Bidan had higher antioxidant capacity than cv. Hort16A and cv. Hayward (11).

AChE and BChE Inhibition of kiwifruits AChE plays a principal role in the termination of nerve impulse transmissions at cholinergic synapses by rapidly breaking down the neurotransmitter acetylcholine into choline and acetate (29). BChE is known to be widely distributed in tissues and plasma in the body (30). Like AChE, BChE can hydrolyze the neurotransmitter acetylcholine (31). Together with AChE, BChE helps to maintain acetylcholine levels for cholinergic neurotransmission. Inhibition of AChE and BChE activity might be a good strategy to prolong neurotransmission in neurodegenerative diseases such as Alzheimer's disease.

We quantitatively investigated whether the five different freeze-dried kiwifruits inhibited AChE activity (Fig. 3). The tacrine standard curve made in a logarithmic function had a linear trend line with a correlation coefficient (r^2) of 0.943 (Fig. 3A). Pretreatment with various freeze-dried kiwifruits showed AChE inhibitory activity (as tacrine equivalents) from 22.8±1.1 to 79.8±7.8 nM, and cv. Haegeum had the highest AChE inhibitory activity among the cultivars tested in

Table 1. Sensory evaluation of freeze-dried kiwifruits

Characteristics	Cultivar				
	cv. Hort16A	cv. Happygold	cv. Haegeum	cv. Hayward	cv. Bidan
Color	4.8±1.9$^{cd1)}$	7.1±1.6^{b}	5.3±1.8^{c}	4.1±1.5^{d}	8.4±0.8^{a}
Texture	4.5±2.1^{b}	6.2±1.9^{a}	6.3±2.1^{a}	5.5±2.2^{ab}	5.7±2.0^{a}
Flavor	4.4±2.0^{b}	5.9±1.7^{a}	5.1±1.8^{ab}	5.0±1.9^{ab}	5.9±1.9^{a}
Sweetness	4.2±1.8^{c}	6.8±1.7^{a}	5.5±2.1^{b}	4.6±1.7^{c}	5.9±1.8^{ab}
Bitterness	4.5±2.4^{c}	6.4±1.8^{a}	5.0±2.3^{bc}	5.1±2.1^{bc}	6.0±2.0^{ab}
Sourness	5.0±2.1^{c}	6.6±1.6^{a}	5.5±1.8^{bc}	4.9±2.2^{c}	6.4±1.5^{ab}
Overall preference	4.4±1.9^{d}	7.2±1.8^{a}	5.8±2.1^{bc}	4.9±2.0^{cd}	6.7±1.9^{ab}

[1)]Data are presented as mean±standard deviation (n=31). The different letters in the same row shows the significant difference by Duncan's multiple range test (p<0.05).

this study (Fig. 3B–3F). At concentrations of 1,000 μg/mL, pretreatment with the five freeze-dried kiwifruits showed AChE inhibitory activity (nM of tacrine equivalents) as follows in decreasing order: cv. Haegeum (79.8±7.8)>cv. Hort16A (54.5±2.2)>cv. Hayward (44.6±9.5) >cv. Happygold (42.9±10.3)>cv. Bidan (27.1±4.9). At the lowest concentration (15.6 mg/mL) used in this study, pretreatment with the five freeze-dried kiwifruits showed AChE inhibitory activity (nM of tacrine equivalents) as follows in decreasing order: cv. Haegeum (53.1±5.5)>cv. Hort16A (46.5±5.5)>cv. Hayward (29.7±4.5)>cv. Happygold (25.0±3.6)>cv. Bidan (22.8±1.1).

We also evaluated the anticholinesterase activity of the five cultivars of freeze-dried kiwifruits on the basis of BChE activity (Fig. 4). To quantitatively evaluate BChE inhibition with treatments of freeze-dried kiwifruits, we applied a tacrine standard curve with a correlation coefficient (r^2) of 0.960 (Fig. 4A). Pretreatment with the five freeze-dried kiwifruits revealed BChE inhibitory activity (as tacrine equivalents) from 4.6±2.3 to 59.8±14.9 nM (Fig. 4B–4F), and cv. Haegeum had the highest BChE inhibitory activity among the cultivars tested in this study. At a concentration of 1,000 μg/mL, pretreatment with the five freeze-dried kiwifruits had BChE inhibitory activity (nM of tacrine equivalents) as follows in decreasing order: cv. Haegeum (59.8±14.9)>cv. Hayward (44.2±7.7)>cv. Hort16A (21.7±8.8)>cv. Happygold (17.3±7.8)>cv. Bidan (14.6±8.5). At the lowest concentration (15.6 mg/mL), pretreatment with the five freeze-dried kiwifruits had BChE inhibitory activity (nM of tacrine equivalents) as follows in decreasing order: cv. Haegeum (13.5±4.3) >cv. Hayward (12.0±2.5)>cv. Bidan (7.6±2.5)>cv. Happygold (7.4±1.9) >cv. Hort16A (4.6±2.3).

Phenolic acids, such as ferulic acid, *p*-coumaric acid, and *p*-hydroxybenzoic acid, work as AChE and BChE inhibitors (15). Flavonoids, such as galangin, quercetin, luteolin, and fisetin, reversibly inhibit human BChE (16). Ethanol extracts of dry kiwifruits, including cv. Hayward, cv. Hort16A, cv. Haegeum, and cv. Bidan, were reported to inhibit both AChE and BChE, and cv. Bidan had the highest inhibition of AChE activity, unlike our results (1). Phenolic acids and flavonoids were previously identified as bioactive compounds in a variety of kiwifruits, including cv. Hayward and cv. Bidan (3,4,32). Hence, the kiwifruits used in this study could serve as AChE and BChE inhibitors to maintain neurotransmitter levels.

Sensory evaluation of freeze-dried kiwifruits The scores from sensory evaluation of the freeze-dried kiwifruits are shown in Table 1. Cv. Happygold received the highest overall preference score followed by cv. Bidan, cv. Haegeum, cv. Hayward, and cv. Hort16A. The highest scores in flavor, sweetness, bitterness, and sourness also were found in cv. Happygold. Cv. Bidan had the highest score in color, and cv. Haegeum received the highest score in texture. In general, the kiwifruits developed in Korea (cv. Happygold, cv. Haegeum, and cv. Bidan) received higher overall preference scores than the exotic cultivars (cv. Hort16A and cv. Hayward).

In conclusion, we comparatively evaluated the total phenolic and flavonoid contents and antioxidants in five cultivars of fresh kiwifruits and their freeze-dried products. Furthermore, we quantitatively investigated the inhibition of cholinesterase (AChE and BChE) by freeze-dried kiwifruits. We used several types of kiwifruits: golden (cv. Happygold, cv. Haegeum, and cv. Hort16A), green (cv. Hayward), and white (cv. Bidan) varieties. The total phenolic and flavonoid contents and the antioxidant capacity of all of the fresh and processed kiwifruits decreased in the following order: cv. Bidan>cv. Happygold>cv. Haegeum>cv. Hort16A>cv. Hayward. Thus, the domestically developed kiwifruits (cv. Bidan, cv. Happygold, and cv, Haegeum) have higher amounts of antioxidants and phenolics than the exotic cultivars we tested (cv. Hort16A and cv, Hayward). Freeze-drying of the kiwifruits decreased their total phenolic and flavonoid content as well as their antioxidant capacity compared to fresh kiwifruits, partly due to the loss of antioxidant phenolics and vitamin C. AChE and BChE were inhibited by the freeze-dried kiwifruit extracts. Our results thus suggest that kiwifruits are a good source of antioxidants and could serve as functional cholinesterase inhibitors. Further study of the identification and quantification of individual phenolic compounds and their inhibition of cholinesterase activity in the fresh and freeze-dried kiwifruits is needed.

Acknowledgments The study was financially supported by a grant (No. 20140322) from Kyung Hee University.

Disclosure The authors declare no conflict of interest.

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