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Short Photoirradiation Induces Flavonoid Synthesis and Increases Its Production in Postharvest Vegetables

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ABSTRACT: It is desirable to increase the flavonoid contents of postharvest vegetables since flavonoids play a beneficial role in human health promotion. In the present study, we show that postharvest vegetables increasingly produced flavonoids when irradiated with light near the absorption wavelength of flavonoids in the plant. Three-day exposure to UV-B for 5 min, 98 μmol m^{-2} s^{-1} per day, increased the contents of jaceidin in spinach, kaempherol glycoside in radish sprout, apigenin glycosides in parsley, and isovitexin in Indian spinach after 6 days of storage in a refrigerator, compared to the contents in plants without irradiation. Six days of storage of unripe green strawberry under green light for 5 min, 98 μmol m^{-2} s^{-1} per day, enabled them to mature and turn red, accompanied by 3.5-fold increased contents of pelargonidin. Elucidation of the mechanism in parsley found the stimulating expression of the flavonoid synthesis gene, PAL, C4H, 4CL, CHS, and FNS, 6 h after exposure to single irradiation with UV−B for 5 min, and the higher expression was maintained for 24 h. After 3 days irradiation during 6 days of storage, parsley did not show adverse changes in the contents of ascorbic acid, β-carotene, chlorophyll, and moisture.

KEYWORDS: *photoirradiation, flavonoids, phenylpropanoids, postharvested vegetables*

INTRODUCTION

The intake of flavonoids, ubiquitous components of plants, has been reported to reduce the risk of degenerative diseases[1] by preventative effects on coronary heart disease,[2] cancer,[3−6] and so on.[7] The contents of flavonoids differ greatly according to culture and weather conditions; for example, vegetables grown in a greenhouse have lower contents than those grown outdoors.[8,9] Plants perceive light with photoreceptors[10,11] and induce production of flavonoids to protect themselves from irradiation stress.[12−14] This indicates that artificial irradiation in the field can increase the flavonoids of human-health benefit in vegetables;[15] however, UV irradiation is usually avoided in the culture field because irradiation stress delays plant growth.[16] When irradiating postharvest vegetables, stress will abolish the problem of growth inhibition. Harvested vegetables take one or more days to deliver from the field to markets and consumers, and the consumers eat them after several days, while usually storing the vegetables in a refrigerator. It would be helpful for our health if the contents of flavonoids increased during delivery and storage.

Light that can induce irradiation stress will induce the plant to synthesize flavonoids to prevent irradiation stress.[12−15] Substrates for flavonoid synthesis are cinnamic acids of phenylpropanoid derived from phenylaranine as shown in the flavonoid synthesis pathway in Figure 1.[17,18] The cinnamic acids possess λ_{max} in a range of 275−320 nm.[19] A wavelength near to the absorption light of cinnamic acids, UV-B at 280−320 nm is better for irradiation to induce flavonoid synthesis. Also, light near the λ_{max} of flavonoids themselves, UV-A at 380−320 nm, is better for irradiating vegetables containing quercetin glycosides with a λ_{max} around 350 nm, kaempherol glycosides with a λ_{max} around 345 nm, and apigenin glycosides with a λ_{max} around 335 nm,[19] and green light at 500−600 nm is better for vegetables containing anthocyanin with a λ_{max} around 503 nm.[20] The appropriate irradiation level is probably near the annual average of sunshine, which globally is between 40 and 900 μmol m^{-2} s^{-1} per day for UV in sunlight irradiation.[21]

In the present study, we set UV-A and UV-B lamps, a green lamp, or a light-emitting diode (LED) of 375 nm in a home refrigerator, radiated vegetables purchased from city markets, and determined the increased contents of flavonoids.

MATERIALS AND METHODS

Chemicals. Standard chemicals to identify plant polyphenols were purchased as follows: phenylpropanoids and flavones were from Nacalai Tesque (Kyoto, Japan), flavonols were from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan), and other flavonoids were from Extrasynthèse (Genay, France). They were dissolved in dimethyl sulfoxide (DMSO) at 10 mM as stock solutions. L-Phenylalanine, β-carotene, and ascorbic acid were from Nacalai Tesque. All other chemicals were of the highest quality commercially available.

Harvested Fresh Vegetables. We had first determined the phenolic contents in various kinds of vegetables and found that the contents had a wide variety for every plant even in the same kind of vegetables. This indicated that the contents should be compared in one plant. In the present study, the contents have to be determined in UV-A and UV-B irradiations and nonirradiation every day for a few days. This required many leaves that contain the similar amounts of phenolics at the beginning of irradiation experiments. Then, we selected the vegetables that possess many leaves in one plant and purchased the following vegetables from local markets in Kobe, Japan: spinach (*Spinacia oleracea* L.), radish (*Raphanus sativum* L.), parsley (*Petroselinum crispum* Mill.), Indian spinach (*Basella rubra* L.), garden pea sprout (*Pisum stivum* L.), and watercress (*Nasturtium officinale* R. Br.).

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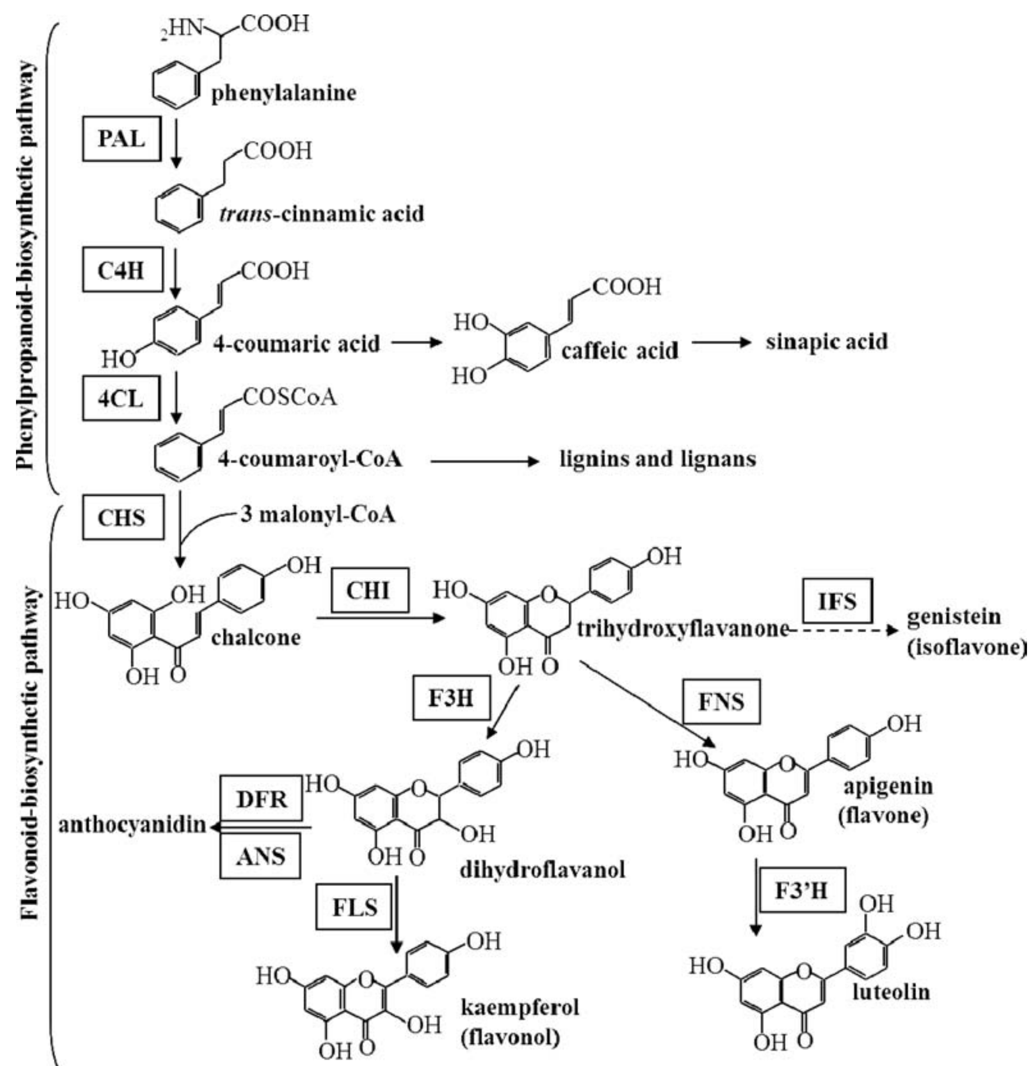

Figure 1. Biosynthetic pathway of flavonoids. Biosynthetic pathway of flavonoids consists of phenylpropanoid- and flavonoid-biosynthetic pathways, and is catalyzed by the following enzymes: PAL, phenylalnine ammonia-lyase; C4H, cinnamic acid 4-hydroxylase; 4CL, 4-coumarate-CoA ligase; CHS, chalcone synthase; CHI, chalcone isomerase; FNS, flavone synthase; F3H, flavonoid 3-hydroxylase; FLS, flavonol synthase; DFR, dihydroflavonol reductase; ANS, anthocyanin synthase; F3′H, flavonoid 3′-hydroxylase; and IFS, isoflavone synthase.

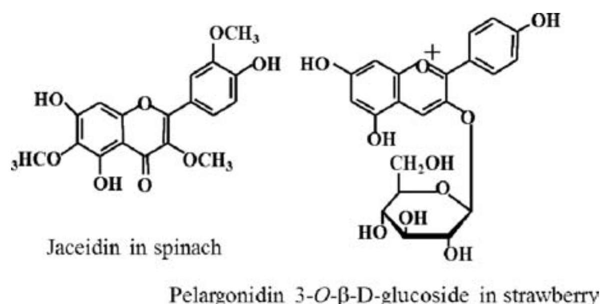

Figure 2. Chemical structures of the increasingly produced flavonoids by irradiation in spinach and strawberry.

Alternatively, strawberry (*Fragaria* x *ananassa* Duch.) was cultured in our garden and was used for green light irradiation.

Extraction of Flavonoids and Phenylpropanoids in Plants. Extraction was performed according to the method described previously.[19] The plants were chopped and homogenized in liquid nitrogen with a homogenizer (Nihonseiki Kaisha Co., Ltd., Osaka, Japan), lyophilized at 0.2 Pa for 48 h, and then extracted with 2 mL of 90% methanol containing 0.5% acetic acid after the addition of 50 nmol of flavone in DMSO solution as an internal standard. After standing in a sonicater for 1 min, the solution was centrifuged at 1,000*g* for 10 min. The extraction was repeated three times, and the supernatant was dried with a centrifugal concentrator (VC-96N; Taitec Co., Saitama, Japan). The resulting residues were dissolved in 0.5 mL DMSO, filtrated through a Millex-LG 0.2 μm pore-sized membrane filter (Millipore Co., Bedford, MA), and subjected to the following HPLC analysis.

HPLC. The HPLC analysis was performed as shown previously,[19] employing an HPLC system (Hitachi, Tokyo, Japan) equipped with model D-7000 chromatography data station software, autosampler D-7200, column oven D-7300, and diode array detection system D-7450 to monitor at all wavelengths from 200 to 600 nm. The analytical column maintained at 35 °C was a Capcell Pak C18 UG120 column (250 × 4.6 mm i.d., S-5, 5 mm; Shiseido Co., Ltd., Tokyo, Japan) and joined with a guard column (10 × 4.0 mm i.d.; Shiseido). The mobile phase was composed of solution A, which was a 1:9 mixture (v/v) of methanol and 50 mM NaH_2PO_4 at pH 3.3, and solution B, which was a 7:3 mixture (v/v) of methanol and 50 mM NaH_2PO_4 at pH 3.3. The gradient method started at 1.0 mL/min from 0 to 30% B in 15 min, and 30 to 35% B in 30 min, 35 to 40% B in 20 min, 40 to 50% A in 5 min, 50 to 100% A in 15 min, and then 100% for 10 min. The injection volume of sample was 10 μL. In all analyses, the column was re-

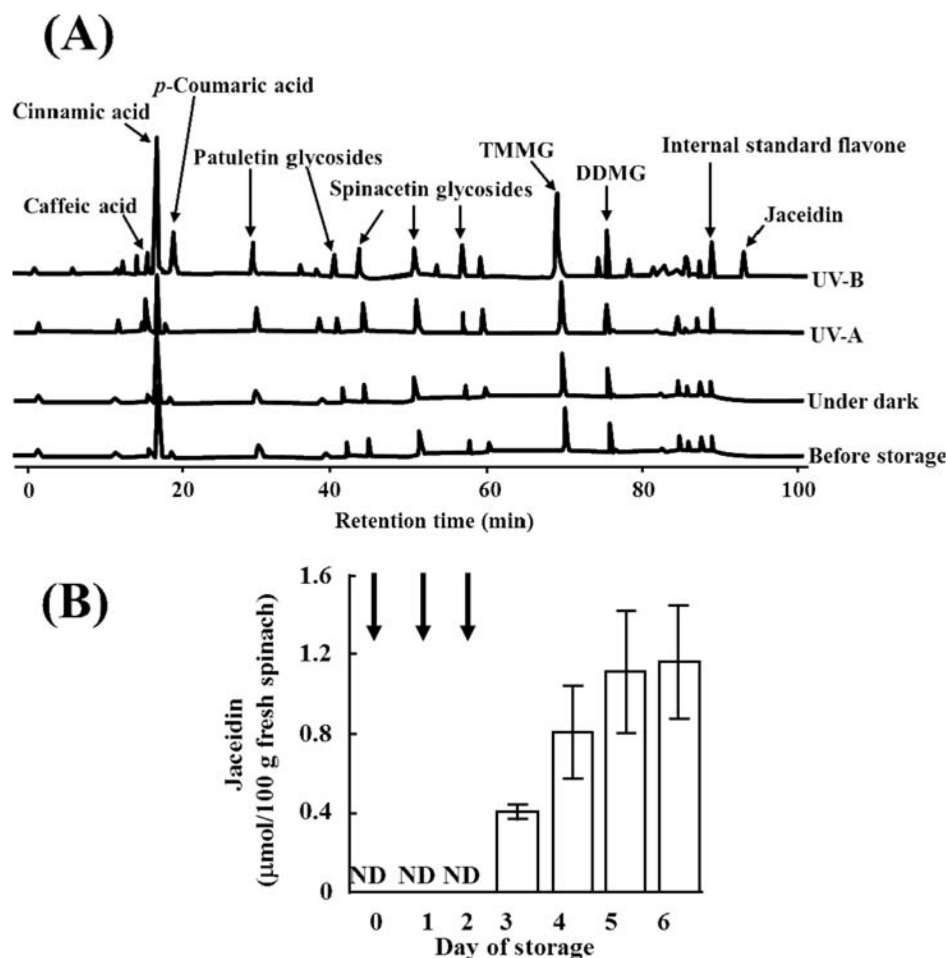

Figure 3. Induction of flavonoid production in spinach by short irradiation in a refrigerator. UV-A and UV-B were irradiated for 5 min, 98 μmol m^{-2} s^{-1} per day, for 3 days during storage for 6 days. Phenolics in vegetables were identified and determined according to our previous method.[17] The experiment was repeated independently 4 times. (A) HPLC of phenolics in spinach on the sixth day after 3 days of irradiation with UV-B (top line), UV-A (second), storage in the dark (third), or HPLC before storage (bottom). Abbreviations: TMMG, 5,3′,4′-trihydroxy-3-methoxy-6:7-methylenedioxyflavone 4′-glucronidemethylester; DDMG, 5,4′-dihydroxy-3,3′dimethoxy-6:7-methylenedioxyflavone 4′-glucronidemethylester. (B) Jaceidin content was determined daily in leaves irradiated with UV-B and compared to the amounts of the internal standard flavone on HPLC. Data are the mean ± SD of triplicate determinations. Arrows show the day when spinach was irradiated with UV-B.

equilibrated between injections with the equivalent of 25 mL of the initial mobile phase.

Determine Contents of Flavonoids and Phenylpropanoids. First, we made a library that accumulated the retention time of phenylpropanoids and flavonoids on the HPLC and their spectra with a diode array detector, and also the calibration curves to determine the contents of phenylpropanoids and flavonoids, as mentioned in our previous report.[19] The plant extracts were analyzed on the HPLC, and the detected peaks were identified by comparing the retention time and spectra to those of standard phenylpropanoids and flavonoids, simultaneously determined using the calibration curves.

When the peaks disagreed with the retention time and spectra of standard chemicals, they were identified from the profile of aglycone forms. To obtain the aglycone profiles of glycoside forms in the plants, 50 mg of lyophilized powder was hydrolyzed with 4 mL of 62.5% aqueous methanol containing 0.5 mg/mL *tert*-butylhydroquinone and 1 mL of 2 N HCl at 90 °C for 2 h. After cooling, the aglycones were extracted with two volumes of ethyl acetate and dried under a nitrogen gas stream. They were dissolved in 0.5 mL of DMSO, filtered through a 0.2 μm membrane filter, and analyzed by the HPLC system. The aglycones were identified by comparing with the retention times and spectra of standard flavonoids, and the amount was determined with the calibration curves.

Three flavonoids in spinach, 5,3′,4′-trihydroxy-3-methoxy-6:7-methylenedioxyflavone 4′-glucronidemethylester, 5,4′-dihydroxy-3,3′di-methoxy-6:7-methylenedioxyflavone 4′-glucronidemethylester, and 5,7,4′-trihydroxy-3,6,3′-trimethoxyflavone (jaceidin) (Figure 2), were identified by comparing the published analysis data[22] and the present spinach sample data with a HPLC-mass spectrometer (LC/MS M-1200H; Hitachi) under atmospheric pressure with chemical ionization and ionizing at +30 eV.

Irradiation in a Refrigerator. UV-A lamps of 380−320 nm, UV−B of 280−320 nm, green light of 500−600 nm, and a light-emitting diode (LED) of 375 nm were set in a home refrigerator (model MR-C46SNF; Mitsubishi Electric Co., Tokyo, Japan) in which humidity was maintained at around 60% and temperature at 10 °C. Irradiation was measured with a Quantum Sensor (model LI-190SA; LI-COR. Inc., Lincoln, NE). In the refrigerator, 2/3 leaves of a plant were wrapped with aluminum foil, the UV-A lamp was switched on for 5 min at 98 μmol m^{-2} s^{-1}, and then, half of the wrapped leaves were exposed, and the bare leaves were newly wrapped followed by illumination with UV-B for another 5 min at 98 μmol m^{-2} s^{-1}. The plants were irradiated for 3 days and stored for 6 days, determining the flavonoid contents daily.

RT-PCR. Total RNA was extracted from 40 mg of powder lyophilized parsley using Sepasol RNA I Super (Nacalai Tesque) according to the manufacturer's protocol. The cDNA synthesis was performed using a first strand cDNA synthesis kit for RT-PCR $[AMV]^+$ (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's protocol. Real-time RT-PCR was performed by a

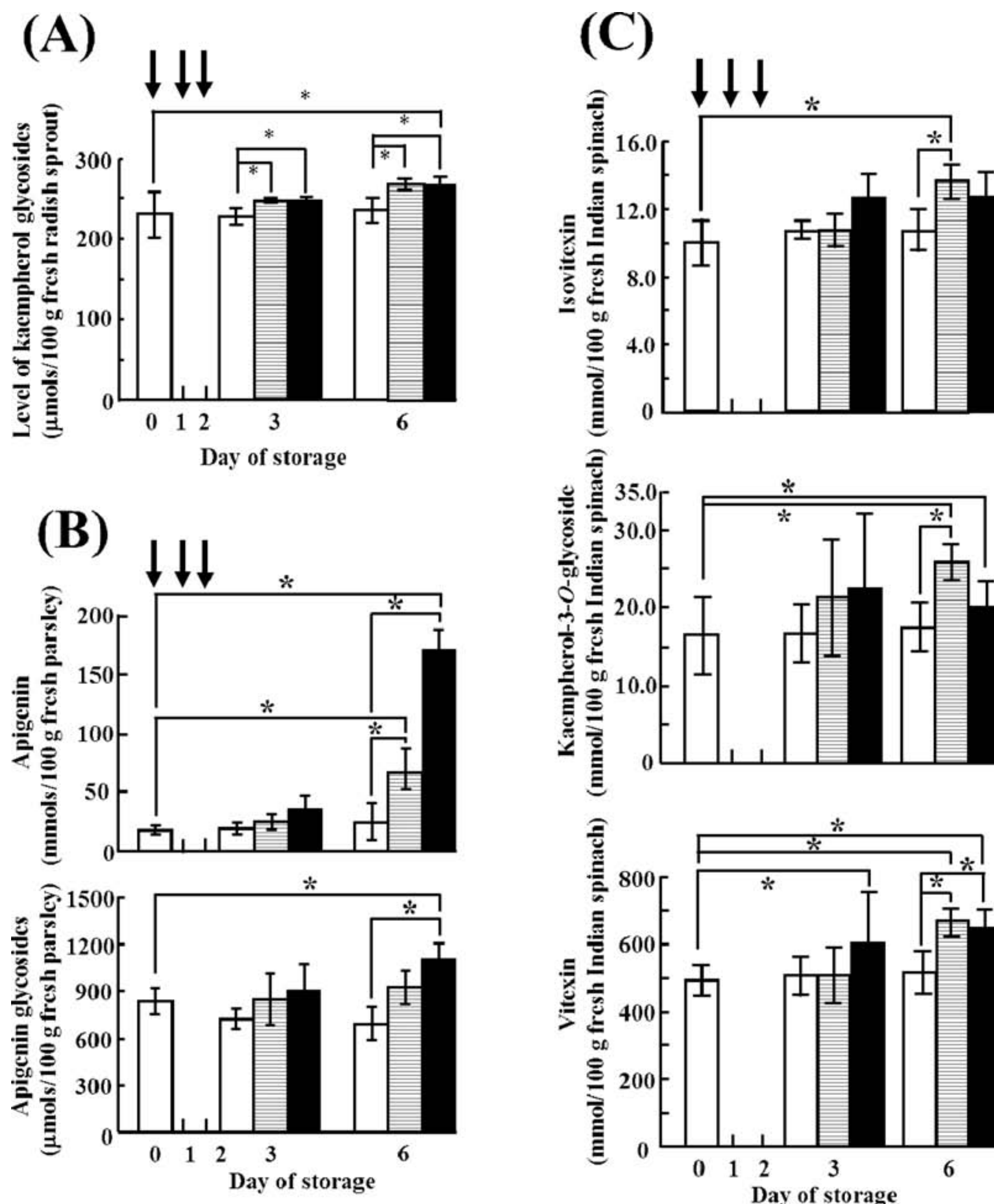

Figure 4. Increased production of flavonoids by short-term illumination of vegetables stored in a refrigerator. Vegetables were irradiated with UV-A and UV-B for 5 min, 98 μmol m^{-2} s^{-1} per day, for 3 days (arrows) and stored for 6 days. Flavonoids in vegetables were determined according to our previous method.[17] The experiment was repeated independently 4 times, and the data are the mean ± SD of triplicate determinations (asterisk indicates $P < 0.05$). (A) Comparison of the contents of kaempherol glycosides in radish sprouts among UV-A (hatching), UV−B (black) irradiation, and storage without irradiation (white). (B) Comparison of the contents of apigenin (upper) and its glycosides (lower) in parsley among UV-A (hatching), UV-B (black) irradiation, and storage without irradiation (white). (C) Contents of isovitexin (top), kaempherol (middle), and vitexin (bottom) in Indian spinach irradiated with UV-A (hatching), UV-B (black), and no irradiation (white).

LightCycler (Roche Diagnosis) equipped with the Roche Molecular Biochemicals LightCycler running LightCycler software using the LightCycler FastStart DNA MasterPLUS SYBR Green I (Roche Diagnosis) according to the manufacturer's protocol. The reaction was performed with the following protocol: 10 min of heat starting at 95 °C, 37 cycles of denaturation at 95 °C for 15 s, annealing at 60 °C for 10 s, and extension at 72 °C for 10 s. Fluorescence A fluorescence detection was performed at 40 °C. The sequences of PCR primers were designed according to the published nucleotide sequence of parsley[23−26] for PAL (5′-TCTGTACAAGTTTGTGAGGG-3′ and 5′-TTCACTAGGGAGAGACATCA-3′), for C4H (5′-TGATGC-GAAGCTTGCGGGTT-3′ and 5′-GGTATCCAGTCCACCCAATT-3′), for 4CL (5′-AGCATCCGGTGATCTTCCC-3′ and 5′-GTTTAT-GATCGGAAAAACC-3′), for CHS (5′-GGAGAAGGTTTG-GATTGGGG-3′ and 5′-TGAGCAAAGCAAAGCAAACC-3′), and for FNS (5′-TTGGCCAAGGAGAAAAGGTT-3′ and 5′-CCAATAA-CACACACCATTG-3′), and they were synthesized by Texas Genomics (Tokyo, Japan).

Assay of Phenylalanine Ammonia-Lyase (PAL) Activity. PAL activity was determined as described previously.[27] Parsley leaf ground in 40−50 mg of liquid nitrogen was homogenized in 0.5 mL of 0.1 M Tris-HCl buffer containing 10 mM mercaptoethanol at pH 8.5 on ice and centrifuged at 11,000g for 10 min at 4 °C. Supernatant 20 μg as protein was incubated with 0.5 mM phenylalanine in 0.4 mL buffer at 30 °C for 60 min. After stopping the enzyme reaction by the addition of 0.1 mL of 0.5 M HCl, the reaction mixture was evaporated with a centrifugal concentrator, dissolved in 40 μL of buffer, and subjected to HPLC analysis under the following conditions: the column was a

Capcell Pak C18 UG120 column (250 mm × 4.6 mm i.d.) maintained at 30 °C and joined with a guard column (10 mm × 4.0 mm i.d.); the mobile phase was 50 mM NaH_2PO_4, pH 3.3 in 55% methanol including 0.5% acetic acid; the flow rate was 1.0 mL/min; the injected sample volume was 10 μL; and monitoring was performed at 280 nm. PAL activity was determined as nmol units of *trans*-cinnamic acid formed per mg of protein. The protein content in the enzyme solution was determined by the Lowry method.

Determination of Nutrients in Parsley. Ascorbic acid in parsley leaf was determined by the previous method[28] with a slight modification. Briefly, 20 mg of lyophilized parsley leaf powder was extracted with 2 mL of 5% metaphosphoric acid three times. After centrifugation at 1,000*g* for 10 min, 2 mL of supernatant was mixed in 3 mL of 5% metaphosphoric acid and passed through a C18-SepPak (Nihon Waters K.K., Tokyo, Japan). Eluat, 200 μL, was incubated with 40 μL of 4% dithiothreitol (Wako Pure Chem. Ind.) at 30 °C for 10 min and immediately subjected to HPLC analysis under the following conditions: column was a Capcell Pak NH_2 (150 mm × 4.6 mm i.d., Shiseido) maintained at 35 °C; the mobile phase was acetonitrile and 50 mM NaH_2PO_4 (60:40, v/v); the flow rate was 1.0 mL/min; the injected sample volume was 10 μL; and monitoring was performed at 254 nm.

For β-carotene, 20 mg of lyophilized powder was extracted with 4 mL of methanol, centrifuged, dried with a centrifugal concentrator, and then dissolved in 1 mL methanol. After filtering through the Millex-LG (Millipore), 10 μL of filtrate was analyzed on the HPLC: column was a Capcell Pak C18 UG120 (250 mm × 4.6 mm i.d.; Shiseido) maintained at 35 °C; the mobile phase was a mixed solvent of methanol, chloroform, and *n*-hexane (10:7:3, v/v/v); the flow rate was 0.5 mL/min; and the detection wavelength was 453 nm.

Chlorophyll was determined according to the previous method.[29] In brief, 20 mg of lyophilized powder was extracted with 4 mL of chloroform for 10 min, centrifuged at 1,000*g* for 10 min, and then the supernatant was measured at 660 and 642.5 nm absorbance. The concentration of chlorophylls in the extract was calculated by a formula, chlorophyll (mg/mL) = $7.12 \times A_{660} + 16.8 \times A_{642.5}$.

Moisture % in parsley leaf was calculated by the difference in the weight lyophilized at 0.2 Pa for 48 h from the weight of homogenate under liquid nitrogen.

Statistical Analysis. The data are reported as the mean ± SD. Student's *t*-test was employed using Microsoft Office Excel (Microsoft, Redmond, WA) and SPSS, Version 13.0 (SPSS Inc., Chicago, IL), and significance was set at $p < 0.05$.

RESULTS

Induction of Flavonoid Production by Short UV-B Illumination of Spinach Stored in a Refrigerator. Spinach includes various phenolics, phenylpropanoids, and flavonoids (Figure 3A) and was irradiated with UV-A or UV-B in the refrigerator. The content of *p*-coumaric acid apparently increased, and jaceidin was newly produced in the leaves by 3 days of irradiation with UV-B during 6 days of storage in the refrigerator, while they remained almost unchanged in leaves irradiated with UV-A, the same as spinach before storage and stored in the dark. *p*-Coumaric acid is a substrate for flavonoid synthesis (Figure 1)[17,30] and possesses absorption λ_{max} in the UV-B area.[19] Jaceidin was detectable on the third day after irradiation and increased daily up to the fifth day (Figure 3B). This indicated that UV-B irradiation induced flavonoid synthesis and produced jaceidin in spinach leaves.

Increased Production of Flavonoids by Short-Term Illumination of Vegetables Stored in a Refrigerator. The included flavonoids were identified to be kaempherol glycosides in radish, apigenin *O*-glycosides in parsley, and apigenin 8-*C*-glycoside vitexin in Indian spinach (chemical structures shown in Figure 1).[19] Radish sprouts and mature leaves of parsley and Indian spinach received 3 days of irradiation with UV-A or UV-B in the refrigerator. Both UV-A and UV-B slightly and significantly increased kaempherol glycoside content in radish sprouts on the third day of storage and maintained this higher value on the sixth day compared to nonirradiation (Figure 4A). In parsley, the aglycone form of apigenin significantly increased by 7-fold with UV-B and by 2.5-fold with UV-A compared to without irradiation, and its glycoside forms significantly accumulated in plants irradiated with UV-B on the sixth day (Figure 4B). Vitexin content in Indian spinach significantly increased on the third day in UV-B irradiation and on the sixth day with both UV lights compared to the content before storage (Figure 4C). A minor flavonoid, kaempherol glycoside, also increased on the sixth day by both UV-B and UV-A, and isovitexin increased by UV-A. The results indicated that light irradiation induced flavonoid production on the third day, and significant amounts had accumulated by the sixth day.

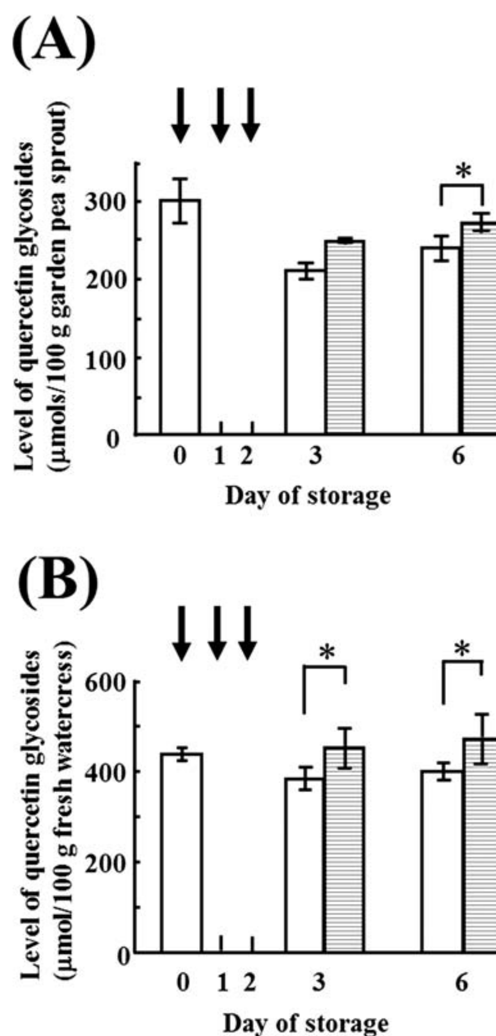

Figure 5. Effects of LED at 375 nm on increased production of quercetin glycosides. Garden pea sprouts (A) and watercress (B) received 160 min of irradiation with 33 μmol m^{-2} s^{-1} per day for 3 days to compare the quercetin glycoside contents without irradiation (white) on the sixth day of storage. The experiment was repeated independently 4 times, and the flavonoids were determined as described in Figure 2. Data are the mean ± SD of triplicate determinations (asterisk indicates $P < 0.05$).

Increased Production of Flavonoids by LED Lighting in a Refrigerator. The major flavonoids are quercetin glycosides in both garden pea sprouts and watercress.[19] They

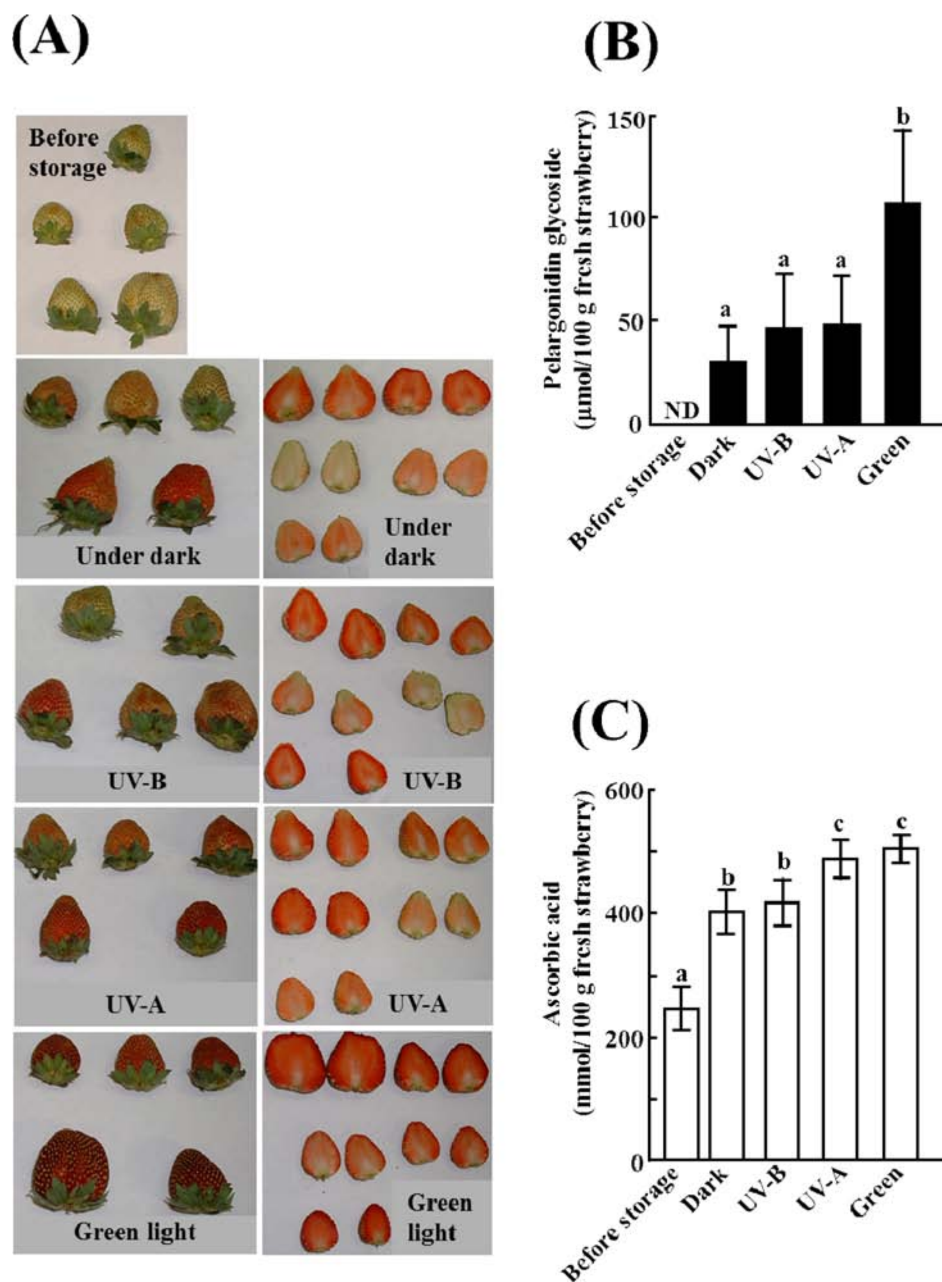

Figure 6. Increased production of anthocyanin color in unripe green strawberries irradiated daily with green light. (A) Four groups of unripe strawberries were irradiated daily with UV-B, UV-A, green light, or without irradiation for 5 min at 98 μmol m^{-2} s^{-1} per day for 6 days in the refrigerator. (B) Amounts of palargonidin glycoside and (C) ascorbic acid on the sixth day of storage. The experiment was repeated independently 3 times, and the data are the mean ± SD of triplicate determinations (different letters indicate significant difference, $P < 0.05$).

were lit with an LED of wavelength at 375 nm for 160 min, 33 μmol m^{-2} s^{-1} per day for 3 days. The contents of quercetin glycosides slightly increased in both garden pea sprout (Figure 5A) and watercress (Figure 5B) on the sixth day of storage. The results indicated that LED radiation could also increase flavonoid production in postharvest vegetables in the refrigerator.

Coloring Strawberry by Short-Term Daily Illumination in a Refrigerator. Unripe green strawberries were collected in our culture garden and irradiated daily with UV-B, UV-A, or green light for 5 min of 98 μmol m^{-2} s^{-1} per day, or without irradiation in the refrigerator for 6 days, and compared for coloration (Figure 6A). The red pigment of strawberry is the anthocyanin pelargonidin 3-*O*-β-D-glycoside (chemical structures shown in Figure 2).[19] The green light induced a deep red color, while UV-B and UV-A colored the strawberries at similar levels to storage without irradiation. The content of pelargonidin glycoside was significantly 3.5-fold higher in strawberries under green light compared to the content in other strawberries (Figure 6B). Green light irradiation also increased ascorbic acid contents as well as UV-A, compared to UV-B and nonirradiation (Figure 6C). This indicated that irradiation with a color complementary to red pigment, green light, facilitated the development of a mature color in harvested strawberries.

Stimulating Expression of mRNAs for Flavonoid Synthesis in Parsley by Single UV-B Irradiation. The genes of flavonoid biosynthesis in parsley have been almost entirely elucidated as shown in Figure 1.[25,31−33] To examine

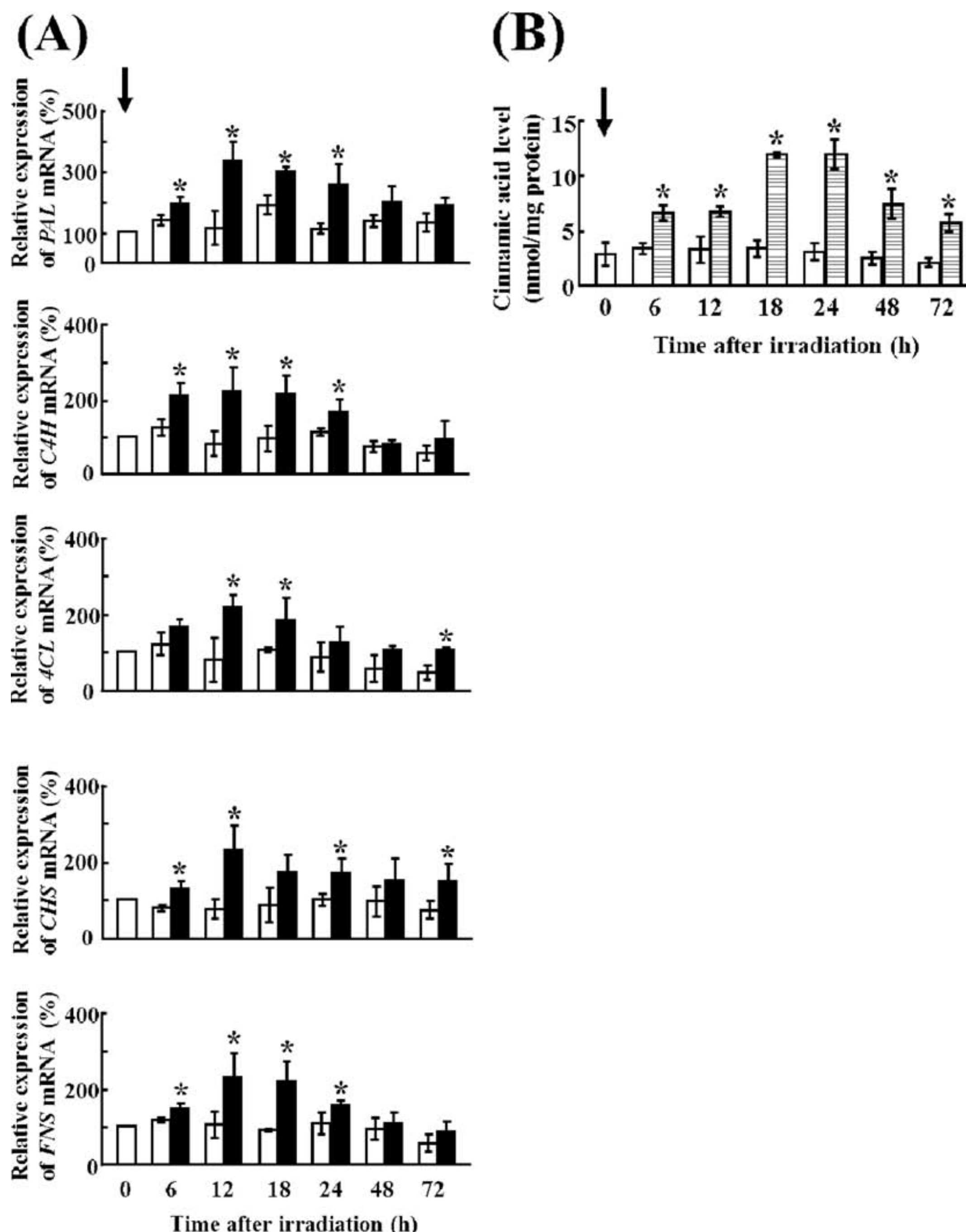

Figure 7. Stimulating expression of mRNAs for flavonoid synthesis by a single irradiation with UV-B. Parsley in the refrigerator at 10 °C and 60% humidity was irradiated with UV-B at 98 μmol m^{-2} s^{-1} for 5 min (arrow). The mRNA expressions were determined at the indicated times, as mentioned in the materials and methods section. Data are the mean ± SD of $n = 9$ in independent triplicate experiments (asterisk indicates $P < 0.05$ vs. storage in the dark). (A) mRNA expressions of PAL, C4H, 4CL, CHS, and FNS in irradiated (black) and nonirradiated parsley (white). (B) Activity of PAL was determined from the amount of cinnamic acid produced in irradiated (hatching) and nonirradiated parsley (white).

when the plant responded to light irradiation, harvested parsley was dosed with a single dose of UV-B irradiation for 5 min at 98 μmol m^{-2} s^{-1} to determine the gene expression (Figure 7A). PAL is the key and start enzyme in flavonoid synthesis and catalyzes the deamination of phenylalanine to *trans*-cinnamic acid.[34] Single irradiation rapidly stimulated the significant expression of PAL mRNA 6 h after the dose, maintained the significant expression for 24 h, and also produced cinnamic acid in the leaves (Figure 7B). PAL expression is followed mainly by cinnamic acid 4-hydroxylase (C4H), 4-coumarate-CoA ligase (4CL), chalcone synthase (CHS), and finally flavone synthase (FNS), and produces aglycones that are mostly stored after glycosylation.[31,33] The expressions of C4H, 4CL, CHS, and FNS were also significant. The parsley produced a higher amount of apigenin glycosides 930 ± 121 μmol/100 g of fresh leaf than 686 ± 23 μmol/100 g of fresh leaf without irradiation 6 days after the single irradiation.

Effects of Irradiation on the Contents of Other Components of Parsley. In order to examine whether an adverse effect occurred, parsley was irradiated with UV-A or

Table 1. Effects of 3 Days of Irradiation during 6 Days of Storage in Refrigerator[a] on Contents of Ascorbic Acid, β-Carotene, Chlorophyll, and Moisture in Parsley Leaf

	contents in 100 g of fresh parsley leaf[b]			
irradiation	ascorbic acid (mmol)	β-carotene (g)	chlorophyll (g)	moisture (%)
nonirradiation	16.2 ± 1.2	7.61 ± 1.98	27.5 ± 5.3	84.8 ± 2.9
UV-A	17.0 ± 3.7	10.54 ± 2.71	32.5 ± 6.3	82.4 ± 4.6
UV-B	16.2 ± 1.3	12.44 ± 2.42*	30.5 ± 6.9	82.9 ± 4.3

[a]Parsley was irradiated with UV-A or UV-B for 5 min, 98 μmol m^{-2} s^{-1} per day for 3 days during storage for 6 days in a refrigerator with humidity maintained around 60% and temperature at 10 °C. [b]Data are expressed as the mean ± SD. Asterisk indicates significant difference from nonirradiation ($n = 4$, $P < 0.05$). The contents before irradiation were 17.0 ± 0.7 mmol/100 g of ascorbic acid, 7.32 ± 0.70 g/100 g of β-carotene, 40.2 ± 2.5 g/100 g of chlorophyll, and 83.5 ± 1.1% moisture.

UV-B for 5 min, 98 μmol m^{-2} s^{-1} per day, for 3 days, and stored for another 3 days. The contents of ascorbic acid, β-carotene, chlorophyll, and moisture were then determined in the parsley leaf and compared to the levels in nonirradiated parsley (Table 1). Ascorbic acid, chlorophyll, and moisture contents did not change after UV-A or UV-B irradiation, and the β-carotene content significantly increased in UV-B-irradiated parsley. Thus, 3 days of irradiation with UV did not show adverse changes in moisture and other phytochemical contents compared to nonirradiated and stored plants.

DISCUSSION

It is desirable to increase the contents of human-health beneficial polyphenols in daily vegetables during handling at the market and/or in a consumer's home. In the present study, we showed that light irradiation in a home refrigerator could easily increase the contents of polyphenols in postharvest vegetables purchased from a city market. The induction of polyphenol production was similar with weak irradiation to daily sunshine,[21] at 98 μmol m^{-2} s^{-1}, and for a short time, 5 min per day for 3 days (Figure 3). The irradiation wavelength was more effective near the absorption wavelength of phenylpropanoids of the substrate for flavonoid synthesis in the plants. Three days of irradiation with UV-B light at 280−320 nm increased the production of a phenylpropanoid, *p*-coumaric acid, and induced the synthesis of flavonoid jaceidin in spinach (Figure 3). A wavelength near to the λ_{max} of flavonoids, UV-A light at 380−320 nm, was also appropriate to increase the contents of flavonoids such as kaempherol glycosides in radish sprouts, apigenin in parsley, and isovitexin, kaempherol glycoside, and vitexin in Indian spinach (Figure 4). LED light has only a narrow wavelength at 375 nm, although it is cheaper than UV lamps. LED light could increase quercetin glycoside contents in garden pea sprouts and watercress (Figure 5). Interestingly, green light increased pelargonidin glycoside content and colored strawberries to a mature deep red, while UV-B and UV-A were not effective (Figure 6). Dixon and Paiva[17] have found that irradiation stress induces the biosynthesis of phenolics in the plants and that the produced flavonoids can vary widely among plant species. The present results clearly indicate that the respective light near to the absorption λ_{max} wavelength of penylpropanoids and/or flavonoids can induce their production; around 310 nm for *p*-coumaric acid, 345 nm for kaempherol glycosides, 335 nm for apigenin, 335 nm for vitexin and isovitexin, 350 nm for quercetin glycosides,[19] and 503 nm for pelargonidin glycoside.[20]

Flavonoid synthesis in plants is induced by perceiving light with photoreceptors and by the expression of phenylpropanoid-biosynthesis genes.[10,11] Among these genes, PAL plays an important role in the first step of the phenylpropanoid-biosynthesis pathway and is activated by UV light. Hahlbrock et al. [35] describe that the promoter on PAL is in Box-P and that Box-P-binding factor 1 is induced by UV stimulation and up-regulates the gene expression of PAL following the binding to Box-P. In UV irradiation of rice seedlings, it has been reported that PAL increases at 12 h to its maximum expression and increases the contents of flavonoids.[36] In the present study, a single irradiation with UV-B stimulated the expression of PAL in postharvest parsley, showing the maximum expression 12−24 h after irradiation (Figure 7A). Box-P is also conserved in the promoter region of the other phenylpropanoid-biosynthesis genes and a UV responsible gene such as AtMYB4 in *Arabidopsis* represses the expression of the CH4 gene, whereas UV irradiation cancels this repression and induces the expression of CH4.[37] In the present study, as shown in Figure 7A, C4H, 4CL, CHS, and FNS of phenylpropanoid biosynthesis-related genes increased and reached maximum expression at 12 h. Cinnamic acid is the first product in the flavonoid biosynthesis pathway and is derived from phenylalanine with PAL (Figure 1).[17,18] A single irradiation with UV-B increased the production of cinnamic acid at 18−24 h for a maximum production that was slightly delayed behind the PAL gene expression (Figure 7B). These results clearly show that light irradiation can stimulate the gene expression of phenylpropanoid biosynthesis-related genes and induce flavonoid synthesis even in postharvest plants, as well as growing plants in the field.

A question remains whether irradiation has adverse effects on vegetables stored in a refrigerator. As shown in Table 1, the contents of ascorbic acid, chlorophyll, and moisture remained unchanged, and β-carotene increased after 3 days of irradiation and 6 days of storage compared to the contents with no irradiation or before irradiation. In strawberries, ascorbic acid content increased after irradiation with UV-A or green light (Figure 6C). Also, the irradiated vegetables remained unchanged visually as compared with those before storage (data not shown). Spinach in Figure 3, radish sprout, parsley, and Indian spinach in Figure 4, garden pea and watercress in Figure 5, and strawberry in Figure 6 were visually similar to those before storage in freshness and color. Controlling moisture to around 60% in the refrigerator seems likely to contribute to the freshness and color of stored vegetables. Irradiation in the refrigerator therefore did not have adverse effects on stored vegetables.

Irradiation is appropriate in a home refrigerator, showcase, and transportation container during the handling of postharvest vegetables and increases the contents of human-health beneficial flavonoids. For example, the increased products jaceidin in Figure 3B and quercetin in Figure 5 possess a

catechol structure. Catechol flavonoids are recognized to exhibit the strong antioxidant potency[38,39] and anti-inflammatory activity in our body.[40] Kaempherol in Figures 4A,C and apigenin in Figure 4B,C are reported to suppress toxicity expression of dioxin.[41] Also, irradiation is commercially useful. For example, regulated irradiation of unripe green strawberries can adjust the time of maturation to red (Figure 6) and thereby adjust the shipping period.

Irradiation with the respective light near to the λ_{max} of penylpropanoids or flavonoids on postharvest vegetables is easy and convenient. Controlled irradiation of postharvest vegetables during handling increased the flavonoid contents and should contribute to human-health promotion and increase the commercial value of agricultural products.

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Notes

The authors declare no competing financial interest.

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