



Physicochemical properties and antioxidant activity of colored peppers (*Capsicum annuum* L.)

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Abstract

Peppers are spices consumed all around the world. This study evaluated the physicochemical properties and antioxidant activities of red, orange, yellow, green, and purple peppers. Total capsaicinoids showed the highest concentration in the green pepper (2416.50 µg/g). Vitamin C showed similar concentrations in all peppers (28.90–30.95 mg/g), except for the purple pepper (25.59 mg/g). Chlorophyll was abundant in the green and the purple peppers (280.36 and 102.13 mg/100 g). Total carotenoid was abundant in the red and the orange peppers (237.04 and 276.94 mg/100 g). Total anthocyanin was detected only in the purple pepper (67.13 mg/100 g). Total flavonoid showed a high concentration in the green and the purple peppers (24.27 and 22.27 CAE mg/g). The yellow pepper showed the highest antioxidant activity according to total polyphenol, DPPH radical scavenging activity, ABTS radical scavenging activity and reducing power assays. Therefore, peppers showed potential for the development of functional food materials.

Keywords Pepper · Color · Physicochemical property · Phytochemical · Antioxidant activity

Introduction

Peppers (*Capsicum annuum* L.) belong to *Solanaceae* family and is valuable in agricultural production as a vegetable and a spice (Carvalho Lemos et al., 2019; Tang et al., 2020). Fresh peppers are an excellent source of vitamins A and C as well as acidic and neutral phenolic compounds, which are important antioxidants used by plant for various defense responses (Howard et al., 2000). Various bioactive compounds (capsaicinoids, polyphenols, flavonoids, and ascorbic acid) affect the antioxidant properties of peppers (Manikharda et al., 2018). Capsaicinoids, which have a pungent taste, the two most important factors for evaluating the quality are the taste and the color (Kim et al., 2002; Srinivasan, 2016).

In recent years, the trend of substituting synthetic for natural compounds in the design and production of healthy functional foods has encouraged research on natural colorants (Di Gioia et al., 2020). Phytochemicals are increasingly purported to exert potent health benefits and may play a role in reducing synthetic drug use for treating metabolic complications (Guil-Guerrero et al., 2006). It has been used to determine the variability of the fruit's smell, flavor, and consumer preference as a source of pigments to enhance or change the food color (Vera-Guzmán et al., 2011). Many studies have demonstrated the color of vegetables is a combination of main groups of pigments: chlorophylls, carotenoids, and anthocyanins (Di Gioia et al., 2020). Chlorophyll, which gives plants their green color, is the principal molecule that absorbs sunlight during photosynthesis (Borovsky and Paran, 2008). Carotenoids, which perform important biological functions as antioxidants, have been used as natural red, orange, and yellow colorants, and β-carotene is a component of the human diet as provitamin A. Anthocyanins are a subgroup of bluish, purple red-colored flavonoid compounds in various plant tissues (Di Gioia et al., 2020).

The economic importance of peppers is due to its diverse range of colors, flavors, and shapes as well as its various nutritional properties (Manikharda et al., 2018). Peppers represent 50.8% of seasoning vegetable growing areas in

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Korea. The Korean peppers are the key ingredient in many processed food products including *kochujang* (fermented red pepper paste) and kimchi (Kim et al., 2002). Color is one of the main characteristics of food materials. With increasing demand for spicy taste, we are seeing more research on different types of peppers, based of consumer preference, that could be used for preparing novel and functional foods. Therefore, it is necessary to study and compare the physicochemical properties and antioxidant activities of colored peppers, which could be used for developing novel products for the global market that would attract new consumers and increase international competitiveness of Korean pepper growers (Huang et al., 2014). Peppers of various colors (e.g., orange, yellow, purple) containing various bioactive compounds have been developed in recent years (Kim et al., 2007). However, studies that analyze and compare the components and antioxidant activities of new colored peppers are insufficient. Therefore, this study evaluates the physicochemical properties including the color value and content of capsaicinoids, vitamin C, phytochemical, and antioxidant activities of red, orange, yellow, green, and purple peppers produced in Korea.

Materials and methods

Plant material

The peppers used in this study were the red pepper (Mesut), orange pepper (E22M-0343), yellow pepper (Surtsey), green pepper (Cheongyang), purple pepper (Deu Syeo Bo Ra F1). The red, orange, yellow, and green peppers were cultivated in Jinju and the purple pepper was cultivated in Chungju, Korea. Every pepper used in this study was grown in the greenhouse and harvested in July 2021 (Fig. 1). After the peppers had been washed, the seeds and stems of the peppers were removed and freeze-dried (MCFD 8508, Ilshin Bio Base, Dong-ducheon, Korea). It was pulverized with a grinder (EV-GB 8000, Everhome, Seoul, Korea) and stored in a deep freezer at $-40\text{ }^{\circ}\text{C}$ (NIHON, SF-53-U, Tokyo, Japan).

Materials and chemicals

Standard capsaicinoids (capsaicin and dihydrocapsaicin), standard vitamin C (L-ascorbic acid), standard gallic acid, standard catechin, metaphosphoric acid, 2,2-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), 2,2-diphenyl-1-picrylhydrazyl, potassium persulfate, and folin-ciocalteu were purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile, trichloroacetic acid, potassium chloride, sodium acetate, sodium carbonate (Na_2CO_3), sodium

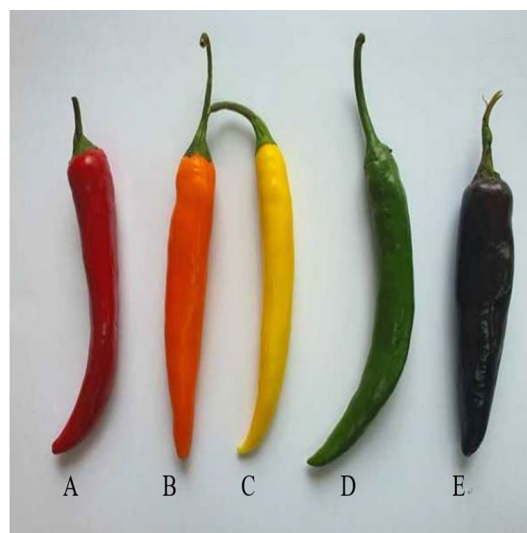


Fig. 1 Colored pepper (*Capsicum annuum* L.) A: red pepper (Mesut), B: orange pepper (E22M-0343), C: yellow Pepper (Surtsey), D: green pepper (Cheongyang), E: purple pepper (Deu Syeo Bo Ra F1)

hydroxide (NaOH), sodium phosphate, and ferric chloride were purchased from Duksan Reagents (Ansan, Korea).

Physicochemical properties

Color value assay

The color value of the peppers was measured using a chromometer (CR-300, Minolta Co., Osaka, Japan) and measured as Hunter's color value L^* value, a^* value, and b^* value. The colorimeter was calibrated with a standard calibration plate of the white surface ($L=93.8$, $a=-0.14$, and $b=3.7$). Three measurements were conducted in each sample. The Hue angle (h°) and Chroma (C^*) were calculated (McGuire, 1992).

$$\text{Chroma} = [(a)^2 + (b)^2]^{1/2}$$

$$\text{Hue angle (h)} = \tan^{-1}(b/a)$$

Capsaicinoids contents assay

0.1 g of the peppers were added to 1 mL of 99% ethanol and homogenized for 5 min at room temperature. After ethanol-treated peppers were centrifuged at $1008\times g$, for 15 min, and at $4\text{ }^{\circ}\text{C}$, the supernatant was filtered through a filter to be used as an analysis sample. UPLC (Ultimate 3000, Dionex, Idstein, Germany) equipped with reverse phase column (Kinetex 2.6 μm C18 100A $100\times 4.60\text{ mm}$, Phenomenex,

Torrance, CA, USA). The separation conditions were followed: 50% acetonitrile solvent; 0–8 min, and the moving flow rate was 1 mL/min. The wavelength was measured at 222 nm. Total capsacinoids are the sum of capsacin and dihydrocapsaicin. The standard material was calculated by the calibration curve with capsacin and dihydrocapsaicin.

Scoville heat unit conversions: The concentration (ppm) of capsacin and dihydrocapsaicin compounds were converted into SHU (ppm) using their coefficient of the heat value with the following formula (Scoville, 1912).

$$\text{SHU} = (\text{capsaicin} \times 16.1) + (\text{dihydrocapsaicin} \times 16.1)$$

Vitamin C contents assay

After 0.5 g of the peppers were added to the 5 mL of 3% trichloroacetic acid, 100 μL of 5% metaphosphoric acid was added and homogenized. The samples were centrifuged at 1008 $\times g$, for 15 min, and at 4 $^{\circ}\text{C}$ and diluted supernatant with 5% metaphosphoric acid solution for 10 times. It was filtered out and used as an analysis sample. UPLC (Ultimate 3000, Dionex, Idstein, Germany) equipped with reverse phase column (X-Terra C_{18} 5 μm 3.9 \times 150 mm, Waters Co., Milford, MA, USA). The mobile phases were A: 0.05 M potassium

$$A \text{ (absorbance value)} = (A_{510\text{nm}} - A_{700\text{nm}}) \text{ at pH } 1.0 - (A_{510\text{nm}} - A_{700\text{nm}}) \text{ at pH } 4.5$$

$$\text{MW (molecular weight of cyanidin - 3 - glucoside)} = 449.2,$$

$$D \text{ (dilution factor)} = \text{dilution ratio of sample}$$

$$\epsilon \text{ (cyanidin - 3 - glucoside molar absorbance)} = 26,900 \text{ M}^{-1} \text{ cm}^{-1}, V = \text{final volume of sample}$$

$$\text{Total anthocyanin content (mg/kg)} = A \times \text{MW} \times D \times 1,000 \div \epsilon \times V$$

monobasic and B: 100% acetonitrile. The separation conditions were followed: solvent B: 0–5 min, 20%; 5–10 min, 80%; 10–20 min, 20%, and the flow rate was 0.8 mL/min. The standard material was calculated by the calibration curve with ascorbic acid, and the absorbance was measured at 254 nm.

Phytochemical components of the colored pepper

Chlorophyll and total carotenoid contents assay

Chlorophyll and total carotenoid contents were according to a modification of the Chappelle et al. (1992). After the peppers were extracted for 24 h in the dark at 30 $^{\circ}\text{C}$ with DMSO (dimethyl sulfoxide), centrifugation was used at 1008 $\times g$, 15 min, and 24 $^{\circ}\text{C}$. The concentrations of the extracted pigments were calculated from the absorbance

values at 664 nm, 648 nm and 470 nm using UV/VIS spectrophotometer (T60UV, PG Instruments, Wibtoft, England).

$$\text{Chlorophyll a } (\mu\text{g/mL}) = 12.25 \times A_{664\text{nm}} - 2.79 \times A_{648\text{nm}}$$

$$\text{Chlorophyll b } (\mu\text{g/mL}) = 21.50 \times A_{648\text{nm}} - 5.10 \times A_{664\text{nm}}$$

$$\text{Total chlorophyll } (\mu\text{g/mL}) = \text{Chlorophyll a} + \text{Chlorophyll b}$$

$$\text{Total carotenoid} = [1000 \times A_{470\text{nm}} - 1.82 \times (\text{chlorophyll a}) - 85.02 \times (\text{chlorophyll b})] / 198$$

Total anthocyanin contents

Total anthocyanins were measured according to a modification of the Hosseinian et al. (2008). After extracting pigments for 24 h by adding 70% ethanol to 1 g of the samples, centrifugation was performed for 10 min at room temperature. One for pH 1.0 using 0.025 M potassium chloride buffer and the other for pH 4.5 using 0.4 M sodium acetate buffer were added to 0.5 mL of each extract and made the final volume of 1 mL. The absorbance of each sample was measured at 520 nm and 700 nm (T60UV, PG Instruments). Total anthocyanin was calculated using the molecular absorption coefficient ($\epsilon = 26,900 \text{ M}^{-1} \text{ cm}^{-1}$) of cyanidin-3-glucoside.

Antioxidant activity

Extraction yield, total polyphenol and total flavonoid contents assay

Two hundred milliliters of 70% ethanol solvent was added to 10 g of pepper powder at room temperature for 20 min, and this was repeated for three times. It was then extracted by ultrasonic (KUS-650, KBT Co., Siheung, Korea) and filtered with a filter paper. The extract was concentrated by using the decompression (N-1200A, EyELA Co., Tokyo, Japan) to prepare dry extracts to be used in each experiment.

The total polyphenol contents of the peppers were measured based on the folin-ciocalteu by Lee et al. (1995). To 150 μL of each extract, 2400 μL of distilled water, 50 μL of 2 N folin-ciocalteu phenol reagent was added. To the resulting solution, 300 μL of 1 N sodium carbonate (Na_2CO_3) was added for 2 h reaction in the dark, after

which absorbance value was measured at 725 nm using a UV/VIS spectrophotometer (T60UV, PG Instruments). Gallic acid was used and calculated as the standard compound, and the results were expressed as milligram gallic acid equivalents (GAE) per gram.

The total flavonoid contents of extracts were measured based on the method by Chang et al. (2002). After adding 100 μL of 99% diethyleneglycol and 100 μL of 1 N NaOH to 1 mL of each extraction, the mixture was stirred. And, the result of the solution left to react at 37 °C for 1 h in a water bath. Absorbance was measured at 420 nm (T60UV, PG Instruments). Catechin was used and calculated as the standard compound, and the results were expressed as milligram catechin equivalents (CAE) per gram extract.

DPPH radical scavenging activity assay

To measure DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activities of the pepper extraction, the method was based by Huang et al. (2014). A 900 μL of each extract was added to 300 μL of DPPH solution (1.5×10^{-4} M), and the mixture was left to react in the dark at room temperature for 30 min. Absorbance value was measured at 517 nm using a UV/VIS spectrophotometer (T60UV, PG Instruments), and the difference in absorbance value with the control group was expressed as a percentage to estimate the DPPH radical scavenging activity. The half maximal inhibitory concentration (IC_{50}) indicating the concentration at which 50% of DPPH radicals were scavenged, and was calculated for each extract.

DPPH radical scavenging activity (%)

$$= (1 - \text{Sample absorbance/Control absorbance}) \times 100$$

ABTS radical scavenging activity assay

To measure 2,2-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) ABTS radical scavenging activities of the peppers extraction the method was based by Otunola and Afolayan (2013). ABTS radical (8 mM) was prepared and mixed with potassium persulfate (2.45 mM). The solution was kept in the dark for 12 h to produce the radical. This solution was diluted with PBS buffer to become 0.70 using in absorbance value of 734 nm (T60UV, PG Instruments). The 900 μL of ABTS⁺ solution were added to 100 μL of each extract and stirred. Absorbance value was measured at 1 min interval. The half maximal inhibitory concentration (IC_{50}) indicating the concentration at which 50% of ABTS radicals were scavenged, and was calculated for each extract.

ABTS radical scavenging activity (%)

$$= (1 - \text{Sample absorbance/Control absorbance}) \times 100$$

Reducing power assay

To measure the reducing power of the pepper extracts, the method was based by Otunola and Afolayan (2013). 1 mL of each extract was mixed with 1 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 1 mL of 1% potassium ferricyanide. The mixture was incubated in a 50 °C water bath for 20 min, followed by the addition of 1 mL of 10% trichloroacetic acid. The resulting solution was centrifuged at 1008 $\times g$, 10 min, 24 °C and 1 mL of the supernatant was added to 1 mL of distilled water. After adding 0.2 mL of 0.1% ferric chloride, absorbance value was measured at 700 nm (T60UV, PG Instruments), and the values were used to express the reducing power.

Statistical analysis

All experimental results were analyzed using the SPSS program (Statistical Analysis Program, version 25, IBM Co., Armonk, NY, USA). The data were evaluated by an analysis of the variance (ANOVA), and a Duncan's test was conducted for a comparison of means ($p < 0.05$). The analysis was carried out in three replicates for all determination and expressed as mean and standard deviation. The correlation was expressed by using Pearson's correlation coefficient.

Results and discussion

Physicochemical properties

Color value

The surface color of peppers is one of the decisive factors that determine consumer preference. The color values of the colored peppers are evaluated (Table 1). The CIE Lab coordinates L^* , a^* , and b^* values were determined, whereby L^* values indicate brightness or luminosity (0, white to 100, black), a^* value is defined as the variation from green (–) to red (+), and b^* value is defined as the variation from blue (–) to yellow (+). The L^* value showed that YP (87.26) had the lightest color followed by OP (71.72), GP (69.01), PP (62.41), and RP (58.32) ($p > 0.001$). The mean value of a^* was lowest (greenest) in GP (–15.02) followed by PP (–6.38) and YP (–3.27) ($p > 0.001$). By contrast, the reddest color corresponded to RP (41.23) followed by OP (23.92). Huang et al. (2014) demonstrated that the $a^* \times L^*$ values characterized the color of pepper, with the samples with $a^* \times L^*$ values above 700 being brilliant red. The $a^* \times L^*$ values of the RP (2404.53) and OP (1715.54) in this study indicate a brilliant red color. The mean value of b^* was highest in OP (66.81), followed by RP (50.20), YP (43.49), GP (27.88), and PP (15.16) ($p > 0.001$). This can be explained

Table 1 Color values of the colored peppers

	RP	OP	YP	GP	PP
L*	58.32 ± 0.90 ^e	71.72 ± 0.51 ^b	87.26 ± 0.22 ^a	69.01 ± 0.35 ^c	62.41 ± 0.44 ^d
a*	41.23 ± 0.93 ^a	23.92 ± 0.23 ^b	-3.27 ± 0.11 ^c	-15.02 ± 0.13 ^e	-6.38 ± 0.12 ^d
b*	50.20 ± 0.65 ^b	66.81 ± 0.71 ^a	43.39 ± 0.40 ^e	27.88 ± 0.28 ^d	15.16 ± 0.12 ^e
C*	64.96 ± 0.10 ^b	70.97 ± 0.75 ^a	43.51 ± 0.39 ^e	31.66 ± 0.30 ^d	16.44 ± 0.12 ^e
h°	50.57 ± 0.95 ^e	70.33 ± 0.40 ^d	94.32 ± 0.18 ^e	118.31 ± 0.69 ^a	112.82 ± 0.10 ^b

RP red pepper (Mesut), OP orange pepper (E22M-0343), YP yellow pepper (Surtsey), GP green pepper (Cheongyang), PP purple pepper (Deu Syeo Bo Ra F1)

All values are mean ± S.D. Different letters in the same row are significantly different by Duncan's multiple range test at $p < 0.05$

by the transformation of chloroplasts into chromoplasts and the new formation of carotenoids that are reddish (Giuffrida et al., 2013). Treated GP and PP powders also exhibited a higher amount of total chlorophyll which is related to the greenness of pepper. Chroma (C*) indicates saturation of color (vividness, degree of departure from gray toward pure chromatic color) (McGuire, 1992). The C* value showed that OP had the brightest color (70.97), followed by RP (64.96), YP (43.51), GP (31.66), and PP (16.44). Manikharda et al. (2018) also reported that orange pepper was the brightest followed by red and green. The hue angle (h°) indicates the tone of the color (e.g., red, orange, yellow, and green). A color wheel subtends 360°, with red–purple traditionally placed at the far right (or at an angle of 0°), and yellow, bluish-green, and blue following counterclockwise at 90°, 180°, and 270°, respectively (McGuire, 1992). An h° of 0° corresponds to pure red, whereas 90° indicates pure yellow. Orange has values between red and yellow, and green has values between 90° and 180°. Therefore, in this study, the hue angle reflected the color of the pepper powders (Fig. 1).

Capsaicinoids contents

Capsaicin, the main component responsible for the hot pungent taste of peppers, is an alkaloid (capsaicinoid) found in the *Capsicum* family. Capsaicin has shown potential clinical value for pain relief in cases of arthritis and post-operative neuralgia, in the treatment of psoriasis and cancer, and as a weight-loss aid. Capsaicinoids include capsaicin, dihydrocapsaicin, nordihydrocapsaicin, homodihydrocapsaicin, homocapsaicin, norcapsaicin, and nornorcapsaicin. Capsaicin and dihydrocapsaicin constitute 90% of capsaicinoids in pepper. Pure capsaicin is a fat-soluble, hydrophobic, colorless, odorless, and crystalline compound (Srinivasan, 2016).

Capsaicin and dihydrocapsaicin showed the highest concentrations in GP, indicating 1854.02 µg/g and 562.58 µg/g, respectively. YP had 1445.58 µg/g and 453.82 µg/g of capsaicin and dihydrocapsaicin, respectively. OP and RP had similar capsaicinoid content and PP exhibited a significantly

lower capsaicin content (85.95 µg/g) shown in Table 2 ($p > 0.001$).

The scoville heat unit (SHU) is used as an indicator of pepper pungency and is related to the capsaicinoid concentration (Manikharda et al., 2018). Weiss (2002) classified the pungency of peppers into five SHU levels: non-pungent (0–700 SHU), mildly pungent (700–3000 SHU), moderately pungent (3000–25,000 SHU), highly pungent (25,000–70,000 SHU), and very highly pungent (> 80,000 SHU). Therefore, according to this scale, GP and YP are highly pungent (38,664.00 and 30,376.34 SHU, respectively), OP and RP are moderately pungent (5767.59 and 5627.54 SHU, respectively), and PP is mildly pungent (1359.20 SHU). Kim et al. (2007) also reported that purple pepper had a very weak spicy taste. Differences among peppers may also depend on the variety, cultivation methods, and environmental factors. Most of the peppers grown in Korea are in the thousands-SHU range; for instance, Cheongyang peppers are generally known to be about 10,000–30,000 SHUs, and regular red pepper powder is known to be about 50,000 SHUs (Lee et al., 2011).

Vitamin C contents

PP exhibited the lowest vitamin C content (25.59 mg/g). RP, OP, YP, and GP showed similar vitamin C contents (28.90–30.95 mg/g) shown in Table 2 ($p > 0.001$). Deepa et al. (2006) reported vitamin C contents of 980–3030 mg/100 g, confirming that the peppers used in this study were high in vitamin C. With regard to recommended daily allowance (RDA), a 100 g serving of fresh peppers could supply more than 100% RDA for vitamin C. In addition to its nutritional potential, vitamin C exhibits antioxidant and strong reducing properties due to its enediol structure, which is conjugated with the carbonyl group in a lactone ring. In the presence of oxygen, vitamin C is degraded to dehydroascorbic acid, which still retains vitamin C activity (Howard and Wildman, 2007). Dehydroascorbic acid and L-ascorbic acid are convertible, but the L-ascorbic acid content is much higher than that of dehydroascorbic

Table 2 Physicochemical properties and phytochemical components of the colored peppers

	RP	OP	YP	GP	PP
Physicochemical properties					
Total capsaicinoid content ($\mu\text{g/g}$)	351.70 ± 18.10^c	360.45 ± 8.84^c	1898.40 ± 0.71^b	2416.50 ± 3.54^a	84.95 ± 2.47^d
Capsaicin content ($\mu\text{g/g}$)	257.20 ± 12.02^c	267.22 ± 9.90^c	1445.58 ± 12.73^b	1854.02 ± 1.41^a	65.20 ± 1.98^d
Dihydrocapsaicin content ($\mu\text{g/g}$)	94.20 ± 6.08^c	93.50 ± 1.06^c	453.82 ± 12.02^b	562.58 ± 2.12^a	19.78 ± 0.50^d
Scoville scale (SHU)	5627.54 ± 289.63^c	5767.59 ± 141.42^c	$30,376.34 \pm 11.31^b$	$38,664.00 \pm .56.57^a$	1359.20 ± 39.60^d
Vitamin C content (mg/g)	28.90 ± 0.57^a	29.76 ± 0.77^a	30.36 ± 1.61^a	30.95 ± 0.99^a	25.59 ± 0.23^b
Phytochemical components (mg/100 g)					
Chlorophyll a content	0.09 ± 0.00^c	0.019 ± 0.00^c	N.D.	280.36 ± 6.86^a	102.13 ± 2.16^b
Chlorophyll b content	0.44 ± 0.02^c	0.30 ± 0.00^c	N.D.	31.01 ± 2.32^a	33.92 ± 1.56^b
Total chlorophyll content	0.53 ± 0.01^c	0.32 ± 0.00^c	N.D.	311.38 ± 6.67^a	136.05 ± 2.87^b
Total carotenoid content	237.04 ± 1.27^b	276.94 ± 1.27^a	14.28 ± 0.64^c	46.54 ± 0.98^c	29.98 ± 1.22^d
Total anthocyanin content	N.D.	N.D.	N.D.	N.D.	67.13 ± 2.27

Refer to Table 1

N.D. not detected

All values are mean \pm S.D. Different letters in the same row are significantly different by Duncan's multiple range test at $p < 0.05$

acid in pepper (Lee et al., 1995). Peppers differ in the contents of ascorbic acid, dehydroascorbic acid, and total vitamin C depending on the cultivar (Huang et al., 2014). For instance, PP grown in Chungju has a lower vitamin C content compared to other peppers. Simonne et al. (1997) reported that the vitamin C contents of purple bell pepper cultivars are lower than those of green, red, and orange bell pepper (62–162 mg/100 g). Also, pepper has a higher vitamin C content than bell peppers. Because of the higher bioavailability of their ascorbic acid, natural sources should be considered more beneficial than ascorbic acid supplements (Simonne et al., 1997).

Phytochemical components of the colored peppers

Chlorophyll and total carotenoid contents assay

Chlorophyll is an indicator of the freshness of vegetables and fruits and is widely used for its physiological activities including appetite stimulation, wound healing, bacterial growth inhibition, and liver function enhancement. Chlorophyll is a green natural pigment distributed in plants and exists in a state combined with proteins, fats, and lipoproteins associated with carotenoids (Lee et al., 2005). Chlorophyll is the principal molecule that absorbs sunlight during photosynthesis. During fruit ripening, the green color is degraded due to the breakdown of chlorophyll into colorless products (Borovsky and Paran, 2008). Chlorophyll was present in GP (311.38 mg/100 g) and PP (136.05 mg/100 g), but it was almost absent in other colored peppers shown in Table 2 ($p > 0.001$). PP changes from green to purple as the pulp ages, with the inside remaining green and the outside becoming purple. It is expected that this color change

affected the chlorophyll content of PP. Chlorophyll b showed a higher content in PP than in GP because chlorophyll a is green–blue, and chlorophyll b is yellow–green. By contrast, the chlorophyll content was hardly measurable in RP, OP, and YP, which do not have a green color (Lee et al., 2005). Among the leafy vegetables known to have a high chlorophyll content, various types of lettuce and romaine lettuce contained an average of 384.98 mg/100 g. GP, which contains the most chlorophyll among colored peppers, also showed a similar content as that of leafy vegetables (Di gioia et al., 2020).

Carotenoid-rich foods are associated with a lower risk of cardiovascular disease and some other types of cancer (Vera-Guzmán et al., 2011). The red color of pepper is due to the various carotenoid pigments (Deepa et al., 2006). Total carotenoids were highest in RP (237.04 mg/100 g), followed by OP (276.94 mg/100 g), YP (14.28 mg/100 g), GP (46.54 mg/100 g), and PP (29.98 mg/100 g) ($p > 0.001$, Table 2). Carotenoids were present in both free form and esterified with fatty acids. When a pepper is at an immature stage, the α -carotene pathway starts from lycopene, and carotenoids are synthesized in the order of α -carotene, zeaxanthin, and lutein. The red carotenoids are mainly capsanthin and capsorubin, which account for 30–60% of total carotenoids, while α - and β -carotene, zeaxanthin, lutein, and β -cryptoxanthin are responsible for the yellow–orange color. Furthermore, the levels of antheraxanthin, zeaxanthin, β -cryptoxanthin, and β -carotene were significantly higher in red pepper than in the other colored peppers. By contrast, the lutein content was higher in green pepper, although lutein was one of the most abundant carotenoids in all the peppers (Guil-Guerrero et al., 2006; Kim et al., 2007; Matsufuji et al., 1998; Schweiggert et al., 2005). The carotenoids and additional pigments

responsible for the purple color have not been characterized yet (Howard and Wildman, 2007). It can be expected that RP and OP contain an abundance of capsanthin and zeaxanthin, whereas GP and PP contain abundant lutein. These differences in the carotenoid content may be due to genotype, growing conditions, and maturity status (Park et al., 2016). Also, Hwang et al. (2015) reported that orange paprika had the highest carotenoid content among colored peppers as it contained an abundance of zeaxanthin. Yellow paprika contained the lowest concentration of total carotenoids, which agreed with the results of this study. In colored sweet potatoes, the total carotenoid content orange sweet potato was significantly higher than in purple or white sweet potato, and β -carotene was predominant (93.41, 2.22, and 1.37 $\mu\text{g/g}$ for orange, purple and white sweet potato, respectively) (Park et al., 2016).

Total anthocyanin contents

Anthocyanin is an important pigment that plays a vital role in the formation of the fruit color of peppers, which appear purple-blue. The synthesis and accumulation of anthocyanin is responsible for the purple color of fruits (Di Gioia et al., 2020; Tang et al., 2020). PP contained 67.13 mg/100 g of total anthocyanin, but this compound was absent in other colored peppers (Table 2). PP is called 'eggplant pepper' because it is the result of hybridization between pepper and eggplant. The purple color of PP is unusual because the pepper is usually due to a mixture of carotenoids and chlorophyll (Schweiggert et al., 2005). Park et al. (2016) reported that the purple color of fruit is related to the high accumulation of cyanidin and delphinidin derivatives. Purple peppers have been reported to contain a high anthocyanin concentration compared to other colored peppers. These anthocyanins include cyanidin, delphinidin, peonidin, pelargonidin, petunidin, and malvidin. The total anthocyanin content of purple eggplant skin was reported to be 1.24 mg/g DW. In addition, nasunin (delphinidin-3-(*p*-coumaroyl-rutinoside)-5-glucoside), is mainly extracted from the peel of the purple eggplant fruit (Di gioia et al., 2020). The purple color of PP

is expected to be due to the accumulation of anthocyanin pigments including delphinidin-3-(*p*-coumaroyl-rutinoside)-5-glucoside and delphinidin-3-(*cis*-coumaroyl-rutinoside)-5-glucoside, whereas no anthocyanins were found in the other colored peppers (Tang et al., 2020).

Antioxidant activities

Extraction yield, total polyphenol & total flavonoid contents assay

The antioxidant extraction yield of RP, OP, YP, GP, and PP was 54.57%, 55.07%, 49.51%, 41.43%, and 44.23%, respectively (Table 3). The highest extraction yield corresponded to RP and OP, and the lowest extraction yield corresponded to GP ($p > 0.001$). Phenol and flavonoids have been recognized to have antioxidant effects on human nutrition and health (Otunola and afolayan, 2013). Phenylpropanoids and phenolic compounds originate from the secondary metabolism of plants (Carvalho Lemos et al., 2019). The total polyphenol content of YP, OP, GP, RP, and PP was 32.17, 31.87, 29.59, 28.32, and 25.06 GAE mg/g, respectively ($p > 0.001$). Škrovánková et al. (2017) reported that the total polyphenol in paprika and pepper was in the range of 14.67–28.78 GAE mg/g, which was similar to the results of this study. The phenolic composition of pepper depends on several other factors such as the ripening stage, cultivar, harvesting location, and year (Carvalho Lemos et al., 2019).

Flavonoids are a family of compounds with a $C_6-C_3-C_6$ skeleton structure. Previous studies on pepper flavonoids have described the quantification of flavonoid aglycones (quercetin and luteolin) obtained after acid hydrolysis (Howard et al., 2000; Lee et al., 1995). Flavanols, flavonols, and anthocyanins are included in the flavonoids group. The total flavonoid content of RP, OP, YP, GP, and PP was 8.19, 8.25, 7.57, 24.27, and 22.27 CAE mg/g, respectively ($p > 0.001$, Table 3). GP and PP contained anthocyanin and chlorophyll in greater concentration than did RP, OP, and YP. The major antioxidant compounds in the lipophilic fraction isolated from peppers

Table 3 Antioxidant activities of the colored peppers

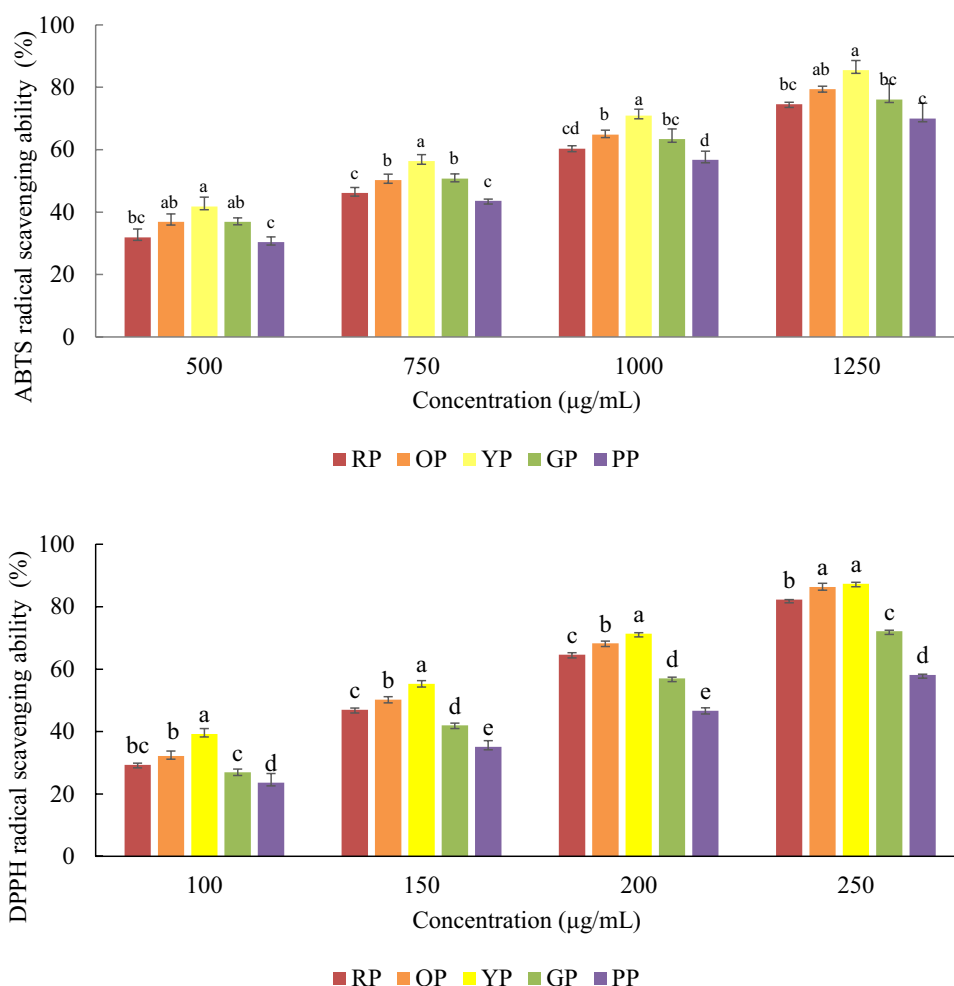
	RP	OP	YP	GP	PP
Yield (%)	54.57 ± 1.20 ^a	55.07 ± 0.90 ^a	49.51 ± 0.81 ^b	41.43 ± 0.51 ^d	44.23 ± 0.21 ^c
Total polyphenol (GAE mg/g)	28.32 ± 0.43 ^c	31.87 ± 1.06 ^a	32.17 ± 0.76 ^a	29.59 ± 0.16 ^b	25.06 ± 0.42 ^d
Total flavonoid (CAE mg/g)	8.19 ± 0.33 ^c	8.25 ± 0.18 ^c	7.57 ± 0.41 ^c	24.27 ± 1.28 ^a	22.27 ± 0.85 ^b
DPPH radical scavenging activity IC ₅₀ ($\mu\text{g/mL}$)	158.74 ± 2.26 ^c	148.04 ± 1.73 ^d	131.29 ± 2.24 ^e	176.28 ± 3.04 ^b	217.63 ± 2.48 ^a
ABTS radical scavenging activity IC ₅₀ ($\mu\text{g/mL}$)	802.05 ± 3.30 ^b	727.80 ± 22.47 ^c	639.34 ± 41.17 ^d	737.96 ± 31.71 ^{bc}	874.05 ± 31.82 ^a
Reducing power	0.52 ± 0.02 ^c	0.58 ± 0.01 ^b	0.62 ± 0.01 ^a	0.46 ± 0.01 ^d	0.35 ± 0.01 ^e

Refer to Table 1

GAE gallic acid equivalent, CAE catechin equivalent

All values are mean ± S.D. Different letters in the same row are significantly different by Duncan's multiple range test at $p < 0.05$

Fig. 2 DPPH & ABTS radical scavenging activities of the colored peppers. Results were expressed the radical scavenging activities as inhibition percent. Values with different letters in a same concentration are significantly different ($p < 0.05$). *RP* red pepper (Mesut), *OP* orange pepper (E22M-0343), *YP* yellow Pepper (Surtsey), *GP* green pepper (Cheongyang), *PP* purple pepper (Deu Syeo Bo Ra F1)



were the flavonoids quercetin and luteolin (Lee et al., 1995). Quercetin has a hydroxyl group at C₃ in the aromatic ring, whereas luteolin does not. Quercetin occurred as quercetin 3-*O*- α -L-rhamnopyranoside-7-*O*- β -D-glucopyranoside, and quercetin 3-*O*- α -L-rhamnopyranoside. Quercetin 3-*O*- α -L-rhamnopyranoside was the main compound in the flavonoid fraction from green pepper. High quercetin 3-*O*-rhamnoside, luteolin, and other flavonoid glycosides were contained in greater amounts in green pepper than in other colored peppers (Materska and Perucka, 2005). The quercetin content in pepper was 2–12 times higher than that of luteolin, and high concentrations of quercetin were typically correlated with high levels of luteolin (Lee et al., 1995). Park et al. (2016) also reported that the flavonoid content of purple sweet potato was four times higher than that of orange and white sweet potato (579.50, 127.12, and 45.41 µg/g, respectively).

DPPH and ABTS radical scavenging activities assay

Antioxidants protect lipids from peroxidation by radicals and may be synthetic or natural. Synthetic antioxidants are

compounds with phenolic structures of various degrees of alkyl substitution, whereas natural antioxidants include vitamins, phenolic compounds, and flavonoids. Natural antioxidants have been extensively studied for their capacity to protect organisms and cells from damage induced by oxidative stress (Nadeem et al., 2011).

When the pepper extract was treated at concentrations of 100, 150, 200, and 250 µg/mg, the DPPH radical scavenging activity tended to increase in a concentration-dependent manner (Fig. 2). The IC₅₀ values obtained from the DPPH assay were 131.29, 148.04, 158.74, 176.28, and 217.63 µg/mL for YP, OP, RP, GP, and PP, respectively ($p > 0.001$, Table 3). When the pepper extract was treated at concentrations of 500, 750, 1000, and 1250 µg/mg, the ABTS radical scavenging activity tended to increase in a concentration-dependent manner (Fig. 2). The ABTS radical scavenging in colored pepper appeared in the order of YP > OP > GP > RP > PP, indicating values of 639.34, 727.80, 737.96, 802.05, and 874.05 µg/mL, respectively ($p > 0.001$, Table 3). Therefore, YP showed the highest antioxidant activity whereas PP showed the lowest. The

Table 4 Correlation coefficients of physicochemical properties and antioxidant activities from colored peppers

L*	a*	b*	C*	h°	CS	DHC	Vit C	TCP	TC	TAC	TPC	TFC	DPPH	ABTS	RP
L*	-0.334														
a*		0.574*													
b*	0.566*		0.868***												
C*	0.105	0.852***													
h°	0.217	-0.991***	-0.664**												
CS	0.528	-0.591	-0.033	-0.253	0.511										
DHC	0.540	-0.568	-0.006	-0.228	0.486	0.999***									
Vit C	0.542	0.015	0.542	0.433	-0.107	0.714*	0.731*								
TCP	-0.292	-0.771**	-0.877***	-0.821**	0.837***	0.315	0.285								
TC	-0.255	0.870***	0.602*	0.887***	-0.855***	-0.554	-0.539	-0.587*							
TAC	-0.407	-0.371	-0.715**	-0.714**	0.460	-0.490	-0.512	0.504	-0.405						
TPC	0.806***	0.145	0.846***	0.595*	-0.258	0.491	0.513	0.864**	0.233	-0.816***					
TFC	-0.272	-0.740**	-0.877***	-0.803**	0.812***	0.416	0.389	0.997***	-0.540*	0.479	-0.540*	0.831**			
DPPH	-0.669*	-0.474	-0.903***	-0.776**	0.587*	-0.325	-0.353	0.808**	-0.432	0.900***	-0.904***	0.252	0.795**		
ABTS	-0.838***	0.264	-0.510	-0.115	-0.146	-0.701*	-0.717*	0.280	0.284	0.606*	-0.756**	0.252	0.795**	-0.983***	
RP	0.700**	0.392	0.929***	0.717**	-0.504	0.284	0.313	0.703*	0.327	-0.807***	0.925***	-0.816***	-0.983***	-0.723**	

CS capsaisin content, DHC dihydrocapsaicin content, TCP total chlorophyll content, TC total carotenoid content, TAC total anthocyanin content, TPC total polyphenol content, TFC total flavonoid content, DPPH DPPH radical scavenging activity IC₅₀, ABTS ABTS radical scavenging activity IC₅₀, RP reducing power

A significant difference at * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$

differences in the DPPH and ABTS scavenging measurements were observed because the DPPH radical is a free radical, whereas the ABTS radical is a cation radical (Wang et al. 1998). Moreover, DPPH and ABTS radicals scavenging show different antioxidant potentials and reaction kinetics when reacting with phenolic compounds. During the reaction, the ABTS radical has to be formed at the start, whereas DPPH is already a stabilized radical by itself (Carvalho Lemos et al., 2019). Otunola and Afolayan (2013) also reported that pepper showed consistently high activity for reducing power, DPPH, ABTS and nitric oxide scavenging.

Reducing power assay

The reducing power was used to evaluate the antioxidant activity of dietary polyphenols and it showed the pepper exhibited a high reducing power compared to other spices (Otunola and Afolayan, 2013). In this study, the reducing power value was in the range of 0.62 to 0.35, in the order of YP > OP > RP > GP > PP. YP exhibited the highest antioxidant power, whereas PP exhibited the lowest ($p > 0.001$, Table 3). Thus, the reducing power of the colored peppers yielded similar results to those of DPPH & ABTS radical scavenging activities, and total polyphenols, with YP exhibiting the highest values. YP showed high vitamin C and capsaicin content, although it had the lowest phytochemical and flavonoid content. OP also showed high antioxidant activity, as well as the highest total carotenoid content and an abundance of polyphenols and vitamin C.

Correlation analysis

Correlation analysis was shown in Table 4. Chroma (C^*) exhibited a strong correlation with the a^* value, b^* value, hue angle, total chlorophyll, and total carotenoid, suggesting that higher chroma is associated with a strong color ($r = 0.852, 0.868, -0.895, -0.821, \text{ and } 0.887$, respectively). Total chlorophyll exhibited a strong negative correlation with the b^* value ($r = -0.877$), suggesting that a higher chlorophyll content is associated with a greenish color. Total carotenoid showed a strong correlation with the a^* value ($r = 0.870$), agreeing with our observations that peppers with a tendency towards red color presented a higher carotenoid concentration. The flavonoid content exhibited a strong correlation with the a^* value, b^* value, and total chlorophyll ($r = -0.740, -0.877, \text{ and } 0.997$, respectively), suggesting that a higher flavonoid content is related to greenish and yellowish color. Vitamin C, which is a strong antioxidant, showed a strong positive correlation with total polyphenol and reducing power ($r = 0.864 \text{ and } 0.703$, respectively), and a strong negative correlation with DPPH and ABTS ($r = -0.729 \text{ and } -0.736$, respectively). Our study

revealed a negative correlation among total polyphenol and total flavonoid, DPPH radical scavenging IC_{50} and ABTS radical scavenging IC_{50} ($r = -0.540, -0.904, \text{ and } -0.756$, respectively), and a strong positive correlation with reducing power ($r = 0.925$). This suggests that the antioxidant activity of peppers was more affected by polyphenols than vitamin C, which agrees with Huang et al (2014).

As the preference for spicy taste increases, the demand for peppers with various colors and functionalities is expected to increase. This study is a basic survey of the physiologically active ingredients of pepper. We expect that it will provide information that can improve the applicability of pepper as a functional food material.

Declarations

Conflict of interest No potential conflict of interest relevant to this article was reported.

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