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Physicochemical and Health Promoting Properties of Everbearing Strawberry Cultivar, 'Dekoruju' Berries

Takeshi Nagai[1,2,3], Norihisa Kai[4], Yasuhiro Tanoue[5] & Nobutaka Suzuki[6]

[1] Graduate School of Agricultural Sciences, Yamagata University, Yamagata, Japan

[2] The United Graduate School of Agricultural Sciences, Iwate University, Iwate, Japan

[3] Graduate School, Prince of Songkla University, Songkhla, Thailand

[4] Graduate School of Engineering, Oita University, Oita, Japan

[5] National Fisheries University, Yamaguchi, Japan

[6] Nagoya Research Institute, Aichi, Japan

Correspondence: Takeshi Nagai, Graduate School of Agricultural Sciences, Yamagata University, Yamagata 9978555, Japan. Tel: 81-235-28-2821. E-mail: nagatakenagatake@yahoo.co.jp; tnagai@tds1.tr.yamagata-u.ac.jp

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Abstract

Overall appearance on everbearing strawberry cultivar, 'Dekoruju' berries were observed. Berries were glossy and colors of skins and pulps were favorable (red-fleshed). An eating quality was good and berries had a favorable balance of sweetness and sourness because of higher sugar-acid ration (14.6) of berries. Berries were foods rich in vitamin B_1 (0.35±0.02 mg/100 g FW) and phenol compounds (80.4±0.6 mg/100 g FW) such as anthocyanins and flavonoids. Extracts prepared from berries, especially in 100% concentration, almost completely inhibited linoleic acid peroxidation under the condition tested. These showed extremely high scavenging activities against specially superoxide anion (92.2±2.16%) and DPPH (> 100%) radicals in comparison with all commercially available natural or synthetic antioxidants tested. These also exhibited the foremost inhibitory activities against not only ACE (62.7±5.1%) but also hyaluronidase (67.6±3.5%), suggesting beneficial effects on anti-hypertension, anti-allergy, and anti-inflammatory. The present study indicated that fresh 'Dekoruju' berries may be considered as good sources of natural antioxidants such as phenol compounds (anthocyanins and flavonoids), and may contribute to maintain and promote health and to help protect body against chronic diseases and an allergy disease.

Keywords: anti-allergic activity, anti-oxidative activity, chemical analysis, 'Dekoruju' berry

1. Introduction

Strawberry (*Fragaria x ananassa* Duch.) is one of popular and attractive vegetables and is eaten as a fresh fruit and a processing ingredient of products such as cakes, ice cream, jams, juices, yogurts, preserves, and liqueurs. For consumers, not only favorable appearances (particularly red or scarlet color) but also desirable flavor, sweetness, and acidity (sourness) are contributed to their initial impression (Mitcham, 2004). In addition, firmness and skin toughness of berries are very important factors that affect these quality maintenance, transportability, and operability in actual demand. In Japan, strawberry is in demand all year round, and mostly is for fresh market in seasons from winter to spring: the June-bearing strawberry cultivars such as 'Tochiotome', 'Toyonoka' and 'Amaou' are on the commercial sales and distribution. However, there is in great request as business purposes for making cakes in seasons from July to October. Flower-bud differentiation of the June-bearing strawberry is inhibited in a hot long-day environment of summer and autumn. So, strawberry is imported about 4,000-5,000 tons per year from foreign countries such as the United State of America to supply demand in domestic off-seasons (Imada, 2006). It draws the highest attention ever in strawberry of summer-autumn production in Japan to be inferior in freshness, taste and flavor, and firmness of the imported strawberries, and to increase demand against domestic products in consumer market.

Since Ohishi had succeeded to cultivate an everbearing strawberry cultivar, 'Ohishi-shikinari 1' in 1954 (Takahashi, 2006), many everbearing strawberry cultivars have been cultivated one after another not only in

Japan (variety names: Miyoshi, Summerberry, Everberry, Pechika, Summerprincess, Summerruby, Natsuakari, and Tochihitomi and so on) but also in England (Serenata), the United State of America (Irvine, Seascape, Capitola, Aromas, Gaviota, Diamante, and UC Albion), and the Kingdom of the Netherlands (Eran) (Yui & Honjo, 2007).

There are many species of everbearing strawberry cultivars, but these characteristics are markedly different according to type of cultivars. Strawberry is an excellent source of nutrients (Sone et al., 1999) and phytochemicals (Yoshida et al., 2002). Especially, phenol compounds in berries possess remarkable antioxidant activity (Basu et al., 2009; Jin et al., 2011; Oszmianski & Wojdylo, 2009). A large quantity of anthocyanin is contained in berries of strawberry cultivars (Lopes-da-Silva et al., 2007). Among them, pelargonidin 3-*o*-glucoside (P3G) is the major predominant and is responsible for fascinating red or scarlet color of fresh berries, followed by cyanidin 3-glucoside, pelargonidin 3-rutinoside, pelargonidin 3-succinate (Bakker et al., 1994), pelargonidin 3-galactoside (Kikoku et al., 1995), and pelargonidin 3-malonylglucoside (Tamura et al., 1995) as minor anthocyanins, flavanols as catechins, and flavonols as quercetin and kaempferol glycosides (Cordenunsi et al., 2005; Gil et al., 1997). In the previous study, it had been proved that intake of strawberries contributed to (1) lower risk of cardiovascular by inhibition of LDL-cholesterol oxidation, (2) promote plaque stability, (3) improve vascular endothelial function, (4) decrease tendency for thrombosis, (5) modulate inflammatory process, (6) block initiation of carcinogenesis, (7) suppress progression and proliferation of tumors (Hannum, 2004), (8) inhibit proliferation of human liver cancer cells (Meyers et al., 2003), (9) inhibit esophageal cancer (Stoner et al., 1999), (10) possess anti-carcinogenic activities to breast and cervical cancer cells (Wedge et al., 2001), (11) inhibit growth and stimulation of apoptosis of human cancer cells (Seeram et al., 2006), and (12) inhibit *Helicobactor pylori* with enhanced susceptibility to clarithromycin (Chatterjee et al., 2004). Intake of fresh fruit as strawberry is believed to have ability to protect human body from some diseases (Huang et al., 2010; Lansky & Newman, 2007; Opara and Al-Ani, 2010).

An everbearing strawberry cultivar, ‘Dekoruju’ was selected from hybrid seedlings between a female parent ‘Pajaro’ (June-bearing strawberry cultivar) and a male parent ‘Morioka No.26’ (everbearing strawberry one) and was registered in 2007 (Okimura et al., 2011). However, as far as we know, there is still lacking of scientific information about nutritional and functional values in these berries. Main objectives of present study try to investigate chemical parameters, vitamins B_1, B_2, and C, total phenols, flavonoids, and anthocyanins, and to clarify medicinal properties as anti-oxidative activity, anti-hypertensive activity, and anti-allergic activity of berries.

2. Materials and Method

2.1 Samples

Fresh everbearing strawberry cultivar, ‘Dekoruju’ berries (weight 12.4-40.3 g, average weight 23.7 g; Figure 1A) were gifted from an independent farmer, Yamagata, Japan, and used in this study.

2.2 Overall Appearance and Sensory Evaluation

Overall appearance of fresh berries was observed. Next, sensory qualities of berries were evaluated on the basis of sweetness, sourness, and firmness by a panel of 4 panelists on a 5-point Hedonic scale.

2.3 Chemical Analysis

Chemical analysis of everbearing strawberry cultivar, ‘Dekoruju’ berries was performed. Moisture was measured using a Moisture Determination Balance (FD-600; Kett Electric Laboratory, Tokyo, Japan). Crude proteins were determined by Kjeldahl method using a conversion factor of 6.25. Crude lipids were analyzed by ether extraction. Crude ashes were determined using an electric furnace (AMI-II; Nitto Kagaku Co. Ltd., Aichi, Japan). Carbohydrates were calculated by difference. The pH was measured using a pH meter (PHL-40; DKK Co. Ltd., Tokyo, Japan). Sugar content was determined using a refractometer (PAL-Pâtissier; Atago Co. Ltd., Tokyo, Japan). Titratable acid content was measured by neutralization titration, and was expressed as percentage equivalent of titratable citric acid. Total vitamin B_1 content was determined by *p*-aminoacetophenone method (Nakamura et al., 1998). Total vitamin B_2 content was measured by lumiflavin fluorescence method (Nakamura et al., 1998). Total vitamin C was determined by α,α’-dipyridyl method (The Vitamin Society of Japan, 1990). Total phenols (Slinkard & Singleton, 1977) and total flavonoids analysis (Kim et al., 2003) was performed by using Folin-Ciocalteu method. These contents were expressed as ellagic acid equivalents and as (+)-catechin equivalent, respectively. Total anthocyanins were measured after an extraction by methanolic hydrochloric solution (1.0% HCl-methanol) (Oki & Suda, 2008). The content was expressed as pelargonidin 3-*o*-glucoside (P3G) equivalent.

2.4 Color Measurement

Color analysis was performed using a colorimeter (NR-11A; Nippon Denshoku Industries Co. Ltd., Tokyo, Japan) with illuminant D65 calibrated to black and white standards. Tristimulus $L^*a^*b^*$ measurement mode was used as the relation to human eye response to color. The L^*, a^*, and b^* scales represents lightness, red-green dimension, and yellow-blue dimension, respectively. Color was determined on ten different spots in each and results were shown as the mean of these measurements.

2.5 Anti-oxidative Activity

Anti-oxidative activities of extracts prepared from everbearing strawberry cultivar, 'Dekoruju' berries were investigated using a linoleic acid oxidation system described by Nagai and Nagashima (2006). Ascorbic acid, *tert*-butyl-4-hydroxyanisole (BHA), 2,6-di-*t*-butyl-4-methylphenol (BHT), α-tocopherol, and trolox were used as positive control, and distilled water was used as negative one.

2.6 Superoxide Anion Radical Scavenging Activity

Superoxide anion radical scavenging activities of extracts from berries were measured using a xanthine/xanthine oxidase system, as described by Nagai and Nagashima (2006). Ascorbic acid, BHA, BHT, α-tocopherol, and trolox were used as positive control, and distilled water was used as negative one. IC_{50} value was defined as concentration of extract to inhibit 50% of superoxide anion radical activity. Moreover, activity was also expressed as micromoles of trolox equivalents per kg of fresh weight of berries [trolox equivalents antioxidant capacity (TEAC); μM TE/kg FW].

2.7 Hydroxyl Radical Scavenging Activity

Hydroxyl radical scavenging activities of extracts from berries were investigated using a Fenton reaction system, as described by Nagai and Nagashima (2006). Ascorbic acid, BHA, BHT, α-tocopherol, and trolox were used as positive control, and distilled water was used as negative one. IC_{50} value was defined as concentration of extract to inhibit 50% of the hydroxyl radical activity. Moreover, activity was also expressed as TEAC (μM TE/kg FW).

2.8 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) Radical Scavenging Activity

DPPH radical scavenging activities of extracts from berries were performed as described by Nagai and Nagashima (2006). Ascorbic acid, BHA, BHT, α-tocopherol, and trolox were used as positive control, and distilled water was used as negative one. IC_{50} value was defined as concentration of extract to inhibit 50% of DPPH radical activity. Moreover, activity was also expressed as TEAC (μM TE/kg FW).

2.9 Angiotensin I-Converting Enzyme (ACE) Inhibitory Activity

ACE inhibitory activities of extracts from berries were measured as described by Nagai and Nagashima (2006). IC_{50} value was defined as protein concentration of extract to inhibit 50% of ACE activity.

2.10 Hyaluronidase Inhibitory Activity

Hyaluronidase inhibitory activities of extracts from berries were investigated by Morgan-Elson method (Elson & Morgan, 1933) with some modifications. A 0.02 ml of extracts and 0.01 ml of hyaluronidase from Ovine testes (1,000U/ml; Wako Pure Chemicals Industries, Ltd., Osaka, Japan) were mixed in an Eppendorf tube and were pre-incubated at 37 ^{o}C for 20 min. Mixture was added 0.02 ml of compound 48/80 solution, and was incubated at the same condition. Enzyme reaction was started by addition of 0.05 ml of hyaluronic acid solution. After incubation at 37 ^{o}C for 40 min, reaction was stopped by addition of 0.02 ml of 1.77 N NaOH and 0.02 ml of 0.8 M boric acid-0.4 N NaOH solution. Mixture was boiled for 3 min, and then cooled in water. Mixture was added 0.6 ml of *p*-dimethylaminobenzaldehyde solution. After incubation at 37 ^{o}C for 20 min, absorbance of mixture was measured at 585 nm, and inhibition rate was calculated by measuring amount of *N*-acetylglucosamine released. Test samples were replaced by buffer solution for control. Enzyme solution was replaced by buffer solution for blank. Percent inhibition was calculated as the following equation.

$$\text{Inhibition (\%)} = [(A - B) - (C - D)]/(A - B) \times 100 \quad (1)$$

Where, A: control OD_{585}, B: control blank OD_{585}, C: sample OD_{585}, D: sample blank OD_{585}.

2.11 Statistical Analysis

Except for color analysis, each assay was repeated 3 times independently and results were reported as means±standard deviation (SD).

3. Results and Discussion

3.1 Overall Appearance

First, overall appearance on everbearing strawberry cultivar, 'Dekoruju' berries was observed. Berries were glossy and colors of skins and pulps of berries were favorable. Particularly, it was red-fleshed and was bright in color. An eating quality was good and berries had a favorable balance of sweetness and acidity (sourness), as mentioned later because of higher sugar-acid ratio of berries. It seemed that taste was superior among other everbearing strawberry cultivars (Nagai et al., 2014; Okimura et al., 2011). Firmness of berries was observed and was fairly higher than other everbearing strawberry cultivars, 'Natsuakari' and 'Summerberry' (Okimura et al., 2011), suggesting high storability and transportability of berries. It is known that firmness of berries is influenced by pectin of cell wall polysaccharides in plants (Watkins et al., 1999). High firmness of 'Dekoruju' berries may be caused by increase of pectin viscosity. Moreover, firmness is directly affected by many factors such as cultivar, planting temperature, land elevation, maturity, size, and moisture content. Firmness is one of important factor for storability and transportability in commercial distribution.

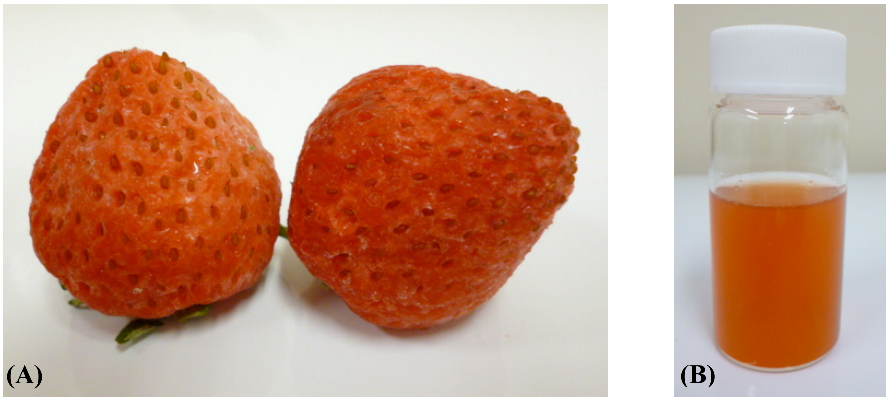

Figure 1. Everbearing strawberry cultivar, 'Dekoruju' berries (A) and extracts (B) prepared from berries

3.2 Chemical Analysis

Chemical parameters of everbearing strawberry cultivar, 'Dekoruju' berries were investigated. Contents of water, crude proteins, crude lipids, carbohydrates, and crude ashes were 86.1, 0.9, 0.1, 12.3, and 0.6%, respectively (Table 1). In comparison to those of other strawberry cultivar berries (Kagawa, 2017; Nagai et al., 2014), 'Dekoruju' berries were remarkable high for contents of carbohydrates.

Berries were weighed, ground in a Potter-Elvehjem homogenizer with a motor-driven Teflon pestle in ice bath, and homogenates were used to the following tests. Specific gravity and pH were tested, and were 1.013 and 3.56 at 20 ^{o}C, respectively (Table 1). Brix% was 11.5% at 20 ^{o}C. Homogenates were then centrifuged at 30,000 ×g for 30 min at 4 ^{o}C. Supernatants were filtered with cheesecloth, and filtrates (extracts) were used to the following investigation. Titratable acidity of extracts was measured, and content was estimated to 0.79% as citric acid equivalent. That is, sugar-acid ratio was calculated to 14.6, suggesting its good eating quality. In comparison with those of everbearing strawberry cultivar, 'Summertiara' berries, Brix% was high, but titratable acid content was low, showing higher sugar-acid ratio (Nagai et al., 2014). Total vitamin B_1, B_2, and C contents of berries were investigated and were as follows: 0.35 mg/100 g FW, 0.0037 mg/100 g FW, and 17.8 mg/100 g FW, respectively (Table 1). Compared to those of other strawberry cultivar berries (Kagawa, 2017; Nagai et al., 2014), vitamin B_1 content was fairly high, but vitamins B_2 and C contents were low. Vitamin C is an important quality attribute of strawberry cultivar berries. Cultivars and pre-harvest climate condition can be defined as crucial factors affecting vitamin C biosynthesis and accumulation (Cordenunsi et al., 2003). Spayed and Morris (1981) reported that strawberry fruit cv. Cardinal had a higher rate of vitamin C accumulation when fruit reached its fully maturity. Olsson et al. (2004), and Kafkas et al. (2007) also reported that ascorbic acid concentration was generally higher in ripe berries compared to unripe ones.

Contents of total phenols, total flavonoids, and total anthocyanins on berries were measured, and results were as follows: 80.4 mg ellagic acid equivalent/100 g FW, 50.6 mg (+)-catechin equivalent/100 g FW, 49.6 mg P3G equivalent/100 g FW, respectively (Table 1). These contents were slightly lower than those of everbearing strawberry cultivar, 'Summertiara' berries (Nagai et al., 2014). Major phenolics of strawberry cultivar berries are ellagitannins, glycosylated dericatives of ellagic acid, *p*-coumaric acids, and anthocyanin (Häkkinen et al., 1999, 2000). Accumulation of phenolic compounds depends on cultivars, geographical position, growing temperature, and moisture contents of berries (Ayala-Zavala et al., 2004). Total anthocyanin contents are one of important parameter for assessing maturity of berries and attracting consumers (Cordenunsi et al., 2005). Contents increase with increasing degree of ripening (Ferreyra et al., 2007). Also, it is reported that greater anthocyanin biosynthesis and accumulation are induced on higher temperature and faster ripening (Ayala-Zavala et al., 2004; Zhang & Watkins, 2005).

Table 1. Chemical parameters of everbearing strawberry cultivar, 'Dekoruju' berries

Parameters		
Water		86.1±0.20%
Proteins		0.9±0.01%
Lipids		0.1±0.03%
Carbohydrates		12.3±0.04%
Ashes		0.6±0.02%
Specific gravity at 20 °C		1.013±0.005
pH at 20 °C		3.56±0.03
Brix% at 20 °C		11.5±0.3
Titratable acid content		0.79±0.04%
Sugar-acid ratio		14.6±0.07
Total vitamin B_1		0.35±0.02 mg/100 g FW
Total vitamin B_2		0.0037±0.0002 mg/100 g FW
Total vitamin C		17.8±0.3 mg/100 g FW
Total phenols		80.4±0.6* mg/100 g FW
Total flavonoids		50.6±0.4** mg/100 g FW
Total anthocyanins		49.6±0.2*** mg/100 g FW
Color parameter		
surface	*L**	26.31±1.01
	*a**	28.42±2.70
	*b**	18.31±1.06
homogenate	*L**	20.63±0.20
	*a**	13.54±0.18
	*b**	11.02±0.15

Note. * ellagic acid equivalent, ** (+)-catechin equivalent, *** P3G equivalent.

Berries were lyophilized and broken into pieces, and powder obtained was added to 10 volumes of boric acid-citric acid-trisodium phosphate buffer (pH 2.0). After extraction for 16 h at room temperature in dark, suspension was centrifuged at 7,740 × g for 10 min. Supernatants were scanned at from 400 to 700 nm using a scanning spectrophotometer. From the result of absorption pattern at the range of 400 to 460 nm, in particular at 430 nm, it indicated presence of P3G as the major anthocyanin in berries as shown in an arrow (Figure 2). Nagai et al. (2014) investigated effect of pH using wide pH range of buffers on anthocyanins from everbearing strawberry cultivar, 'Summertiara' berries. They reported presence of P3G as the major anthocyanin of berries in the absorption pattern at 430 nm (pH 2.0). Also, they reported changes in structures of P3G with increasing pH.

3.3 Color Measurement

Colors on skins of everbearing strawberry cultivar, 'Dekoruju' berries were measured in twenty berries using a colorimeter. Colors were as vivid red (Figure 1A) and parameter was as follows: *L** = 26.31, *a** = 28.42, *b** = 18.31 (Table 1). Color of homogenate was also vivid red (Figure 1B). Its parameter was as follows: *L** = 20.63, *a** = 13.54, *b** = 11.02 (Table 1). Compared to the colors of everbearing strawberry cultivar, 'Summertiara' berries (Nagai et al., 2014), the mean *a* * and *b** values on surface (skins) of berries and the mean *L** value were higher in homogenate, but the mean *a* * in homogenate were lower, suggesting with deep red color on surface (skins) and less redness in central portion of berries.

3.4 Anti-oxidative Activity

Anti-oxidative activities of extracts from berries were determined to evaluate inhibition effects at initiation stage of linoleic acid peroxidation. Extracts showed anti-oxidative activities and activities increased with an increasing concentration of extracts (Table 2). Activity of 1.0% extract was the same as that on 1.0 mM ascorbic acid.

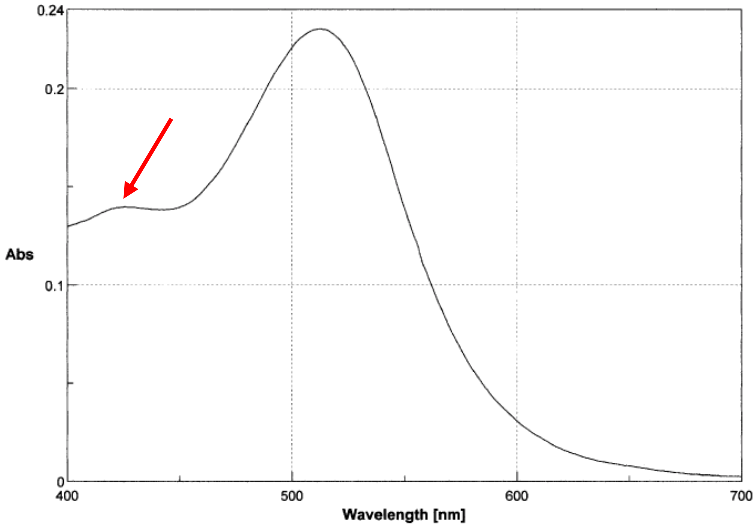

Figure 2. Visible rays absorption spectrum of extracts from everbearing strawberry cultivar, 'Dekoruju' berries

Table 2. Antioxidative activities of extracts prepared from everbearing strawberry cultivar, 'Dekoruju' berries

Samples	Absorbance at 500 nm		
	Time (min)		
	50	100	200
'Dekoruju' extracts (%)			
0.1	0.220±0.011	0.464±0.024	1.059±0.055
1.0	0.103±0.005	0.189±0.012	0.419±0.018
10	0.051±0.004	0.100±0.009	0.207±0.010
100	0.023±0.003	0.021±0.003	0.038±0.007
Ascorbic acid (mM)			
1.0	0.022±0.001	0.135±0.006	0.469±0.027
5.0	0.016±0.001	0.032±0.003	0.090±0.008
BHA (mM)			
0.01	0.084±0.005	0.120±0.008	0.245±0.012
0.1	0.056±0.003	0.090±0.006	0.165±0.010
1.0	0.054±0.002	0.057±0.003	0.100±0.006
BHT (mM)			
0.01	0.082±0.003	0.112±0.009	0.248±0.011
0.1	0.058±0.004	0.108±0.005	0.173±0.008
1.0	0.044±0.002	0.051±0.003	0.093±0.005
α-Tocopherol (mM)			
1.0	0.006	0.025±0.001	0.028±0.002
Trolox (mM)			
0.01	0.084±0.005	0.094±0.006	0.262±0.013
0.1	0.038±0.002	0.051±0.003	0.123±0.008
1.0	0.011±0.001	0.031±0.002	0.032±0.002
Control	0.379±0.008	0.715±0.025	1.406±0.041

Activity on 10% extract was higher than those on 0.01 mM BHA and BHT, but was lower than those on 0.1 mM BHA and BHT. The 100% extract almost completely inhibited linoleic acid peroxidation; activity on 100%

extract was remarkably higher than those on 1.0 mM BHA and BHT and 5.0 mM ascorbic acid, and was almost as high as those on 1.0 mM α-tocopherol and trolox (Table 2).

3.5 Superoxide Anion Radical Scavenging Activity

Superoxide anion radical scavenging activities of extracts from berries were investigated. As a result, activities on 0.1 and 1.0% extracts were fairly low; these were not up to those on 0.01 mM BHT and 1.0 mM ascorbic acid (Table 3). On the other hand, activity on 10% extract was higher than that on 0.1 mM BHA, but was slightly lower than those on 0.1 mM BHT and 0.01 mM trolox. The 100% extract showed the highest activity (92.2%) in comparison with each positive control sample tested (Table 3). IC_{50} value was calculated about 12.02% as concentration of extracts from berries. Also, TEAC against this radical was estimated to 5.93×10^4 μM TE/kg FW.

3.6 Hydroxyl Radical Scavenging Activity

Hydroxyl radical scavenging activities of extracts from berries were measured. The 0.1% extract did not shown this activity. For 10% extract, activity was low about 7.9% compared with those on 1.0 or 5.0 mM ascorbic acid (Table 3). Activity for 10% extract was about 30.8%, and even for 100% extract activity was not up to those of BHA, BHT, α-tocopherol, and trolox tested. IC_{50} value was calculated to about 16.35% as concentration of extracts from berries. TEAC against this radical was 1.19×10^5 μM TE/kg FW.

Table 3. Superoxide anion radical, hydroxyl radical, and DPPH radical scavenging activities of extracts prepared from everbearing strawberry cultivar, 'Dekoruju' berries

Samples	Scavenging activity (%)		
	Superoxide anion radical	Hydroxyl radical	DPPH radical
'Dekoruju' extracts (%)			
0.1	0.4±0.06	0.0	24.7±0.37
1.0	4.5±0.11	7.9±0.19	33.7±0.89
10	41.6±0.95	30.8±0.71	79.5±1.93
100	92.2±2.16	54.2±2.29	>100
Ascorbic acid (mM)			
1.0	14.7±0.20	13.2±0.21	3.1±0.04*
5.0	89.9±5.31	17.6±0.71	34.1±2.01**
BHA (mM)			
0.01	29.3±0.52	59.1±0.78	5.5±0.04
0.1	36.4±0.91	93.3±1.39	17.5±0.36
1.0	51.9±1.36	95.2±1.44	72.7±3.58
BHT (mM)			
0.01	11.7±0.19	82.8±0.91	3.9±0.03
0.1	46.6±1.02	97.6±1.55	7.9±0.08
1.0	48.4±1.17	>100	31.7±0.76
α-Tocopherol (mM)			
1.0	52.6±4.18	67.6±4.34	87.6±2.75
Trolox (mM)			
0.01	46.4±0.98	81.5±0.63	0.1±0.01
0.1	58.1±1.12	91.8±1.17	17.9±0.20
1.0	76.1±1.89	>100	86.3±3.27

Note. * 0.1 mM ascorbic acid; ** 1.0 mM ascorbic acid.

3.7 DPPH Radical Scavenging Activity

DPPH radical scavenging activities of extracts from berries were determined. As a result, each sample exhibited activity and these activities increased with increasing concentration of extracts. Activity for 0.1% extract was fairly higher than those on 1.0 mM ascorbic acid, and 0.01 and 0.1 mM BHA, BHT, and trolox (Table 3). For 1.0% extract possessed the same activity as those on 5.0 mM ascorbic acid and 1.0 mM BHT. Activity for 10%

extract was higher than that on 1.0 mM BHA, although activity did not reach those on 1.0 mM α-tocopherol and trolox. On the contrary, activity of 100% extract extremely high in comparison to each positive control sample tested. Extracts perfectly inhibited this radical. IC_{50} value was calculated to about 5.21% as concentration of extracts from berries. TEAC against this radical was 1.07×10^7 μM TE/kg FW. It is known that DPPH radical scavenging activity is correlated with total phenol contents. It suggests that highest activity against DPPH radicals of the berries attribute to high contents of total phenols (Table 1).

3.8 ACE Inhibitory Activity

ACE inhibitory activities of extracts prepared from berries were measured. Extract for 0.1% did not exhibited activity at all. However, activities tended to increase extract concentration-dependently. Extract for 100% had strong activity about 62.7% against ACE (Table 4). Protein content of extract was measured by method of Lowry et al. (1951). IC_{50} value against ACE activity was calculated to 2.79 mg protein/ml.

3.9 Hyaluronidase Inhibitory Activity

Patients with allergy are increasing yearly. Many species of allergic symptoms exists as follows: alimentary allergy (food allergy), asthma bronchiale, atopic dermatitis, and pollinosis (hay fever). It is known four classes from type I to IV as allergic responses on mechanism of immunological involvement. Hyaluronidase exists in some organs and fluids of body, and is relased from mast cells with histamin as chemical mediators. It hydrolyzes hyaluronic acid in extracellular matrix of connective tissue. Hyaluronidase is involved not only in action that induces inflammation but also in permeability of vascular system. Hyaluronidase is responsible for process causing allergic reactions and inflammation. It is well known that hyaluronidase inhibitory activity and suppression of histamine relase have strong positive correlation. So, effect of hyaluronidase inhibition is used to evaluate anti-allergic activity. At present, research on the phytochemical compositions and anti-allergic activities of food materials are progressing (Kaneda et al., 1998; Yamamoto et al., 2004). Hyaluronidase inhibitory activities of extracts from berries were determined. Surprisingly, extracts below 50% did not shown activities at all, but extract for 100% possessed activity about 67.6% (Table 4). Sodium cromoglicate is used as one of commercially available anti-allergic drugs (chemical mediator release inhibitors) against atopic dermatitis based on food allergies, allergic rhinitis, allergic conjunctivitis and so on. Hyaluronidase inhibitory activity was measured using sodium cromoglicate (purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan) using the same method, and then a standard curve was prepared. As a result, activity for 100% extract corresponded to that of 0.783 μM (0.783 μM sodium cromoglicate equivalent). These results indicated that anti-allergic and anti-inflammatory effects were expected by intake of fresh 'Dekoruju' berries.

Table 4. ACE and hyaluronidase inhibitory activities of extracts prepared from everbearing strawberry cultivar, 'Dekoruju' berries

Extracts (%)	ACE inhibition (%)	Hyaluronidase inhibition (%)
0.1	0.0	0.0
1.0	9.5±0.2	0.0
10	27.0±2.1	0.0
50	46.2±3.0	0.0
100	62.7±5.1	67.6±3.5

From the present study, it found that everbearing strawberry cultivar, 'Dekoruju' berries were foods rich in vitamins B_1 and C and phenol compounds such as anthocyanins and flavonoids. Vitamin C and phenol compounds are one of powerful scavengers against reactive oxygen species and free radicals such as superoxide anion radicals, hydroxyl radicals, hydrogen peroxide, and singlet oxygen. In fact, extracts prepared from berries possessed remarkable high scavenging activities against superoxide anion radicals, hydroxyl radicals, and DPPH radicals, compared to commercially available natural or synthetic antioxidants such as BHA, BHT, and α-tocopherol. Formation and increase of these radicals in human body directly or indirectly cause degenerative diseases and chronic diseases such as cancer, cardiac affection, diabetes, and hypertension. Anti-oxidative function to scavenge reactive oxygen species plays the most important role in early stages of biological defense mechanism to suppress carcinogenesis in body. Extracts showed the highest inhibitory activity against linoleic acid peroxidation. Moreover, extracts exhibited the foremost inhibitory activities against not only ACE but also hyaluronidase, suggesting beneficial effects on anti-hypertension and anti-allergy. It is hereafter deeply anxious to maintain and promote health, and to rejuvenate body or to prevent and treat diseases through conventional daily meals more and more.

4. Conclusion

Everbearing strawberry cultivar, 'Dekoruju' berries had a favorable balance of sweetness and sourness because of higher sugar-acid ration of berries. These were foods rich in vitamin B_1 and phenol compounds such as anthocyanins and flavonoids. Extracts prepared from berries, especially in 100% concentration, almost completely inhibited linoleic acid peroxidation under the condition tested. These showed extremely high scavenging activities against specially superoxide anion and DPPH radicals in comparison with all commercially available natural or synthetic antioxidants tested. These also exhibited the foremost inhibitory activities against not only ACE but also hyaluronidase, suggesting beneficial effects on anti-hypertension, anti-allergy, and anti-inflammatory. The present study indicated that fresh 'Dekoruju' berries may be considered as good sources of natural antioxidants such as phenol compounds (anthocyanins and flavonoids), and may contribute to maintain and promote health and to help protect body against chronic diseases and an allergy disease.

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