Research Article Open Access

Characterization and functional properties of new everbearing strawberry (*Fragaria x ananasa* Duch.) cultivar, 'Summertiara' berries

Takeshi Nagai[1,2,3]*, Misuzu Tamai[4], Masato Sato[5], Yasuhiro Tanoue[6], Norihisa Kai[6], and Nobutaka Suzuki[7]

[1]Graduate School of Agricultural Sciences, Yamagata University, Yamagata 9978555, Japan; [2]The United Graduate School of Agricultural Sciences, Iwate University, Iwate 0208550, Japan; [3]Graduate School, Prince of Songkla University, Songkhla 90112, Thailand; [4]Yamagata University, Yamagata 9978555, Japan; [5]The Farm Village Industry Federation of JA Kushibiki Agricultural Cooperatives, Yamagata 9970341, Japan; [6]National Fisheries University, Yamaguchi 7596595, Japan; [7]Nagoya Research Institute, Aichi 4701131, Japan

Corresponding author: *Takeshi Nagai, PhD, Professor, Graduate School of Agricultural Sciences, Yamagata University, Yamagata 9978555, Japan

Submission date: November 29, 2013; Acceptance date: January 12, 2014; Publication date: January 24, 2014

ABSTRACT

Background: In recent years, a new everbearing strawberry cultivar, 'Summertiara' was cultivated to supply the strawberries in pre-harvest season from July to October in Japan. For highly research and development of processing of this cultivar, 'Summertiara' berries, the objective of this study was to characterize these berries, with relation to chemical parameters, total phenols, total flavonoids, total vitamin C, and total anthocyanins, and was to investigate the solubility and the stability of anthocyanins from the berries. Moreover, the functional properties such as antioxidative activity, active oxygen species scavenging activity, and antihypertensive activity were also evaluated.

Methods: Chemical analysis, colour measurement, and sensory evaluation of new everbearing strawberry cultivar, 'Summertiara' berries were performed. Next, the solubility of anthocyanins from the berries and stability of these against pH, temperature, and an incandescent lighting were investigated. Moreover, functional properties of the extracts prepared from berries were elucidated using 5 different methods.

Results: The contents of water, proteins, lipids, carbohydrates, and ash were the same as those of other cultivar berries. The sugar-acid ratio in the berries was low; these were acidulous. By sensory evaluation, the main factors were vivid red colour, aroma, and acidity. The berries were rich in phenols, flavonoids, vitamin C, and anthocyanins. The anthocyanins of the berries became unstable by heat treatment and light exposures such as visible rays. On the other hand, the extracts prepared from the berries showed the functionalities such as antioxidant activity, active oxygen species scavenging activities, and antihypertensive activity.

Conclusions: The strawberry cultivar, 'Summertiara' berries were the most suitable for processing ingredient of strawberry-derived products with superior health promoting functionalities.

Keywords: Summertiara, everbearing strawberry cultivar, characterization, sensory evaluation, color and storage, functional property

INTRODUCTION

Strawberry (*Fragaria x ananassa* Duch.) is a cold-resistant and perennial plant, and is a non-climacteric fruit. It belongs to Rosoideae and is widely cultivated in all parts of the world. The preservation term of the berries at the best quality is very short. After harvesting the berries are stored at low temperatures and modified atmospheres in order to preserve freshness, and then their shelf life can be expanded to at least one week. Strawberry is used not only as fresh fruit, but also as a processing ingredient of strawberry-derived products such as juices, liqueurs, ice cream, yogurts, cakes, preserves, and jams. At present most of them on the commercial sales and distribution are June-bearing strawberry cultivars in Japan. The flower bud initiation of these cultivars is inhibited under high temperature and long photoperiod. So it become to pre-harvest season from July to October in Japan, and the imported strawberries are used to produce the processed foods. Since Ohishi had succeeded to cultivate an everbearing strawberry cultivar, 'Ohishi-shikinari 1' in 1954 [1], many everbearing strawberry cultivars (such as 'Summerberry', 'Natsuakari', 'Dekoruju', 'Kaho', 'Summercandy', 'Summerruby', and so on) have been cultivated in many area of Japan. In recent year, a new everbearing strawberry cultivar, 'Summertiara' was cultivated in Yamagata Prefecture, Tohoku region of Japan.

The presence of bioactive compounds such as flavonoids and ellagic acid derivatives makes the consumption of strawberry suitable for potential health benefits. Dietary intake of flavonoids has been associated to lower risk of heart disease as well as cancer, probably related to the antioxidative activity of these compounds. From the recent studies, the compounds in strawberries have potential power as follows: lower risk of cardiovascular by inhibition of LDL-cholesterol oxidation, promotion of plaque stability, improved vascular endothelial function, and decreased tendency for thrombosis, modulation of the inflammatory process, blocking initiation of carcinogenesis, suppressing progression and proliferation of tumors [2], inhibition of proliferation of human liver cancer cells [3]), inhibition of esophageal cancer [4], anticarcinogenic activities to breast and cervical cancer cells [5], and inhibition of growth and stimulation of apoptosis of human cancer cells [6]. Moreover, the extracts from strawberry showed antithrombotic effect [7], and inhibition of *Helicobactor pylori* with enhanced susceptibility to clarithromycin [8].

Strawberry cultivar' berries contains large amounts of anthocyanins, the major pigment of strawberry. Particularly, pelargonidin 3-*o*-glucoside (P3G) is predominant and is responsible for the brights red colour of fresh berries, followed by cyanidin 3-glucoside (C3G), pelargonidin 3-rutinoside, pelargonidin 3-succinate [9], pelargonidin 3-galactoside [10], and pelargonidin 3-malonylglucoside [11] as minor anthocyanins, flavanols (catechins), and flavonols (quercetin and kaempferol glycosides) [12,13]. Besides appearance, sweetness, aroma, and acidity, the development of red, scarlet, or vivid red colour is one of the most important characters affecting the quality of strawberry. Also it contributes to a consumer's initial impression and influences their acceptance. Therefore, for highly research and development of processing of new everbearing strawberry cultivar, 'Summertiara' berries, the objective of this study was to characterize these berries, with relation to chemical parameters, total phenols, total flavonoids, total vitamin C, and total anthocyanins, and was to investigate the solubility and the stability of anthocyanins from the berries. Moreover, the functional properties such as antioxidative activity, active oxygen species scavenging activity, and antihypertensive activity were also evaluated.

MATERIALS AND METHODS

Materials

The fresh everbearing strawberry cultivar, 'Summertiara' berries (weight 3.6-7.4 g, average weight 4.9 g; Fig. 1A) were gifted from the Farm Village Industry Federation of JA Kushibiki Agricultural Cooperatives, Yamagata, Japan, transported to our laboratory, and used in the following tests. Ellagic acid dihydrate, 2,6-di-*t*-butyl-4-methylphenol (BHT), ACE from bovine lung (1U), 2,2'-azobis(2-amidinopropane)dihydrochloride (AAPH), α,α'-dipyridyl, chlorogenic acid, 2-deoxy-D-ribose, ethylenediaminetetraacetic acid disodium salt (EDTA), 1,1-diphenyl-2-picrylhydrazyl (DPPH), ethyl acetate for spectrochemical analysis grade, hippuryl-L-histidyl-L-leucine as substrate peptide, linoleic acid, nitroblue tetrazolium salt (NBT), α-tocopherol, and

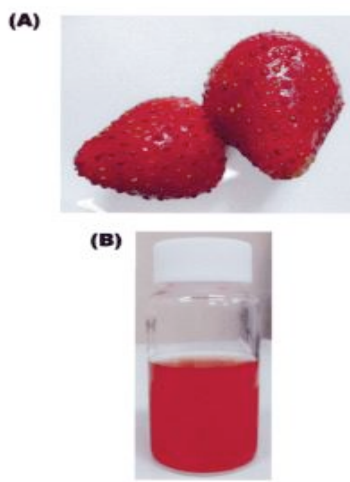

Figure 1. (A) New everbearing strawberry cultivar, 'Summertiara' berries, (B) extracts prepared from the berries

xanthine were purchased from Wako Pure Chemicals Industries, Ltd. (Osaka, Japan). *tert*-Butyl-4-hydroxyanisole (BHA) and (+)-catechin were from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Xanthine oxidase from buttermilk (XOD; 0.27U/mg powder) was from Oriental Yeast

Co., Ltd. (Tokyo, Japan). Trolox was from Cayman Chemical Company (MI, USA). Pelargonidin 3-*o*-glucoside (P3G) chloride was from Extrasynthese (Genay cedex, France). All other reagents were of analytical grade.

Chemical analysis of new everbearing strawberry cultivar, 'Summertiara' berries

AOAC method [14] were used for chemical analysis of new everbearing strawberry cultivar, 'Summertiara' berries. Moisture content was measured using a Moisture Determination Balance (FD-600; Kett Electric Laboratory, Tokyo, Japan). Protein content was analyzed by the Kjeldahl method using a conversion factor of 6.25. Lipid content was determined by ether extraction. Ash content was analyzed using a furnance. Sugar content and pH were measured using a refractometer (PAL-Patissier; Atago Co. Ltd., Tokyo, Japan) and a pH meter (PHL-40; DKK Co. Ltd., Tokyo, Japan), respectively. Total phenolic components were determined at 760 nm using ellagic acid as standard [15]. Total flavonoid contents were measured at 510 nm using (+)-catechin as standard [16]. Total vitamin C contents were determined using the α,α'-dipyridyl method [17]. Total anthocyanin contents were measured using P3G chloride as standard [18].

Colour measurement of new everbearing strawberry cultivar, 'Summertiara' berries

Colour analysis was performed using a Nippon Denshoku Industries Co., Ltd. colorimeter NR-11A (Tokyo, Japan) with illuminant D65 calibrated to black and white standards. The tristimulus L^* a^* b^* measurement mode was used as the relation to human eye response to colour. The L^* variable represents lightness (0 for black, 100 for white)], the a^* scale represents the red-green dimension (-a for greenness, +a for redness), and the b^* scale represents the yellow-blue dimension (-b for blueness, +b for yellowness). Colour was measured on three different spots in each and the results were shown as the mean of these measurements.

Sensory evaluation of new everbearing strawberry cultivar, 'Summertiara' berries

The sensory qualities of new everbearing strawberry cultivar, 'Summertiara' berries were evaluated on the basis of appearance, colour, aroma, sweetness, acidity, and overall acceptability on a 7-point Hedonic scale [19] by a panel of 6 panelists consisting of staffs of our laboratories in the Graduate School of Agricultural Sciences, Yamagata University.

Solubility of anthocyanins from new everbearing strawberry cultivar, 'Summertiara' berries

The berries were lyophilized and the powders were obtained by crushing into pieces using a mortar. To investigate the solubility of anthocyanins from berries, the lyophilized powders were added to 10 volumes of each concentration of ethanol (pH3.5). After 16 h at room temperature in the dark condition, the suspension was centrifuged at 7,740 x g for 10 min. The absorbance of the supernatants was measured at 510 nm.

Effect of pH on anthocyanins from new everbearing strawberry cultivar, 'Summertiara' berries

The berries were lyophilized and the powders were added to 10 volumes of wide pH range of buffer (pH 2.0-12.0; boric acid-citric acid-trisodium phosphate buffer). After 16 h at room temperature in the dark condition, the suspension was centrifuged at 7,740 x g for 10 min. The supernatants were used to scan at from 400 to 700 nm.

Stability of anthocyanins from new everbearing strawberry cultivar, 'Summertiara' berries

The lyophilized powders were added to 10 volumes of 60 % ethanol (pH 3.5). After 16 h at room temperature in the dark condition, the suspension was centrifuged at 7,740 x g for 10 min. The supernatants were collected and used as following stability tests.

Effect of heat treatment

The supernatants were heated at each temperature (20, 60, 80, and 100°C) for 1 day in the dark condition. The absorbance of the solution was measured at 510 nm and was expressed as the residual quantities of anthocyanins.

Effects of storage temperatures and periods

The supernatants were preserved at each temperature (4, 10, and 30°C) for 3 months in the dark condition. The absorbance of the solution was measured at 510 nm and was expressed as the residual quantities of anthocyanins.

Effect of an incandescent lighting

The supernatants were preserved at room temperature under an incandescent lighting for 3 months. The absorbance of the solution was measured at 510 nm and was expressed as the residual quantities of anthocyanins. The intensity of illumination measured using a digital LUX meter (HEM-DDLM5-K, TSdrena, China) was about 730 LUX.

Functional properties of the extracts prepared from new everbearing strawberry cultivar, 'Summertiara' berries

The berries were weighed, ground in a Potter-Elvehjem homogenizer with a motor-driven Teflon pestle, and centrifuged at 30,000 x g for 30 min at 4°C. The supernatants were filtrated with cheesecloth, and then were used to the following investigation.

Antioxidative activity

The antioxidative activities of the extracts prepared from new everbearing strawberry cultivar, 'Summertiara' berries were measured in a linoleic acid oxidation system described by Nagai and Nagashima [20] with a slight modification of the method of Mitsuda *et al*. [21]. A 0.083 ml of the extract and 0.208 ml of 0.2 M sodium phosphate buffer (pH 7.0) were mixed with 0.208 ml of 2.5% (w/v) linoleic acid in ethanol in an Eppendorf tube. The oxidation was initiated by the addition of 20.8 µl of 0.1 M AAPH and carried out at 37°C for 200 min in the dark. The degree of oxidation was measured according to the thiocyanate method for measuring peroxides by reading the absorbance at 500 nm after colouring with $FeCl_2$ and ammonium thiocyanate. BHA (0.01, 0.1, and 1.0 mM), BHT (0.01, 0.1, and 1.0 mM), trolox (0.01, 0.1, and 1.0 mM), ascorbic acid (1 and 5 mM), and α-tocopherol (1 mM) were used as positive control. Distilled water was used as negative control.

Superoxide anion radical scavenging activity

The superoxide anion radical scavenging activities of the extracts made from new everbearing strawberry cultivar, 'Summertiara' berries were evaluated as described by Nagai and Nagashima [20] with a slight modification of the method of Rosa *et al*. [22]. This system contained 0.48 ml of 0.05 M sodium carbonate buffer (pH 10.5), 0.02 ml of 0.15% of BSA, 0.02 ml of 3 mM EDTA, 0.02 ml of 0.75 mM NBT, 0.02 ml of 3 mM xanthine, and 0.02 ml of the extract in an Eppendorf tube. After pre-incubation at 25°C for 10 min, the reaction was started by adding 6 mU XOD and carried out at 25°C for 20 min. The reaction stopped by adding 0.02 ml of 6 mM CuCl. The solution was centrifuged at 12,000 rpm for 5 min, and the absorbance of the reaction mixture was measured at 560 nm and the inhibition rate was calculated by measuring the amount of formazan that was reduced from NBT by superoxide. BHA (0.01, 0.1, and 1.0 mM), trolox (0.01, 0.1, and 1.0 mM), and ascorbic acid (1 and 5 mM) were used as positive control. Distilled water was used as negative control. The IC_{50} value was defined as the concentration of the extract required to inhibit 50% of superoxide anion radical activity. Moreover, the activity was also expressed as moles of trolox equivalents per kg of fresh weight of strawberries [trolox equivalents antioxidant capacity (TEAC); μmol TE/kg FW].

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activities of the extracts prepared from new everbearing strawberry cultivar, 'Summertiara' berries were assayed using the deoxyribose method [20] with a slight modification of the method of Chung *et al*. [23]. The reaction mixture contained 0.45 ml of 0.2 M sodium phosphate buffer (pH 7.0), 0.15 ml of 10 mM 2-deoxy-D-ribose, 0.15 ml of 10 mM $FeSO_4$-EDTA, 0.525 ml of distilled water, and 0.075 ml of the extract in an Eppendorf tube. The reaction was started by the addition of 0.15 ml of 10 mM H_2O_2. After incubation at 37°C for 4 h, the reaction was stopped by adding 0.75 ml of 1.0% (w/v) of 2-thiobarbituric acid in 50 mM NaOH and 0.75 ml of 2.8% (w/v) trichloroacetic acid. The solution was boiled for 10 min, and then cooled in water. The solution was centrifuged at 12,000 rpm for 5 min, and the absorbance of the supernatants was measured at 520 nm. Hydroxyl radical scavenging activity evaluated as the inhibition rate of 2-deoxy-D-ribose oxidation by hydroxyl radicals. BHA (0.01, 0.1, and 1.0 mM), trolox (0.01, 0.1, and 1.0 mM), and ascorbic acid (1 and 5 mM) were used as positive control. Distilled water was used as negative control. The IC_{50} value was defined as the concentration of the extract required to inhibit 50% of hydroxyl radical activity. Moreover, the activity was also expressed as TEAC (μmol TE/kg FW).

DPPH radical scavenging activity

The DPPH radical scavenging activities of the extracts prepared from new everbearing strawberry cultivar, 'Summertiara' berries were measured as described by Nagai and Nagashima [20] with a slight modification of the method of Okada and Okada [24]. The assay mixture contained 0.03 ml of 1.0 mM of DPPH radical solution in ethanol, 0.24 ml of 99% of ethanol, and 0.03 ml of the extract in an Eppendorf tube. The mixture was rapidly mixed and after 30 min the absorbance of the mixture was measured at 517 nm. BHA (0.01, 0.1, and 1.0 mM), trolox (0.01, 0.1, and 1.0 mM), and ascorbic acid (1 and 5 mM) were used as positive control. Distilled water was used as negative control. The IC_{50} value was defined as the concentration of the extract required to inhibit 50% of DPPH radical activity. Moreover, the activity was also expressed as TEAC (μmol TE/kg FW).

ACE inhibitory activity

The ACE inhibitory activities of the extracts prepared from new everbearing strawberry cultivar, 'Summertiara' berries were performed as described by Nagai and Nagashima [20] with a slight modification of the method of Cushman and Cheung [25]. Twenty-five microliters of the extracts and 95 μl of 0.1 M sodium borate buffer (pH 8.3) containing 4.61 mM hippuryl-L-histidyl-L-leucine as substrate and 1.0 M NaCl in an Eppendorf tube were pre-incubated at 37°C for 5 min. The mixture was incubated with 5 μl of 0.1 M sodium borate buffer (pH 8.3) containing 1 mU ACE and 1.0 M NaCl at 37°C for 60 min. By adding 125 μl of 1.0 M HCl the reaction was stopped. The resulting hippuric acid was extracted with 750 μl of ethyl acetate by violently mixing for 15 s. After centrifugation at 6,000 rpm for 3 min, 500 μl of the upper layer was transported into the other tube and evaporated at 80°C for 2 h. The hippuric acid was dissolved in 500 μl of distilled water, and then the absorbance was measured at 228 nm. The IC_{50} value was defined as the concentration of the extract required to inhibit 50% of the ACE activity.

Statistical analysis

Each assay was repeated 3 times independently and the results were reported as means ± standard deviation (SD).

RESULTS AND DISCUSSION

Chemical parameters

First, chemical parameters of new everbearing strawberry cultivar, 'Summertiara' berries were investigated. As a result, the contents of water, proteins, lipids, carbohydrates, and ash were 89.9, 0.9, 0.1, 8.5, and 0.6 %, respectively (Table 1). These results were the same as those of other strawberry cultivar berries [26].

The berries were weighed, ground, and centrifuged at 30,000 x g for 30 min at 4°C. The supernatants were filtrated with cheesecloth, and then were used to the following investigation. The specific gravity and the pH of the extract were 0.996 and 4.24 at 20°C, respectively (Table 1). The contents of sugars (Brix%) were 7.3% at 20°C. The acid contents of the extracts were measured by titrimetry and the result was indicated as citric acid equivalent. The content was estimated to 1.19% and then the sugar-acid ratio was calculated to 6.13 (Table 1). Sone *et al*. [27] was investigated the relationship between stability of eating quality of 43 strawberry cultivars and their sugar and organic acid contents. As a result, it reported that the acid contents of these cultivars were from 0.61 to 1.17 % (average 0.81%) as citric acid equivalent. Miyazaki *et al*. [28] investigated the characteristics of a new processing strawberry cultivar, 'Benihibari' and compared with those of other cultivars, 'America' and 'Hokowase'. These Brix% and acid contents were as follows: 7.3, 7.4, and 8.0, respectively and 0.74, 072, and 0.46, respectively. The sugar-acid ratios also were calculated to 9.86, 10.28, and 17.39, respectively. On the other hand, Okimura *et al*. [29] measured the contents of sugars and acid in new everbearing strawberry cultivars, 'Natsuakari' and 'Dekoruju', and 'Summerberry' as a female parent of 'Natsuakari'. As a result, these sugar contents (Brix%) were as follows: 8.7, 8.4, and 8.7%, respectively. The acid contents were as 0.74, 0.72, and 0.99, respectively. That is, the sugar-acid ratios were calculated to 11.8, 11.8, and 8.8, respectively. It was suggested that new everbearing strawberry cultivar, 'Summertiara' berries in eating quality were acidulous in comparison with other cultivars and everbearing cultivars.

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

Parameters		
Water		89.9±0.1(%)
Proteins		0.9±0.01(%)
Lipids		0.1±0.02(%)
Carbohydrates		8.5±0.02(%)
Ash		0.6±0.01(%)
Specific gravity		0.996±0.003
pH at 20°C		4.24±0.02
Brix% at 20°C		7.3±0.1
Titratable acid content		1.19±0.05 (%)
Sugar-acid ratio		6.13±0.05
Total phenols		94.5±0.4*(mg/100g FW)
Total flavonoids		67.3±0.1**(mg/100g FW)
Total vitamin C		62.2±0.2(mg/100g FW)
Total anthocyanins		55.3±0.1***(mg/100g FW)
Colour parameter		
surface	*L**	26.57±0.10
	a*	22.14±0.09
	b*	15.51±0.11
central portion	*L**	40.05±0.13
	a*	17.44±0.19
	b*	10.01±0.14
homogenate	*L**	14.66±0.04
	a*	19.80±0.03
	b*	10.33±0.03

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

Strawberry is not only a good source of flavonoids and vitamin C [30], but also a very rich source of vitamin E and β-carotene [31]. Next, the contents of total phenolic components, total flavonoids, and total vitamin C of new everbearing strawberry cultivar, 'Summertiara' berries were measured and were as follows: 94.5 mg ellagic acid equivalent/100 g FW, 36.2 mg (+)-catechin equivalent/100 g FW, and 62.2 mg/100 g FW, respectively (Table 1). In the recent report, Akutsu *et al*. [32] investigated the total phenols in the cultivar, 'Tochiotome' berries. As a result, the contents of unripe berries (272 mg gallic acid equivalent/100 g FW) were higher than those of full-ripe ones (234 mg gallic acid equivalent/100 g FW). Then, they also measured the total flavonoid contents in these berries as (+)-catechin equivalent. It found that the unripe berries (4.7 mg/100 g FW) contained more flavonoids than the full-ripe ones (3.6 mg/100 g FW). Moreover, free ellagic acid contents were very low as follows: 0.85 mg/100 g FW (full-ripe berries) and 1.09 mg/100 g FW (unripe ones), respectively. Häkkinen *et al*. [33] measured the content of ellagic acid in strawberry cultivars, 'Jonsok' and 'Senga' to investigate the storage condition in a domestic freezer or refrigerator. As a result, the content of ellagic acid of 'Jonsok' was 40.3 mg/100 g FW. Ellagic contents of two strawberry cultivars reduced to about 40% during the 9 months of storage at -20°C. Moreover, the content in strawberry jam, one of the processed foods, was about 80% of that in unprocessed strawberries (fresh berries). Williner *et al*. [34] determined the ellagic acid content in 5 strawberry cultivars, 'Camarosa', 'Sweet Charly', 'Chandler', 'Osa Grande', and 'Milsei'. The contents were the range of about 2.9 to 6.8 mg/100 g FW and were significantly lower than that of 'Summertiara'. The content of ellagic acid in each cultivar reduced with rising the ripening degree. To investigate the variations in ascorbic acid content among strawberry cultivars on different harvest dates in 1995, Sone *et al*. [35] measured the ascorbic acid content in 293 cultivars berries. The contents ranged from 15.9 to 114.8 mg/100 g FW (the average was 59.1 mg/100 g FW). Moreover, in 1996, the contents in 149 cultivars ranged from 24.0 to 88.9 mg/100 g FW (the average was 48.1 mg/100 g FW). From these results, it found that the content of vitamin C in 'Summertiara' berries was slightly higher than those from other strawberry cultivars.

The total anthocyanin contents of new everbearing strawberry cultivar, 'Summertiara' berries were investigated and were calculated to 55.3 mg P3G equivalent/100 g FW (Table 1). Yoshida *et al*. [36] determined the changes in concentration and composition of anthocyanins during color development in fruit of 20 strawberry (*Fragaria x ananassa* Duch.) cultivars. As a result, P3G was the predominant pigment about 66-94% of total anthocyanins. The contents were the range of 1.75 to 6.94 mg P3G equivalent/100 g FW. On the other hand, Akutsu *et al*. [32] measured total anthocyanin contents extracted from lyophilized strawberry cultivar, 'Tochiotome' berries using 50% acetic acid at room temperature for 24 h. The contents of the anthocyanins in full-ripe and unripe berries were estimated to 17.5 and 9.1 mg P3G equivqlent/100 g FW, respectively. In our present investigation, it was shown that the anthocyanin contents of 'Summertiara' berries were fairly high compared with other strawberry cultivars.

Colour measurement

The colours of the surface (peels) and the central portion (inside) of new everbearing strawberry cultivar, 'Summertiara' berries and the extracts prepared from the berries were investigated by a colorimeter using 10 berries. As shown in Fig. 1A, the colours of the peels of the berries were as vivid red (colour paramater L^* = 26.57, a^* = 22.14, b^* = 15.51; Table 1). After the berries were cut lengthwise in half, the colours of the central portion of berries were measured. The paramater was as follows: L^* = 40.05, a^* = 17.44, b^* = 10.01 (Table 1). On the other hand, the berries were homogenized with a Potter-Elvehjem homogenizer with a motor-driven Teflon pestle, and then centrifuged at 30,000 x g for 30 min at 4°C. The supernatants were filtrated with cheesecloth, and were used as colour measurement. As a result, the colour of the extracts from berries was also as vivid red (Fig. 1B) (colour paramater L^* = 14.66, a^* = 19.80, b^* = 10.33; Table 1).

Besides sweetness, acidity, and aroma, a red or scarlet colour is one of the most important characteristics affecting fruits quality of strawberry cultivars berries. Moreover, it contributes to a consumer's initial impression. It is well known that anthocyanins are the major pigments of strawberry cultivars berries. Among them, P3G is predominant, followed by C3G [9]. In fact, the total anthocyanin contents were very high (55.3 mg P3G equivalent/100 g fresh berries) in this cultivar, 'Summertiara' berries. It suggested that high P3G contents contributed to the vivid red colour of the berries.

Sensory evaluation

The sensory tests of new everbearing strawberry cultivar, 'Summertiara' berries were performed on a 7-point Hedonic scale on the basis of 6 parameters by a panel of 6 panelists consisting of staffs of our laboratories in the Graduate School of Agricultural Sciences, Yamagata University. As a result, the main factors were found in intensities and characteristics of colour, aroma, and acidity (Fig. 2). From the result of the sugar- acid ratio (Table 1), the 'Summertiara' berries were aromatic flavorful and acidulous and it might be suitable not for eating in the raw but for processing. It is known that more than 360 volatile compounds such as esters, acids, alcohols, aldehydes, ketones, and lactones exists in the strawberries [37]. These compounds are responsible for the floral and fruity aroma in the berries. In particular, aldehydes and furanones play an important role of these aromas [38].

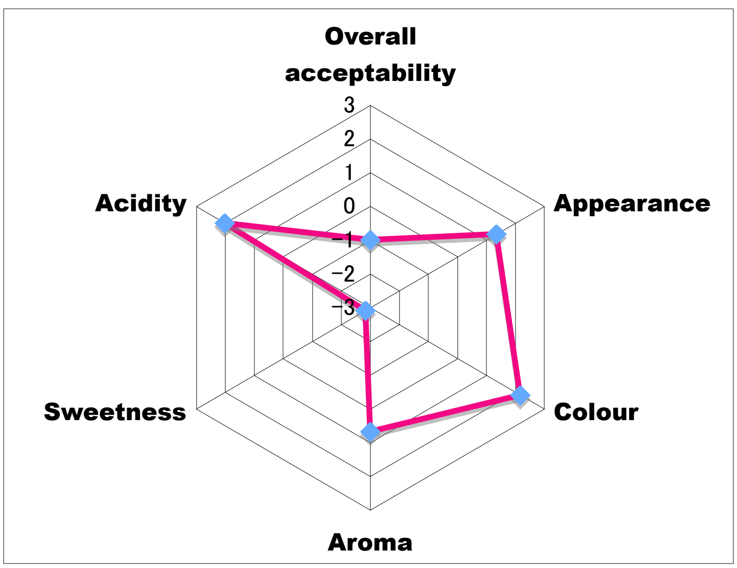

Figure 2. Sensory evaluation of new everbearing strawberry cultivar, 'Summertiara'

Solubility of anthocyanins in ethanol solution

The solubility of anthocyanins from new everbearing strawberry cultivar, 'Summertiara' berries was investigated in each concentration of ethanol solution. As a result, it found that 60 % ethanol was most effective and was as followed in the order 80% > 40% > 90% > 20% (Fig. 3). Surprisingly, the anthocyanins were solubilized more by distilled water (pH 3.5) than by 20, 40, and 90% ethanol solutions. It indicated that the result was useful for berries production of processed foods making use of its colour.

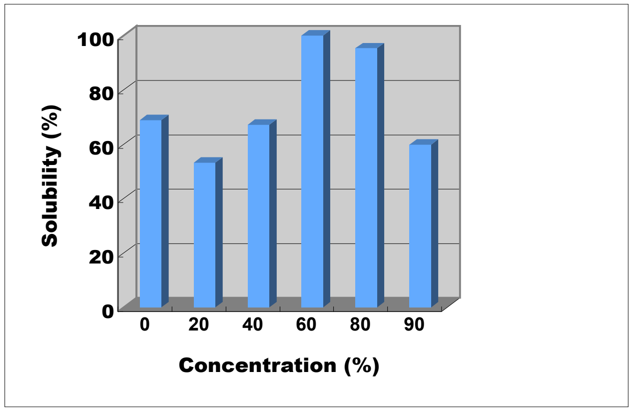

Figure 3. Solubility tests of anthocyanins from new everbearing strawberry cultivar, 'Summertiara' berries. The lyophilized powsers were added to 10 volumes of each concentration of ethanol (pH 3.5). After 16 h at room temperature in the dark condition, the absorbance of the supernatants was measured at 510 nm.

Effect of pH

Anthocyanins may degrade due to various factors such as pH, thermal treatments, light, oxygen, and enzymes [39]. Also the stabilities of anthocyanins may be influenced by other components, particularly the interaction with ascorbic acid, resulting in mutual degradation as reported in strawberry [40]. The absorbance patterns of the extracts prepared from new everbearing strawberry cultivar, 'Summertiara' berries using wide pH range of buffers were investigated by scanning at from 400 to 700 nm. As a result, the stability of anthocyanins was best at pH 2.0, but reduced with rising pH of buffer (Fig. 4). In the absorption pattern at pH 2.0 at the range of 400 to 460 nm, particularly at 430 nm (as shown in an arrow), it indicated the presence of P3G (Fig. 5) as the major anthocyanin in this berries.

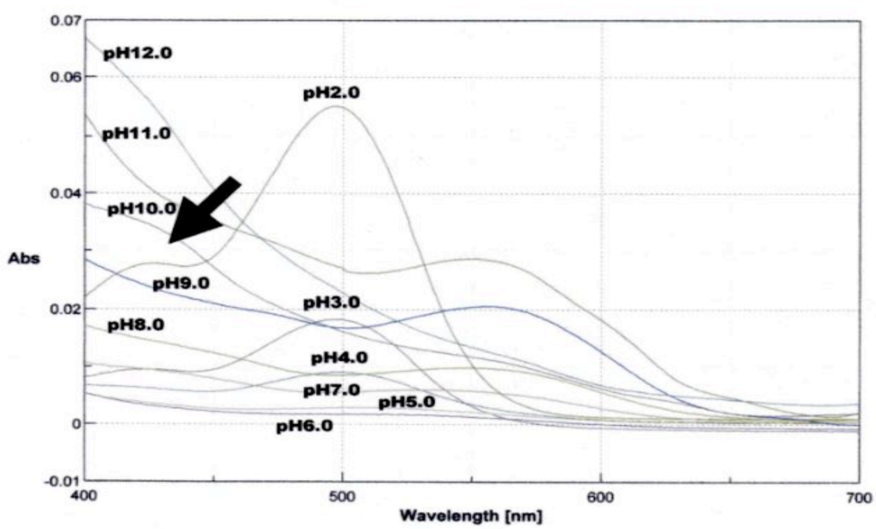

Figure 4. Effects of pH on anthocyanins from new everbearing strawberry cultivar, 'Summertiara' berries. The lyophilized powders were added to 10 volumes of wide pH range of buffer (pH 2.0-12.0). After 16 h at room temperature in the dark condition, the supernatants were used to scan at from 400 to 700 nm.

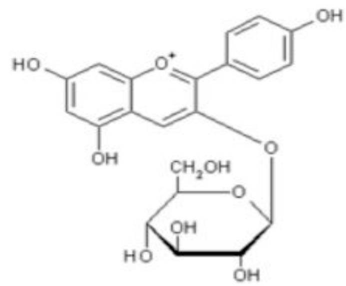

Figure 5. Structure of pelargonidin 3-*o*-glucoside

Effect of heat treatment

After the extracts prepared from new everbearing strawberry cultivar, 'Summertiara' berries were heated at each temperature until 1 day in the dark condition, these were immediately cooled in water, and then the absorption of the extracts were measured at 510 nm. As a result, the anthocyanins in the berries were almost stable for 1 day at 20°C (Fig. 6). These were comparably stable for 4 h at 60-100°C, but became suddenly unstable after 4 h at each temperature. In particular, the anthocyanins became drastically unstable by 100°C treatment. The anthocyanin contents after heating for 1 day were as follows: about 68% (60°C), 58% (80°C), and 46% (100°C), respectively (Fig. 5). Akutsu *et al*. [32] investigated the changes of total polyphenol contents in nonstandard strawberry cultivar, 'Tochiotome' berries by heat treatment (60, 80, and 100°C). As a result, the decreasing degrees of polyphenols were low under low temperature and short time conditions. The content after heating at 80°C for 60 min was about 78%, and the decreasing patterns of polyphenols were no difference among the sample species (standard berries, nonstandard berries, full-ripe berries, and mid-ripe berries). Moreover, the anthocyanin contents after same treatment was about 70%, and the decreasing patterns were the same as the patterns of total polyphenols.

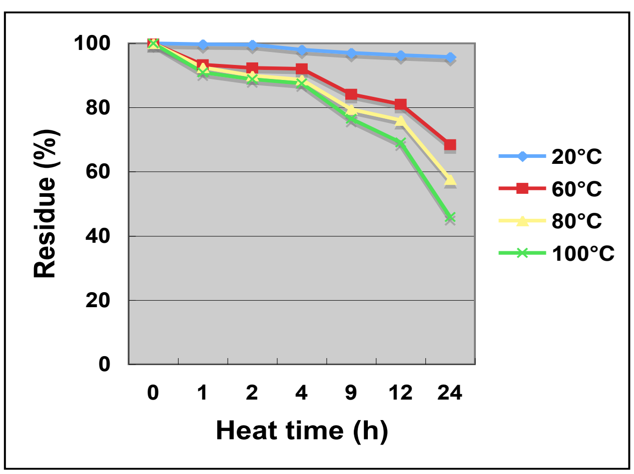

Figure 6. Effects of heat treatment on anthocyanins from new everbearing strawberry cultivar, 'Summertiara' berries. The lyophilized powders were added to 10 volumes of 60% ethanol (pH 3.5). After 16 h at room temperature in the dark condition, the supernatants were heated at each temperature for 1 day in the dark condition. The absorbance of the solution was measured at 510 nm and was expressed as the residual quantities of anthocyanins.

Effects of storage temperatures and periods

To investigate the effects of storage temperatures on the extracts prepared from new everbearing strawberry cultivar, 'Summertiara' berries, extract were preserved at each temperature for 3 months in the dark. As a result, the anthocyanins were relatively stable for 7 days at 4°C (about 98%), but were quickly unstable till 14 days (about 89%), and thereafter were gradually decomposed (about 71% after 90 days) (Fig. 7).

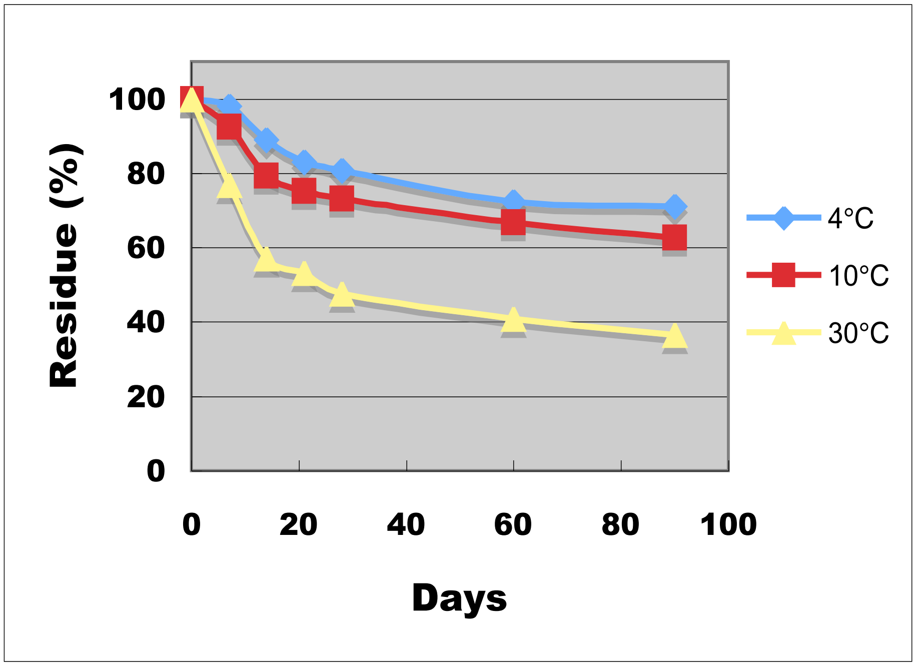

Figure 7. Effects of storage temperatures and periods on anthocyanins from new everbearing strawberry cultivar, 'Summertiara' berries. The lyophilized powders were added to 10 volumes of 60% ethanol (pH 3.5). After 16 h at room temperature in the dark condition, the supernatants were preserved at room temperature (4, 10, and 30°C) for 3 months in the dark condition. The absorbance of the solution was measured at 510 nm and was expressed as the residual quantities of anthocyanins.

These tendencies were the same as those of the storage at 10°C. At 30°C the anthocyanin contents were linearly decreased till 14 days (about 57%), and were gradually decreased (about 37% after 90 days). On the contrary, Torreggiani [41] investigated the anthocyanin resistance of frozen strawberry juice. As a result, the anthocyanin contents decreased by 68.8% and 69.7% after 3 and 8 months, respectively. From these investigations it found that temperature is one of the important factors for the stabilities of anthocyanins [42].

Effect of an incandescent lighting

The extracts prepared from new everbearing strawberry cultivar, 'Summertiara' berries were tested to investigate the effect of an incandescent lighting. As a result, the anthocyanins were relatively stable for 1 day, but thereafter the anthocyanin contents suddenly decreased till 5 days (about 69%) (Fig. 8). Moreover, the decreasing rates of the anthocyains became slowly after 5 days, and thereafter the residual contents were as follows: about 41% (30 days), 33% (60 days), and 24% (90 days), respectively. From these investigations, it found that the anthocyanins contained in new everbearing strawberry cultivar, 'Summertiara' berries easily decompose by visible rays such as incandescent lighting.

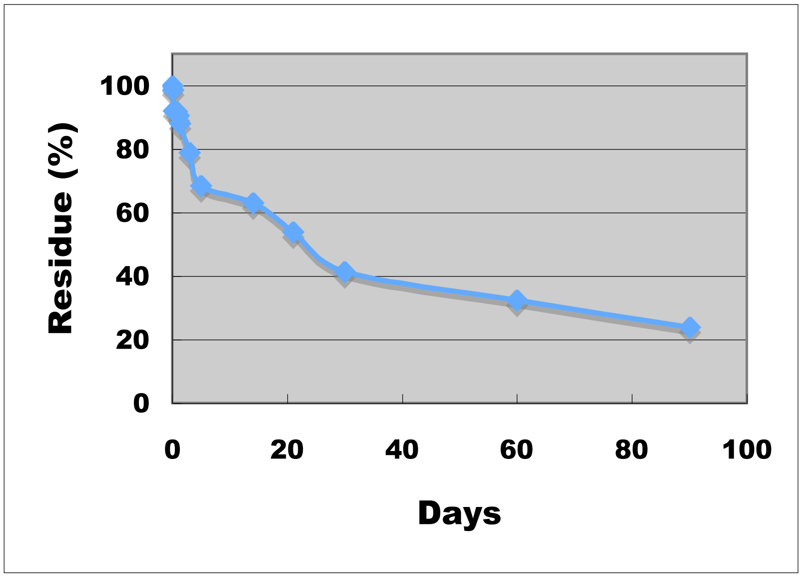

Figure 8. Effects of an incandescent lighting on anthocyanins from new everbearing strawberry cultivar, 'Summertiara' berries. The lyophilized powders were added to 10 volumes of 60% ethanol (pH 3.5). After 16 h at room temperature in the dark condition, the supernatants were preserved at room temperature under an incandescent lighting with 730 LUX for 3 months. The absorbance of the solution was measured at 510 nm and was expressed as the residual quantities of anthocyanins.

Antioxidative activity

The antioxidative activities of the extracts prepared from new everbearing strawberry cultivar, 'Summertiara' berries were investigated to evaluate the inhibition effects at the initiation stage of linoleic acid peroxidation. As a result, each sample species possessed antioxidative activity and the activity increased with an increasing the concentration of the sample (Table 2). The activity for 1% extract was higher than that of 1 mM ascorbic acid, but was lower than those of 0.01 mM BHA, BHT, and trolox. The activity for 10% extract was similar to that of 0.1 mM trolox. On the other hand, the activity for 100% extract was extremely high and was remarkably higher than those of 1 mM trolox and α-tocopherol and 5 mM ascorbic acid; 100% extract perfectly inhibited a linoleic acid peroxidation for 200 min (Table 2).

Superoxide anion radical scavenging activity

Superoxide anion radical scavenging activities of the extracts prepared from new everbearing strawberry cultivar, 'Summertiara' berries were determined using xanthine/xanthine oxidase system. As a result, the activity for 0.1% extract was low as the same as that of 1 mM ascorbic acid (Table 3). This for 1.0% extract was higher than that of 0.01 mM BHA, but was lower than those of 0.1 mM BHA and 0.01 mM trolox. The activity for 10% extract was much higher than those of 1 mM BHA and trolox.

Table 2. Antioxidative activities of the extracts prepared from new everbearing strawberry cultivar, 'Summertiara' berries

Sample	Absorbance (500 nm)		
	Time (min)		
	50	100	200
Summertiara (%)			
0.1	0.141±0.010	0.408±0.015	1.198±0.021
1.0	0.080±0.004	0.168±0.007	0.387±0.005
10	0.006±0.001	0.016±0.002	0.120±0.006
100	0.000	0.000	0.007±0.001
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
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
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
α-Tocopherol (mM)			
1.0	0.006	0.025±0.001	0.028±0.002
Control	0.379±0.008	0.715±0.025	1.406±0.041

The extract for 100% extract possessed highest scavenging activity as much as 5 mM ascorbic acid (Table 3), The IC_{50} value was calculated to about 4.94% as the extracts prepared from the berries. Moreover, the TEAC against superoxide anion radical was 2.03 x 10^4 μmol TE/kg FW. It is known that this radical was effectively scavenged by vitamin C. These results indicated that high scavenging activity against this radical contributed to high concentration of vitamin C in the extracts prepared from the 'Summertiara' berries (Table 1).

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

Sample	Scavenging activity (%)		
	Superoxide anion radical	Hydroxyl radical	DPPH radical
Summertiara (%)			
0.1	12.2±0.21	9.7±0.12	12.3±0.15
1.0	33.6±1.23	13.0±0.14	30.9±1.73
10	87.6±3.51	18.9±0.22	87.3±3.00
100	89.8±4.52	52.1±2.17	90.4±3.52
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
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	>100.0	86.3±3.27
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**

*0.1 mM ascorbic acid; **1.0 mM ascorbic acid

Hydroxyl radical scavenging activity

Using the Fenton reaction system, hydroxyl radical scavenging activities of the extracts prepared from new everbearing strawberry cultivar, 'Summertiara' berries were measured. The activity for 1% extract was the same as that of 1 mM ascorbic acid (Table 3). For 10% the activity was about 19% similar to that of 5 mM ascorbic acid. On the other hand, 100% extract showed the middle activity about 52%; that did not amount to any of other antioxidants except for ascorbic acid (Table 3). The IC_{50} value was calculated to about 93.9% as the extracts prepared from the berries. Moreover, the TEAC against hydroxyl radical was 4.92 x 10^4 μmol TE/kg FW.

DPPH radical scavenging activity

DPPH radical scavenging activities of the extracts prepared from new everbearing strawberry cultivar, 'Summertiara' berries were investigated. Each extract exhibited the scavenging activity and the activity tended to increase with an increasing degree of the concentration of the extract. The activity for 0.1% extract was fairly high compared with 0.01 mM BHA, and trolox, 0.1 mM BHA, and 1 mM ascorbic acid (Table 3). One percent of extract showed the same activity as that of 5 mM ascorbic acid. The activity for 10% extract was extremely higher than that of 1 mM BHA. Moreover, this was almost similar to that of 1 mM trolox. On the contrary, 100% extract had highest scavenging activity among the samples tested. The IC_{50} value was calculated to about 93.9% as the extracts prepared from the berries. Moreover, the TEAC against DPPH radical was estimated to 1.00 x 10^7 μmol TE/kg FW. The DPPH radical scavenging activity is correlated with the total phenol contents. As mentioned above (Table 1), the 'Summertiara' berries contained high concentration of the phenols. It suggested that highest DPPH radical scavenging activity of the berries attributed to high contents of total phenol compounds.

ACE inhibitory activity

ACE inhibitory activities of the extracts prepared from new everbearing strawberry cultivar, 'Summertiara' berries were determined. The protein content was measured by the method of Lowry *et al*. [43]. As a result, the activity increased with an increasing the concentration of the extract. The activities for 0.1 and 1% extracts were low about 16% (Table 4). Fifty percent of the extract inhibited the ACE activity about 62%. Moreover, 100% extract was the highest activity and its activity was perfectly inhibited the ACE activity (Table 4). The IC_{50} value against ACE activity was measured and was calculated to 1.10 mg protein/ml.

Table 4. ACE inhibitory activities of the extracts prepared from new everbearing strawberry cultivar 'Summertiara' berries

Sample (%)	Activity (%)	IC_{50}(%) (mg protein/ml)
		1.10
0.1	13.5±0.2	
1	15.5±0.5	
10	27.0±2.2	
50	62.3±4.7	
100	>100.0	

In the present study, the chemical parameters of new everbearing strawberry cultivar, 'Summertiara' berries were investigated for highly research and development of processing of the berries. As a result, the berries were very rich in phenols, flavonoids, vitamin C, and anthocyanins. The Brix% was relatively low and titratable acid contents were high; that is, the berries were acidulous. By the sensory evaluation, the main factors of the berries were vivid red colour, aroma, and acidity. It contained a large amount of P3G in the berries; high P3G contents contributed to the colour of the berries. It found that new everbearing strawberry cultivar, 'Summertiara' berries might be suitable not to eat in the raw, but to process strawberry-derived products.

The main flavonoids present in strawberries are anthocyanins, which are responsible for the attractive colour development of the fruits, besides being very important for the quality and the evaluation of many fresh and processed fruits. Though they are a good source of natural antioxidants, anthocyanins are quite unstable during processing and storage. Particularly, pH, temperature, time of processing and storage, and light exposure have been found to exert a great influence on anthocyanin stability. Moreover, it is known that the loss of anthocyanins has been attributed to many factors such as phenolic compounds, sugars and sugar degradation products, oxygen, ascorbic acid, fruit maturity, and thawing time. By heat treatment tests, anthocyanins of 'Summertiara' berries were comparably stable until 4 h heating at each temperature (20, 60, 80, and 100°C), but above 60°C became drastically unstable with an increasing degree of the temperature. The effects of storage temperatures and periods were investigated for 3 months in the dark. The anthocyanins were relatively stable for 7 days at 4°C, but were quickly unstable till 14 days, and thereafter gradually decomposed. On the other hand, the anthocyanins contents were linearly decreased till 14 days at 30°C, and were gradually decreased for 90 days.

It is well known that anthocyanins are also easily decomposed by light exposure. The stabilities of the anthocyanins of the berries were tested for 90 days under an incandescent lighting with about 730 LUX. The anthocyanins were relatively stable for 1 day, but thereafter suddenly decomposed till 5 days. Then, the decreasing rates of the anthocyanins became slowly, and the residual contents of the anthocyanin were about 24% after 90 days. The anthocyanins contained in new everbearing strawberry cultivar, 'Summertiara' berries decomposed easily by visible rays as incandescent lighting. Melgarejo *et al*. [44] reported the effects of different temperatures (5 and 25°C) and light exposures (daylight and darkness) on anthocyanin contents and colour development of jams made from the pomegranate cultivar, 'Mollar' during five months. The results indicated the stabilities of anthocyanin were closely related with not light exposures but the storage temperatures.

Among anthocyanin derivatives, P3G is the major anthocyanin in strawberry *Fragaria x ananassa* (153-652 mg/kg FW) [45,46]. On average about 83% of total anthocyanin in strawberry extracts was P3G, and followed by pelargonidin 3-rutinoside (about 8%) and C3G (about 7%). Goiffon *et al*. [47] analyzed the contents of P3G and C3G in five strawberry varieties, and the percentages of these components were 89-95% and 4-11%, respectively. Ellagic acid is a dietary hydroxybenzoic acid and a hydrolytic product of ellagitannins that may occur in the free form in plants [48]. Ellagic acid is the main phenolic compound in the berries of the family *Rosaceae*, forming 77-88% of the phenolic compounds, and in the genus *Fragaria* 51% of the content of phenolic compounds [49]. There are health benefits such as antioxidant, antimutagenic, and anticarcinogenic effects in ellagic acid as well as other phenolic acids [50,51]. From the reasons, *in vitro* antioxidative activity was investigated in the extracts prepared from everbearing strawberry cultivar, 'Summertiara' berries. The extracts exhibited extremely high antioxidative activity (Table 2), suggesting that a large amount of anthocyanins and phenols such as P3G and ellagic acid contributed to the activity. Active oxygen species scavenging activities were investigated and the extracts possessed the strongest activities: 2.03×10^4 μmol TE/kg FW (superoxide anion radical scavenging), 4.92×10^4 μmol TE/kg FW (hydroxyl radical scavenging), and 1.00×10^7 μmol TE/kg FW (DPPH radical scavenging), respectively. Moreover, the extracts exhibited high ACE inhibitory activity. Overall, the functionalities of new everbearing strawberry cultivar, 'Summertiara' berries are associated with high contents of phenols, flavonoids, vitamin C, and anthocyanins.

CONCLUSION

In recent years, new everbearing strawberry cultivar, 'Summertiara' was cultivated to supply the strawberries in pre-harvest season from July to October in Japan. The sugar-acid ratio in the berries was low; these were acidulous. By sensory evaluation, the main factors were vivid red colour, aroma, and acidity. The berries were rich in phenols, flavonoids, vitamin C, and anthocyanins. The anthocyanins of the berries became unstable by heat treatment and light exposures such as visible rays. The extract prepared from the berries showed the functionalities such as antioxidant activity, active oxygen species scavenging activities, and antihypertensive activity. From these investigations, it indicated that the strawberry cultivar, 'Summertiara' berries are the most suitable for processing ingredient of strawberry-derived products with superior health promoting functionalities.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

All authors contributed to this study.

Acknowledgements and Funding

The authors wish to express their gratitude to the help of Yamagata University Regional Industry, Academia and Government Cooperation Project (2012).

REFERENCES

1. Takahashi H: Present states and prospects of everbearing strawberry breeding in Northern Japan. Hort Res (Japan), 2006,5:213-217 (in Japanese).
2. Hannum SM: Potential impact of strawberries on human health: A review of science. Crit Rev Food Sci Nutr 2004,44:1-17.
3. Meyers KJ, Watkins CB, Pritts MP, Liu RH: Antioxidant and antiproliferative activities of strawberries. J Agric Food Chem 2003,51:6887-6892.
4. Stoner GD, Kresty LA, Carlton PS, Sliglin JC, Morse MA: Isothiocyanates and freeze-dried strawberries as inhibitors of esophageal cancer. Toxicol Sci 1999,52(Supplement):95-100.
5. Wedge DE, Meepagala KM, Magee JB, Smith SH, Huang G, Larcom LL: Anticarcinogenic activity of strawberry, blueberry, and raspberry extracts to breast and cervical cancer cells. J Med Food 2001,4:49-51.
6. Seeram NP, Adams LS, Zhang Y, Lee R, Sand D, Scheuller HS, Heber D: Blackberry, black raspberry, blueberry, cranberry, red raspberry, and strawberry extracts inhibit growth and stimulate apoptosis of human cancer cells in vitro. J Agric Food Chem 2006,54:9329-9339.
7. Naemura A, Mitani T, Ijiri Y, Tamura Y, Yamashita T, Okimura M, Yamamoto J: Anti-thrombotic effect of strawberries. Blood Coag Fibrin 2005,16:501-509.
8. Chatterjee A, Yasmin T, Badchi D, Stohs SJ: Inhibition of *Helicobacter pylori in vitro* by various berry extracts, with enhanced susceptibility to clarithromycin. Mol Cell Biochem 2004,265:19-26.

9. Bakker J, Bridle P, Bellworthy SJ: Strawberry juice colour: A study of the quantitative and qualitative pigment composition of juices from 39 genotypes. J Sci Food Agric 1994, 64:31-37.
10. Kikoku Y, Fukuhara K, Saito I, Oota H: Identification and high performance liquid chromatographic determination of strawberry anthocyanin pigments. J Jap Soc Food Sci Tech 1995,42:118-123 (in Japanese).
11. Tamura H, Takada M, Yoshida Y: Pelargonidin 3-*o*-(6-*o*-malonyl-β-D-glucoside) in *Fragaria x ananassa* Duch. Cv. Nyoho. Biosci Biotech Biochem 1995,59:1157-1158.
12. Cordenunsi BR, Genovese MI, Nascimento JRO, Hassimotto NMA, Dos Santos RJ, Lajolo FM: Effects of temperature on the chemical composition and antioxidant activity of three strawberry cultivars. Food Chem 2005,91:113-121.
13. Gil MI, Holcroft DM, Kader AA: Changes in strawberry anthocyanins and other polyphenols in response to carbon dioxide treatments. J Agric Food Chem 1997,45:1662-1667.
14. AOAC 2005. Official Methods of Analysis of AOAC International. 18th edn. Association of Official Analytical Chemists, Arlington.
15. Slinkard K, Singleton VL: Total phenol analysis. Am J Enol Viticul 1977,28:49-55.
16. Kim D-O, Chun OK, Kim YJ: Quantification of polyphenolics and their antioxidant capacity in fresh plums. J Agric Food Chem 2003,51:6509-6515.
17. The Vitamin Society of Japan: Vitamin Handbook. Kyoto: Kagakudojin; 1990:139-140.
18. Oki T, Suda I: Anthocyanin. In Science of Functional Foods (Science of Functional Foods Editorial Committee, ed.) Tech Information s.c.; 2008:1057-1066.
19. Amerine MA, Pangborn RM, Roessler EB: Principles of sensory evaluation of food, London, Academic Press; 1965.
20. Nagai T, Nagashima T: Functional properties of dioscorin, a soluble viscous protein from Japanese yam (*Dioscorea opposita* Thunb.) tuber mucilage *tororo*. Z Naturforsch C 2006,61:792-798.
21. Mitsuda H, Yasumoto K, Imai K: Antioxidative action of indole compounds during the autoxidation of linoleic acid. Eiyo to Shokuryo 1966,19:210-214.
22. Rosa GD, Duncan DS, Koen CL, Hurley LS: Evaluation of negative staining technique for determination of CN^--insensitive superoxide dismutase activity. Biochim Biophys Acta 1979,566:32-39.
23. Chung S-K,Osawa T, Kawakishi S: Hydroxyl radical scavenging effects of species and scavengers from brown mustard (*Brassica nigra*). Biosci Biotechnol Biochem 1997,61:118-123.
24. Okada Y, Okada M: Scavenging effects of water soluble proteins in broad beans on free radicals and active oxygen species. J Agric Food Chem 1998,46:401-406.
25. Cushman DW, Cheung HS: Spectrophotometric assay and properties of the angiotensin-I converting enzyme of rabbit lung. Biochem Pharmacol 1971,20:1637-1648.
26. Standard Tables of Food Composition in Japan 2010. Tokyo:Kagawa Nutrition University Publishing Division; 2012.

27. Sone K, Mochizuki T, Noguchi Y: Relationship between stability of eating quality of strawberry cultivars and their sugar and organic acid contents. J Japan Soc Hort Sci 2000,69:736-743 (in Japanese).
28. Miyazaki M, Sato H, Oku M, Goto T: Characteristics of a new processing strawberry cultivar, 'Benihibari'. J Japan Soc Hort Sci 1995,63:811-817.
29. Okimura M, Okamoto K, Honjo M, Yui S, Matsunaga H, Ishii T, Igarashi I, Fujino M, Kataoka S, Kawazu Y: Breeding of new everbearing strawberry cultivars, 'Natsuakari' and 'Dekoruju'. Hort Res (Japan), 2011,10:121-126 (in Japanese).
30. Wang H, Cao G, Prior RL: Total antioxidant capacity of fruits. J Agric Food Chem 1996,44:701-705.
31. Oszmianski J, Wojdylo A: Comparative study of phenolic content and antioxidant activity of strawberry puree, clear, and cloudy juices. Eur Food Res Technol 2009,228:623-631.
32. Akutsu S, Tsutsui T, Oyama T, Ito K: Effective compounds of farm products in Tochigi Prefecture and behavior of these in the heating etc.: polyphenol composition and heating behavior in strawberries. Rep Ind Tech Cent Tochigi Pref 1999, 7:75-79 (in Japanese).
33. Häkkinen S, Kärenlampi SO, Mykkänen HM, Heinonen IM, Törrönen AR: Ellagic acid content in berries: Influence of domestic processing and storage. Euro Food Res Technol 2000,212:75-80.
34. Williner MR, Pirovani ME, Güemes DR: Ellagic acid content in strawberries of different cultivars and ripening stages. J Sci Food Agric 2003,83:842-845.
35. Sone K, Mochizuki T, Noguchi Y: Variations in ascorbic acid content among strawberry cultivars and their harvest times. J Japan Soc Hort Sci 1999,68:1007-1014 (in Japanese).
36. Yoshida Y, Koyama N, Tamura H: Color and anthocyanin composition of strawberry fruit: Changes during fruit development and differences among cultivars, with special references to the occurrence of pelargonidin 3-malonylglucoside. J Japan Soc Hort Sci 2002,71:355-361.
37. Forney CF: Horticultural and other factors affecting aroma volatile composition of small fruit. Hort Technol 2001,11:529-538.
38. Bood KG, Zabetakis I: The biosynthesis of strawberry flavor (II): Biosynthetic and molecular biology studies. J Food Sci 2002,67:2-8.
39. Wang W, Xu S: Degradation kinetics of anthocyanins in blackberry juice and concentrate. J Food Eng 2007,82:271-275.
40. Skrede G, Wrolstad RE, Lea P, Enersen G: Colour stability of strawberry and blackcurrant syrups. J Food Sci 1992,57:172-177.
41. Torreggiani D: Modification of glass transition temperature through carbohydrates addition: effect upon colour and anthocyanin pigment stability in frozen strawberry juices. Food Res Inter 1999,32:441-446.
42. Withy LM, Nguyen TT, Wrolstad RF, Heatherbell DA: Storage changes in anthocyanin concentrate of red raspberry juice content. J Food Sci 1993,58:190-192.

43. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: Protein measurement with the Folin phenol reagent. J Biol Chem 1951,193:265-275.
44. Melgarejo P, Martínez R, Hernáadez Fca, Martínez JJ, Legua P: Anthocyanin content and colour development of pomegranate jam. Food Bioproducts Process 2011,89:477-481.
45. García-Viguera C, Zafrilla P, Tomás-Barberán FT: The use of acetone as an extraction solvent for anthocyanin from strawberry fruits. Phytochem Anal 1998,09:274-277.
46. Lopes-da-Silva F, Escribano-Bailón MT, Pérez Alonso JJ, Rivas-Gonzalo JC, Santos-Buelga C: Anthocyanin pigments in strawberry. LWT- Food Sci Technol 2007,40:374-382.
47. Goiffon JP, Mouly PP, Gaydou EM: Anthocyanic pigment determination in red fruit juices, concentrated huices and syrups using liquid chromatography. Anal Chim Acta 1999, 382:39-50.
48. Clifford MN, Scalbert A: Ellagitannins-nature, occurrence and dietary burden. J Sci Food Agric 2000,80:1118-1125.
49. Häkkinen S, Heinonen M, Kärenlampi S, Mykkänen H, Ruuskänen J, Törrönen R: Screening of selected flavonoids and phenolic acids in 19 berries. Food Res Inter 1999,32:345–353.
50. Maas JL, Galletta GJ, Stoner GD: Ellagic aicd, an anticarcinogen in fruits, especially in strawberries: a review. HortScience 1991,26:10-14.
51. Maas JL, Wang SY, Galletta GJ: Evaluation of strawberry cultivars for ellagic acid content. HortScience 1991,26:66-68.