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Research Report

Anthocyanin content, bioactive compounds and physico-chemical characteristics of potential new strawberry cultivars rich in-anthocyanins

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Abstract.
BACKGROUND: High anthocyanin content and the presence of other bioactive compounds are attractive characteristics of strawberry fruits for healthy consumption.
OBJECTIVES: To characterize the anthocyanin content and the presence of other bioactive compounds, including anthocyanin (total and predominant types) andantioxidant activity; and to determine the physico-chemical fruit quality parameters of two new strawberry cultivars.
METHOD: Fruits of two new hybrids were extracted and total anthocyanin and antioxidant activity were determined usinga UV-Vis spectrophotometer. Individual anthocyanins and vitamin C were measured using an HPLC. Physico-chemical characteristics of fruits were analyzed.
RESULTS: Hybrid No. 4 line 5 and hybrid No. 4 line 26 are two potential new strawberry cultivars that are rich in anthocyanins. The total anthocyanin contents of these two hybrids were approximately 31–38 mg/100 g FW with no significant differences between them. Cyanidin 3-glucoside and pelargonidin 3-glucoside were foundat amounts of approximately 15–24 mg/kgFW and 332–478 mg/kg FW, respectively. Total phenolic compounds and FRAP activity of the two hybrids were approximately 2295–2579 mg GAE/kgFWand 27–30 mmol Fe^{2+}/kg FW, respectively.
CONCLUSION: The two new hybrid strawberry lines, hybrid No. 4 line 5 and No. 4 line 26, when compared to the parents, had higher levels of bioactive compounds, especially anthocyanins, total phenolics, and FRAP, together with improved physico-chemical quality, and higher vitamin C content. These results indicate a considerable potential of these hybrids for commercial cultivation in Thailand and other production regions.

Keywords: Strawberry, bioactive compounds, anthocyanins, cyanidin-3-glucoside, pelargonidin-3-glucoside

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1. Introduction

Highconsumption of fruit and vegetables is considered an effective way to increase the intake of bioactive compounds and to enhance the nutritional values of a human diet. Berry fruits, especially strawberry, represent one of the most important sources of bioactive compounds with high antioxidant capacity. Therefore, increasing the consumption of berries that are high in 'healthy compounds' seems to be an appropriate strategy for improving human health [1].

Presently, strawberries are one of the most popular fruits in the world [2] since they are valued for flavor, fragrance and richness in natural bioactive compounds, especially anthocyanins. Anthocyanins play an important role in color of the berries, while lowering the risk of cardiovascular disease, and reducing the risk of cancer in humans [3]. Moreover, they can reverse age-related neurodegenerative decline [4], improve gluco regulation [5, 6], protect brain tissue from hypoxia [7], improve visual functions [8], and protect against DNA damage [9]. In addition, it has been shown that a diet rich in these bioactive compounds may prevent hyperlipidemia [10], inhibit antimutagenic activity [11], stimulatethe production of insulin in pancreatic cells [5], protect against liver damage [12], and reduce inflammation and oxidative stress in the brain leading to beneficial effects against neurodegenerative processes such as Parkinson's or Alzheimer's disease [13].

Anthocyanins are well-known polyphenolic compounds and quantitatively the most important in strawberry [14]. The two major anthocyanin compounds in strawberry are pelargonidin-3-glucoside (89–95%of total anthocyanin content) and cyanidin-3-glucoside (3.9–10.6%) [15, 16]. Breeding programs have been typically focused on developing new and improved cultivars for specific agronomic, qualitative and sensorial traits. However, the interest in breeding cultivars with specific health-related phytochemicals is increasing. This development of phytochemically rich fruits can potentially benefit not just consumers but may also benefit farmers and processors through increased returns for higher-value products [17].

In Thailand, strawberries are widely grown in the northern provinces under cool weather at high elevation including in Chiangmai, Chiangrai and other provinces of northern Thailand. The most popular strawberry cultivars include Praratchatan No. 50, Praratchatan No. 72 and Akihime, which are highly desired by consumers. Provided that these cultivars have standout features such as good aroma, sweetness, redness, firmness and high yield, they are attractive for being consumed both fresh and processed [18]. However, strawberry cultivars in Thailand generally have a low anthocyanin content [19] and until now, there has not been anyresearch that specifically focuses on the development of cultivars that are high in anthocyanin. Therefore, the aim of this study was to determine the anthocyanin content, the antioxidant activity of bioactive compounds and the physicochemical characteristics of fruit of two new potential new strawberry cultivars that would enhance the quality and nutritional value of this fruit in Thailand.

2. Materials and methods

2.1. Chemicals

All chemical reagents for antioxidant activity analysis, including folin-Ciocalteu reagent, sodium carbonate (anhydrous), 3,4,5-trihydroxybenzoic acid (gallic acid), sodium hydroxide, sodium acetate trihydrate, glacial acetic acid, 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), hydrochloric acid, 2,4,6-tripyridyl-s-triazine (TPTZ),and Iron(III) chloride hexahydrate, were purchased fromat Sigma-Aldrich (USA). For HPLC analysis, HPLC grade ortho-phosphoric acid, potassium dihydrogen phosphate, meta-phosphoric acid, ascorbic acid, water, methanol, ethanol and acetonitrile, were used. Anthocyanins standards, including pelargonidin 3-glucoside and cyanidin 3-glucoside, were purchased from Sigma-Aldrich (Switzerland).

Table 1
List of the strawberry parents involved in the cross combinations for the study of fruit nutritional quality

Hybrid pair	Progenitors		
number	Female		Male
1	Praratchatan No. 50	×	Praratchatan No. 50
2	Praratchatan No. 72	×	Praratchatan No. 50
3	Akihime	×	Praratchatan No. 50
4	Praratchatan No. 50	×	Praratchatan No. 72
5	Praratchatan No. 72	×	Praratchatan No. 72
6	Akihime	×	Praratchatan No. 72
7	Praratchatan No. 50	×	Akihime
8	Praratchatan No. 72	×	Akihime
9	Akihime	×	Akihime

2.2. Source of strawberry cultivars

2.2.1. Parent cultivars

Sixty mature plantlets from crosses between three parental strawberry cultivars were obtained from The Royal Project Foundation, Chiangmai, northern Thailand (latitude: 18.812369, longitude: 98.884381) and cultivated in plastic pots (20 plantlets per cultivar). These plantlets were maintained under approximately 18.8°C and 64.3% RH ina greenhouse at the Highland Agricultural Research and Development Center, Khao Kho, Phetchabun, lower northern Thailand (latitude: 16.588400, longitude: 100.960130).

2.2.2. The breeding process, selection and cultivation of hybrid strawberry lines

The new hybrid strawberries were developed at The Highland Agricultural Research and Development Center, Khao Kho. This program involved nine cross combinations (Table 1).

Each hybrid pair of strawberry was crossed using ten flowers of each cultivar using standard methods. Seeds from ripe fruits from each cross were separated using a blender [20]. The seeds were air-dried at room temperature for one month, chilled at 4°C for a month and then placed on a piece of moist filter paper in Petri dishes. A 2% metalaxyl fungicide solutionwas sprayed on the seeds for disease control.

A total of 2,700 seeds of nine hybrid pairs were selected and cultivated in peat moss containing plastic trays at approximately 25°/22°C (day/night) and 71.2% RH, for 5 months in a greenhouse at Faculty of Agriculture Natural Resources and Environment, Naresuan University, Phitsanulok, Thailand (latitude: 16.746360, longitude: 100.196050). From the initial seeds, 1,591 vigorous hybrids were selected for growing on.

These plantlets were transferred a greenhouse at 15°C and 80% RH at Doi Wawee, Chiangrai, north Thailand (latitude: 19.917925, longitude: 99.495306), where 10 fruits from each of the parents and from the hybrid strawberry lines were collected at the full maturity stage (30 day after anthesis) during the season, from the beginning of January to the end of March 2017. Samples were collected, placed in plastic PE cold bags (size 4 × 6 cm) and stored at –20°Cuntil the samples were analyzed at the laboratory (Department of Agricultural Sciences, Faculty of Agriculture Natural Resources and Environment, Naresuan University). Ten fruit samples were individually analyzed for anthocyanin content, antioxidant activity of bioactive compounds and for determining physico-chemical characteristics.

2.3. Anthocyanin content analysis of strawberry fruits

2.3.1. Total anthocyanin content

Strawberry fruit peel was extracted for total anthocyanins analysis as previously described [21] with some modifications. Anthocyanin was extracted from the fruit peel (2 g) by homogenizing the peel with 5 ml HCl (1%)

methanol solution. The extract was filtered through a piece of number 1 filter paper (Whatman™ diam. 110 mm). A 5 ml sample of the supernatant was measured for absorbance at 520 nm, using a UV mini-1240 UV-Vis spectrophotometer (Shimadzu, Japan). Total anthocyanin content was expressed as milligrams of pelargonidin-3-glucoside equivalents per 100 g fresh weight.

2.3.2. Content of individual anthocyanins

Individual anthocyanins, including cyanidin-3-glucoside and pelargonidin-3-glucoside were analyzed as previously described [22] with slight modifications. A 0.1 g sample of freeze-dried strawberry powder was extracted with 5 ml acidified methanol (0.1% HCl in MeOH). The 5 ml aliquots from each sample were dried down in a Labconco Centrivap Concentrator at 30°C (Labconco, Kansas City, MO, USA). The samples were then resuspended with 0.1% HCl in MeOH (1000 µl) and passed through a 0.45 µm Nylon Syringe Filter prior to analysis by HPLC. A 60 µl sample was analyzed using a Shimadzu analytical HPLC system (Isocratic system) incorporating a specific column (Inertsil®ODS-3 5 µm 4.6 × 250 mm, guard column Inertsil®ODS-3 4.0 × 10 mm). The mobile phase consisted of acetonitrile + 0.1% formic acid (A solution), and acetonitrile/water/formic acid (5 : 94.9 : 0.1) (B solution). The elution profile that consisted of a linear gradient from solvent A was 0% at zero time and ramped linearly to 20% at 20 min, 30% at 26 min, 50% at 28.5 min, 50% at 28.5 min, 95% at 32 min and back to 0% at 35 min. The total run time was 35 min. The monitoring was performed using a 520 Photodiode Array Detector (PDA) at a flow rate of 0.8 ml/min and a column temperature of 35°C. Anthocyanin was quantified and identified using external Cy-3-glc and Pg-3-glc calibration curves and calculated as milligrams per kilogram fresh weight (mg/kg FW).

2.4. Bioactive compounds analysis of strawberry fruits

2.4.1. Total phenolic content analysis (TPC)

Total phenolic content was determined using the Folin-Ciocalteu assay [23]. The extraction procedure was carried out as described previously [17, 24] with some modifications. A 100 mg sample of strawberry powder was initially extracted with 3 ml of extracting solution (80% MeOH, 19% H2O and 1% formic acid). The mixture was vortex mixed for 2 h in darkness and under cold (4°C) conditions and then shaken at 300 rpm under the same conditions. The extracts were then centrifuged at 5000 rpm for 15 min at 8°C. The supernatant was re-extracted with a further 2 ml of the extracting solution. This supernatant was combined with the first supernatant and stored at –20°C before analysis. The combined supernatant of fruit extracts was directly assayed at 750 nm, using a UV mini-1240 UV-Vis spectrophotometer (Shimadzu, Japan) with gallic acid serving as a standard. Results were expressed as milligrams of gallic acid equivalents per kilogram fresh weight (mg GAE/kg FW).

2.4.2. Ferric reducing antioxidant power analysis (FRAP)

The FRAP assay was carried out as described previously [25], with minor modifications. The extraction procedure was conducted using the same procedure as described for total phenolic analysis. The supernatant extract was directly determined at 595 nm, using a UV mini-1240 UV-Vis spectrophotometer (Shimadzu, Japan). FRAP values were obtained by comparing the absorption values of the samples against those obtained from a calibration curve provided by iron (II) sulfate heptahydrate as the reference standard. All values were calculated as millimoles of Fe^{2+} equivalents per kilogram fresh weight of strawberry (mmol Fe^{2+} /kg FW).

2.5. Physico-chemical analysis of strawberry fruits

2.5.1. Size, weight, firmness and color

At each sampling, the fruit width, length (using a vernier caliper) and fruit fresh weight (by fine balance) were measured. Firmness was measured using a texture analyzer (model QTS 25, Brookfield, USA) and expressed in Newtons (N) according to the modified method [21]. The peel and flesh color of the fruit were assessed using a

colorimeter (CR-20, Minolta Co., Tokyo, Japan) and expressed as L*, a*, b* and hue angle values. The colour of the outer whole fruit at a central point on the fruit circumference was measured. For inner flesh colour, the fruits were cut into a half longitudinal section and then measured (duplicate measurements per fruits).

2.5.2. HPLC determination of vitamin C content

Vitamin C (ascorbic acid) analysis was assessed using an HPLC according to a modified method [27], using a specific column (Inertsil®ODS-3 5 μm 4.6 × 150 mm with guard column Inertsil®ODS-3 4.0 × 10 mm, Shimadzu analytical) (Isocratic system), a UV detector 244–262 nm, with a mobile phase: 3 mM potassium dihydrogen phosphate in 0.35% v/v ortho-phosphoric acid, and a flow rate of 0.8 ml/min. Each 10 g sample was cut into small pieces, wrapped in cheesecloth, and squeezed by hand. A 2 ml of the clear juice sample was then diluted with 2 ml 3% meta-phosphoric acid, filtered through a 0.45 μm Nylon Syringe Filter before a 60 μl sample was injected into the HPLC system at a column temperature 40°C. Ascorbic acid was quantified using an L-ascorbic acid calibration curve. The values were calculated as milligrams per kilogram fresh weight (mg/kg FW).

2.5.3. Total soluble solids content, titratable acidity, pH, soluble solids content to titratable acidity

Total soluble solids content (TSS), titratable acidity (TA) and pH were determined as described previously [26]. A 10 g portion of fruit was cut into small pieces and hand squeezed through cheesecloth. The clear juice was used for analysis. Juice TSS was measured with a pocket refractometer (PAL-1, Atago, Japan) and expressed as a percentage (%). TA was determined by dilutingeach 2 ml aliquot of strawberry juice in 40 ml distilled water and titrating to pH 8.2 with 0.1 NNaOH using an automatic titrator (East Plus Titation, Mettler Toledo). The results were expressed as the percentage equivalentof citric acid. The ratio of soluble solids content to titratable acidity was calculated. Juice pH was measured with a pH meter (Satorious, Docu pH Meter).

2.6. Morphological characteristics of plant and strawberry fruits

After anthocyanin-rich strawberry hybrids were selected, they were subjected to micropropagation and cultivation. The morphological characteristics of plants and fruits were recorded with digital camera (Panasonic Model No. DMC-TZ30 Panasonic Co., Japan). Triplicate fruits per line were measured.

2.7. Data and statistical analysis

Data were analyzed using SPSS software version 17.0 for determining the analysis of variance ($p < 0.05$). Differences among treatment means were analyzed using Duncan's multiple range test (DMRT) at $p < 0.05$.

3. Results

3.1. Total anthocyanin content

Two plants, each from one of the nine hybrid pairs, were found to have anthocyanin concentrations up to two-fold higher than either their parents or Akihime ($p < 0.05$). These were hybrid No. 4 line 5 and hybrid No. 4 line 26. Strawberries from the crosses where Akihime was a parent had the lowest anthocyanin concentrations (Table 2).

Table 2
Comparison of two strawberry hybrids and the parents for total anthocyanin content in the fruit

Cultivars	Line	Total anthocyanin content (mg/100gFW)
Parental		
Praratchatan No. 50	–	$20.80 \pm 0.10^{b1/}$
Praratchatan No. 72	–	19.18 ± 0.17^{b}
Akihime	–	15.57 ± 0.20^{b}
Hybrid strawberry		
Praratchatan No. 50 × Praratchatan No. 72	5	38.49 ± 0.13^{a}
Praratchatan No. 50 × Praratchatan No. 72	26	31.68 ± 0.11^{a}

[1/] Means values (±SD); $n = 10$; different superscripted lowercase letters within the column indicate significant difference ($p < 0.05$).

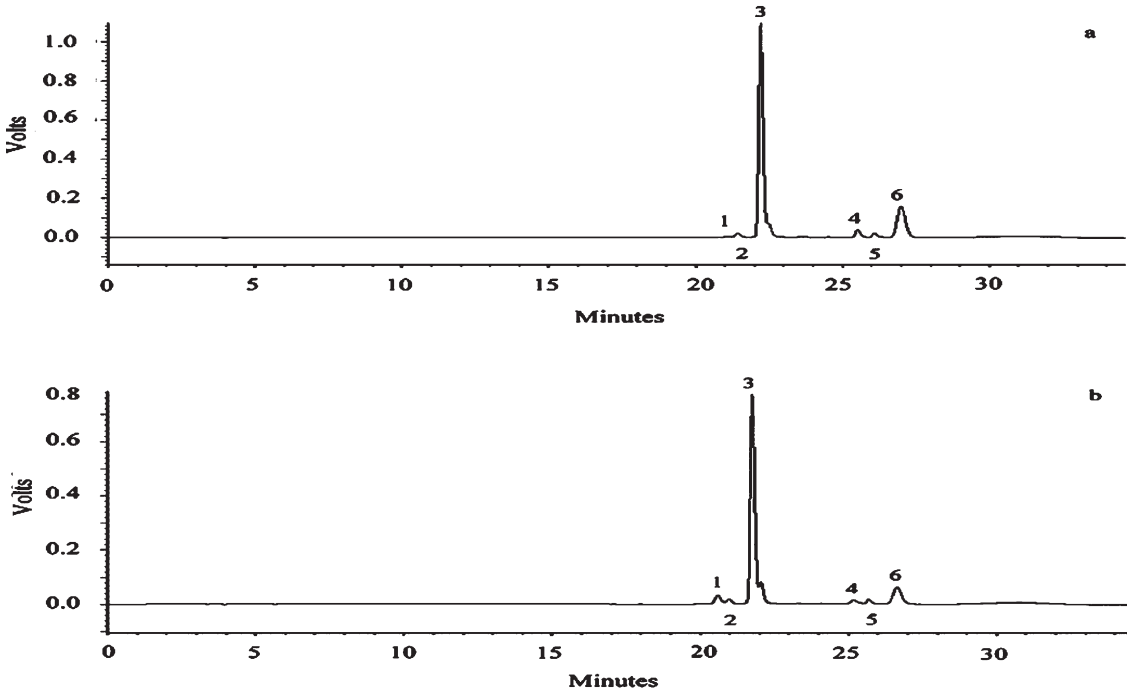

Fig. 1. Anthocyanin-rich profile of hybrid strawberry fruits; (a) Praratchatan No. 50 × Praratchatan No. 72 line 5, (b) Praratchatan No. 50 × Praratchatan No. 72 line 26 determined by HPLC. Peak identification was as follows; (1) cyanidin 3-glucoside, (2) unknown 1, (3) pelargonidin 3-glucoside, (4) unknown 2, (5) unknown 3, (6) unknown 4.

3.2. Individual anthocyanins and bioactive compounds analysis

Values of cyanidin-3-glucoside (Cy-3-glc), total phenolics and FRAP were typically significantly higherin the two selected hybrid lines than in the parents and Akihime. Pg-3-glcand Cy-3-glc were major components found

Table 3
Anthocyanins (Pg-3-glc, Cy-3-glc), total phenolics and FRAP concentrations in the assessed parental strawberry cultivars and in two selected hybrid strawberry lines

Parental/Hybrid pairs	Line	Pg-3-glc (mg/kgFW)	Cy-3-glc (mg/kg FW)	Total phenolics (mg GAE/kg FW)	FRAP (mmol Fe^{2+}/kgFW)
Praratchatan No. 50	–	294.72 ± 0.24c[1/]	12.92 ± 0.17d[1/]	1940.65 ± 0.28d[1/]	16.11 ± 0.26d[1/]
Praratchatan No. 72	–	132.66 ± 0.12d	17.43 ± 0.19b	2071.55 ± 0.22c	17.75 ± 0.14c
Akihime	–	99.12 ± 0.15e	10.52 ± 0.23e	1601.40 ± 0.21e	14.96 ± 0.18e
Praratchatan No. 50 × Praratchatan No. 72	5	478.15 ± 0.11a	15.10 ± 0.12c	2579.12 ± 0.08a	30.24 ± 0.25a
Praratchatan No. 50 × Praratchatan No. 72	26	332.28 ± 0.06b	24.90 ± 0.16a	2295.73 ± 0.13b	27.96 ± 0.17b

[1/] Means values (±SD); $n = 10$; with the different superscripted in lowercase letter within the same column are significantly different ($p < 0.05$).

in these strawberry fruits (Fig. 1). Pg-3-glc values were the highest in hybrid No. 4 line 26 but the lowest in hybrid No. 4 line 5. Within the parents, Akihime consistently had the lowest values, and Praratchatan No. 50 the highest (Table 3 and Fig. 1).

3.3. Fruit size, weight, firmness and color

Fruit size, weight and firmness were highest in hybrid No. 4 line 26, with significant differences compared to parents and other hybrids (Table 4). The L*, b* and hue angle values for both the peel and flesh of fruit from both hybrid No. 4 line 5 and hybrid No. 4 line 26 were typically lower, and a* values higher, than those values for the parental lines. Differences among the parental lines were small and inconsistent (Table 6). However, L*, a*, b* and hue angle of peel and flesh from fruits of the two lines were significantly different from those of Akihime.

3.4. Total soluble solids content, titratable acidity, ratio (TSS/TA) and pH

Fruit from hybrid No. 4 line 26 had the highest TSS content and the lowest TA, hence the highest ratio ($p < 0.05$). The two hybrids had different physico-chemical characteristics, especially a higher ratio (27.2–30.3) than either the parents or Akihime (Table 4).

The pH of hybrid No. 4 line 5 was significantly different from hybrid line 26, the parents or Akihime. The hybrid No. 4 line 26 had the lowest value while hybrid No. 4 line 5 had the highest (Table 4).

3.5. Vitamin C content

Hybrid No. 4 line 5 and hybrid No. 4 line 26 both had higher values ($p < 0.05$) of vitamin C content when compared to the parents orAkihime. The amounts of vitamin C in lines 5 and 26 were between 81.0 and 82.5 mg/100 g FW (Table 5).

3.6. Morphological characteristics of plant and strawberry fruits

Fruit shapes were long conic in hybrid lines 5 and 26. Fruit size tended to be slightly smaller in line 5 compared to line 26 (Fig. 2). Both hybrids had larger fruit than their parents or Akihime ($p < 0.05$) (Table 4).

Table 4
Physico-chemical quality of parental and two lines of strawberry cultivars

Parental/Hybrid pairs	Line	Fruit width (cm)	Fruit length (cm)	Fruit fresh weight (g/fruit)	Firmness (N)	Total soluble solids (%)	Titratable acidity (%)	TSS/TA	pH
Praratchatan No. 50	–	3.03 ± 0.28b[1/]	5.53 ± 0.23a[1/]	16.50 ± 0.14ab[1/]	2.64 ± 0.10b[1/]	9.86 ± 0.13ab[1/]	0.74 ± 0.20c[1/]	13.19 ± 0.19b[1/]	3.82 ± 0.22b[1/]
Praratchatan No. 72	–	2.38 ± 0.20c	3.13 ± 0.15c	14.29 ± 0.29ab	3.17 ± 0.22ab	8.86 ± 0.15ab	0.84 ± 0.23b	10.51 ± 0.21bc	3.71 ± 0.26c
Akihime	–	2.70 ± 0.17bc	4.36 ± 0.18b	13.12 ± 0.12b	1.20 ± 0.24c	7.26 ± 0.29c	1.15 ± 0.27a	6.37 ± 0.12c	3.62 ± 0.18d
Praratchatan No. 50 × Praratchatan No. 72	5	3.56 ± 0.15a	5.78 ± 0.13a	16.71 ± 0.17a	4.11 ± 0.06a	11.00 ± 0.11a	0.40 ± 0.16d	27.27 ± 0.04a	3.91 ± 0.19a
Praratchatan No. 50 × Praratchatan No. 72	26	3.87 ± 0.14a	5.93 ± 0.05a	17.33 ± 0.08a	4.14 ± 0.18a	11.13 ± 0.10a	0.36 ± 0.07d	30.35 ± 0.09a	3.53 ± 0.11e

[1/] Means values (±SD); $n = 10$;with the different superscripted in lowercase letter within the same column are significantly different ($p < 0.05$).

Table 5
Vitamin C content of parental and two lines of strawberry cultivars

Parental/Hybrid pairs	Line	Vitamin C(mg/kg FW)
Praratchatan No. 50	–	70.71 ± 0.11c[1/]
Praratchatan No. 72	–	77.85 ± 0.14b
Akihime	–	42.42 ± 0.12d
Praratchatan No. 50 × Praratchatan No. 72	5	82.51 ± 0.07a
Praratchatan No. 50 × Praratchatan No. 72	26	81.04 ± 0.10a

[1/] Means values (±SD); $n = 10$; with the different superscripted in lowercase letter within the same column are significantly different ($p < 0.05$).

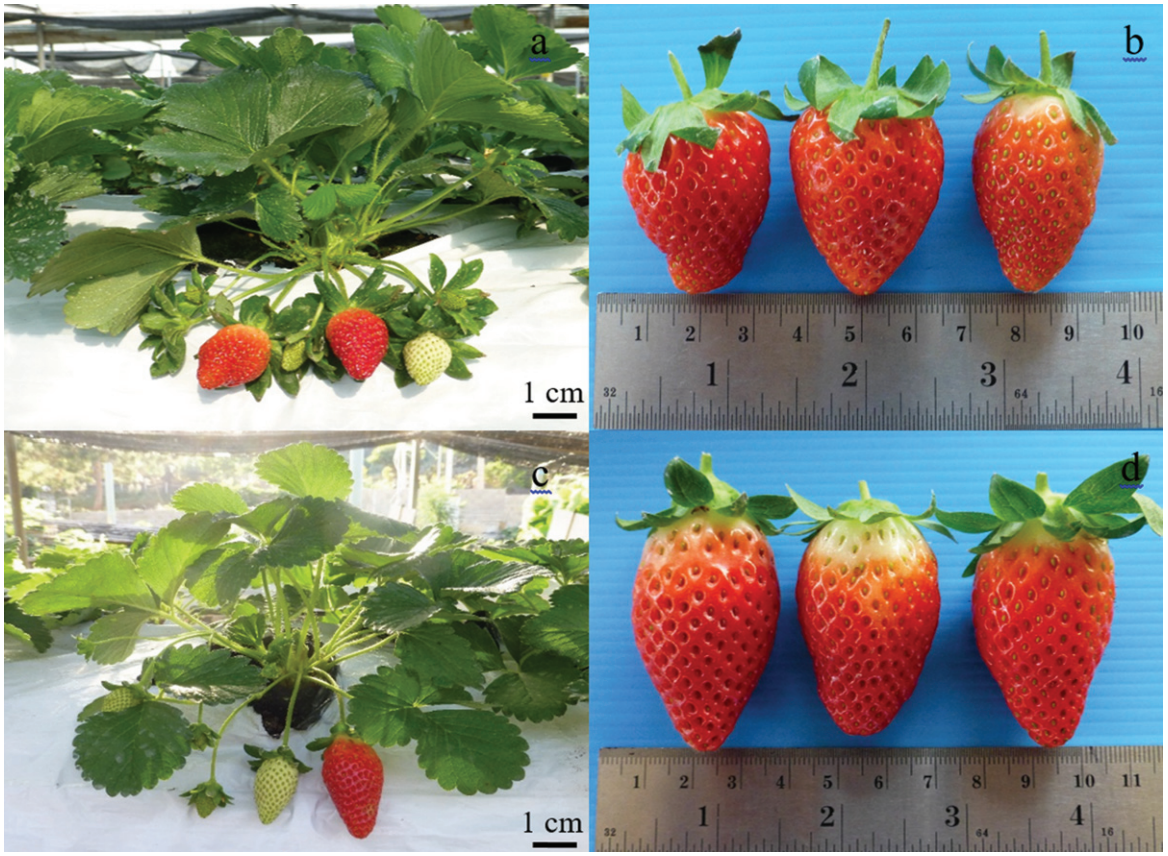

Fig. 2. Morphological characteristics of plants and fruits of (a,b) hybrid No. 4 line 5; (c,d) hybrid No. 4 line 26.

4. Discussion

Strawberry breeding programs have previously been mainly focused on the improvement of agronomic and qualitativetraits, and disease resistance. Recently,however, breeders have become increasingly interested in developing new cultivars focused on sensorial and nutritional characteristics for human health [1, 17, 28].

Table 6
Peel and flesh color of parental and of two lines of strawberry cultivars

Parental/Hybrid pairs	Line	Peel color				Flesh color			
		L*	a*	b*	Hue angle	L*	a*	b*	Hue angle
Praratchatan No. 50	–	31.93 ± 0.28bc[1/]	38.80 ± 0.21bc[1/]	18.92 ± 0.19c[1/]	26.20 ± 0.20b[1/]	56.60 ± 0.29bc[1/]	30.33 ± 0.15d[1/]	25.13 ± 0.17ab[1/]	39.00 ± 0.05b[1/]
Praratchatan No. 72	–	35.57 ± 0.08ab	38.13 ± 0.02c	29.39 ± 0.28a	35.30 ± 0.23a	57.93 ± 0.21b	36.22 ± 0.10c	25.90 ± 0.25a	36.03 ± 0.16bc
Akihime	–	39.20 ± 0.19a	36.93 ± 0.20c	24.92 ± 0.11b	33.60 ± 0.26a	72.20 ± 0.02a	6.95 ± 0.13e	10.83 ± 0.15d	58.57 ± 0.18a
Praratchatan No. 50 × Praratchatan No. 72	5	31.68 ± 0.03bc	40.70 ± 0.12b	15.92 ± 0.23d	22.73 ± 0.16c	50.71 ± 0.15c	41.48 ± 0.24b	24.23 ± 0.06bc	35.47 ± 0.04bc
Praratchatan No. 50 × Praratchatan No. 72	26	31.44 ± 0.16c	43.69 ± 0.03a	13.52 ± 0.25e	21.67 ± 0.18c	50.27 ± 0.11c	51.63 ± 0.07a	23.12 ± 0.10c	33.80 ± 0.08c

[1/] Means values (±SD); $n = 10$; with the different superscripted in lowercase letter within the same column are significantly different ($p < 0.05$).

The results show that hybrid No. 4 line 5 and hybrid No. 4 line 26 had higher total anthocyanin content than either of their parents or Akihime (Table 2). The concentrations of total anthocyanin in these two new hybrid cultivars were higher than those in 27 strawberry cultivars grown in Norway [29], and approximately two times higher than that in 15 strawberry genotypes selected from a breeding program in British Columbia and Canada [30].

The results indicate that hybrid No. 4 line 5 and hybrid No. 4 line 26 have the potential to be used in the development of phytochemically rich strawberry cultivars. The hybrid No. 4 line 26 produced approximately 24.9 mg/kg FW of Cy-3-glc, which was five-fold higher than in 'Sugarbaby', reported as a high anthocyanin-containing strawberry cultivar [17]. In addition, hybrid No. 4 line 5 produced approximately 15.1 mg/kg FW of Cy-3-glc, which is almost fifteen-fold higher thanin either 'Seyhun' or 'Osmanli' [31].

For Pg-3-glc content, the hybrid No. 4 line 26 and hybrid No. 4 line 5 produced 332.2 and 478.1 mg/kg FW, respectively(Table 3 and Fig. 1), which was higher than that reported in 'Camarosa', 'Seyhun', 'Osmanli' and 'Tudnew' [31, 32].

There are more than two types of individual anthocyanin (Cy-3-glc, Pg-3-glc) in strawberry fruits (Fig. 1), which should be further investigated. However, anthocyanin content has been shown to depend on many factors including genotype, harvest period, climatic conditions and pH [33–36]. The higher anthocyanin levels of these two new cultivars indicates that they have the potential to be commercially important.

In this study, hybrids No. 4 line 26 and No. 4 line 5 showed a lower hue angle and L* in both peel and flesh than occurred in either the parents or inAkihime. Consequently, fruit color was a deeperred. Previous findings have shown that differences in L* and hue angle depend on cultivar, year and harvest period [37]. In another study, Camarosa showed lower hue angle and L* in both years that were investigated, consistent with a redder and darker color. Total anthocyanin content among six genotypes was in the decreasing order: Ovation < Puget Reliance < 2384-1 < 2273-1 < Totem <1723-2 [38]. The selection 1723-2 had the highest anthocyanin content and the lowest L* and h° values while Ovation had the lowest anthocyanin content and the highest L* and h° values. Moreover, hue angle and anthocyanin content were correlated and could be used as a screening tool for total anthocyanin content [17]. Our study provided a similar relationship, where hue angle in both peel and flesh of the two hybrid lines were the lowest and anthocyanin content was the highest in the fruit tested (Tables 2 and 6).However, pH is one factor which may impact on these results. Anthocyanins have been shown to be more stable at low pH (acidic conditions), which results in a dark red pigment, primarily as cyanidin [39]. Athigher pH (alkalinity condition) anthocyanin provides colors such as bright red pigment, primarily as pelargonidin. These colors also depend on a direct relationship with the number of hydroxyl groups and an indirect relationship with the number of methoxyl groups. This corresponds with our results, which show that hybrid No. 4 line 26 has a low pH, corresponding with the highest cyanidin-3-glucoside concentration. The hybrid No. 4 line 5 had a higher pH than either of the parents or of Akihime, and the Pg-3-glc contentwas also the highest.

Therefore, the elevated levels of anthocyanin content obtained in both hybrids have a potential significance for human health benefits. The anthocyanin increase was related to the genetic background of the selected parents used in the cross combinations, which were chosen because they had already been identified as having high anthocyanin content in comparison with other cultivars [1]. The results show that Praratchatan No. 50 and Praratchatan No. 72, which were the parents of these hybrid strawberry lines, produced higher anthocyanin content in the progeny compared with the other parent tested.

For bioactive compound analysis, as measured by antioxidant activity (Table 3), hybrid No. 4 line 5 had the highest total phenolic and FRAP concentrations (2579.12 mg GAE/kgFW and 30.24 mmol Fe^{2+}/kgFW, respectively). These results are similar to the total phenolics and FRAP values of 2525 mg GAE/kg FW and 27.9 mmol Fe^{2+}/kg FW of the new breeding line BL 2006-221-8 [17] and similar to those from three cultivars from selections of *Fragaria* × *ananassa* and one cultivar (F1) selection from an inter-specific cross *F.* × *ananassa* × *F.virginiana* spp. *glauca* (1800–3200 mg GAE/kg FW) [40]. Similarly, 20 selections derived from a strawberry interspecific backcross breeding program had approximately 1381–2992 mg GAE/kg FW of total phenolics [41]. Our two new hybrid cultivars are within those reported ranges (2295–2579 mg GAE/kgFW). Total phenolic compounds

of these two new strawberry cultivars (Table 3) were clearly higher than that in three cultivars from back-crossing of BC1-FVG × *F.* × *ananassa* (1964.33 mg GAE/kg FW) derived from 8 families. Two cultivars that originated from *F.* × *ananassa* intra-species crossinghad the lowest value of total phenolics (1363.16 mg GAE/kg FW) [1]. FRAP values of hybrid lines 5 and 26 (Table 3) were almost twice as highthanin six other hybrid pairs of strawberry [40]. However, antioxidant activity is not only determined by FRAP but also by other metabolites. Therefore, the measurement of other antioxidant activity by methods including DPPH, ABTS and ORAC would be useful for compiling antioxidant information about the two new strawberry hybrids in this study and of hybrids in other studies. In this study, the physico-chemical characteristics of the hybrid strawberries were significantly better than those of their parents or of Akihime, especially with regard to fruit width, length, fresh weight, firmness, TSS/TA and vitamin C concentration (Tables 4 and 5). The highest TSS/TA ratio of the two newhybrid strawberry cultivars would indicate more sweetness and less sourness, which would possibly improve berry flavor. The balance between TSS and acidity (ratio) is an indicator of consumer acceptance [42]. Hence, specific sugar-acid ratios are used as maturity and quality standards in marketing, especially in international trade [43].

5. Conclusions

The results obtained in this work show that the inclusion of parent strawberry cultivars such as Praratchatan No. 50 and Praratchatan No. 72 can be useful in improving the nutritional quality of fruit. However, the two new hybrid strawberry lines, specifically hybrids No. 4 line 5 and No. 4 line 26 have a promising basis for producing strawberries with higher levels of bioactive compounds and improved morphological characteristics, which would make them particularly attractive to consumers. Therefore, these two new cultivars have commercial potential in Thailand and in other regions of the world with similar growing conditions.

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Conflict of interest

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