Variation in Concentration and Composition of Anthocyanins among Strawberry Cultivars

Yuichi Yoshida1* and Hirotoshi Tamura2
1Faculty of Agriculture, Okayama University, Okayama 700 – 8530
2Faculty of Agriculture, Kagawa University, Miki, Kagawa 761 – 0795

Summary

Japanese and European strawberry (Fragaria × ananassa Duch.) cultivars were analyzed for their differences in their concentration and composition of anthocyanins. Within a fruit, the concentration of total anthocyanins is higher in epidermal tissues than in inner cortex or pith for all cultivars, but among cultivars, differences in the concentration were greater in the inner flesh compared to the epidermal tissues. The pigment in inner flesh was significantly less concentrated in the newly released Japanese cultivars than in the European cultivars, ‘Elsanta’ and ‘Darselect’, or the old Japanese cultivar ‘Hokowase’. Pelargonidin 3- glucoside was the predominant pigment except for achenes. In achenes, the compositions of cyanidin derivatives and malonlated anthocyanins were extremely high compared to the other tissues. Considerable amounts of cyanidin 3- malonylglucoside, which has not been reported previously and pelargonidin 3- malonylglucoside occurred in achenes of all cultivars. The epidermal tissues of ‘Toyonoka’, ‘Sachinoka’ and ‘Ai-Berry’ contained only a trace level of malonlated anthocyanins; the achenes also had low concentration of the pigments compared to other cultivars. With these results, the functions and expression of Amg, the single dominant gene that supposedly controls the synthesis of malonlated anthocyanins in the strawberry receptacle, are discussed.

Key Words: achenes, cyanidin, Fragaria × ananassa, malonlated anthocyanins, pelargonidin.

Introduction

Anthocyanins are the major pigment of strawberry (Fragaria × ananassa Duch.), among which, pelargonidin 3- glucoside (PG) is predominant. Usually, it was followed in quantity by cyanidin 3- glucoside (CG) (Bakker et al., 1994; Lokton et al., 1955; Robinson and Robinson, 1932; Sondeheimer and Karash, 1956; Sondeheimer and Kertesz, 1948). However, the authors isolated pelargonidin 3- malonylglucoside (PMG) in fresh fruits of 'Nyoho' (Tamura et al., 1995), and found it in 10 of 19 other Japanese cultivars (Yoshida et al., 2002). The amount of PMG often exceeds that of CG, but it could not be detected in fruits of the other 9 cultivars. We also reported that the synthesis of PMG may be controlled by a single dominant gene (Yoshida et al., 2000). However, in a successive experiment, the level of PMG was less than 1% of total anthocyanins in some cultivars and some selfed progenies of ‘Nyoho’ (Yoshida, 2000). Whether the synthesis of PMG in strawberry fruits is controlled by one gene or more may be a subject for genetic research.

Usually, fruit skin color is redder than inner flesh of strawberry. Holcroft and Kader (1999) reported that not only the concentration of the pigments but also their composition differs between outer and inner tissues for the ‘Selva’. Red color development, a result of anthocyanin accumulation, usually occurs in achenes followed by epidermis and finally in inner receptacular tissue. As CG is the major anthocyanin at the early stage of color development (Yoshida et al., 2002), it was postulated that the differences in the composition of anthocyanins among strawberry cultivars or genotypes may reflect the difference within a fruit and its parts. We tested this postulation by analyzing 10 widely grown Japanese and European cultivars.

Materials and Methods

Runner plants of 10 cultivars (Table 1), that were started in polyethylene pots were transplanted on November 15, 1999, and covered with PVC film on February 10, 2000. Five fully ripe fruits that flowered in early March were harvested in April from each cultivar. Achenes on the equatorial part of the receptacle were removed, and the epidermal tissue, less than 1.5 mm thick, was excised. Subsamples of the epidermal tissue and achenes, inner cortex and the pith, including some vascular tissues, were prepared. Each tissue, except achenes, was weighed and soaked in aliquots, 3–10
Table 1. Differences in the concentration of total anthocyanins among fruit tissues in Japanese and European strawberry cultivars.

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>PMG</th>
<th>Epidermis</th>
<th>Cortex</th>
<th>Pith</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>European cultivars</strong></td>
<td>&amp;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elsanta (1981)</td>
<td>+</td>
<td>2.249 a</td>
<td>1.004 a</td>
<td>0.451 a</td>
</tr>
<tr>
<td>Darselect (1995)</td>
<td>+</td>
<td>1.821 b</td>
<td>0.524 b</td>
<td>0.423 ab</td>
</tr>
<tr>
<td><strong>Japanese cultivars</strong></td>
<td>&amp;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hokowase (1960)</td>
<td>+</td>
<td>2.545 a</td>
<td>1.058 a</td>
<td>0.368 b</td>
</tr>
<tr>
<td>Nyoho (1984)</td>
<td>+</td>
<td>1.852 b</td>
<td>0.221 cd</td>
<td>0.075 de</td>
</tr>
<tr>
<td>Toyonoka (1984)</td>
<td>-</td>
<td>0.932 d</td>
<td>0.077 de</td>
<td>0.038 de</td>
</tr>
<tr>
<td>Ai - Berry (1985)</td>
<td>-</td>
<td>1.790 b</td>
<td>0.188 cde</td>
<td>0.085 cde</td>
</tr>
<tr>
<td>Akihime (1990)</td>
<td>+</td>
<td>1.414 c</td>
<td>0.063 de</td>
<td>0.012 e</td>
</tr>
<tr>
<td>Sachinoka (1996)</td>
<td>-</td>
<td>1.504 be</td>
<td>0.048 e</td>
<td>0.054 de</td>
</tr>
<tr>
<td>Tochiotome (1996)</td>
<td>+</td>
<td>1.724 be</td>
<td>0.285 c</td>
<td>0.087 cd</td>
</tr>
<tr>
<td>Asuka - Ruby (1997)</td>
<td>+</td>
<td>1.785 b</td>
<td>0.210 cde</td>
<td>0.154 c</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td>&amp;</td>
<td>1.761</td>
<td>0.368</td>
<td>0.175</td>
</tr>
<tr>
<td>C. V. %</td>
<td>&amp;</td>
<td>0.250</td>
<td>1.022</td>
<td>0.974</td>
</tr>
<tr>
<td>Max/Min*</td>
<td>&amp;</td>
<td>2.7</td>
<td>22.0</td>
<td>36.1</td>
</tr>
</tbody>
</table>

* Values in parentheses indicate release years.
* Occurrence of pelargonidin 3'-malonylgalacoside within whole fruits in the previous study (Yoshida, 2000); + 4.9 - 28.3%, - less than 0.01% of total anthocyanins, respectively.
* Different letters indicate significant differences within columns by LSD test, 5% level.
* Coefficient of variation.
* Ratio of maximum (bold letters) to minimum (italic letters) values.

Fig. 1. Relationship between spectrophotometric absorbance at 510 nm (10 mm path length) and total peak area of HPLC chromatogram for epidermal tissue extracts of strawberry receptacle. ○, 2.0 > OD_{510} × , 2.0 ≤ OD_{510}.

Times of tissue FW (v/v), of the extracting solution containing CH_3CN : CH_3COOH : H_2PO_4 : H_2O = 5 : 4 : 1.5 : 89.5 (v/v). As vascular bundle tissues attached to achenes could not be removed completely, nearly 100 well-developed achenes were separated from 5 fruits by pooling and their anthocyanins were extracted with 5 ml of the extracting solution. For anthocyanin extraction, the samples were reciprocally shaken at 100 times·min^{-1} for 24 hr for achenes and 2 hr for the other tissues. A preliminary examination showed that a 2-hr of extraction was sufficient except for achenes. The absorption of the filtrate was measured spectrophotometrically at 510 nm (OD_{510}, 10 mm pass length).

The anthocyanins were separated and analyzed with a HPLC as described previously (Yoshida et al., 2002). The resulting chromatograms were analyzed with a data processing program (BORWIN, JASCO, Tokyo). When OD_{510} was more than 2.0, there was no linear relationship between calculated total peak areas of anthocyanins and OD_{510} (Fig. 1). The concentration of total anthocyanins in the tissues (C, μmol·g^{-1}FW) was calculated as PG equivalent, based on its coefficient of absorbance (C_{λ}, 40.8 OD_{510}·mmol^{-1}), with the following equation using peak areas (A, mV·sec) of each chromatogram and injection volume (V_i, μl), not spectrophotometrically.

\[ C = \left( A_i / V_i \times 0.0174 + 0.0711 \right) \times (V_E + W) / W / C_λ \]

Where W = fresh weight of tissues (g); V_E = volume of extracting solution (ml).

**Results and Discussion**

Concentration of total anthocyanins was highest in epidermis and least in pith, irrespective of cultivar (Table 1). The concentration in epidermis ranged from a low of 0.93 μmol·g^{-1}FW in 'Toyonoka' to a high of 2.55 μmol·g^{-1}FW in 'Hokowase'. Only 'Toyonoka', in which albino fruits with less or no color development, often occurred in mid-winter (Fushihara and Takao, 1991), exhibited a minimal value. The concentration in cortex was highest in 'Hokowase' (1.06 μmol·g^{-1}FW) followed by 'Darselect' (1.00 μmol·g^{-1}FW), and least in 'Sachinoka' followed by 'Akihime' and 'Toyonoka',
0.05, 0.06 and 0.08 $\mu$mol·g⁻¹FW, respectively. In the cortex, ‘Hokowase’ and ‘Darselect’ had more than 20 fold of anthocyanins compared to that in ‘Sachinoka’. However in pith, the concentration was the highest in ‘Darselect’ (0.45 $\mu$mol·g⁻¹FW) followed by ‘Elsanta’ (0.42 $\mu$mol·g⁻¹FW), and least in ‘Akihime’ (0.01 $\mu$mol·g⁻¹FW). In 7 Japanese cultivars, except ‘Hokowase’, the concentration in the pith was significantly the lowest compared to that of the other 3 cultivars, being 0.09 $\mu$mol·g⁻¹FW.

When variations among cultivars were expressed as the ratio of maximum to minimum values, the difference in the concentration of anthocyanins among cultivars was larger in the inner flesh than that in the epidermis. The concentrations in cortex or pith were significantly less in newly released 7 Japanese cultivars, excluding ‘Hokowase’, than those in European cultivars, ‘Elsanta’ and ‘Darselect’ (P<0.01, LSD-test). This difference may reflect a cultural preference that the color of inner flesh is less important in Japan where the whole intact strawberries are consumed fresh, whereas in Europe the berries are frequently cut and set on the table or processed.

When extracts of achenes were analyzed with HPLC, a large peak No. 3 which had not been identified previously, was detected for all cultivars (Fig. 2A). The peak was supposedly cyanidin 3-malonylglucoside (CMG), based on its retention time. The differences in retention time for peak No. 3 - No. 1 (CG) and No. 4 (PMG) - No. 2 (PG) were 2.81 and 2.89 min, respectively. To examine the hypothesis, an aliquot of 2% HCl was added to the same volume of achene extracts for some cultivars, and the mixtures containing 1% HCl were hydrolyzed at 80°C. The area of peaks No. 3 and No. 4 decreased within 2 hr of hydrolysis, while peaks No. 1 and No. 2 increased concurrently (Fig. 2B, 3).

Another new small peak appeared just before PMG that also increased slightly. However, the peak No. 3 was estimated as CMG because of the increase in the area of CG.

The composition of cyanidin derivatives, CG and CMG, was extremely higher in achenes compared to the other tissues (Fig. 4), ranging from 36% of total anthocyanins in ‘Asuka·Ruby’ to 78% in ‘Toyonoka’; in the epidermis, cortex and pith, it was only 1.9–8.3%, 0.4–1.3% and 0.6–3.3%, respectively. Consequently, the composition of pelargonidin derivatives in these tissues was higher, ranging from 86 to 97%, compared to achenes in which it ranged from 19 to 62%. Usually, color development of achenes precedes that of epidermis. Thus, high CG composition of achenes may result in the high CG composition of ‘Nyoho’ in the early stage of fruit coloring observed previously (Yoshida et al., 2002).

In achenes, composition of malonylated anthocyanins in total anthocyanins was also higher than that in the other tissues (Fig. 4). The compositions of CMG and PMG were highest in ‘Darselect’ (44.0%) and ‘Asuka·Ruby’ (29.6%), respectively; they were 4.1 and 0.6%, respectively, in ‘Aii·Berry’. Considerable amounts of CMG (0.6–2.7%) and PMG (4.1–6.8%) were detected from achenes of ‘Toyonoka’, ‘Sachinoka’ and ‘Aii·Berry’, whereas PMG could not be detected in the whole fruits previously (Yoshida et al., 2002). For these 3 cultivars, the compositions of CMG and PMG were less than 0.2% in the epidermis in which the concentrations of total anthocyanins were significantly higher than the other parts. The concentration of PMG was much lower than PG in extracts of the whole fruit of 3 cultivars; a small peak of PMG could not be detected by the usual data processor or a recorder.

For the three cultivars, the ratio of PMG/PG in

![Fig. 2. HPLC chromatograms for an achene extract of 'Elsanta' strawberry before (A) and after (B) hydrolyzation with 1% HCl at 80°C for 2 hr.](image)

![Fig. 3. Variations in anthocyanin composition of achene extracts of strawberry cultivars before (left) and after (right) hydrolyzation with 1% HCl at 80°C for 2 hr. CG, cyanidin 3-glucoside; CMG, cyanidin 3-malonylglucoside; PG, pelargonidin 3-glucoside; PMG, pelargonidin 3-malonylglucoside.](image)
epidermis, cortex and pith was less than 0.001, and the ratio of PMG/PG and CMG/CG in achenes was also less than 0.1 (Fig. 5). On the contrary, the ratio of PMG/PG and CMG/CG in achenes was larger than 0.37 and 0.25, respectively, for the 7 other cultivars in which considerable amount of PMG occurred in epidermis or inner flesh. These results indicate that the acylation of anthocyanins with malonic acid by malonyltransferase may commonly occur in achenes of strawberry. Although the activity of the enzyme differs among cultivars and parts of fruit, the enzyme may catalyze the malonylation of PG and CG equally as reported by Ino et al. (1993). The observation that the ratio of PMG/PG and CMG/CG was similar within achenes or epidermis of 9 cultivars examined, except ‘Darselect’ (Fig. 5), seems to confirm this proposal.

Considerable and trace levels of PMG occurred in achenes and receptacle, respectively, in cultivars containing only trace levels of the pigment in the whole fruit, such as ‘Toyonoka’ (Yoshida et al., 2002). ‘Redgiantlet’ and ‘Cambridge Vigour’ fruits that weighed from 4.4 - 40.6 g had 100 - 500 achenes on their receptacles (Abott et al., 1970), fruits of ‘Toyonoka’ and ‘Nyoho’ had relative weights of 58 - 65 mg per achene (Mori, 1998). Mean fresh weight of well-developed achenes ranged 7 - 10 mg (unpublished data). It is not evident, in their experiment, whether the calyx is included in fruit weight. However, it is supposed that achenes make up 10 - 15%, no more than 20%, of fresh weight of strawberry fruit excluding the calyx. Furthermore, the concentration of total anthocyanins in achenes is much lower than that of the epidermis for all cultivars; it never exceeded 0.07 μmol·g⁻¹FW, less than 1/10 of epidermis, when the values were estimated using the previous FW of 10 mg. These results suggest that the occurrence of a trace level of PMG, less than 0.4% of total anthocyanins, in the whole fruit of some selfed progenies of ‘Nyoho’ (Yoshida, 2000), may originate from the pigment in the achenes, but not in the epidermis which accumulates a much higher amount of anthocyanins than the other parts. Thus, the amount of malonylated anthocyanins, mainly PMG in epidermis, produced by the whole fruit of a strawberry phenotype is assumed to be controlled by a single dominant gene as reported previously (Yoshida et al., 2000). The gene, controlling the synthesis of malonylated anthocyanins,
may be referred to Amg. The Amg gene may be a regulatory factor of DNA coding anthocyanin malonol-
tranferase, mainly in epidermis and partly in achenes, as the enzyme commonly occurred in achenes of straw-
berry cultivars.

**Literature Cited**


**Fig. 5.** Variations in the ratio of anthocyanins in achen (A), epidermis (E), cortex (C) and pith (P) of strawberry cultivars. CG, cyanidin 3-glycoside; CMG, cyanidin 3-malonylglycoside; PG, pelargonidin 3-glycoside; PMG, pelargonidin 3-malonylglycoside; Cs, cyanidin derivitives (CG+CMG); Ps, pelargonidin derivitives (PG+PMG). Cultivars followed by (+) and (−) indicate occurrence and absence of PMG in a whole fruit, respectively; see Table 1.
イチゴ果実中アントシアニン濃度と組成の部位および品種による差異

吉田裕一 1・田村啓敏 2

1岡山大学農学部 700-8530 岡山市津島中
2香川大学農学部 761-0795 香川県木田郡三木町

摘　要

イチゴ果実の部位によるアントシアニン濃度と組成の差異を日本とヨーロッパの品種について調査した。全アントシアニン濃度は、いずれの品種においても果肉が最も高く、皮層や種は低かった。品種間の濃度差は果皮より内部の果肉組織の方が大きかった。80年代以降に公表された日本の主要品種は、ヨーロッパの品種（'Elanta'，'Darselect'）や‘宝交早生’と比較して果肉中の濃度が低かった。果肉以外の組織では、pelargonidin 3-glucoside（PG）の比率が他の色素と比較して著しく高かったが、10品種すべてにおいて果実中のシアニジン系およびマロニル化したアントシアニンの比率が果肉部分と比較して著しく高く、果実中にはpelargonidin 3-malonylg glucoside と cyanidin 3-malonylg glucoside がかなり多量に含まれていた。ただし、果皮中にマロニル化アントシアニンをわずかしか含まない'愛ベリー'，'とよのか'，'きたのか'の3品種においては、果実中のマロニル化アントシアニンの比率が他の品種と比較して低かった。これらの結果に基づき、イチゴ果実組織へのマロニル化アントシアニン蓄積を支配すると考えられる遺伝子Ang の機能と発現について考察した。