Anthocyanin Pigment Composition of Red Radish Cultivars as Potential Food Colorants

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ABSTRACT

Red radish (*Raphanus sativus* L.) cultivars were evaluated with respect to qualitative and quantitative anthocyanin (ACN) pigment content. Radishes were grown at 2 locations (Corvallis and Hermiston, OR) and harvested at 2 maturity stages. Pigment content was dependant on cultivar, root weight and location, higher amounts being obtained at Hermiston. Spring cultivars (n=22) had pigmentation in the skin, ranging from 39.3 to 185 mg ACN/100g skin. Red-fleshed Winter cultivars (n=5) had pigment content ranging from 12.2 to 53 mg ACN/100g root. ACN profiles were similar for different cultivars, the major pigments being pelargonidin-3-sophoroside-5-glucoside, mono- or di-acylated with cinnamic and malonic acids; individual proportions varied among cultivars. Estimated pigment yields ranged from 1.3 to 14 kg/ha.

Key Words: anthocyanins, red radish, *Raphanus sativus* L., cultivars, pigment profile

INTRODUCTION

RADISH (*RAPHANUS SATIVUS* L.) IS AN EASY vegetable to grow and can be harvested within 4 to 6 wk (Hartmann et al., 1988). Radishes occur in a variety of shapes: global, oval, long, flattened and pear-shaped roots; and colors: white, red, purple, yellow and black (George and Evans, 1981). The anthocyanin (ACN) pigments responsible for red and purple radishes have been characterized by different researchers (Ishikura and Hayashi, 1962, 1965; Harborne 1963; Fuleki, 1969), and the presence of pelargonidin- and cyanidin-derivatives was reported in red and purple radishes, respectively. Red radish ACN were characterized as pelargonidin-3-sophoroside-5-glucoside (Pg-3-soph-5-glu, or raphanusin) derivatives, with cinnamic acids attached to the glycosidic moieties. The presence of this basic structure has been confirmed in red radish cultivar (cvs) Fuego and an additional acylating group on this basic structure was also detected and identified as malonic acid (Giusti and Wrolstad 1996a).

Interest in ACN has increased because they are potential natural alternatives to artificial colorants. The use of red radish ACN extract for coloring maraschino cherries was evaluated (Giusti and Wrolstad, 1996b) and the color characteristics of radish-colored brined cherries were extremely close to those with FD&C Red No. 40 for ≥ 6 mo storage at room temperature. The high color and pigment stability were attributed to the presence of acylating groups attached to the ACN moiety.

Our objective was to determine which red radish cvs may be more appropriate for potential colorant production. We analyzed different cvs to determine those with higher pigment contents and examined the qualitative pigment composition to make inferences regarding hue and chroma. The effect of planting location and maturity on pigment yield were also evaluated by comparing results from 2 locations, and by harvesting at 2 maturity stages. Based on radish yield obtained and published data, we calculated potential yields of pigment/ha and pigment/ha-day, to determine the viability of pigment production on a commercial scale.

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MATERIALS & METHODS

Plant material

A total of 27 red radish cvs were evaluated. Seeds were obtained from Daehnfeldt Inc. (Albany, OR); Nunhems International (Haelen, Holland); W. Atlee Burpee Co. (Warmenster, PA); Rogers Seed Co. (Boise, ID); Stokes Seeds Inc. (Buffalo, NY); Known-You Seed Co. (Kaohsiung, Taiwan); Tokita Seed Co. (Nakagawa, Japan); American Takii Inc. (Salinas, CA); Sakata Seed America (Morgan Hill, CA). Additional seeds were provided by Mah Dong King (Tianjin Processing Center of Agricultural Products, Tianjin, P.R. China) (Table 1).

During the first year (Summer 1994) 23 red radish cvs were planted at the Oregon State University (OSU) Lewis-Brown Horticultural Farm (Corvallis, OR) and screened for pigment content and profile and from those, 11 were selected for second year (Summer 1995) analyses. For the second year trials, radishes from the selected cvs were planted at 2 locations: the OSU Lewis-Brown Horticultural Farm (Corvallis, OR) and the OSU Hermiston Agricultural Research and Extension Center (Hermiston, OR). Two cvs that showed high pigment content in previous years, as well as 4 additional Chinese cvs were planted only at Corvallis and analyzed during a third year (Summer 1996).

Radishes were harvested, leaves manually cut, and roots washed with cold water using a vibratory spray washer (A.B. McLauchlan, Co., Salem, OR) to eliminate extraneous matter. Radishes were weighed and refrigerated at 1°C until analyzed. Sample size was 1 kg root or 4 units, whichever was larger. Radish cvs with pigment only in the skin were manually peeled, the epidermal tissue was frozen in liquid nitrogen and stored at −23°C until analyzed. Radish cvs with pigmentation in the flesh were cut into small cubes (about 1 cm^3 each), the cubes were mixed, and a sample of 200 g was frozen and stored at −23°C until analyzed.

Pigment extraction

The extraction followed the procedure described by Giusti and Wrolstad (1996b). Frozen epidermal tissue (for skin pigmented radishes) was liquid nitrogen powdered using a stainless steel Waring Blendor. Powdered samples were blended with 1 volume of acetone and filtered on a Buchner funnel using Whatman #1 paper. For radishes with red flesh, frozen radish cubes were directly blended with 1 volume of acetone and filtered as described. The filter cake residue was re-extracted with aqueous acetone (30:70 v/v) until a clear solution was obtained. Filtrates were combined, shaken in a separatory funnel with 2 volumes of chloroform and stored overnight at 1°C. The aqueous portion was collected and placed on a Büchi rotavapor at 40°C until all residual acetone was evaporated (5 to 10 min) and brought to a known volume with distilled water.

Monomeric anthocyanin content

Monomeric ACN content was determined using the pH-differential method (Wrolstad et al., 1982). A Shimadzu 300 UV spectrophotometer and 1 cm pathlength disposable cells were used for spectral measurements at 510 and 700 nm. Pigment content was calculated as pelargonidin-3-glucoside, using an extinction coefficient of 31,600 L cm^{-1} mol^{-1} and a molecular weight of 433.2 g mol^{-1} (Wrolstad, 1976).

Anthocyanin purification

The aqueous extract was passed through a C-18 Sep-Pak cartridge (Waters Assoc., Milford, MA), previously activated with methanol followed by 0.01% aqueous HCl (Wrolstad et al., 1990). Anthocyanins (and other phenolics) were adsorbed onto the mini-column; sugars, acids and other water-soluble compounds were removed with 2 volumes of 0.01% aqueous HCl and ACN were subsequently eluted with methanol containing 0.01% HCl (v/v). The methanolic extract was then concentrated using a Büchi rotavapor at 35°C and pigments were dissolved in distilled deionized water containing 0.01% HCl.

Alkaline hydrolysis of anthocyanins

Purified pigment (ca. 10 mg) was hydrolyzed (saponified) in a screw-cap test tube with 10 mL of 10% aqueous KOH for 8 min at room temperature in the dark, as described by Hong and Wrolstad (1990). The solution was neutralized using 2N HCl, and the hydrolysate was purified using a C-18 Sep-Pak cartridge, as described.

High performance liquid chromatograph (HPLC)

Apparatus. A high performance liquid chromatograph Perkin-Elmer Series 400, equipped with a Hewlett-Packard 1040A photodiode array detector, Gateway 2000 P5-90 computer with a Hewlett-Packard HPLC^{2D} ChemStation software and a Beckman 501 autosampler with a 50 μL loop was used, with simultaneous detection at 280, 310 and 520 nm. The spectra (detection wavelengths from 250 to 600 nm) were recorded for all peaks.

Columns and mobile phase. *System I.* A PLRP-S column (5 μ) 250 × 4.6 mm i.d. (Polymer Labs, Amherst, MA), fitted with a Polymer Labs, 1.5 cm × 4.6 mm i.d. guard column was used. The solvents used were A: 100% HPLC grade acetonitrile and B: 4% phosphoric acid (aqueous).

System II. A Supelcosil LC-18 column (5 micron), 250 × 5 mm i.d. (Supelco, Inc.), fitted with an ODS-10, 4 cm × 4.6 mm i.d., Micro-Guard column (Bio-Rad Laboratories) was used. The solvents used were A: 100% HPLC grade acetonitrile and B: 1% phosphoric acid (concentrated), 10% acetic acid (glacial), 5% acetonitrile (v:v:v) in water. Flow rate: 1 mL/min. Solvents and samples were filtered through a 0.45 μL Millipore filter type HA (Millipore Corp., Bedford, MA).

Conditions for anthocyanin analysis

Radish ACN and saponified radish ACN were separated using Systems I and II. System I used a linear gradient from 12 to 22% A in 50 min. System II used a linear gradient from 0 to 30% A in 30 min. The identity of ACN was verified by the 2 different HPLC systems.

Conditions for phenolic acid analysis

Phenolic acids, obtained from radish ACN saponification, were separated using the 2 different HPLC systems. System I used a linear gradient from 10 to 15% A in 15 min, a linear gradient from 15 to 25% A in 15 min and isocratic at 25% A for 5 min. System II was run isocratic at 5% A, with simultaneous detection at 280 and 320 nm. The identity of acyl groups was confirmed by the 2 different HPLC systems. Retention times and spectra were compared to pure standards. Caffeic acid, ferulic acid and *p*-coumaric acid standards were purchased from Sigma Chemical Co. (St. Louis, MO).

Statistical analysis

The data were analyzed using regression analyses and analysis of variance (ANOVA) as a complete randomized block (location) design. Significance of differences was defined at $p \leq 0.05$.

RESULTS & DISCUSSION

Monomeric anthocyanin content of radish cultivars

Different red radish cvs (23) were screened during the first year for pigment content (Table 1) and other horticultural characteristics. Radish cvs are often grouped according to their time to mature and season when grown. We examined both Spring and Winter radish types for cvs that could provide high ACN concentration. Rapid-growing and quick-maturing Spring cultivars are commonly used for salads, usually small, globally shaped with mild pungency, but a few Spring cultivars have larger and longer roots (Hartmann et al., 1988). All Spring cvs analyzed showed red pigmentation only on the skin, and most were round and small, with exception of cvs Flamboyant Sabina, Jumbo, Long Red and Picolo that had an elongated shape. Later-maturating Winter radish varieties are usually more pungent, larger, and well adapted for storing, with considerable variations in size, color (internal and external) and shape (Hartmann et al., 1988). Winter cvs analyzed included red-skinned radishes (cvs Joyce) and those with red pigmentation in the flesh (white/green epidermal color), all with large size and round shape.

The different cvs (Table 1, 1994) showed marked differences in monomeric ACN content, ranging from 11.1 to 60.4 mg ACN/100g root. Spring cvs Arabic Red, Easter Egg and Picolo, and Winter cvs Joyce showed the lowest pigment content, <20 mg ACN/100g root. The highest pigment content per total root weight, >42 mg ACN/100g root, was found in Winter flesh pigmented cvs. The Red Baron, Arista and Fuego cvs showed the highest pigment content among Spring cvs with monomeric ACN content >32 mg ACN/100g root.

From the 23 cvs screened, we selected 8 which showed higher pigment content and 3 based on size, shape or growing characteristics—Crimson Giant because of larger size (see Table 2), Flamboyant Sabina because of smooth elongated shape, and Long Red because of large and elongated root—for further analyses of pigment content and ACN profile.

Anthocyanin content of different cultivars as related to planting seasons

During the second season (Table 1, 1995) cvs were planted at 2 different locations. Results showed good consistency with the previous year results (1994) for Spring cvs, but were more variable on the results obtained for Winter cvs. Lower ACN content was found for Misato Red and Chinese Round Red Cored and higher ACN content for Chinese Red Meat. The ACN content per 100g of epidermal tissue of Spring cvs ranged from 65.7 to 193 mg ACN/100g skin, and the ACN content based on total root weight ranged from 16.5 to 76.9 mg ACN/100g root. The highest pigment content was obtained with Spring cvs Fuego and Winter cvs Red Meat, and the lowest pigment content corresponded to cvs Flamboyant Sabina.

A third year planting (Table 1, 1996) of Fuego and Red Meat cvs showed good reproducibility for Fuego as compared with the previous years, while high variability was found with cvs Red Meat. During this season, additional Chinese cvs were evaluated. Among those, all Spring cvs had a pigment content much lower than that of cvs Fuego. The Winter cvs Mah Tong Hong showed pigment content higher than that obtained from Chinese Red Meat during the same season.

Comparing the pigment content at two maturity stages

Spring cvs radishes from the first planting were harvested at 2 maturity stages: 4 or 7 wk after seeding (Table 2). Changes in radish weight were monitored for all cvs, and changes in pigment content were monitored in 8 cvs randomly selected to evaluate the effects of maturity on radish and pigment yield. All cvs showed an increase in weight when grown for an additional 3 wk (avg. 5.7 times increase), but not all showed the same weight increments with time. The root weight of cvs Arista, Fuego, and Red Pak increased 2.6–2.7 times, while cvs Arabic Red and Long Red increased in weight almost 10 times. Cultivars Crimson Giant, Danra, and Leda also showed a large root weight increment during the additional 3 wk in the field, with weights between 7.2–8.5 times the original weight.

The skin pigment content did not significantly change (p>0.1) with maturity. The average pigment content / kg root decreased when radishes were left in the field for a longer time, with exception of Crimson Giant and Picolo cvs, where pigment contents slightly increased. The average pigment content / kg root of Spring cvs was reduced by 30% during this time (Table 2). If the average root weight increased 5.7 times and the pigment content / kg root decreased 30%, there would be a 4-fold increase in total pigment from the same number of radishes after allowing them to grow for 3 additional wk. The changes in weight and pigment content observed in the Spring radish cvs suggest that a more efficient pigment yield would result from allowing the roots to grow for a longer period.

Effect of growing location on pigment yield

During the second year Spring (8) and Winter cvs (3) were planted at 2 locations and the monomeric anthocyanin content was evaluated (Table 3). Since maturity affected monomeric anthocyanin content, the weight of the radishes was also recorded at time of harvesting. Planting location and weight of radishes had a significant effect on pigment content (p<0.01), which was also dependant on cultivar (p<0.01). Planting location (Corvallis and Hermiston, OR) had a significant effect on total pigment content and the highest amounts of ACN from either Spring or Winter cvs sample were from those harvested at Hermiston. However, the effect of location was more clear for Winter cvs which showed a higher pigment content in those grown at Hermiston. Winter cvs Chinese Red Meat showed monomeric ACN content substantially higher when grown at Hermiston, as compared to Corvallis, and much higher than the other two red-flesh cvs (Table 3b). We also observed that pigment content showed high variability within red meat cvs.

Several factors affecting differences in

Table 1—Monomeric anthocyanin content in red radish cvs (mg ACN/100g)

Source	Cultivars	1994		1995		1996		Average	
	Spring cvs	Skin	Root	Skin	Root	Skin	Root	Skin	Root
1	Arabic Red	98.7	16.7					98.7	16.7
2	Arista	158	33.3	149	33.3			154	33.3
3	Cherry Bomb	119	25.0					119	25.0
3	Crimson Giant	113	20.9					113	20.9
1	Crimson Giant	122	29.0	146	32.0			134	30.5
1	Danra	122	22.6					122	22.6
3	Easter Egg	62.3	11.6					62.3	11.6
1	Flamboyant Sabina	127	23.7	92.3	16.5			110	20.1
4	Fuego	161	32.0	193	42.6	202	41.9	185	38.8
5	Jumbo	113	22.3					113	22.3
2	Leda	127	24.4					127	24.4
1	Long Red	91.2	25.1	65.7	17.4			78.5	21.3
2	Picolo	42.6	11.1					42.6	11.1
4	Red Baron	163	37.5	179	39.2			171	38.4
4	Red Pak	131	30.2					131	30.2
4	Red Prince	126	29.3					126	29.3
2	Robijn	144	32.0	128	30.3			136	31.2
1	Saxa "Korto"	163	30.7	116	29.5			140	30.1
4	Scarlet Knight	102	21.9					102	21.9
10	Red Harvest #1					39.3	4.7	39.3	4.7
10	Wu Ying Shui					77.8	8.6	77.8	8.6
10	Cherry Radish					91.4	15.3	91.4	15.3
	Winter cvs	**Skin**	**Root**		**Root**		**Root**	**Skin**	**Root**
6	Joyce	52.8	12.2					52.8	12.2
7	Chinese Round Red Cored		48.9		36.0				42.5
8	Chinese Red Meat		42.8		76.9		39.3		53.0
9	Misato Rose Flesh		60.4		28.1				44.3
10	Man Tang Hong						48.7		48.7

[a]Source: 1=Daehnfeldt; 2=Nunhems; 3=Burpee; 4=Rogers; 5=Stokes; 6=Known-You; 7=Tokita; 8=Takii; 9=Sakata; 10=Mah Dong King.

Table 2—Comparing skin and root weight and ACN content of red radishes harvested at 2 maturity stages[a]

Source[b]	Cultivar	4 Weeks Root[c] wt (g)	4 Weeks g Skin/ kg root	4 Weeks mg ACN/ 100g Skin	4 Weeks mg ACN/ 100g Root	7 Weeks Root[c] wt (g)	7 Weeks g Skin\ kg root	7 Weeks mg ACN\ 100g Skin	7 Weeks mg ACN\ 100g Root
	Spring cvs								
1	Arabic Red	27.5	169.3	98.7	**16.7**	250.0			
2	Arista	22.8	210.9	158	**33.3**	61.1			
3	Cherry Bomb	17.0	209.4	119	**25.0**	69.1			
3	Crimson Giant	34.1	185.3	113	**20.9**	148.2	203.0	106.0	**21.6**
1	Crimson Giant	28.4	238.5	122	**29.0**	204.4	206.4	103	**21.3**
1	Danra	25.0	185.3	122	**22.6**	181.9			
3	Easter Egg	25.0	186.2	62.3	**11.6**	159.1			
1	Flamboyant Sabina	37.3	187.2	127	**23.7**	191.1	132.2	90.3	**11.9**
4	Fuego	19.2	198.6	161	**32.0**	54.0	134.3		
5	Jumbo	26.3	196.8	113	**22.3**	159.2	115.5	117	**13.6**
2	Leda	17.1	192.5	127	**24.4**	143.0	164.6		
1	Long Red	46.4	275.4	91.2	**25.1**	435.0	246.5	53.5	**13.2**
2	Picolo	26.5	260.4	42.6	**11.1**	172.1	234.4	58.0	**13.6**
4	Red Baron	23.8	230.0	163	**37.5**	73.0			
4	Red Pak	18.4	230.6	131	**30.2**	48.6	236.3	96.4	**22.8**
4	Red Prince	21.3	232.4	126	**29.3**	66.2			
2	Robijn	28.6	221.4	144	**32.0**	136.1			
1	Saxa "Korto"	23.8	188.6	163	**30.7**	127.5	210.7	109	**22.9**
4	Scarlet Knight	14.6	215.8	102	**21.9**	64.2			
	Winter cvs								
6	Joyce[d]					71.4	231.3	52.8	**12.2**
7	Chinese Round Red Cored					129.2			**48.9**
8	Chinese Red Meat					125.9			**42.8**
9	Misato Rose Flesh					152.7			**60.4**
	Avg of Spring cvs	**25.4**	**118.4**	**119.0**	**95.6**	**105.3**	**113.2**	**111.7**	**105.5**
	Standard deviation	7.7	96.3	80.4	80.7	84.8	85.5	82.8	83.3
	Avg Winter red flesh cvs					**106.4**			**106.0**
	Standard deviation					86.7			85.1

[a]Same planting (1994) harvested at 2 maturity stages: 4 and 7 wk after seeding.
[b]Source: Same as Table 1.
[c]average root weight for 1 kg (small roots) to 3 kg (large roots) samples of radish.
[d]winter cv Joyce with pigmentation in the skin only was not included for this average.

radish and pigment yields from different locations may include differences in temperature, illumination and soil characteristics. Soil levels of phosphorous have a great effect on root yields of radish (Sanchez et al., 1991). Wilcox and Pfeiffer (1990) determined that temperature had a significant effect on seed germination, growth and root development of radishes, when at 10°C or lower. The two locations evaluated were close geographically but had differences in latitude (44°37' and 45°49', respectively) and elevation (70 and 189 m above sea level, respectively). Typically, the Hermiston location has higher temperatures and more growing days (at 10°C) than the Corvallis area (Oregon Climate Service, 1997). These factors may have been important for higher radish yields from Hermiston as compared with Corvallis. For Summer 1995, Hermiston maximum temperatures during the day (29.7°C) were 3.8 degrees higher and minimum temperatures (13.1°C) 2.4 degrees higher than Corvallis (Oregon Climate Service, 1997). Anthocyanin biosynthesis is a photomorphogenetic phenomenon controlled by phytochromes (Guruprasad and Laloraya, 1980). The effects of light on the light-mediated synthesis of many compounds, like anthocyanins, depends on the intensity and quality of absorbed light (Buschmann and Lichtenthaler, 1982). Differences in number of light hours and temperatures may have caused some of the differences in pigment contents.

Anthocyanin profiles

We used the ACN profile of cvs Fuego as a reference since it had been previously described (Giusti and Wrolstad, 1996a). The ACN profiles were very similar for different red radish cvs, the major pigments being Pg-3-soph-5-glu (raphanusin), mono- or di-acylated with cinnamic acids (ferulic, *p*-coumaric and caffeic acids) and malonic acid (Fig. 1).

The relative amounts of pigments (Table 4) after deacylation with subsequent HPLC analysis of saponified ACN showed that all cvs had the raphanusin structure. The non-acylated raphanusin structure (pigment 1) was present in low levels that ranged from 0.6 to 2.5% of the total pigment area, while acylated ACN (pigments 2-6) represented the major proportion of ACN in all cvs analyzed. The proportions of mono- and di-acylated ACN (cinnamic acid only, or cinnamic plus malonic acids) were different. For the Spring cvs Arista, Flamboyant Sabina, Fuego, and Saxa‘Korto’ di-acylated ACN (Pks 5 and 6) represented 60–80% of the total ACN content (based on HPLC peak area). Winter cvs showed a lower proportion of these di-acylated ACN, representing 52–59% of total pigment. Additional unidentified di- or poly-acylated raphanusin derivatives, represented between 1.8–27 % of the total area at 520 nm. Those ACN were present in larger amounts in all Winter cvs and Spring cvs Long Red.

Table 3—Monomeric anthocyanin content in red radish cvs grown at 2 locations in Oregon (1995)

(a) Anthocyanin content in red radish epidermal tissue (mg ACN/100g skin)

Source[a]	Cultivar	Corvallis[b] I	Corvallis[b] II	Hermiston[c]	Average
	Spring cvs				
2	Arista	123	131	195	150
1	Crimson Giant	139	149	151	146
1	Flamboyant Sabina	103	84.6	88.9	92
4	Fuego	164	206	210	193
1	Long Red	76.0	56.1	64.9	66
4	Red Baron	161	186	192	180
2	Robijn	120	131	133	128
1	Saxa “Korto”	133	119	94.5	116

(b) Anthocyanin content in whole red radish roots (mg ACN/100g root)

Source[a]	Cultivar	Corvallis[b] I	Corvallis[b] II	Hermiston[c]	Average
	Spring cvs				
2	Arista	31.3	28.6	40.1	33.3
1	Crimson Giant	37.4	30.0	28.7	32.0
1	Flamboyant Sabina	19.7	15.6	14.1	16.5
4	Fuego	40.5	36.0	51.2	42.6
1	Long Red	21.8	16.0	14.3	17.4
4	Red Baron	36.6	31.0	50.0	39.2
1	Robijn	33.5	26.4	30.8	29.4
1	Saxa “Korto”	31.6	23.8	33.0	29.4
	Winter cvs (red flesh)				
7	Chinese Round Red	16.2	30.4	37.8	28.1
8	Chinese Red Meat	37.7	25.1	168	76.9
9	Misato Rose Flesh	26.1	16.0	66.1	36.1

[a]Source: Same as Table 1.
[b]Corvallis I and II: Two different plantings at the Lewis Brown Horticultural Farm, Corvallis, OR.
[c]Hermiston: Grown at the OSU Hermiston Agricultural Research & Extension Center, Hermiston, OR.

Table 4—Relative anthocyanin composition[a] of red radish cultivars

Source[b]	Cultivar	Pigment 1	2	3	4	5	6	Others
	Spring cvs							
2	Arista	0.6(0.3)	7.2(0.6)	6.7(3.7)	2.0(1.3)	59.7(7.3)	20.8(2.1	3.2(4.5)
1	Crimson Giant	1.3(0.3)	14.8(0.7)	4.9(0.6)	5.4(0.9)	26.2(3.5)	33.9(0.8)	13.5(3.2)
1	Flamboyant Sabina	0.7(0.9)	7.0(2.5)	6.9(4.9)	2.0(0.7)	62.5(7.7)	17.2(0.1)	3.9(3.8)
4	Fuego	0.6(0.6)	4.6(0.6)	5.5(0.6)	5.5(2.8)	39.1(2.8)	41.4(1.6)	3.5(2.2)
1	Long Red	1.1(0.6)	7.4(2.3)	4.9(0.5)	3.6(1.3)	29.4(6.9	32.5(5.0)	21.3(7.2)
4	Red Baron	0.9(1.0)	7.7(2.5)	7.9(7.4)	4.8(3.5)	41.0(1.6)	31.1(12.7)	6.7(4.9)
2	Robijn	1.0(0.8)	8.7(3.7)	7.1(4.9)	2.1(0.4)	52.8(6.9)	25.1(11.4)	3.5(2.2)
1	Saxa “Korto”	0.8(0.5)	l 8.0(2.9)	5.3(2.0)	3.5(3.1)	56.7(1.7)	24.0(2.8)	1.8(1.8)
	Winter cvs							
7	Chinese Red Round Cored	2.1(0.3)	7.3(3.3)	9.1(2.3)	7.0(1.3)	22.5(7.6)	33.4(6.5)	18.7(13.6)
8	Chinese Red Meat	2.0(0.3)	7.7(0.1)	5.1(0.8)	5.9(2.5)	18.3(0.8)	33.8(5.7)	27.4(8.0)
9	Misato Rose Flesh	2.5(0.5)	7.0(0.5)	8.0(1.3)	7.4(2.8)	26.8(1.1)	31.8(2.5)	16.6(0.4)

[a]%Peak area at 520 nm. 1: Pg-3-soph-5-glu. 2, 3 and 4: same as 1, acylated with caffeic, *p*-coumaric, and ferulic acids, respectively. 5 and 6, same as 3 and 4, with additional malonic acid, respectively. Standard deviations in parentheses.
[b]Source: Same as in Table 1.

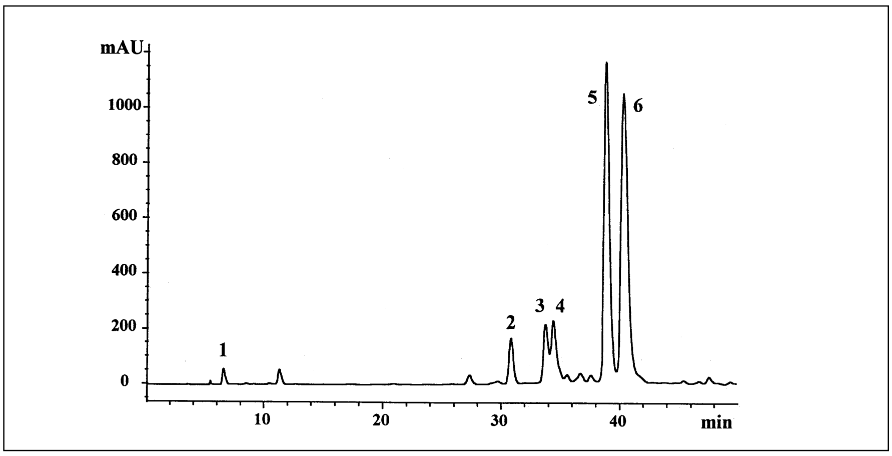

Fig. 1—HPLC separation of red radish anthocyanins. Polymer Labs PLRP-S, 250 × 4.6 mm i.d. column. Solvent A: 100% acetonitrile, B: 4% phosphoric acid. Linear gradient from 12 to 22% A in 50 min. Flow rate: 1 mL/min. Injection volume: 50 L. Peak assignments: 1: Pg-3-soph-5-glu; 2: same, acylated with caffeic acid; 3: same as 1, acylated with *p*-coumaric acid; 4: same as 1, acylated with ferulic acid; 5: same as 3, with additional malonic acid acylation; 6: same as 4 with additional malonic acid acylation.

The analysis of phenolic acids from ACN saponification revealed the presence of *p*-coumaric, caffeic and ferulic acids (Table 5). The major phenolic acids present as acylating groups in Fuego cvs ACN were *p*-coumaric and ferulic acids, in similar concentrations, and caffeic acid was present in low levels. Flamboyant Sabina and Arista cvs showed a much higher proportion of *p*-coumaric acids as the acylating group. Cultivar Crimson Giant showed the highest amount of caffeic acid (14.8%) as the acylating group, while other cvs showed concentrations between 4.6 and 8.7% of caffeic acid.

Many studies have reported (Francis, 1989; Lu et al., 1992, Dangles et al., 1993; Giusti and Wrolstad, 1996b) that acylation improved color and pigment stability of anthocyanins. Acylation with aromatic acids also caused a bathochromic shift to higher wavelengths of maximum absorbance, which caused differences in perceived color (Dangles et al., 1993). The differences found in the acylation pattern of ACN from the various red radish cvs may have an effect on the color and pigment stability of the extracts when evaluated as potential natural red food colorants.

Table 5—Acylating groups[a] on anthocyanins of red radish cultivars[b]

Source	Cultivar	Acylated[c] ACN	Caffeic acid	*p*-Coumaric acid	Ferulic acid	Malonic acid
	Spring cvs					
2	Arista	99.6(3.3)	7.2(0.6)	66.7(5.5)	22.9(1.7)	80.8(4.7)
1	Crimson Giant	85.2(1.6)	17.4(0.7)	36.5(2.1)	46.1(0.9)	70.5(2.2)
1	Flamboyant Sabina	95.6(3.3)	7.3(2.5)	72.6(6.3)	20.1(0.4)	83.4(3.9)
4	Fuego	96.1(1.8)	4.8(0.6)	46.4)1.7)	48.8(2.2)	83.8(2.2)
1	Long Red	77.8(3.9)	9.5(2.3)	44.1(3.7)	46.4(3.2)	79.6(6.0)
4	Red Baron	92.5(5.4)	8.3(2.5)	52.9(4.5)	38.8(8.1)	77.9(7.2)
2	Robijn	95.8(4.9)	9.1(3.7)	62.5(5.9)	28.4(5.9)	81.3(9.2)
1	Saxa “Korto”	97.5(2.4)	8.2(2.9)	63.6(1.9)	28.2(3.0)	82.8(2.3)
	Winter cvs					
7	Chinese Red Round Cored	79.3(5.8)	9.2(3.3)	39.8(5.0)	50.9(3.9)	70.5(7.1)
8	Chinese Red Meat	70.8(3.0)	10.9(0.1)	33.1(0.8)	56.1(4.1)	73.6(3.3)
9	Misato Rose Flesh	81.0(1.4)	8.6(0.5)	43.0(1.2)	48.4(2.7)	72.3(1.8)

[a]Acylating groups: % of total acylated anthocyanins that are acylated with that acid.
[b]Expressed as % of total peak area at 520 nm. Source: Same as in Table 1. Standard deviation in parentheses.
[c]Acylated ACNs: % of total anthocyanins corresponding to acylated anthocyanins.

Table 6—Estimated radish pigment yield

	Radish				Red cabbage
		Fuego		Red Meat	
	3 wk[a]	4 wk[b]	7 wk[b]	7 wk[b]	14 wk[c]
Row area (m^2)		16.7	16.7	50.2	
Rows/ha		598	598	199	
Yield (kg/ha)	4300	28000	39400	19500	56000
Pigment content (mg ACN/100g)	30	32	40	40	25
Pigment yield (kg/ha)	1.3	9.0	15.8	7.8	14.0
Pigment yield (kg/ha-day)	0.06	0.32	0.32	0.16	0.14

[a]Reported radish yield (Florida Agricultural Statistics, 1996) and experimental pigment content for radish at commercial maturity.
[b]Based on experimental data for row spacing, radish yield, and pigment content.
[c]Reported cabbage yield (Florida Agricultural Statistics, 1996) and pigment content (Timberlake, 1988).

Red radish ACN yields

The potential of food plants as commercial sources of anthocyanins is generally limited by availability of raw material and economic considerations (Jackman and Smith, 1996). From an economic perspective, the best potential commercial sources of anthocyanins are those from which the pigment is a by-product of manufacture of other value-added product, e.g. grape and grape skin extract. The availability of highly pigmented low cost crops such as red cabbage makes viable the use of these crops as possible sources for commercial food colorants (Jackman and Smith, 1996). We evaluated the potential yield and availability of radish pigment and compared it to those estimated for red cabbage.

The yield of anthocyanins from red radishes was estimated using average values from experimental and published data (Table 6). Yields of radish obtained in the OR experimental stations for radishes harvested 4 or 7 wk (28 and 39.4 MT/ha, respectively) after seeding were considerably higher than those reported for marketable radishes produced in Florida (4.3 MT/ha). This is probably due to the weight increase produced with longer growing times and very low levels of discarded material. Sanchez et al. (1991) reported marketable yields of radishes cvs Red Baron between 6 and 10 MT/ha when using phosphorus fertilization, and losses between 2 and 47%. Radish losses would be much lower when used for pigment production since size and cracks would not be criteria for selection.

Radishes harvested at a commercial stage would yield about 1.4 kg ACN/ha. However, radishes left in the ground for longer time resulted in higher pigment yields. Cultivar Fuego could produce between 9 and 16 kg ACN/ha when they were grown for 4 or 7 wk, respectively. Chinese Red Meat cvs showed an estimated yield of anthocyanin of 7.8 kg ACN/ha. Similar calculations were used to determine the potential pigment yield from a commodity already used for pigment production, red cabbage. Calculations based on data for red cabbage yields in Florida (Florida Agricultural Statistics, 1996), and reported pigment yield (Timberlake, 1988) showed that 14 kg ACN/ha could be obtained. The estimated yields for radishes and red cabbage suggests similar potentials for pigment production.

The maturing times of these crops should be considered to make a more valid comparison. Typical growing periods for commercial radishes are 3 wk, and we also used 4 and 7 wk to increase pigment yields. Typical growing periods for cabbage are in the order of 12–15 wk, depending on climate and horticultural practices (Hunt and Bortz, 1986). Another way of comparing yields for commodities with different maturing times is to report yields/ha per day of growing period. For radishes grown for 4 and 7 wk yields on that basis were the same, and more than twice as much as that obtained for red cabbage (Table 6). Estimated values would vary depending on different horticultural practices and growing conditions, but pigment production from radishes at a commercial scale should be feasible.

CONCLUSIONS

RADISH CVS SHOWED DIFFERENT MONOmeric ACN contents after accounting for factors such as planting location, maturity stage and root weight. Chinese Red Meat cvs had the highest pigment content (53 mg ACN/100g root). High variability within Winter cvs and between planting locations was found. Cultivar Fuego had the highest pigment content (39 mg ACN/100g root) among Spring cvs. All radish cvs showed similar ACN profiles: pelargonidin-3-sophoroside-5-glucoside with 1 or 2 acylating groups. Differences among pigment profiles of cvs were in the relative amounts at which the different acylating groups were found. Estimation of pigment yields suggest that production of pigment at commercial scale could be viable.

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