'Cyanidin volumetric index' and 'chromaticity coordinates ratio' to characterize red raspberry (*Rubus idaeus*)

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Abstract

The object of this work is presented in a larger research project concerning 'New indexes to evidence the nutritional quality of small fruits' in progress at the Analytical Food Research Laboratories, University of Milan. The present paper contains data that contribute to the analytical characterization of 12 varieties of red raspberry through the high-performance liquid chromatography determination of the aglycon 'cyanidin' derived from chemical hydrolysis of berries. Even more interesting results are the proposal of the 'cyanidin volumetric index', by which it is possible to compare different red raspberry varieties with higher meaningfulness. A new possible correlation between the ratio of chromaticity coordinates '*a/b*' and the cyanidin content of red raspberries has been identified.

Keywords: *Anthocyanins, cyanidin, raspberry, Rubus idaeus, small fruits*

Introduction

Preliminary notes and purpose of the research

FAO sources in 2002 reported that the worldwide production of red, black and hybrid raspberries amounted to 414,000 tonnes/year. In Europe, the production of Poland, Hungary, United Kingdom, and Germany jointly totalize about 155,000 tonnes/year: in these countries, the raspberry is considered a full-scale crop and not just a marginal one, and the greater part of the production of the aforesaid countries is earmarked for processing.

In Italy the red raspberry alone, and not the black raspberry, has found favour with consumers: the overall area, in 2003, already exceeded 164 ha, with a production of about 1400 tonnes, and during the recent 3-year period the imports to Italy reached an average total of 400 tonnes/year. The regions most involved in production are Trentino Alto Adige, Piemonte, Lombardia, Friuli, and Emilia Romagna, and the plantations are now expanding in the direction of a number of southern regions. The Region of Alto Adige (Südtirol) has registered the largest area of cultivation (88 ha in 2003) for raspberries destined for fresh consumption: this is their most remunerative use.

The nutritional value of the raspberry as fresh fruit is due to its connection with polyphenols rather than in consideration of the vitamins and any additional fresh fruit ingredients also contained in other berries. The anthocyanins, in particular, have a protective, anti-inflammatory effect at a capillary level; indeed, the production of

berry concentrate is of commercial significance because of its extensive use in processed foods, whose colour depends on the anthocyanin content.

Colour has often been considered the index with the most immediate nutritional quality characterization, because it is often associated with the idea of anthocyanin content, and tristimulus colorimetry has been considered the best method to estimate visible colour. But the various experiments so far performed have shown that correlation is never simple or direct, because various factors influence the colouring power: for example, acidity and pH are both influential factors in determining the colour of anthocyanin solutions. In addition to the contribution of colour by the monomeric anthocyanins, there can be a contribution of colour from polymerized anthocyanins and brown pigments arising from enzymatic and non-enzymatic browning: raspberries have been shown to contain many polyglycosylated anthocyanins, and various papers describe the distinctive anthocyanin pattern of the raspberry (Barritt and Torre 1975, Hong and Wrolstad 1990, Garcìa-Viguera et al. 1990, Rommel et al. 1990, Goiffon et al. 1991, Boyles and Wrolstad 1993, Versari et al. 1997).

In all cases cyanidin is the only aglycon derived from hydrolysis, and it has been correct to refer to the aglycon cyanidin content in order to quantify, with simple data, the flavonoid class represented by anthocyanins. For such a small fruit as the raspberry, the opportunity has been taken in this paper to define a new quality index that refers to the cyanidin content present in the 'consumption unit'; that is, the number of berries that occupy a specific 'apparent' volume.

In analysing different red raspberry varieties produced by the Azienda Agraria 'Francesco Dotti' of Arcagna, a farm that belongs to Milan State University (Italy), we have compared 12 varieties from the nutritional point of view, evaluating the cyanidin content and other data such as chromaticity coordinates, sugar/acidity ratio, and the mean number of berries/1000 ml and the mean weight of berries.

The cyanidin level (mg) referred to fruit weight (g), which has an apparent volume of 1000 ml, has been named the 'cyanidin volumetric index' (CVI). This index makes it possible to compare the varieties of raspberry more conclusively than the simple cyanidin level expressed as mg/kg berries.

Materials and methods

Samples

Twelve varieties of red raspberries have been considered, deriving from 2004 production by Azienda Agraria 'Francesco Dotti' of Arcagna, which belongs to Milan State University. Data refer to the varieties 'Glen Lyon', 'Glen Ample', 'Glen Garry', 'Heritage', 'Autumn Bliss', 'John Squire', 'Polana', 'Ruby', 'Tulameen', 'Caroline', 'EM6505/5', and 'Anne': the first four were planted in 1999 and the others in 2000 with the exception of the last three, which were planted in 2003. 'Anne' is a raspberry variety with a very low level of anthocyanins: the colour of this fruit is yellow.

Reagents and standards

The reagents used for the determination of cyanidin were analytical-grade water, methanol, hydrochloric acid and acetic acid (Merck KGaA, Darmstadt, Germany). Standard cyanidin chloride was from Extrasynthese (Genay Cedex, France). The

standard of cyanidin-3-O-β-galactoside was from Polyphenols Laboratories (Sandnes, Norway).

For the measure of titratable acidity, the sodium hydroxide solution ConvoL® (BDH Laboratory Supplies, Poole, UK) was used.

Apparatus

High-performance liquid chromatography (HPLC) analysis of cyanidin aglycon was performed on a Shimadzu apparatus (Shimadzu, Milan, Italy) equipped with two pumps (10AD vp), a Diode Array Detector (DAD) (SPD-M10A vp), a system controller (SCL-10A vp), and an Rheodyne 20 μL injection loop (Cotati, CA, USA). The HPLC pumps and diode array system were controlled by computer using a CLASS VP Version 5.032 Workstation program.

The analytical column employed was a Discovery™ 505064 RP-AMIDE C16 (25cm ×0.46 cm^2, particle size 5 μm), manufactured by Supelco (Bellefonte, PA, USA).

The content of dry soluble solids was estimated with an Abbe type refractometer (S.A.V.I., Milan, Italy), reading directly to the nearest 0.1% sucrose. Acid correction has been applied following method number 8 IFFJP 1991.

A Colorimeter Minolta Chroma Meter CR-100 was used to measure lightness and chromaticity coordinates in the $L\star a\star b\star$ colour space (CIELAB), where $L\star$ indicates the lightness and $+a\star$, $-a\star$, $+b\star$, and $-b\star$ are the chromaticity coordinates, respectively, for the red, green, yellow and blue directions.

Titratable acidity was measured by refractometer titration with standard volumetric sodium hydroxide solution to a pH value of 8.1.

Sample preparation and HPLC analysis of cyanidin

For sample preparation, the method suggested by Nyman and Kumpulainen (2001) was adopted. The water content of fresh fruit was first detected. The fresh fruit sample (ca. 50 g) was finely grounded by an electrical blender and an amount of 2 g (exactly weighted) was put into a 50 ml screw-cap test tube, and then 20 ml of 2 M HCl in methanol were added. The test tube was sealed tightly, and hydrolysis was carried out at 90°C in a water bath for 50 min. The cooled sample was decanted and 1 ml was drawn-out and diluted to 6 ml with 2 M HCl in methanol. Then the sample was filtered throughout a 0.2 μm millipore filter, and the filtrate was recovered in a vial and injected into the HPLC/DAD.

The HPLC operative conditions and the column type were derived from the authors' experiences of setting-up. The mobile phase consisted of (A) water/acetic acid (95:5 v/v) and (B) methanol/acetic acid (98:2 v/v).

The operating conditions were as follows: 0–10 min isocratic elution 20%B; linear gradient from 20%B to 46%B, 10–20 min; isocratic elution 46%B, 20–50 min; and isocratic elution follows for 30 min. To re-equilibrate, 10-min isocratic elution 20%B is enough. The flow rate was 0.5 ml/min. The injection volume was 20 μl for all samples and standard solutions.

The identity of the sample peak was confirmed by comparison with the retention time and ultraviolet spectra of the standard cyanidin.

The wavelength used for quantification was 530 nm. The external standard method was used for calibration: the calibration curve was made by standard solutions of cyanidin ranging from 508 to 10,160 µg/L (ppb).

The quantitative calculation was processed as follows:

$$C_c = C_e \times D \times (20 + W) = P$$

where C_c is the concentration of cyanidin resulting in the sample (mg/kg), C_e is the concentration of cyanidin obtained from the calibration curve (µg/l), D is the dilution factor (4–6), W is the water content (ml) in the sample weight (g) P, and 20 is the volume (ml) of 2 M HCl in methanol used for hydrolysis.

Method validation

The aglycon cyanidin was used for calibration, and a series of dilutions was prepared in 2 M HCl in methanol at concentrations from 508 to 10,160 µg/l. For the recovery test, glycoside was dissolved in methanol. Standard solutions were stored in darkness at 4°C.

The detector response was linear over the tested concentration ranges for the 'aglycon' cyanidin: the coefficient of correlation was 0.993. The limit of detection (LOD) in full value was 30 µg/kg and the limit of quantification (LOQ) was assumed to be 90 µg/kg (i.e. three times the LOD).
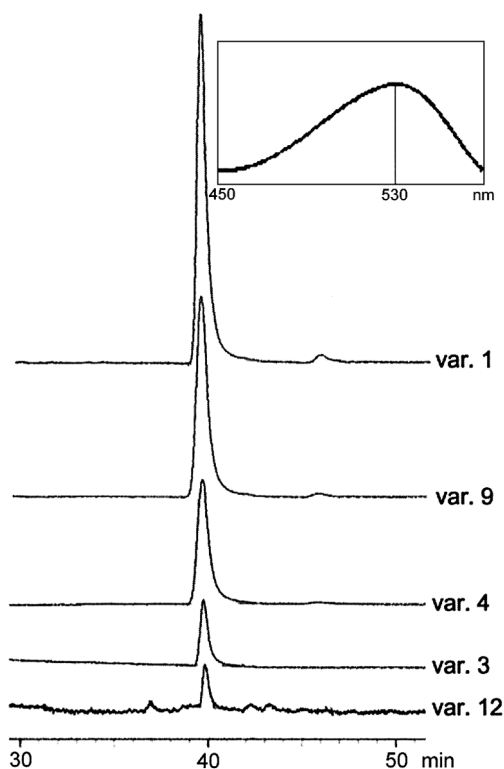

Figure 1. HPLC traces (530 nm) of cyanidin peak in five extracts produced following the method described in the text. The dilution factor was 6 for all samples. All peaks were graphically reported in this figure from HPLC traces having the same y-axis (0–100 mAU) with the exception of peak 12, derived from the trace having y-axis 0–10 mAU.

Table I. Comparison of general properties for twelve varieties of raspberries.

	Variety	Mean raspberry weight (g)	N_L	°Bx corrected for acidity	Titratable acidity (g/100 g)	°Bx/acidity ratio	$L^\star$	$a^\star$	$b^\star$
1	'Autumn Bliss'	2.5	220	9.45	1.25	7.56	28.59	22.92	6.28
2	'Glen Ample'	3.8	150	6.87	1.33	5.16	30.56	26.54	8.77
3	'Glen Garry'	3.4	150	9.59	1.45	6.62	36.87	32.92	13.34
4	'Glen Lyon'	2.2	350	6.11	1.07	5.71	31.51	29.90	11.24
5	'Heritage'	1.7	230	9.02	1.58	5.71	31.60	28.22	9.48
6	'John Squire'	2.6	170	9.11	1.56	5.84	36.07	30.53	11.17
7	'Polana'	2.5	190	7.60	1.49	5.10	29.19	25.14	7.98
8	'Ruby'	2.6	180	8.29	1.43	5.80	28.83	23.17	7.43
9	'Tulameen'	2.7	190	7.76	1.28	6.06	32.21	24.73	8.90
10	'Caroline'	2.4	170	9.39	1.44	6.52	29.92	24.23	7.15
11	'EM 6505/5'	4.3	130	10.50	1.50	7.00	34.27	29.93	12.58
12	'Anne'	3.2	160	9.61	1.55	6.20	55.50	7.95	28.34

N_L, number of berries that occupy an apparent volume of 1000 ml. Titratable acidity as monohydrate citric acid (g/100 g sample). $L^\star$, $a^\star$ and $b^\star$, lightness and chromaticity coordinates.

The recovery of the aglycon subjected to 2 M HCl hydrolyses was 96–102%. The repeatability was inspected by measuring the peak area: the coefficient of variation CV% ranged from 5.1% to 2.3% for the concentration data limits of 0.5–10.0 mg/l used for the calibration curve.

Results and discussion

Figure 1 shows some examples of the HPLC response obtained with the method previously described: the extract diluted six times produced from 'Anne' (trace 12) has the lowest cyanidin level of 0.30 mg/l. The extracts from 'Glen Garry' (trace 3), 'Glen Lyon' (trace 4), 'Tulameen' (trace 9) and 'Autumn Bliss' (trace 1) showed cyanidin levels, respectively, of 2.95 mg/l, 6.18 mg/l, 8.97 mg/l and 10.05 mg/l.

Table I reports the essential analytical data, including chromaticity coordinates, for the 12 red raspberry varieties. For these samples, Table II presents the cyanidin level (expressed as mg/kg fruit) and the corresponding CVI values derived from the formula:

Table II. Cyanidin level (mg/kg), cyanidin volumetric index (mg/1000 ml) and $a^\star/b^\star$ ratios for twelve varieties of raspberry.

	Variety	Cyanidin level (mg/kg)	CVI (mg/1000 ml)	$a^\star/b^\star$ ratio
1	'Autumn Bliss'	684	376	3.65
2	'Glen Ample'	415	237	3.03
3	'Glen Garry'	294	150	2.47
4	'Glen Lyon'	366	282	2.66
5	'Heritage'	420	164	2.98
6	'John Squire'	463	205	2.73
7	'Polana'	655	311	3.15
8	'Ruby'	592	277	3.12
9	'Tulameen'	564	289	2.78
10	'Caroline'	570	233	3.39
11	'EM 6505/5'	174	97	2.38
12	'Anne'	18	9	0.28

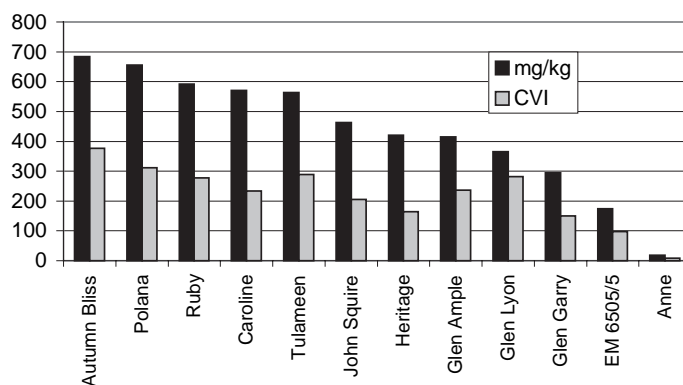CVI, cyanidin content in fruit weight having an apparent volume of 1000 ml.

Figure 2. Comparison of cyanidin level (mg/kg) and cyanidin volumetric index for twelve varieties of red raspberry (data detailed in Tables I and II).

$$CVI = N_{L} \times M_{W} \times Cy{=}1000$$

where N_{L} is the number of berries that occupy an apparent volume of 1 l, M_{w} is the mean weight (g) of a single berry (calculated as the mean of 50–100 berries), and Cy is the cyanidin level (mg/kg) calculated by HPLC analysis.

Figure 2 shows, for each variety, the comparison of the cyanidin level with the index CVI. In practice, if a volume defined as a 'common measure' (or consumption unit) is assumed, the quantitative intake of anthocyanin is more easily valuable from the CVI value. More than for other types of small fruits, the CVI of red raspberry are correlated with the fruit morphology.

To show an example of practical utility of CVI, we can compare the results presented in Table III. The data offer, for example, some immediate nutritional information: eating 35 berries of 'Glen Lyon' we assume to be a very similar quantity of anthocyanins than eating 18 berries of 'Ruby'; and eating 22 berries of 'Autumn Bliss' we assume contains a quantity of anthocyanins that is 35.7% greater than that corresponding to 18 berries of 'Ruby'.

Regarding the identification of a possible correlation between Hunter values and anthocyanin contents in fruits, other authors have exposed the results of different experiences (Hong and Wrolstad 1990).

The most interesting result is the one we obtained plotting the $a^{\star}/b^{\star}$ ratio values reported in Table II versus the cyanidin level (mg/kg). In the case of our experiences concerning red raspberry, we have evidenced the possible correlation between these parameters. Figure 3 shows that the best correlation exists for the exponential ($R^{2} = 0.9381$) and not for the linear tendency ($R^{2} = 0.7231$). The authors consider the two

Table III. Example of comparison between cyanidin content and CVI in three varieties of red raspberry.

Variety	Cyanidin (mg/100 g)	CVI/10	$N_{L}/10$
'Glen Lyon'	36.6	28.2	35
'Ruby'	59.2	27.7	18
'Autumn Bliss'	68.4	37.6	22

CVI/10, cyanidin level in fruit weight having an apparent volume of 100 ml; $N_{L}/10$, number of berries that occupy an apparent volume of 100 ml.
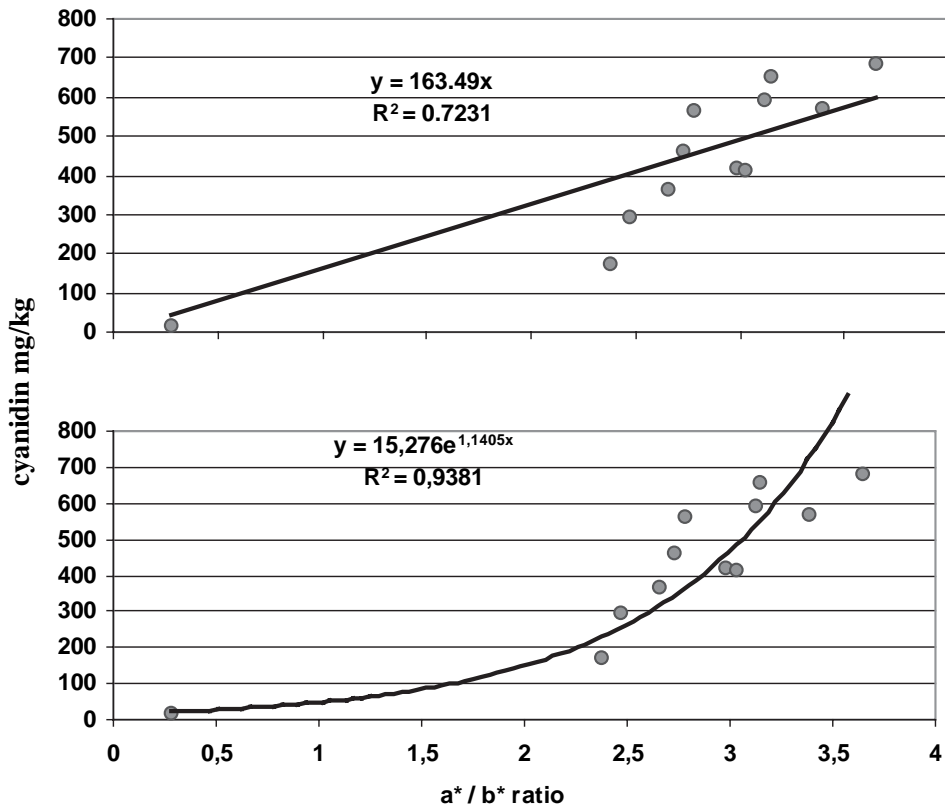
Figure 3. Cyanidin level (mg/kg) vs. chromaticity coordinates ratio $a\star/b\star$ for twelve raspberry varieties. The best correlation exists for exponential ($R^2 = 0.9583$) and not for linear tendency ($R^2 = 0.7225$).

indexes presented in this work to be worthy of attention and of future, more extended, experiences.

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