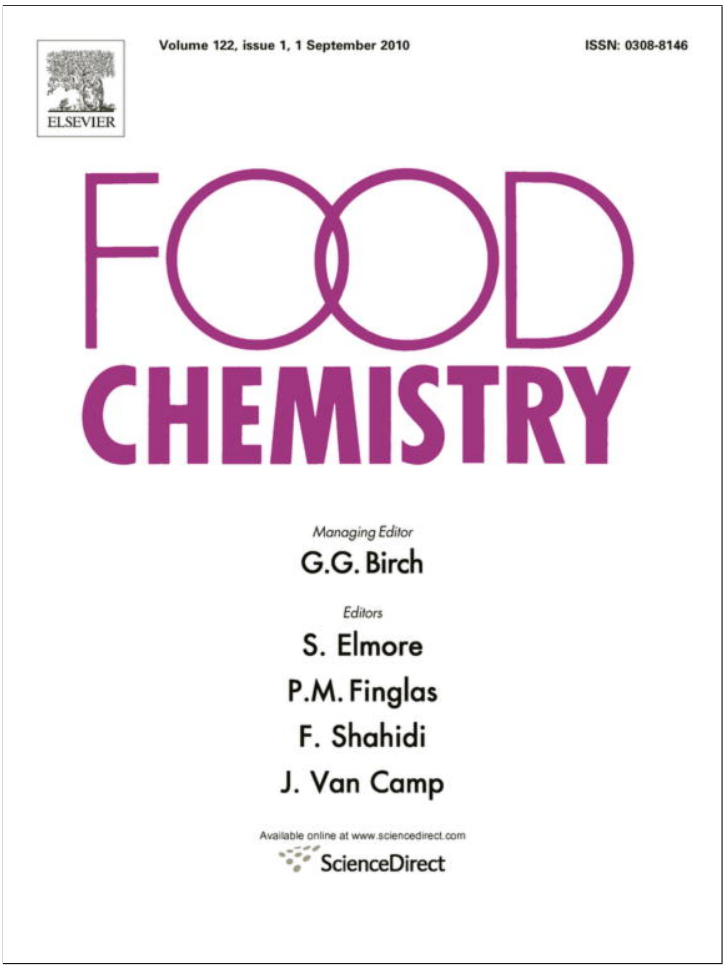

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Characterisation of major taste and health-related compounds of four strawberry genotypes grown at different Swiss production sites

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ABSTRACT

Individual sugars, organic acids, anthocyanins and vitamin C were quantified in strawberry fruits of four newly-bred cultivars grown at two production sites in Switzerland with different soil, climatic conditions and altitudes (1060 and 480 m above sea level). All the measured compounds were significantly influenced by genotype. Pelargonidin-3-glucoside was the main anthocyanin present in all cultivars, while the presence of other pelargonidin derivatives was genotype-dependent. Differences of about 2-fold were observed among the studied cultivars for their vitamin C content. In the mountain region, where plants produced a higher fruit yield over a shorter period, the concentration of both health and taste-related compounds was detrimentally affected. In particular, the vitamin C content in the fruits was negatively related to the average yield per day. However, the compositional variations of strawberry fruits in response to different production sites were genotype specific. Within the four cultivars studied, cv. Antea was most affected by the production site, showing generally lower contents of all analysed compounds when cultivated at higher altitudes, whereas cv. Clery seemed to have the more consistent chemical composition, regardless of production site. The results presented in this work corroborate the dominant role of strawberry genotype over environmental factors.

1. Introduction

Epidemiological studies suggest that consumption of fruit and vegetables contributes towards reducing risk of certain types of human cancer and cardiovascular diseases (Bazzano et al., 2002). Among fruits, berries are popular because of their good taste and their known nutritional value. Indeed, strawberry fruits, when compared to other fruits and vegetables, have been shown to contain high amounts of vitamin C and phenolic compounds, which are known to provide protection against free radicals when tested *in vitro* (Meyers, Watkins, Pritts, & Liu, 2003). Several studies have identified a wide range of phenolic compounds in strawberry fruits (Aaby, Ekeberg, & Skrede, 2007; Seeram, Lee, Scheuller, & Heber, 2006), but anthocyanins remain quantitatively the most important type in ripe fruits. Anthocyanins belong to the flavonoid group and are responsible for the bright red colour of strawberry fruits. Despite a great number of anthocyanins being identified in strawberry fruits, pelargonidin-3-glucoside (pg 3-gluc), pelargonidin 3-rutinoside (pg 3-rut) and cyanidin-3-glucoside (cya 3-gluc) represent over 95% of the total anthocyanin bulk present in most strawberry fruits (Lopes Da Silva, Escribano-Bailón, Pérez Alonso, Rivas-Gonzalo, & Santos-Buelga, 2007).

Nowadays, a large number of research studies have been conducted with the aim of elucidating the mechanisms for increased synthesis of bioactive compounds in the fruits and hence potentially healthier berries. This said, there seems to be a lack of information which addresses how genotype and cultivation systems affect the concentration of sugars and acids in the fruit, since they can act as an index of consumer acceptability (Azodanlou, Darbellay, Luisier, Villettaz, & Amadò, 2003; Jouquand, Chandler, Plotto, & Goodner, 2008; Keutgen & Pawelzik, 2008; Pelayo-Zaldivar, Ebeler, & Kader, 2005). Sugars in strawberry fruits are mainly mono- and disaccharides (*viz.* glucose, fructose and sucrose) (Giné Bordonaba & Terry, 2009; Kallio, Hakala, Pelkkikangas, & Lapvetelainen, 2000; Perez, Olias, Espada, Olias, & Sanz, 1997; Terry, Chope, & Giné Bordonaba, 2007) and the relative proportion of these individual sugars is important for governing the perception of sweetness (Keutgen & Pawelzik, 2008).

To date, there is substantial evidence that reveals genotype as the main source of variation in composition, specifically for both anthocyanin and sugar contents, of berry fruits (Capocasa, Scalzo, Mezzetti, & Battino, 2008; Giné Bordonaba & Terry, 2008, 2009; Kosar, Kafkas, Paydas, & Baser, 2004; Tulipani et al., 2008;

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Yoshida, Koyama, & Tamura, 2002). However, commercially available cultivars are changing rapidly and hence constant updating of information is required to quantify important taste- and health-related compounds in fruits from newly released cultivars. In addition to differences among genotypes, numerous works have shown that exogenous factors, such as environmental parameters (*viz.* light conditions, temperature, irrigation, fertilisation or cultivation systems) can affect the concentration of anthocyanins and antioxidant activity in strawberries and other berry crops (Crespo, Ançay, Carlen, & Stamp, 2009; Davik, Bakken, Holte, & Blomhoff, 2006; Terry et al., 2007; Wang & Zheng, 2001). A recent study conducted on blackcurrant berries (Zheng, Yang, Tuomasjukka, Ou, & Kallio, 2009) demonstrated that genotype crucially influenced the composition of fruits as a response to weather conditions at different latitudes. The effect of altitude at the same latitude was analysed for wild populations of common elderberries and bilberries (Rieger, Muller, Guttenberger, & Bucar, 2008). Nevertheless, thus far no other studies have elucidated the effect that production site, principally differing in altitude, may have on strawberry fruit composition. Given that strawberry production in middle or Eastern Europe, including countries such as Italy, Switzerland and Turkey is carried out in mountain regions, understanding the relationship between genotype and environmental conditions may be crucial to optimising the cultivation of high quality fruits which can satisfy the requirements of the market. Accordingly, this study aimed to characterise the fruit quality of four newly released June-bearing cultivars (*viz.* Antea, Asia, Clery and Matis) when plants were grown on two different production sites at different altitudes.

2. Materials and methods

2.1. Cultivation sites

The trials were conducted at two different sites in Switzerland (Conthey, 46°12′N/7°18′E and Bruson, 46°04′N/7°18′E) during 2008, characterised by different soil and climatic conditions but principally differing in elevation above sea level (Table 1) and different harvest periods.

Table 1
Soil and weather conditions, flowering and harvest periods at the two different production sites under investigation.

	Site 1: Conthey	Site 2: Bruson
Altitude	480 m	1060 m
Latitude/longitude	46°12′N/7°18′E	46°04′N/7°18′E
Total (cumulated) rainfall from flowering to sampling	31.8 l/m^2	86.1 l/m^2
Average temperature from flowering to sampling	16.4 °C	14.6 °C
Average temperature 10 days before sampling	16.8 °C	17.6 °C
Average radiation from flowering to sampling	19.19 MJ m^{-2}	15.35 MJ m^{-2}
Granulometric soil composition	23% Clay 44% Silt 33% Sand	16% Clay 35% Silt 49% Sand
Soil fertility	Phosphorus: optimum Potassium: above optimum Magnesium: above optimum	Phosphorus: above optimum Potassium: optimum Magnesium: optimum
Soil pH	7.7	6.8
Soil organic matter (%)	3.60	3.60
Flowering start (earliest cvs.)	29.04.08	13.05.08
Harvest begin (earliest cvs.)	12.05.08	20.06.08

2.2. Plant materials and cultivation practices

The cultivars used in this study were selected for their genetic diversity (different parentages) and growing commercial importance. A+ frigo plants from cvs. Antea (FB6L-3 × Onebor, Salvi Vivai, Italy), Clery ((Elsanta × FB6L-3) × (Agathe × Sweet Charlie), Salvi Vivai, Italy) and Matis (Mara Des Bois × (Douglas × Belrubi), Marionnet, France) were planted at the beginning of June 2007 in Bruson and one month later in Conthey. The earlier plantation in Bruson was done due to the earlier start of winter in the mountain region. For cv. Asia (Maya × selection, New Fruits, Italy), plug plants were planted beginning of August for both sites. Plants were planted on raised beds covered with black plastic mulch at a density of 4 plants per m^2. Distance between the raised beds was 1.25 m (centre to centre) and strawberries were planted in one row at a distance of 0.2 m (Crespo et al., 2009). For each cultivar four replications of 28 plants were planted at both production sites. During plant growth and fruit production the same fertigation and phytosanitary treatments were applied at both sites. Water and nutrients were given by fertigation with drip irrigation at a flow of 1 l/h with emitters spaced 0.2 m apart (T-Tape, T-Systems International Inc., San Diego, CA). Nutrients were applied once a week during the growing period based on the recommendations for strawberries with a yield of 2 kg/m^2 (total nutrients applied in kg per ha: 100 N, 60 P_2O_5, 180 K_2O and 35 Mg). Vacuum gauge tensiometers (Irrometer Co., Riverside, CA) were used to schedule irrigation at 200 hPa measured at 0.2 m soil depth in the middle of the raised beds below the drip tube. Applications of phytosanitary treatments were made according to the Swiss Integrated Production System (Steffek et al., 2003), to control spider mites, powdery mildew and grey mould.

2.3. Fruit sampling

Fruits were harvested three days a week and the yield at each harvest recorded. The total leaf area of five consecutive plants per replication was measured at the end of the harvest with an I-3100 area meter (Li-COR Biosciences Inc., Lincoln, NE). Samples for analysis were taken 14 days after the first harvest and mainly consisted of secondary and tertiary fully ripe fruits (when fully red). The samples were prepared for further analysis as described by Tulipani et al. (2008). Briefly, within three hours following harvesting, the samples (whole fruit) were stored at −20 °C for one month. The frozen berries were then dipped into liquid nitrogen and milled with a pre cooled laboratory blender (IKA A 11 Basic, Staufen, Germany). The obtained powder was subsequently stored at −80 °C until analysis. The determination of total antioxidant capacity and vitamin C was conducted on wet samples, whereas determination of individual anthocyanins, organic acids and sugars was performed on freeze-dried samples, as described by Terry et al. (2007).

2.4. Analysis of sugars

Sugars were extracted using 62.5% (v/v) aqueous methanol as described by Terry et al. (2007). Sugar content in strawberry extracts was determined using an Agilent 1200 series HPLC binary pump system (Agilent, Berks., UK), equipped with an Agilent refractive index detector (RID) G1362A. Strawberry extracts (20 μl) were diluted (1:10), and injected into a Rezex RCM monosaccharide Ca+ (8%) column of 300 mm × 7.8 mm diameter (Phenomenex, Torrance, CA) with a Carbo-Ca^{2+} guard column of 4 mm × 3 mm diameter (Phenomenex). Temperature of the column was set at 80 °C using a G1316A thermostated column compartment. The mobile phase used was HPLC-grade water at a flow rate of 0.6 ml/min (Giné Bordonaba & Terry, 2008; Terry

et al., 2007). Temperature of the optical unit in the detector was set to 30 °C and temperature of the autosampler to 4 °C using an Agilent cooled autosampler G1330B. The presence and abundance of fructose, glucose and sucrose were automatically calculated by comparing sample peak area to standards (0.025–2.5 mg/ml) using ChemStation Rev. B.02.01.

The sweetness index was calculated by multiplying the sweetness coefficient of each individual sugar (glucose = 1, fructose = 2.3 and sucrose = 1.35), as described by Keutgen and Pawelzik (2007).

2.5. Analysis of malic, citric and ascorbic acid

Extracts for organic acids determination were prepared as described elsewhere (Giné Bordonaba & Terry, 2008; Terry et al., 2007). Briefly, freeze-dried strawberry extracts (50 mg) were dissolved in 3 ml of HPLC-grade water. Samples were kept at room temperature (25 °C) for 10 min and then filtered through a 0.2-μm syringe-driven filter (Millipore Corporation, Billerica, MA). Citric, malic and ascorbic acid contents in extracts were detected at 210 nm using the same HPLC system as described earlier, in this case equipped with an Agilent DAD G1315B/G1365B photodiode array with multiple wavelength detector. Extracts (20 μl) were injected into an Alltech Prevail Organic Acid column (250 mm × 4.6 mm diameter, 5 μm particle size; Alltech, Carnforth, UK) with an Alltech Prevail Organic Acid guard column of 7.5 mm × 4.6 mm diameter. The mobile phase was analytical grade degassed 0.2% (w/v) metaphosphoric acid in water (Giné Bordonaba & Terry, 2008). The flow rate of the mobile phase was 1.0 ml/min under isocratic conditions and the column temperature was set to 35 °C. The presence and quantity of ascorbic, citric and malic acid was calculated against a calibration curve obtained by using external standards for each acid (0.02–2.0 mg/ml) using ChemStation Rev. B.02.01.

2.6. Analysis of total vitamin C

The vitamin C was determined in strawberry samples before freeze drying (fresh frozen). The determination of vitamin C included the ascorbic acid (AsA) and the dehydroascorbic acid (DHAA) form. Vitamin C was extracted with a phosphate buffer solution (36 mM, pH 5.0) containing 1 g/l DL-dithiothreitol (Sigma–Aldrich, Buchs, Switzerland) for one hour at room temperature allowing the reduction of DHAA into ASA (Brause, Woollard, & Indyk, 2003). Extracts were filtered through a 0.45-μm filter and injected on a Varian Prostar 230 HPLC pump system equipped with a diode array detector (Varian Prostar 335) and a reverse-phase C18 column (Nucleodur C18, 4.5 × 250 mm, Macherey–Nagel, Düren, Germany) with a flow rate of 0.6 ml/min. The mobile phase consisted of the same buffer adjusted to a pH value of 2.5 to maintain AsA in the reduced form. The absorbance was measured with a UV-detector at 254 nm and the AsA peak area was quantified with the software Galaxie Chromatography Data System Vers.1.9 (Rev. 2) on the basis of an external standard calibration curve (0–60 mg/l).

2.7. Analysis of individual anthocyanins

Individual anthocyanins were extracted as described elsewhere (Giné Bordonaba & Terry, 2008; Terry et al., 2007) by mixing 150 mg of freeze-dried sample with 3 ml of 70% (v/v) methanol and 0.5% (v/v) HCl in HPLC-grade water. The slurry obtained was held at 35 °C in a water bath with constant shaking for 1.5 h; mixing the samples every 15 min. Finally, the solution obtained was filtered through a 0.2-μm Millex-GV syringe-driven filter unit and the clear extract analysed by HPLC. The anthocyanin profile of strawberry fruits was determined according to the method described by Giné Bordonaba, Crespo and Terry (submitted for publication). Briefly, the separation was performed on an Agilent 1200 Series system as described for organic acids determination. Strawberry diluted (1:5 v:v) extracts were injected (10 μl) into a Zorbax Eclipse XDB-C18 column of 250 mm × 4.6 mm diameter, 5 μm particle size with an XDB-C18 guard column of 12.5 mm × 4.6 mm diameter. The mobile phase consisted of 2% (v/v) acetic acid in HPLC-grade water (A) and 2% (v/v) trifluoroacetic in methanol (B). Flow rate, column temperature and temperature of the autosampler were set up at 1 ml/min, 35 °C, and 4 °C, respectively. Finally, eluted anthocyanins were detected at 520 nm. The presence and abundance of cya 3-gluc and pg 3-gluc was calculated by comparing peak area against a calibration curve obtained by using external standards of cya 3-gluc and pg 3-gluc, respectively (Extrasynthèse, Lyon, France) and Agilent ChemStation Rev. B.02.01. Unknown peaks were quantified using the external calibration curve of pg 3-gluc.

2.8. Extraction and quantification of total antioxidant capacity

Antioxidants were extracted according to the method of Tulipani et al. (2008) with slight modifications. Briefly, 5 g of snap-frozen strawberry powder (wet) were weighed in 25 g of the extraction solution containing 80% methanol and 1% formic acid in water (v:v). The obtained slurry was sonicated in a cooled water bath for 15 min then centrifuged 5 min at 10,000 g. The supernatant was filtered through an LS 14 filter (Schleicher and Schuell, GmbH, Dassel, Germany).

The determination of antioxidant capacity with 2,2-diphenyl-1-picrylhydrazyl (DPPH) is based on the properties of DPPH, which in its radical form has an absorption band at 517 nm and disappears upon reduction by an antiradical compound. The determination of antioxidant capacity was performed according to the method described by Brandwilliams, Cuvelier, and Berset (1995). All chemicals used in this section were purchased from Sigma–Aldrich (Buchs, Switzerland). Briefly, 100-μl extracts were added to 10 ml of a 0.1 mM DPPH solution stirred well and leaved to react at room temperature. The absorption at 517 nm was measured after 30 min against a blank. Quantification was performed with a Trolox standard calibration curve (0–2.4 μM) and the results were calculated in μmol Trolox Equivalents (TE)/g FW.

2.9. Data analysis

All statistical analyses were carried out using XLSTAT Version 2007.5 (Addinsoft, Paris, France). All data were subjected to a two-way analysis of variance and the means were compared using Tukey test at a significance level of 95% ($p = 0.05$). Relationships between factors were analysed by simple linear regression and by the coefficient of determination (r^2), calculated from the Pearson Product Moment Correlation Coefficient (r).

3. Results and discussion

3.1. Variation among cultivars in taste- and health-related compounds

In the present study, strawberry compounds directly related to taste- or health-related properties of the fruit strongly differed between genotypes (Table 2). On a FW basis, cv. Asia showed the highest total sugar content (51.8 mg/g; mean of both sites) followed by Clery (48.3 mg/g), Antea (41.4 mg/g) and Matis (40.9 mg/g). Three main sugars (*viz.*, fructose, glucose and sucrose) were quantified in each cultivar with fructose being quantitatively the most important. The proportion of each individual sugar to the total sugar concentration was similar in cvs. Antea and Asia. Lower concentrations of sucrose as compared to the rest of the cultivars

Table 2
Sugars content of strawberry fruits on a fresh weight (FW) and dry weight (DW) basis from different cultivars and production sites. The sweetness index was calculated on a fresh weight basis.

Cultivar	Site	Sucrose (mg/g FW)	Glucose (mg/g FW)	Fructose (mg/g FW)	Total sugars (mg/g FW)	(Fru + glu)/sucrose (mg/g FW)	Sweetness index
Antea	Conthey	14.7 a	16.0 a	18.3 a	49.0 a	2.6 b,c	77.9 a
	Bruson	12.0 a,b,c	10.1 b	11.6 b	33.7 b	1.8 c	53.1 b
Asia	Conthey	14.0 a,b	18.1 a	19.6 a	51.5 a	2.9 b,c	81.9 a
	Bruson	17.9 a	16.2 a	17.8 a	52.0 a	1.9 c	81.5 a
Clery	Conthey	11.9 a,b,c	18.2 a	20.6 a	50.9 a	3.3 b	83.4 a
	Bruson	13.1 a,b	15.3 a	17.2 a	45.6 a,b	2.5 b,c	72.6 a
Matis	Conthey	7.2 b,c	16.0 a	18.2 a	41.4 a,b	4.9 a	67.6 a,b
	Bruson	6.3 c	16.0 a	18.0 a	40.3 a,b	5.4 a	65.9 a,b
	Cultivar (C)	***	***	***	**	***	**
	Site (S)	ns	***	***	*	*	**
	C × S	ns	*	**	*	ns	*
		Sucrose (mg/g DW)	Glucose (mg/g DW)	Fructose (mg/g DW)	Total sugars (mg/g DW)		
Antea	Conthey	175.3 a,b	194.1 c	221.9 c	591.3 a,b		
	Bruson	189.3 a,b	159.4 d	183.9 d	532.6 b		
Asia	Conthey	155.2 a,b,c	211.4 a,b,c	230.9 b,c	597.5 a,b		
	Bruson	213.0 a	194.5 c	213.1 c,d	620.6 a,b		
Clery	Conthey	149.3 b,c	234.4 a,b	264.8 a,b	648.5 a		
	Bruson	177.3 a,b	208.5 b,c	234.5 b,c	620.3 a,b		
Matis	Conthey	107.2 c	238.9 a,b	271.3 a,b	617.4 a,b		
	Bruson	97.1 c	245.4 a	275.1 a	617.6 a,b		
	Cultivar (C)	***	***	***	*		
	Site (S)	*	**	**	ns		
	C × S	ns	*	ns	ns		

Sweetness index = (glucose × 1) + (fructose × 2.3) + (sucrose × 1.35).
Different letters in the same column indicate significant differences. Significant parameters are indicated as follows.
ns, Not significant.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

were encountered in cv. Matis; this resulted in fruits from this genotype having the greatest monosaccharide/disaccharide ratio (5.2 relative units). Despite differences in the sugar distribution between the cultivars, the cultivar ranking for the sweetness index was similar to the cultivar ranking for the total sugar content ranging from 65.5 to 81.7 relative units.

Sugar content is an important taste attribute for strawberries and is highly correlated with consumer acceptance (Azodanlou et al., 2003; Jouquand et al., 2008). To date, no information is available on the sugar and acid content of fruits from these newly released cultivars (*viz*., Antea, Asia, Clery, Matis); however, fructose and glucose contents were comparable to those reported for other cultivars (Kallio et al., 2000; Perez et al., 1997; Wang, Zheng, & Galletta, 2002; Skupien & Oszmianski, 2004; Davik et al., 2006; Giné Bordonaba & Terry, 2009; Giné Bordonaba & Terry, accepted for publication; Keutgen & Pawelzik, 2008). This said, the concentrations of sucrose described herein were higher than those found in some of the above-mentioned studies. Besides differences among cultivars, greater sucrose content may be partially attributed to differences in the extraction procedures used between this and other studies; the greater solubility of sucrose in methanol as compared to fructose and glucose has already been pointed out by others (Giné Bordonaba & Terry, 2009; Terry et al., 2007).

Taste in strawberry fruits is, however, not only influenced by sugars. Acids within the fruit are part of the soluble solids pool and are also important contributors to strawberry taste and flavour (Cordenunsi, Do Nascimento, Genovese, & Lajolo, 2002). In the present study, three major organic acids were found within the cultivars studied: citric, malic and ascorbic acid (Table 3). Citric acid was the major acid, with concentrations on a FW basis ranging from 3.8 mg/g (cv. Matis) to 5.7 mg/g (cv. Asia) and accounting for 62.7% (cv. Clery) to 71.7% (cv. Matis) of total acid content, and was in agreement with values found in the literature (Giné Bordonaba & Terry, accepted for publication; Keutgen & Pawelzik, 2008; Perez et al., 1997; Terry et al., 2007). Similarly, malic acid concentrations were *ca*. 24% of the total acid concentration and significantly differed between cultivars. Fruits from cv. Antea had 1.5-fold greater malic acid content than fruits from cv. Matis. As compared to other acids, ascorbic acid (AsA) was present in all cultivars in lower actual amounts (*ca*. 7% of total acids). The AsA contents were also highly variable between the genotypes studied: the greatest content of AsA was encountered in fruits from cv. Antea (0.6 mg/g FW), showing 1.7 more AsA than cv. Matis. Similarly, up to 2-fold difference was found by Tulipani et al. (2008), when comparing nine strawberry cultivars and selections, with contents varying from 0.3 mg/g FW to 0.5 mg/g FW. Variations in organic acid metabolism have been reported for many fruits (Zheng et al., 2009) and several genetic studies have shown that the accumulation of organic acids (i.e., malic acid) is controlled by genes which differ not only between species but also between cultivars (Saradhuldhat & Paull, 2007).

Because of its effect on perceived sweetness the ratio between sugar and acids in strawberries and other berries can act as an important indicator of fruit taste (Giné Bordonaba & Terry, 2008; Terry, White, & Tigwell, 2005), fruit ripeness (Perez et al., 1997) or even as an index of consumer acceptability (Keutgen & Pawelzik, 2007). Values from this study were comparable to those found in the literature (Davik et al., 2006; Terry et al., 2007). This said, the sugar/acid ratios reported by Davik et al. (2006) were slightly lower than those reported in this study (between 5.4 and 6.5), which was mainly due to higher acid contents founds in their cultivars (*viz*., Polka Korona, Aurora, Babette, Carmen, Hannibal and four Norwegian advanced selections) or maybe related to different growing conditions.

In this study, the vitamin C content in frozen fruit samples was determined after reducing DHAA into AsA. Strong variations were

Table 3
Organic acids and vitamin C contents of strawberry fruits on a fresh weight (FW) and dry weight (DW) basis from different cultivars and production sites.

Cultivar	Site	Malic acid (mg/g FW)	Citric acid (mg/g FW)	Ascorbic acid (mg/g FW)	Total acids (mg/g FW)	Vitamin C (mg/g FW)[A]	Sugar/acid ratio
Antea	Conthey	1.9 a	4.8 a,b,c	0.7 a	7.4 a,b,c	0.9 a	6.7 a,b,c
	Bruson	1.8 a	3.6 c,d	0.5 a,b	6.0 b,c,d	0.7 b	5.7 c
Asia	Conthey	1.8 a	6.1 a	0.5 b	8.4 a	0.7 b	6.2 b,c
	Bruson	1.8 a	5.3 a,b	0.5 b	7.6 a,b	0.6 b,c	6.9 a,b,c
Clery	Conthey	1.2 a,b	4.6 b,c,d	0.5 b	6.3 b,c,d	0.7 b	8.2 a,b
	Bruson	1.4 a,b	3.9 c,d	0.4 b,c	5.7 c,d	0.5 b,c,d	8.0 a,b
Matis	Conthey	1.5 a,b	4.1 b,c,d	0.3 c,d	5.8 c,d	0.4 c,d	7.1 a,b,c
	Bruson	1.1 b	3.5 d	0.2 d	4.8 d	0.4 d	8.6 a
	Cultivar (C)	**	***	***	***	***	***
	Site (S)	ns	***	**	**	***	ns
	C × S	ns	ns	ns	ns	*	ns
		Malic acid (mg/g DW)	Citric acid (mg/g DW)	Ascorbic acid (mg/g DW)	Total acids (mg/g DW)	Vitamin C (mg/g DW)	
Antea	Conthey	22.7 a,b	57.6 a,b	8.4 a	88.8 a,b	11.2 a	
	Bruson	28.6 a	56.4 a,b	8.5 a	93.5 a	11.0 a	
Asia	Conthey	20.9 a,b	70.9 a	5.7 a,b	97.5 a	7.5 b	
	Bruson	21.6 a,b	63.6 a,b	5.5 b	90.7 a,b	6.8 b	
Clery	Conthey	15.7 b	58.2 a,b	5.8 a,b	79.6 a,b	8.4 a,b	
	Bruson	19.5 b	52.9 b	5.3 b	77.8 a,b	7.4 b	
Matis	Conthey	21.7 a,b	60.9 a,b	4.7 b	86.8 a,b	6.4 b	
	Bruson	16.5 b	52.8 b	3.4 b	72.8 a	6.4 b	
	Cultivar (C)	***	**	***	**	***	
	Site (S)	ns	*	ns	ns	ns	
	C × S	*	ns	ns	ns	ns	

Different letters in the same column indicate significant differences. Significant parameters are indicated as follows.
ns, Not significant.
[A] Vitamin C = ascorbic + dehydroascorbic acid as described in Section 2.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

found in the vitamin C content of the different cultivars (Table 3). The vitamin C content in fruits results from a balance between synthesis and degradation. The *in situ* synthesis occurs in strawberries from D-galacturonic acid, a component of cell wall pectins during fruit ripening and the final concentration in the fruits depends on the expression of the gene GalUR as well as the availability of the substrate D-galacturonic acid (Agius et al., 2003). The first step of vitamin C degradation is the oxidation of AsA into DHAA; however, both forms of vitamin C are nutritionally available for utilisation as ascorbate (Welch et al., 1995).

Strawberry fruits are important sources of health-related compounds, from which, in the present work, special attention was given to anthocyanins and vitamin C concentrations. The HPLC method described herein allowed separation of five anthocyanin compounds (Fig. 1). Cya 3-glu and pg 3-gluc were identified and quantified by comparing retention time and UV spectra with external standards. Other unknown peaks, mainly peaks 1, 2 and 3, were quantified as pg derivatives using pg 3-gluc as standard and given the similarities of their UV spectra with that of pg 3-glucoside standard (data not shown). Differences existed not only in the actual amounts of anthocyanins detected among different genotypes but also in their anthocyanin profile (Table 4; Fig. 1). Pg 3-gluc remained quantitatively the main anthocyanin found in the strawberry extracts. Its concentration varied from 129 μg/g FW (cv. Antea) to 182 μg/g FW (cv. Asia) and represented between 75% and 93.8% of the total anthocyanin content. Pg derivative 2 concentration in cvs. Matis and Clery reached 23.7 and 54.8 μg/g FW, respectively (13.7% and 23.1% of the total anthocyanin content). This said, this specific anthocyanin was not detected in cvs. Antea and Asia. Similarly, pg derivative 3 was only detected in cv. Asia and not in the others. Pg derivative 1 was ubiquitously present in all the cultivars studied representing a marginal 3% of the total anthocyanin concentration. Cv. Clery showed the highest total anthocyanin content among the cultivars investigated with 1.7-fold greater concentrations than that of cv. Antea which, indeed, showed the lowest total content (Table 4).

Reported anthocyanin concentrations for strawberry fruits differ markedly (Hernanz, Recamales, Melendez-Martinez, Gonzalez-Miret, & Heredia, 2007; Lopes Da Silva et al., 2007; Terry et al., 2007). The total anthocyanin contents found in this study were lower than the contents reported in five strawberry cultivars by Lopes Da Silva et al. (2007), but slightly higher than the values reported by Hernanz et al. (2007). Despite the fact that different genotypes were used in each study, the higher values obtained by Lopes da Silva et al. (2007) may be partially explained by the different extraction procedure used, as the extraction was repeated several times until complete removal of the colour was achieved. In contrast, in the present study, and as earlier reported by Hernanz et al. (2007), only one extraction step was performed, despite still achieving total removal of the colour from the extracts. Further pelargonidin derivatives such as pg 3-rutinoside, pg 3-malonylglucoside and pg 3-acetylglucoside have been identified in strawberries by other authors (Aaby et al., 2007; Hernanz et al., 2007; Lopes da Silva et al., 2007; Yoshida et al., 2002). The elution order of the pelargonidin derivatives and their occurrence in strawberry cultivars reported by others strongly suggest pg derivative 1 to be pg 3-rutinoside. However, to further corroborate this assumption, the identification of the substituting sugar by mass spectra analysis would be necessary.

The total antioxidant capacity measured in fruits depends on the presence of oxygen radical scavengers such as phenolic compounds and vitamin C presents in the fruit tissues. Antioxidant

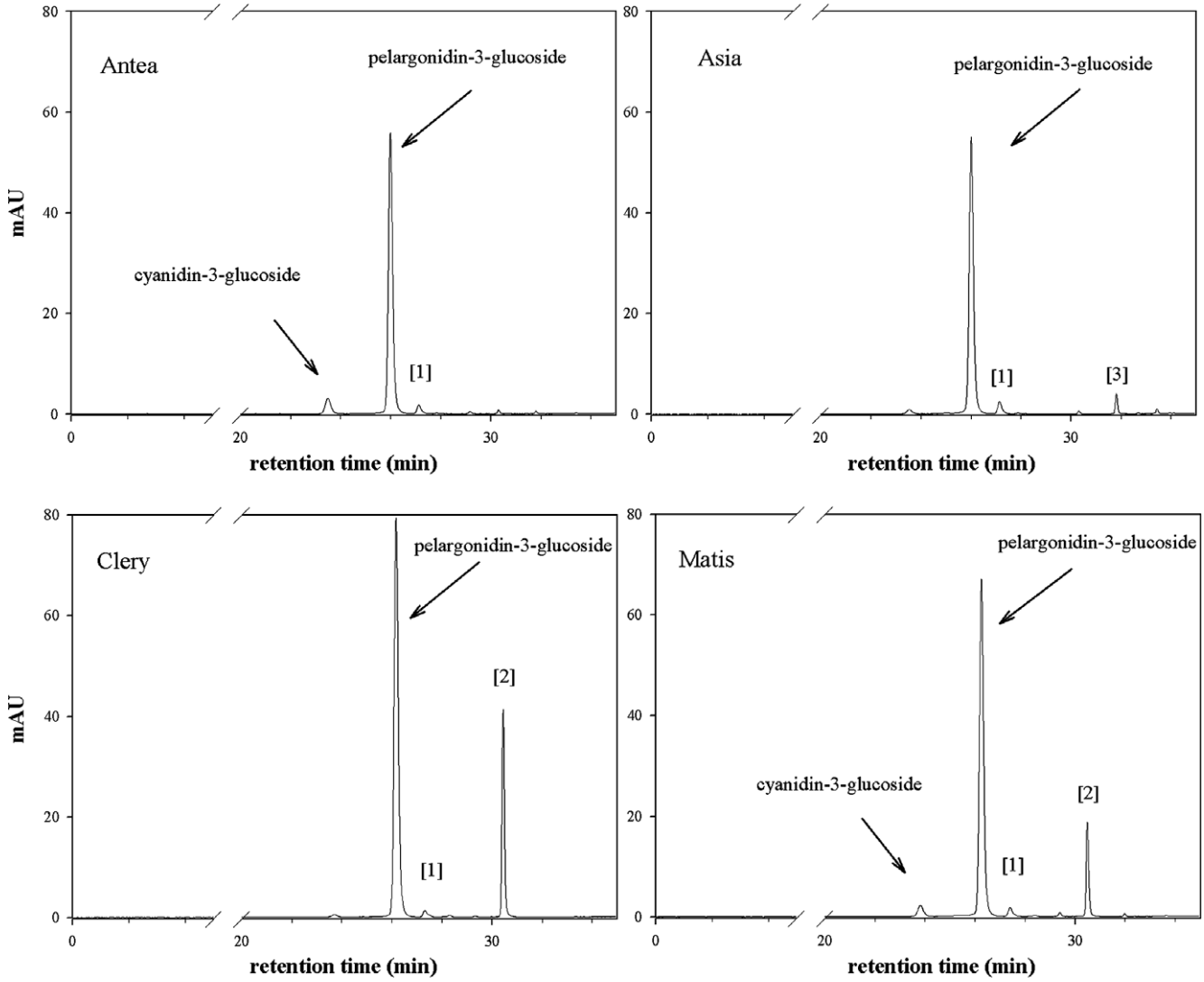

Fig. 1. Detected anthocyanins in four strawberry cultivars by means of HPLC–DAD (520 nm). Unknown peaks [1], [2] and [3] were identified as three pg derivatives according to their UV spectra.

capacity, on a FW basis, was highest in the fruits from cv. Antea (13.3 μmol TE/g) followed by Asia and Clery (both 9.8 μmol TE/g) and lowest in cv. Matis (7.6 μmol TE/g) (Table 4). The antioxidant capacity correlated strongly with the total vitamin C content of the samples (r^2 = 0.84) but not with their total or individual anthocyanin content (r^2 < 0.1).

3.2. Effect of production sites on agronomical traits and fruit taste- and health-related compounds

Agronomical traits such as yield, harvest duration and leaf area per plant were significantly affected by the production site (Table 5). Generally plants grown in the mountain region of Bruson had a higher mean yield (133 g more per plant), a shorter harvest period (4.7 days less) and a larger leaf area (525 cm^2 more per plant), compared to the plants grown in Conthey. Furthermore, cvs. Antea, Clery and Matis showed a higher leaf area/yield ratio when cultivated in Bruson. However, the higher leaf area/yield ratio in Bruson did not increase the content of sugars in the fruits compared to Conthey (Table 2). Despite the soluble solid content not always being well correlated with actual sugar concentrations in strawberry (Giné Bordonaba & Terry, 2009) or other berry fruits (Giné Bordonaba & Terry, 2008), this finding was in contrast with earlier findings by Carlen, Potel, and Ançay (2007), where leaf area/yield ratio of strawberry cultivars was positively related to their fruit soluble solid content. Cloudier weather with more rain and less sunshine during the ripening period (Table 1) or the higher yield per day (Table 5) observed in Bruson, may also account for the discrepancies between this and earlier works (Carlen et al., 2007). These trends were consistent for all the cultivars except for cv. Asia, where high leaf area/yield ratio was recorded in both production sites, probably due to the low yield of this cultivar and the late plantation of plug plants.

The production site had a significant effect on the content of monosaccharides in the different cultivars investigated. This said, such an effect was observed to be genotype specific (Table 2) with greater differences between production sites encountered in cv. Antea. Specifically, fruits from this cv. showed significantly lower glucose and fructose content when plants were grown in the mountain region and hence potentially could have resulted in lower sweetness of the fruits (Table 2). Similarly, other studies (Giné Bordonaba & Terry, accepted for publication; Terry et al., 2007) have shown that preharvest factors generally resulted in changes in the monosaccharide, but not the sucrose concentration of the

Table 4
Anthocyanins contents and antioxidant capacity of strawberry fruits on a fresh weight (FW) and dry weight (DW) basis from different cultivars and production sites.

Cultivar	Site	Cya 3-gluc (μg/g FW)	Pg 3-gluc (μg/g FW)	Pg derivative 1 (μg/g FW)	Pg derivative 2 (μg/g FW)	Pg derivative 3 (μg/g FW)	Total anthocyanins (μg/g FW)	Antioxidant capacity (μmol TE/g FW)
Antea	Conthey	7.1 a	146 a,b,c	5.1 b	0	0	158 c,d	15.2 a
	Bruson	2.1 c,d	112 c	3.2 c	0	0	118 d	11.4 b
Asia	Conthey	0.0 d	177 a,b	8.4 a	0	3.8 a	189 b,c	9.8 b,c,d
	Bruson	0.9 d	187 a	8.2 a	0	4.9 a	201 a,b,c	9.8 b,c,d
Clery	Conthey	0.0 d	167 a,b	5.1 b,c	50.0 a	0	222 a,b	10.6 b,c
	Bruson	0.0 d	187 a	3.8 b,c	59.6 a	0	251 a	8.9 c,d,e
Matis	Conthey	4.9 a,b	140 b,c	4.1 b,c	22.5 b	0	172 b,c,d	8.3 d,e
	Bruson	3.7 b,c	141 b,c	4.1 b,c	24.9 b	0	174 b,c	6.9 e
	Cultivar (C)	***	***	***	***	***	***	***
	Site (S)	**	ns	**	*	ns	ns	***
	C × S	***	ns	ns	ns	ns	*	*
		Cya 3-gluc (μg/g DW)	Pg 3-gluc (μg/g DW)	Pg derivative 1 (μg/g DW)	Pg derivative 2 (μg/g DW)	Pg derivative 3 (μg/g DW)	Total anthocyanins (μg/g DW)	Antioxidant capacity (μmol TE/g DW)
Antea	Conthey	87.6 a	1796 a,b	63.5 b	0	0	1947 b	187 a
	Bruson	35.0 b,c	1775 b	51.1 b	0	0	1862 b	183 a
Asia	Conthey	0	2051 a,b	98.2 a	0	44.1 a	2193 b	114 b
	Bruson	11.3 c	2247 a,b	97.8 a	0	60.0 a	2417 b	118 b
Clery	Conthey	0	2103 a,b	64.1 b	630 b	0	2797 a,b	134 a,b
	Bruson	0	2587 a	51.6 b	822 a	0	3460 a	123 b
Matis	Conthey	73.6 a	2105 a,b	60.8 b	338 c	0	2577 a,b	124 b
	Bruson	57.2 a,b	2163 a,b	62.4 b	383 c	0	2665 a,b	106 b
	Cultivar (C)	***	*	***	***	***	***	***
	Site (S)	*	ns	ns	*	ns	ns	ns
	C × S	**	ns	ns	ns	ns	ns	ns

Antioxidant capacity is expressed in μmol Trolox equivalents (TE) per g fresh weight or dry weight.
Different letters in the same column indicate significant differences. Significant parameters are indicated as follows.
ns, Not significant.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

Table 5
Strawberry yield, harvest duration, yield per day, leaf area, leaf area/yield ratio of different cultivars and production sites.

Cultivar	Site	Yield (g/plant)	Harvest duration (days)	Yield per day (g/day)	Leaf area per plant (cm^2)	Leaf area/yield ratio (cm^2/g)
Antea	Conthey	309 b,c,d	24 b,c	13.2 b,c	2056 c	6.6 c
	Bruson	546 a,b	20 c	27.9 a	7086 a	13.1 a,b
Asia	Conthey	173 d	22 c	8.1 c	3238 c	18.9 a
	Bruson	249 c,d	20 c	12.7 b,c	4119 b,c	17.0 a
Clery	Conthey	487 a,b,c	29 a,b	16.9 a,b,c	4209 b,c	9.3 b,c
	Bruson	436 a,b,c d	22 c	20.2 a,b	7035 a	16.3 a
Matis	Conthey	386 b,c,d	32 a	11.9 b,c	1927 c	5.2 c
	Bruson	661 a	26 b,c	26.0 a	6222 a,b	9.6 b,c
	cultivar (C)	***	***	**	*	***
	site (S)	**	***	***	***	**
	C × S	*	ns	*	**	*

Different letters in the same column indicate significant differences. Significant parameters are indicated as follows.
ns, Not significant.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

fruit. Thus, such changes may indicate the impact of preharvest conditions on respiratory metabolism, in which sugars, including glucose and fructose, are the main substrates.

Organic acid content of the fruits was generally affected by production site however no genotype × production site interaction was found and the observed differences were mainly in the fresh matter (Table 3). Significant differences among production sites were found for citric acid, in which for all cvs. lower values (4.1 mg/g FW *vs.* 4.9 mg/g FW, mean values for all cultivars) were observed in fruits from plants grown in the region of Conthey. There was a clear trend towards greater malic acid content, on a DW basis, in fruits obtained in the mountain region, except for cv. Matis, where the opposite was observed (Table 3). Similarly, Zheng et al. (2009) found significant differences in both malic and citric acid but not for ascorbic and total acid content in blackcurrant berries grown at two different latitudes; the observed effects were also cultivar dependent. In addition the same authors found citric and total acid concentrations to be negatively correlated with high humidity. Davik et al. (2006) found similar results with the acid content of strawberries being positively influenced

by the radiation and the number of sunshine hours the day before the harvest.

Vitamin C is often regarded as one of the main health-related compounds present in strawberry fruits and is mainly responsible for the antioxidant capacity of strawberry fruits as shown earlier. Its content was detrimentally affected when plants were grown in the mountain region of Bruson, with values *ca.* 1.2-fold lower than those obtained in the lower region (Table 3). Furthermore in Bruson, the plants produced a higher yield during a shorter period (Table 5). Thus, a negative correlation was found between strawberry yield per day (g/day) and the total vitamin C in fresh weight for each cultivar (Antea, $r^2 = 0.92$; Asia, $r^2 = 0.57$; Clery, $r^2 = 0.74$; Matis, $r^2 = 0.69$). The differences between production sites levelled off when considering vitamin C content on a dry matter basis (Table 3) suggesting a dilution of vitamin C in the fruits.

The relative distribution of the anthocyanin compounds present in each cultivar was consistent for the different production region. This result suggests that anthocyanin profile is mainly genetically inherited rather than being affected by external environmental factors. Similar results were found by Carbone et al. (2009) when the flavonoid composition of different strawberry genotypes grown at different locations within Italy was compared. The authors found that the general variation for anthocyanins in strawberry ripe fruits was affected largely by genetic background rather than by environmental factors.

The concentration of minor anthocyanins on a fresh weight basis such as cya 3-gluc, pg derivative 1 and pg derivative 2 was significantly affected by the production site (Table 4). The differences were still significant when considering the concentration in the dry matter for cya 3-gluc and pg derivative 2 but not for pg derivative 1. Cv. Antea showed lower cya 3-gluc contents in both fresh and dry matter when cultivated in Bruson, while higher contents of pg derivative 2 in the dry matter were observed in cv. Clery. In contrast, the content of the main strawberry anthocyanin, pg 3-gluc and accordingly the total anthocyanin content was not particularly affected by the location where the plants were grown. To date no other studies have analysed the impact that production sites, principally differing in altitude, have on strawberry anthocyanins. Nevertheless, a similar study was conducted on wild populations of *Vaccinium myrtillus* (Rieger et al., 2008), in which lower amounts of anthocyanins were found in the berries collected at higher altitude regions.

The variability in the composition of fruits from different cultivars grown in different regions strongly suggests that cultivars should be carefully selected for each production site. Based on one year data, the results presented in this work suggested that the newly released cv. Clery may be a suitable cultivar for its high anthocyanin levels as well as for the great stability of its chemical composition regardless of the cultivation site. In this context, the selection of environmentally-adaptable cultivars such as cv. Clery, may be of crucial importance given the expected effects that general climate changes and yearly climatic variation may have on berry production within Europe or elsewhere. Similarly, the high ascorbic acid content of cv. Antea could be a promising attribute; however its potential sensitivity to production site has to be taken into consideration when selecting this cultivar. Finally, the results presented in this work corroborate the dominant role of strawberry genotype over environmental factors. Further work should address the impact that other environmental factors, as well as the year-to-year variation, may have on the final quality of strawberry fruits.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.foodchem.2010.02.010.

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