

Morphological, nutraceutical and sensorial properties of cultivated *Fragaria vesca* L. berries: influence of genotype, plant age, fertilization treatment on the overall fruit quality

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Sucrose, glucose, fructose, citric, malic, ascorbic (AA) and dehydroascorbic (DHAA) acids, total polyphenols (TP), radical scavenging activity (RSA), physicochemical and sensorial properties were determined on *F. vesca* Alpine (ALP) and Regina delle Valli (RDV) berries in relation to plant age and fertilisation treatment (Effective Microorganism Technology, EMT vs. traditional fertilization, TFT). ALP berries had a sum of AA and DHAA about 20% lower and TPs about 30% higher than RDV. Plant age affected most physicochemical parameters, sugars and organic acids, as well as sensorial appreciation, being them generally higher in berries produced in the second year. TPs were not affected by plant age. EMT produced an increase of 50%, 70% and 20% for TP, organic acids and RSA, respectively. Although changes in berry quality are expected with plant age, EMT cultivation of ALP should be preferred to the growth of RDV under TFT, to obtain fruits more valuable from the nutraceutical viewpoint.

Key words: total polyphenols, radical scavenging activity, vitamin C, organic acids, strawberry

Introduction

Wild strawberry (*Fragaria vesca* L.) is a spontaneous species widely diffused in the temperate areas of Eurasia and North America. Although official data on its cultivations are not currently available, an Italian annual production of few hundred tons, mainly with the cultivar Regina delle Valli (RDV) and, secondarily, with Alpine (ALP), can be estimated; other varieties (e.g. “Sara” and “Valitutto”) are seldom adopted for cultivation in Italy (Doumett et al. 2011).

F. vesca berries show peculiar sensorial attributes, compared to *Fragaria x ananassa* fruits; among them, a much more intense flavour, sweetish-aroma and some astringent and bitter impressions were evidenced by Descriptive Sensory Analysis with trained panels (Ulrich et al. 2007).

Beyond sensorial traits, the growing interest in the production of *F. vesca* berries, is related to their valuable nutritional and nutraceutical properties. In fact, previous studies indicated that *F. vesca* fruits have mean sugar concentrations twice or three times higher than those observed for *Fragaria x ananassa* strawberries (Ruiz-Nieto et al. 1997, Caruso et al. 2004a, Crespo et al. 2010, Doumett et al. 2011). Mean concentrations of citric acid determined in *F. vesca* berries resulted similar or higher than those found for *Fragaria x ananassa* fruits (Perez et al. 1997, Basson et al. 2010, Crespo et al. 2010, Doumett et al. 2011); a similar trend was also observed for malic acid (Perez et al. 1997, Crespo et al. 2010). Conversely, total vitamin C in *F. vesca* was found to be 30–70% lower than in *Fragaria x ananassa* (Aaby et al. 2007, Corral-Aguayo et al. 2008, Basson et al. 2010, Crespo et al. 2010, Doumett et al. 2011).

A quite comprehensive picture of polyphenolic compounds of *F. vesca* berries was recently reported by different authors (Del Bubba et al. 2012, Vrhovsek et al. 2012, Sun et al. 2014), highlighting a very complex composition. According to several studies, mean concentration of total polyphenols of *F. vesca* is higher than that found for *Fragaria x ananassa* fruits (Halvorsen et al. 2002, Pellegrini et al. 2003, Milivojevic et al. 2011, Mikulic-Petkovsek et al. 2012, Milivojevic et al. 2013, Zugic et al. 2014); similar patterns were found for antiradical activity (Jablonska-Rys et al. 2009, Doumett et al. 2011).

It is well-assessed that pre-harvest factors such as cultivar, cultural practice and plant age have a significant effect on attributes associated to sensorial traits (e.g. berry size, colour, taste), as well as on nutritional and nutraceutical components (e.g. sugars, organic acids, polyphenols) of various fruits (Mattheis and Fellman 1999, Lamperi et al. 2008, Giordani et al. 2011), including the cultivated strawberry *Fragaria x ananassa* (Alvarez-Suarez et al. 2014).

Recently, the impact of various pre-harvest factors on the quality of *Fragaria vesca* berries have been also evaluated. Del Bubba et al. (2012) and Caracciolo et al. (2013) compared the fruit quality of different cultivars and biotypes (i.e. “Sara” vs. RDV; “Valitutto” vs. ALP; RDV vs. “Fragolina di Maletto” vs. “Fragolina di Ribera”), evidencing the effect of genotype on fruit characteristics, polyphenol composition and antioxidant activity. Environment and cultivation practices have been also highlighted as factors affecting the content of bioactive compounds in wild strawberry, with variations in vitamin C, total polyphenols, antiradical activity and especially sugars and organic acids (Doumet et al. 2011).

Among cultural practices, Effective Microorganism Technology (EMT) is a fertilization technique based on the addition to soil of a mixed culture of bacteria, yeasts, actinomycetes and fermented fungi, aiming at improving yields and quality of crops by enhancing soil microbial biodiversity (Higa and Wididana 1991a, Siqueira et al. 1993, Higa and Parr 1994). EMT has been successfully tested on spinach and tomato (Higa and Wididana 1991b), as well as capsicum, bean and carrot (Siqueira et al. 1993). In this regard, it should be highlighted that a literature overview performed with different research engines (i.e. Scopus, SciFinder, Google Scholar) did not evidence any information on the effect of EMT on strawberry production and quality.

According to the aforementioned considerations, the aim of this paper was the determination of morphological characteristics, sensorial properties and major nutritional and nutraceutical components (sugars, organic acids, vitamin C and polyphenols), together with antiradical activity, in ALP and RDV berries obtained in the same location, in three consecutive years and under two different fertilization treatments. One commercial sample (CS) of unknown cultivar was also included in the study. To the best of the author’s knowledge, this study is the first one giving information on a very wide spectrum of physical, chemical, nutritional, nutraceutical and sensorial parameters of cultivated *F. vesca* berries, in relation to genotype, plant age and fertilisation treatment and it is planned to increase the knowledge in the field of wild strawberry cultivation, providing original and significant information on the effect of the investigated pre-harvest parameters on the overall quality of fruits.

Materials and methods

Cultivation system, study design and fruit sampling

Commercially certified ever-bearing “Alpine” (ALP) and “Regina delle Valli” (RDV) *F. vesca* plants were planted at the end of June 2012 and cultivated under organic regime until the end of 2014 in a private farm located in Cireglio (Tuscany, Italy; N43°44.105'; E012°06.013') at 657 m a.s.l. Soil had a silt-loam texture, acidic reaction (pH = 5.8 in H₂O; pH = 4.4 in KCl 1M), low cationic exchange capacity (90 meq kg⁻¹) and a good P availability (50 mg kg⁻¹); the content of organic macro-elements, determined by elemental CHN flash combustion microanalysis with a Carlo-Erba NA 1500 (Milan, Italy) analyser, resulted 2.9% for C, 0.78% for H and 0.26% for N. Before planting, soil was ploughed and fertilized with 1.5 kg m⁻² of guano pellet (Naturfert, PK 6/12), drawn in six 30 cm high cambered rows distanced by 1.2 m and covered by a mulching net. A randomized block design consisting of 16 plots (8 planted with ALP and 8 with RDV) of 12 plants each, surrounded by 2 guard plant rows for each block side, was adopted for the trial. For each cultivar, 4 blocks were treated with EMT by distributing 100 ml of a 10% EMA + 3 g l⁻¹ of EM-X ceramic powder (Lorch 2008) per plant every year, whereas the other 4 blocks were untreated (following defined as “traditional fertilization treatment” – TFT).

Fruits considered “marketable” (ripen ready-to-eat berries, characterized by a red colour all over the fruit) were collected during the maximum productive peaks, which occurred in the last week of September 2012 and June 2013 for the first and second crop cycle, respectively. An additional fruit collection was planned for 2014, but fruit production was very low and not sufficient to carry out the scheduled measures. Hence, the following treatments were investigated: ALP-2012-TFT, ALP-2012-EMT, RDV-2012-TFT, RDV-2012-EMT, ALP-2013-TFT, ALP-2013-EMT, RDV-2013-TFT and RDV-2013-EMT.

Photosynthetically active radiation (PAR), air temperature (T) and relative humidity (H) were hourly determined using a WatchDog 2700 Weather Station (Spectrum Technologies, Aurora, IL, USA); monthly mean values regarding the years 2012–2014 are reported in Table 1.

For each experimental unit, approximately 400 g (fresh weight, f.w.) of strawberries (the result of a random sampling from the 4 blocks) were collected and used for the sensorial evaluation and the analysis.

Table 1. Mean daily values of photosynthetically active radiation (PAR, MJ m⁻² d⁻¹), temperature (T, °C) and relative humidity (H, %) measured in the proximity of the *Fragaria vesca* cultivation plant; n.d. = data not determined

	PAR			T			H		
	2012	2013	2014	2012	2013	2014	2012	2013	2014
Jan	n.d.	3.7	3.8	n.d.	8.6	8.1	n.d.	84.4	87.8
Feb	n.d.	4.8	4.4	n.d.	2.5	1.9	n.d.	74.0	81.2
Mar	n.d.	5.9	6.2	n.d.	5.6	4.8	n.d.	79.4	74.2
Apr	n.d.	9.4	9.1	n.d.	11.7	11.4	n.d.	72.4	77.9
May	n.d.	10.3	11.1	n.d.	12.4	12.9	n.d.	79.6	71.3
Jun	n.d.	12.5	12.1	n.d.	16.4	16.9	n.d.	77.5	68.9
Jul	15.7	14.0	n.d.	21.4	19.7	n.d.	61.3	70.6	n.d.
Aug	13.9	13.1	n.d.	23.5	23.9	n.d.	52.7	50.1	n.d.
Sep	9.5	9.1	n.d.	18.7	18.4	n.d.	68.0	70.2	n.d.
Oct	5.3	5.1	n.d.	13.1	12.7	n.d.	85.2	83.4	n.d.
Nov	4.2	3.8	n.d.	11.1	13.4	n.d.	84.3	76.7	n.d.
Dec	3.5	3.1	n.d.	9.0	9.2	n.d.	78.5	72.3	n.d.

Each fruit sample was split into four sub-samples for the analysis of the following groups of parameters:

Group 1: weight, size, flesh hardness and skin colour of whole berries

Group 2: refractometric solid residue (RSR), pH and total titratable acidity (TA) of fruit juice

Group 3: sugars, organic acids, total polyphenols (TP) and radical scavenging activity (RSA)

Group 4: fruit sensorial parameters as described by panel taste analysis

All the aforementioned parameters were also measured on one CS of *F. vesca* berries purchased in a local market in 2012.

Analysis of Group 1 parameters

Weight (g) was measured with a Sartorius TE 150/2s balance (Sartorius AG, Göttingen, Germany) and diameter (mm) with a hand calliper; colour (L, a, b coordinates) was determined with a Minolta Chromameter CR200 (Konica Minolta, Tokyo, Japan) electronic colorimeter; flesh hardness was measured with a TR 53205SW (TR Turoni, Forlì, Italy) hard-meter. All the above-mentioned parameters were measured on 10 fruits for each experimental unit.

Analysis of Group 2 parameters

RSR, pH and TA were assessed on three samples, each one obtained from 15 fruits for each experimental unit. TA (expressed as mg citric acid kg⁻¹ f.w.) was determined at pH 8 with a 0.1 M solution of NaOH, adopting a Basic 20-Crison (Barcelona, Spain) pH meter; RSR (°Brix) was quantified with a hand refractometer (Atago N1-Atago Co., Tokyo, Japan). RSR/TA ratio was calculated and expressed throughout the manuscript as 10·RSR/TA, to make it comparable with literature (Keutgen and Pawelzik 2007).

Analysis of Group 3 parameters

When strawberries were delivered to the laboratory for biochemical analyses (about one hour after harvesting), about 200 g f.w. of fruits free of defects were immediately frozen in liquid nitrogen, freeze-dried to constant weight and finally kept at -80 °C until analyses were performed. The analysis of each sample was replicated five times.

Reagents, solvents and materials

Standards (purity ≥ 99%) of (+)-catechin, glucose, fructose, sucrose and citric, L-malic, L-ascorbic (AA), dehydroascorbic (DHAA) and D-isoascorbic acids were supplied from Sigma-Aldrich (St. Louis, MO, USA).

Hexadecyltrimethylammonium bromide and 1,2-phenyldiamine were purchased from Sigma-Aldrich. HPLC grade methanol, acetic acid and water were purchased from J.T. Baker (Deventer, Holland). Sodium fluoride, sodium carbonate, sodium hydroxide, orthophosphoric acid, sodium dihydrogen phosphate, EDTA, Folin-Ciocalteu (F-C) reagent and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical were obtained from Merck (Darmstadt, Germany). Ultrapure water was taken from a MilliQ system supplied by Millipore (Billerica, MA, U.S.A.). Polytetrafluoroethylene (PTFE) filtration membranes (porosity 0.2 μm) were obtained from Sartorius. Supelclean LC-18 SPE tubes (6 ml, 1 g) were supplied by Supelco (Bellefonte, Pa, USA).

Analytical procedures

Extraction and HPLC analysis of the two forms of vitamin C (AA and DHAA) were performed according to Zapata and Dufour (1992) and following the experimental specifications of Doumett et al. (2011). Under the chromatographic conditions herein adopted the recovery percentage, calculated by means of D-isoascorbic acid as reference standard, was 96.1 ± 5.8 .

The extraction of sugars, organic acids and TP was simultaneously carried out according to Lamperi et al. (2008). Sugars and organic acids were determined by HPLC after application of 500 μl -aliquots of the extracts on the top of Supelclean LC-18 SPE Tubes (preconditioned with 10 ml of methanol and 10 ml of ultrapure water acidified at $\text{pH} = 4$ with acetic acid) and eluted with 2.5 ml of water. Recoveries of the extraction procedure for sugars, organic acids and TP were evaluated with the following procedure: five sequential extractions, each one with 15 ml-aliquots of methanol/water solution 8/2 (v/v) containing 10 mM NaF, were carried out on five test samples and sugars, organic acids and F-C TPs were determined in each extract, after fractionation on C18 tubes. The results showed that the fifth extraction accounted for less than 5% of the whole recovery, for TP and organic acids, while sugars were completely recovered after three extractions. Hence, four sequential extractions were chosen for the recovery of analytes.

The chromatographic analysis of sugars and organic acids was performed according to Doumett et al. (2011).

TPs were spectrophotometrically determined using (+)-catechin as a reference standard according to Doumett et al. (2011), expressing the results as mg of (+)-catechin per 100 g of dry weight (d.w.).

RSA was spectrophotometrically tested using the free radical DPPH determination according to Brand-Williams et al. (1995), as modified by Doumett et al. (2011).

Analysis of “Group 4” parameters

Fruits were evaluated by a trained panel of 9 judges (three men and six women). Panellists were selected in relation to their tasting practice and experience in fruit field. During preliminary sessions, panellists were trained, following the ISO 3972:2011 for taste tests, and the ISO 5496:2006 for olfactory analysis. In the initial sessions different kind of berries (strawberry, blueberry, raspberry, blackberry, currant), including two samples of *F. vesca* were presented to panellists, in order to determine and refine appropriate descriptors, using a consensus method. During the formal rating sessions, panellists were asked to evaluate the *F. vesca* samples, presented in a randomized block design, in two replicates.

The following fruit samples were included in the Descriptive Sensory Analysis of this study: ALP-2012-TFT, ALP-2012-EMT, RDV-2012-TFT, RDV-2012-EMT, ALP-2013-TFT, RDV-2013-TFT and CS. A profile sheet based on a 9 point discontinuous scale was elaborated considering the evaluation of colour hue (considered from orange to purple), overall fruity odour, consistency, graininess, sour and sweet sensory attributes, together with an overall appreciation score.

Statistical analysis

One-way analysis of variance (ANOVA), Duncan and Dunnett T3 multiple comparison tests, as well as principal component analysis (PCA) and linear univariate correlations between variables were performed on the original physicochemical, nutritional and nutraceutical data by using the statistical package SPSS, version 20.0 for Windows (SPSS Inc., Chicago, IL, USA).

Panel performance was assessed using PanelCheck (PC, Matforsk) software and included analysis of variance for the effect of sample, judge, as well as presentation replicate and their interactions, degree of agreement with the panel mean, and degree of discrimination across samples (Tomic et al. 2007, Dahl and Næs 2009). PCA of mean sensorial parameters was also performed.

Results

Overall quality of ALP, RDV and CS berries

Physicochemical analyses

Physicochemical properties exhibited a different variability, depending on the considered parameter (Table 2). Hardness, f.w., TA, as well as the RSR-to-TA ratio, showed by far the highest values of relative standard deviation (RSD = 35–46%), being them respectively in the ranges of 26–59 g, 0.8–2.0 g, 3.14–8.45 g citric acid kg⁻¹ and 10.2–30.3 °Brix g⁻¹ citric acid kg. The fruit diameter ranged from 10.1 to 17.7 mm, thus evidencing a lower, even though significant, variability (RSD = 23%). The CIELAB spatial coordinates measured on strawberry skin were in the ranges of 34–42, 29–39 and 21–40 for brightness (L), green to red (a) and blue to yellow (b) axes. These latter coordinates corresponded to a fully red zone of the CIELAB colour chart (see Fig. S1). Much more homogeneous data were measured for RSR (8.1–9.5 °Brix) and pH (3.3–4.1), for which RSD values of 7–8% were observed. Physicochemical data of the CS were in the range found for ALP and RDV berries, with the exceptions of hardness and RSR, which exhibited much higher values (Table 2).

Biochemical analyses

Total sugar concentrations found in the ALP and RDV samples ranged from 1.90 to 2.30 mmol g⁻¹ d.w. with the CS well included in this range (Table 3). In all samples investigated, the concentrations of the individual measured carbohydrates were in the order fructose > glucose >> sucrose.

Concentrations of citric acid were found to be significantly higher than those of malic acid. Concentrations determined in ALP and RDV berries for malic acid and, especially, citric acid were characterized by a high variability (RSD equal to 23% and 32%, respectively). The CS evidenced a citric acid concentration similar to the highest values found in ALP and RDV berries, whereas a much higher abundance was determined for malic acid. Citric acid concentrations, as well as malic acid ones and their sum, were linearly related with TA values ($r^2 \geq 0.57$; $p \leq 0.001$).

Figure 1 shows the values of the sugar-to-acid ratio calculated from the concentrations of individual carbohydrates and acids reported in Table 3.

For ALP and RDV berries the sum of AA and DHAA concentrations (following indicated as “total vitamin C”) was in the range of approximately 1.3–1.8 mg g⁻¹ d.w. AA represented the main contribution, accounting for about 65% of total vitamin C. Surprisingly, the CS evidenced an extremely low content of both AA (0.18 mg g⁻¹ d.w.) and DHAA (0.12 mg g⁻¹ d.w.). TP ranged from about 29 to 60 mg catechin equivalent g⁻¹ d.w., thus evidencing a high variability among the eight ALP and RDV samples analyzed (RSD = 28%). TP measured in the CS were found in the lower limit of the above-reported range. DPPH-RSA showed a great variability, as well (RSD = 33%). RSA was strongly related with TP ($r^2 = 0.97$; $p < 0.001$).

Sensorial analysis

According to ANOVA, the investigated samples showed statistically significant differences ($p < 0.05$) for all the sensorial parameters evaluated. Graphical results of PCA are illustrated in Figure 2.

The first two components accounted for 88.9% of the total variance and can be therefore considered as representative of the whole data set. PC1 was mainly described by “colour hue”, “consistency” (negative correlation) and “fruity odour” (positive correlation), whereas for PC2 the main contributions were given by the positive correlations with “graininess”, “sourness”, “sweetness” and “overall appreciation”. Overall “appreciation” resulted positively related ($r^2 = 0.96$) with “sweetness” and uncorrelated to “sourness” ($r^2 = 0.004$). A similar sensorial test, performed elsewhere on *Fragaria x ananassa* cultivars, highlighted that “overall fruit liking” had a strong association with “sweetness intensity” ($r^2 = 0.74$), and an almost null correlation ($r^2 = 0.008$) with “sourness” (Schwieterman et al. 2014).

Table 2. Mean values ± standard deviation of selected physicochemical parameters, determined in Alpine (ALP) and Regina delle Valli (RDV) *F. vesca* strawberries (fresh weight; maximum diameter; hardness; CIELAB spatial coordinates: L – brightness axis, a – green to red axis, b – blue to yellow axis; pH; refractometric solid residue, RSR; titratable acidity, TA; and 10·RSR/TA ratio) harvested in two consecutive years (2012 and 2013) and grown under two different fertilisation treatments (EMT – Effective Microorganism Technology; TFT – Traditional Fertilisation Treatment). Data of one commercial sample (CS) are also presented. For each measured parameter, general mean ± standard deviation and relative standard deviation (RSD%) are also reported (n=80 or n=24 for fruits or juices, respectively). Within each parameter, values with at least one common letter are not statistically different according to the Duncan multiple comparison test ($p < 0.05$). All values referred to fresh weight.

Sample	Fresh weight (g) (n=10)	Max diameter (mm) (n=10)	Hardness (g) (n=10)	L (n=10)	a (n=10)	b (n=10)	pH (n=3)	RSR (°Brix) (n=3)	TA (g citric acid kg ⁻¹) (n=3)	10 (RSR/TA) (n=3)
ALP-2012-TFT	1.0±0.1 (a)	11.3±0.5 (a)	34±6 (ab)	37±1 (ab)	32±3 (a)	26±5 (a)	3.6±0.1 (ab)	9.5±0.1 (a)	3.14±0.06 (a)	30.3±1.6 (a)
ALP-2012-EMT	1.0±0.1 (a)	10.9±0.6 (a)	26±5 (a)	34±1 (a)	29±4 (b)	22±6 (b)	4.1±0.1 (a)	9.5±0.1 (a)	4.10±0.13 (b)	23.2±1.5 (b)
RDV-2012-TFT	1.0±0.1 (a)	11.0±0.5 (a)	29±6 (a)	34±1 (a)	32±3 (a)	25±3 (c)	3.9±0.1 (a)	9.5±0.1 (a)	3.46±0.06 (a)	27.5±1.5 (a)
RDV-2012-EMT	0.8±0.1 (a)	10.1±0.4 (a)	26±5 (a)	34±1 (a)	29±7 (b)	21±9 (d)	3.8±0.1 (ab)	9.5±0.1 (a)	5.76±0.06 (c)	16.5±1.5 (c)
ALP-2013-TFT	2.0±0.2 (b)	17.7±0.3 (b)	58±5 (c)	44±1 (c)	38±3 (c)	36±4 (e)	3.4±0.1 (b)	9.1±0.3 (ab)	8.45±0.13 (e)	10.8±1.5 (d)
ALP-2013-EMT	1.4±0.1 (c)	14.8±0.7 (c)	56±5 (c)	38±1 (b)	37±3 (d)	31±4 (f)	3.3±0.1 (b)	8.3±0.3 (b)	7.74±0.06 (d)	10.7±1.5 (d)
RDV-2013-TFT	2.2±0.2 (b)	16.7±0.8 (b)	47±4 (bc)	42±1 (c)	39±2 (c)	40±9 (g)	3.3±0.1 (b)	8.1±0.3 (b)	7.94±0.26 (d)	10.2±1.5 (d)
RDV-2013-EMT	1.8±0.2 (b)	16.3±0.7 (b)	59±5 (c)	40±2 (bc)	37±6 (d)	34±9 (h)	3.4±0.1 (b)	8.3±0.2 (b)	8.02±0.32 (de)	10.4±1.5 (d)
General mean	1.4±0.5 (38%)	14±3 (23%)	42±15 (35%)	38±4 (10%)	34±4 (12%)	29±7 (24%)	3.6±0.3 (8%)	9.0±0.6 (7%)	6.08±2.24 (37%)	17.4±8.1 (46%)
CS	1.1±0.2 (ac)	11.2±0.9 (a)	72±24 (c)	43±6 (bc)	32±3 (a)	29±4 (de)	3.7±0.2 (ab)	12.2±0.2 (c)	7.04±0.65 (d)	17.3±1.6 (c)

Table 3. Mean values ± standard deviation (n=5) of sucrose, glucose, fructose, total sugars (TS) citric acid, malic acid, ascorbic acid (AA) dehydroascorbic acid (DHAA) and total polyphenol (TP, expressed as (+)-catechin) concentration, and DPPH radical scavenging activity (RSA) of Alpine (ALP) and Regina delle Valli (RDV) *F. vesca* strawberries harvested in two consecutive years (2012 and 2013) and grown under two different fertilisation treatments (EMT - Effective Microorganism Technology; TFT - Traditional Fertilisation Treatment). Data of one commercial sample (CS) are also presented. For each measured parameter, general means ± standard deviation and relative standard deviation (RSD%) are also reported. All data referred to dry weight. Within each parameter, values with at least one common letter are not statistically different according to the non-parametric Dunnett T3 multiple comparison test ($p < 0.05$).

Sample	Sucrose (mg g ⁻¹)	Glucose (mg g ⁻¹)	Fructose (mg g ⁻¹)	TS (mmol g ⁻¹)	Citric acid (mg g ⁻¹)	Malic acid (mg g ⁻¹)	AA (mg g ⁻¹)	DHAA (mg g ⁻¹)	Total Vitamin C (mg g ⁻¹)	TP (mg g ⁻¹)	RSA (µg DPPH inhibited µg ⁻¹)
ALP-2012-TFT	66±9 (a)	158±12 (a)	178±14 (a)	2.1±0.1 (a)	23±2 (a)	4.7±0.5 (a)	0.89±0.08 (ab)	0.40±0.08 (a)	1.29±0.11 (a)	32.9±5.1 (ac)	0.23±0.02 (a)
ALP-2012-EMT	73±10 (a)	151±11 (a)	171±20 (a)	2.0±0.1 (a)	26±3 (a)	6.2±0.8 (abc)	0.96±0.07 (ab)	0.41±0.09 (ac)	1.37±0.11 (a)	59.8±5.6 (b)	0.54±0.04 (b)
RDV-2012-TFT	61±11 (a)	147±11 (a)	163±16 (a)	1.9±0.2 (a)	22±2 (a)	4.7±0.6 (a)	0.94±0.07 (ab)	0.67±0.08 (b)	1.61±0.11 (b)	28.5±3.1 (a)	0.22±0.02 (a)
RDV-2012-EMT	68±8 (a)	140±14 (a)	171±12 (a)	1.9±0.1 (a)	35±2 (b)	5.1±0.5 (ac)	1.15±0.14 (a)	0.65±0.09 (bc)	1.80±0.17 (b)	38.5±9.2 (abc)	0.34±0.11 (ac)
ALP-2013-TFT	75±7 (a)	177±18 (a)	198±20 (a)	2.3±0.2 (a)	46±3 (c)	6.6±0.9 (ad)	0.85±0.06 (ab)	0.41±0.09 (ac)	1.26±0.11 (a)	36.0±0.6 (a)	0.31±0.02 (c)
ALP-2013-EMT	83±8 (a)	169±19 (a)	189±20 (a)	2.2±0.2 (a)	53±8 (c)	8.3±1.0 (bd)	0.89±0.05 (ab)	0.41±0.06 (a)	1.30±0.08 (a)	51.4±2.7 (b)	0.41±0.02 (d)
RDV-2013-TFT	72±10 (a)	168±11 (a)	186±14 (a)	2.2±0.2 (a)	42±6 (c)	6.5±0.7 (cd)	0.95±0.06 (ab)	0.59±0.10 (ab)	1.54±0.11 (b)	33.4±1.8 (a)	0.27±0.01 (c)
RDV-2013-EMT	76±10 (a)	161±19 (a)	182±21 (a)	2.1±0.2 (a)	48±5 (c)	6.7±1.4 (ad)	0.96±0.09 (b)	0.55±0.07 (ab)	1.51±0.11 (b)	38.5±0.3 (c)	0.30±0.01 (c)
General Mean	70±10 (14%)	155±18 (12%)	175±19 (11%)	2.1±0.2 (10%)	37±12 (32%)	6.1±1.4 (23%)	0.92±0.11 (12%)	0.50±0.14 (28%)	1.42±0.18 (13%)	40±11 (28%)	0.33±0.11 (33%)
CS	70±6 (a)	161±8 (a)	179±4 (a)	2.1±0.1 (a)	49±3 (c)	11±1 (e)	0.18±0.01 (c)	0.12±0.01 (e)	0.30±0.01 (c)	29.1±2.0 (a)	0.22±0.09 (ac)

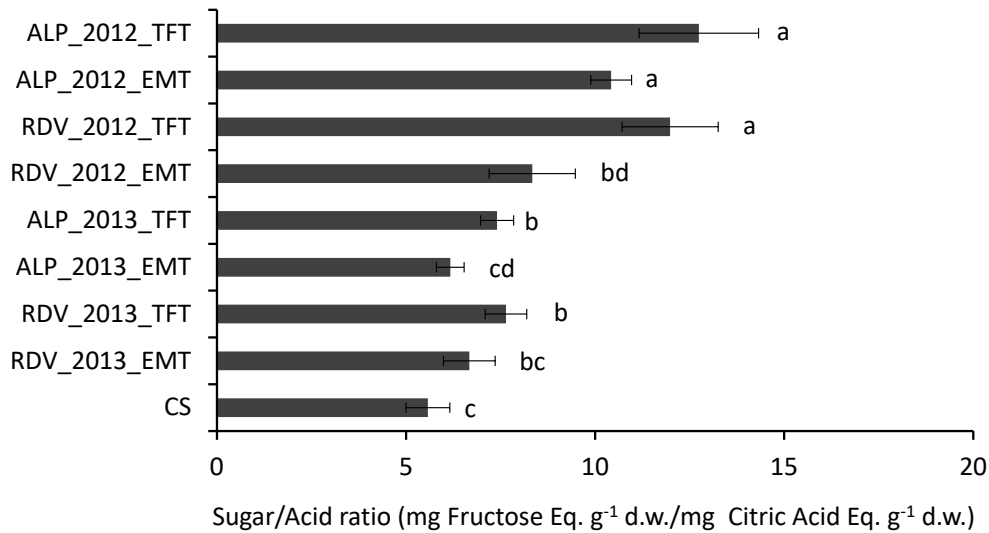


Fig. 1. Sugar to acid ratio determined in Alpine (ALP) and Regina delle Valli (RDV) *F. vesca* strawberries harvested in two consecutive years (2012 and 2013) and grown under two different fertilisation treatments (EMT = Effective Microorganism Technology; TFT = Traditional Fertilisation Treatment), in comparison with a commercial sample (CS, unknown cultivar). All data referred to dry weight. Values with at least one common letter are not statistically different.

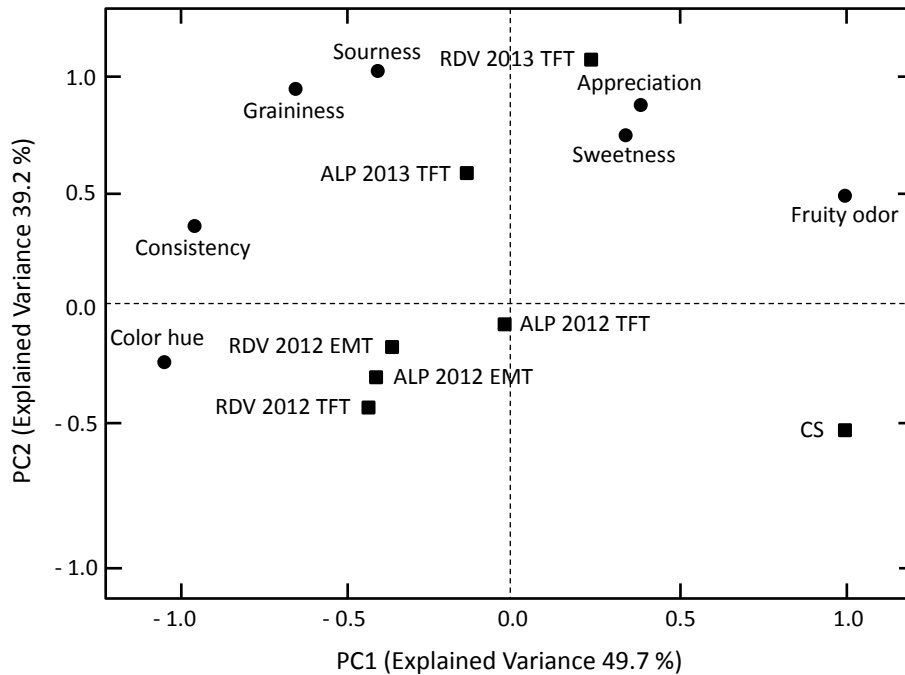


Fig. 2. Score and loading bi-plot in the Cartesian plane of the first two principal components obtained from the factor analysis of the seven *F. vesca* samples and the seven sensorial variables investigated within the panel test analysis

Discussion

Overall berry quality and consumer appreciation

Relationships among the above-reported physicochemical, nutritional and nutraceutical quality parameters and the sensorial attributes were complex and not easy to interpret. However, some considerations regarding overall berry quality can be conducted.

In this research we analysed strawberries obtained under very different pre-harvest conditions. Accordingly, most physicochemical properties measured (i.e. f.w., diameter, hardness, TA and the RSR-to-TA ratio) were affected by a high variability (Table 2); high RSD values, approximately equal to 30%, were also found for citric acid, DHAA, TP and RSA (Table 3).

The data range observed for fruit f.w. and diameter was larger than the elsewhere published data (1.0–1.8 g and 11.8–17.5 mm) regarding various cultivars grown in Italy under different cultural practices (Caruso et al. 2011, Doumett et al. 2011, Caracciolo et al. 2013).

No comparison with literature was possible for fruit hardness, which was determined by us through a hard-meter, whereas elsewhere published data were obtained using the penetrometer technique (Caracciolo et al. 2013).

The wide range of TA values determined was fully included in the one found out from the literature (0.6–13.3 g citric acid kg⁻¹) (Doumett et al. 2011, D'Anna et al. 2012, Caracciolo et al. 2013, Yildiz et al. 2014). More in detail, extremely different TA values have been reported also within a same variety, such as RDV (1.2–11.3 g citric acid kg⁻¹) (Doumett et al. 2011, D'Anna et al. 2012, Caracciolo et al. 2013) and “Fragolina di Ribera” (1.3–13.3 g citric acid kg⁻¹) (D'Anna et al. 2012, Caracciolo et al. 2013). Similar considerations can be drawn by RSR/TA values, which were in our case the most variable, even though included in the very wide range calculated from literature data (7.8–85.9) (Doumett et al. 2011, D'Anna et al. 2012, Caracciolo et al. 2013).

The mean value of fruit skin brightness (L) determined was 38 ± 4 , that is close to that observed by D'Anna et al. (2012) and much higher than the data reported by Caracciolo et al. (2013) in berries from plants cultivated in open field in Sicily (South Italy) (41.5 and 27.6, respectively). Our CIELAB “a” and especially “b” coordinates resulted higher than the values observed by D'Anna et al. (2012) for “Fragolina di Ribera” and RDV varieties (a = 22–36; b = 10–13). Hence, strawberries evaluated in this study exhibited a redder skin colour than fruits grown in Sicily. This finding could be associated to the effect of lower air temperatures of Cireglio, compared to those of Sicily, that may promote the anthocyanin expression in skin of *Fragaria* berries (Ferreyra et al. 2007). Further comparisons were unfortunately not possible, since in other studies only Chroma Index was reported, without specifying the hue angle (Caracciolo et al. 2013). It should be however noted that the CS analysed in this study located well within the group of ALP and RDV samples in the CIELAB “a” and “b” Cartesian plane (Suppl. Fig. 1).

RSR and pH were the only physicochemical properties which showed a little RSD. However, RSR general mean herein measured (9.0 ± 0.6 °Brix) was lower than the mean values reported for RDV (10.3–15.9 °Brix) (Doumett et al. 2011, D'Anna et al. 2012, Caracciolo et al. 2013) and ALP (9.5–12.1 °Brix) fruits grown in different Italian environments (Doumett et al. 2011). As regards pH, the only comparison that can be done is with published data on fifteen Turkish wild *F. vesca* accessions (Yildiz et al. 2014), which showed pH values generally lower than those measured herein.

Sugars are without doubts metabolites very important for assessing fruit quality and consumer appreciation. RSR is commonly adopted as an index of the sugar level in fruit, since its determination can be performed rapidly and directly in the field. However, when RSR values were plotted as a function of total sugars, a weak but inverse linear correlation (i.e. negative slope) was found, thus evidencing that the refractometric method does not allow the accurate evaluation of the sugar content in wild strawberry. This finding was previously highlighted for other fruits such as persimmon (Del Bubba et al. 2009) and explained by the presence in the fruit juice of other components, such as soluble tannins, that have been found at significant concentrations in *F. vesca* (Najda et al. 2014) and may affect the refractometric measure (Sugiura et al. 1983).

In all samples investigated, concentration of sucrose was found to be significantly lower than those of glucose and fructose, in accordance with findings previously reported in both spontaneous and cultivated *F. vesca* berries (Caruso et al. 2011, Mikulic-Petkovsek et al. 2012, Blanch et al. 2015). However, the difference among sucrose and monosaccharides varied greatly, depending on the study considered; furthermore, very similar concentrations of the three carbohydrates were occasionally highlighted (Doumett et al. 2011).

In this study TS concentrations were among the highest, when compared to those reported in the overall scientific literature. More in detail, total sugar concentrations found in the ALP and RDV samples, as well as in the CS, resulted higher than values reported by various authors for Serbian (Milivojevic et al. 2011) and Slovenian (Mikulic-Petkovsek et al. 2012) spontaneous *F. vesca* fruits (1.09 and 1.34 mmol g⁻¹ d.w., respectively), hydroponically grown ALP (1.67 mmol g⁻¹ d.w.) (Caruso et al. 2011) and open field cultivated “Mara des Bois” berries (1.75 mmol g⁻¹ d.w.) (Blanch et al. 2015). Conversely, concentrations herein obtained were lower than those elsewhere determined on various Italian ALP and RDV samples obtained with different rural practices (2.67 and 3.03 mmol g⁻¹ d.w., respectively) (Doumett et al. 2011).

Our data, together with earlier findings, suggest that cultivated genotypes supply a higher total sugar content than spontaneous plants; this may be associated to both the genotype selection effect and environmental conditions of plant growth.

Also for organic acids, which represent another class of metabolites closely related to the fruit quality and consumer appreciation, the concentrations found in cultivated ALP and RDV fruits were higher than the ones measured in wild grown berries (Mikulic-Petkovsek et al. 2012), suggesting a more efficient primary metabolism of cultivated plants. The higher expression of citric acid, compared to malic acid, was in agreement with data reported for both *F. vesca* (Caruso et al. 2011, Doumett et al. 2011, Mikulic-Petkovsek et al. 2012) and *F. x ananassa* berries (Davik et al. 2006, Mikulic-Petkovsek et al. 2012).

Samples with high scores of sweetness in the sensorial evaluation (RDV-2013-TFT and ALP-2013-TFT) were also the richest in TS, as well as glucose and fructose, the latter being perceived as the sweetest among soluble sugars, and showed the highest values of the sweetness index (data not shown) (Keutgen and Pawelzik 2007, Akhatou and Fernández Recamales 2014). The same samples showed also high values for “sourness” and, accordingly, they were characterized by the highest concentrations of organic acids and especially TA. Moreover, these two samples resulted as the most appreciated. For these samples, 10·RSR/TA values were 10.2–10.8, fully included in the range of 8.5–14 that is commonly considered an appropriate balance of sweet-tart flavour for human palatability (Keutgen and Pawelzik 2007).

Conversely, all the other investigated samples were characterized by RSR-to-TA ratios (16.5–30.3) much higher than the upper limit of the aforementioned range and, accordingly, showed a poor appreciation (Fig. 2). This finding was also observed for the CS, which however behaved as a clear outlier, compared to all the other samples, notwithstanding its RSR-to-TA ratio (16.5) was in between the whole interval of data determined in this research.

Sugar-to-acid ratio (Fig. 1) is another commonly adopted indicator of consumer acceptability. It may act as an important evaluation tool of fruit taste (Crespo et al. 2010) and was reported to be highly related to overall consumer appreciation for *Fragaria x ananassa* berries (Keutgen and Pawelzik 2007). Sugar-to-acid ratios were found to be linearly related with RSR/TA data ($r^2 = 0.79$). However, similarly to the sensorial analysis, CS was an outlier within this correlation and a strong increase of the determination coefficient ($r^2 = 0.96$) was observed when it was excluded from the regression. Hence, sugar-to-acid ratio was capable to clearly differentiate the CS from the others, in agreement with the sensorial analysis.

The abundance of antioxidant secondary metabolites represents an additional element of quality of the investigated strawberries.

ALP and RDV samples supplied a good amount of total vitamin C (20–30 mg 100 g⁻¹ f.w.), mainly as AA. Comparable values of total vitamin C and a prevalence of its oxidized form were reported by Doumett et al. (2011) on ALP, RDV and “Sara” cultivars, grown under various agricultural and environmental conditions. It should be however noted that, in the “Valitutto” variety, the same authors reported DHAA as the highly prevalent form, representing it approximately 90% of total vitamin C. AA concentrations found herein for ALP and RDV were in agreement with literature data concerning *F. vesca* samples (Nuñez-Mancilla et al. 2013, Yildiz et al. 2014) and were included in the very wide range (0.55–2.20 mg g⁻¹ d.w.) observed by Caruso et al. (2011), according to the fruit production cycle of ALP berries grown under hydroponic conditions.

TP values found in the eight ALP and RDV samples analysed, even though characterized by high variability, were included in the range of concentrations reported in literature for wild (Cheel et al. 2007, Mikulic-Petkovsek et al. 2012, Yildiz et al. 2014) and cultivated berries (Doumett et al. 2011, Nuñez-Mancilla et al. 2013) (approximately 20–60 mg g⁻¹ d.w.). The high variability observed both in our and already published data, clearly evidenced the number of genetic and environmental factors that affect the synthesis of polyphenols, and agrees with the complex role of phenolic compounds in plant response to biotic and abiotic stress factors (Balasundram et al. 2006, Del Bubba et al. 2013, Fuoco et al. 2013, Ancillotti et al. 2015).

As already assessed in previous studies (Cheel et al. 2007, Doumett et al. 2011), TP measured by the F-C are well-related with DPPH-RSA, confirming the role of polyphenols for explaining the antiradical activity of *F. vesca* fruits. Conversely, all other primary and secondary metabolites investigated did not show any statistically significant relation with RSA. In contrast with findings elsewhere observed (Doumett et al. 2011), the lack of correlation involved also citric acid. Comparison of RSA data herein obtained for ALP and RDV berries with the ones reported in literature was in some cases hindered by the measuring units adopted for expressing RSA. When the comparison

was possible, the RSA data herein obtained were found to be similar to the ones reported on various cultivated *F. vesca* genotypes (Doumett et al. 2011, Nuñez-Mancilla et al. 2013). However, antiradical activities one magnitude order lower than those observed in this study were reported by Yildiz et al. (2014) for the above-mentioned fifteen wild accessions, in agreement with the low polyphenolic content of their samples.

The CS appeared as a sample of minor interest as source of antioxidant secondary metabolites, since it supplies the lowest dose of total vitamin C and among the lowest quantities of polyphenols and RSA.

Cultivar effect

The effect exerted by genotype on the parameters of Groups 2–4 can be highlighted by comparing the results obtained for the following sample pairs: ALP-2012-EMT vs. RDV-2012-EMT, ALP-2012-TFT vs. RDV-2012-TFT, ALP-2013-EMT vs. RDV-2013-EMT and ALP-2013-TFT vs. RDV-2013-TFT. These comparisons did not show any cultivar effect, since statistically significant variations were observed only occasionally and without a clear trend (Table 2). It is also remarkable that no genotype effect was highlighted during the whole trial also for yield, being the observed means equal to 19 and 17 g/plant, for ALP and RDV respectively (data not shown).

Genotype effect was investigated only in very few literature studies, neither of which regarding the comparison of ALP and RDV berries. In these studies, statistically significant differences were found for f.w., RSR and TA by comparing RDV with local Sicilian varieties (Caracciolo et al. 2013); moreover, RSR, TA and their ratio were found to be influenced by genotype when fruits of the cultivars “Sara” vs RDV and “Valitutto” vs ALP grown in Italy, were compared (Doumett et al. 2011).

Concentrations of sucrose, glucose and fructose were not affected by the genotype (Table 3). The effect of cultivar on carbohydrate composition of *F. vesca* berries was previously investigated by Doumett and co-workers (Doumett et al. 2011), highlighting very similar sugar concentrations for “Sara” vs. RDV, whereas a very different composition pattern was observed for “Valitutto” vs. ALP. In this regard, it should be noted that also in *Fragaria x ananassa* the influence of genotype on sugars was significant in some cases (e.g. “Mazi” vs. “Oso Grande”) and insignificant in others (e.g. “Dover” vs. “Campineiro”) (Cordenunsi et al. 2002).

Concentration values found for citric and malic acids did not evidence significant differences between the two cultivars with the only exception of the former in the 2012-EMT samples. These findings were in agreement with those evidenced in a previous study regarding ALP and RDV berries grown in different Italian regions and under different rural practices (Doumett et al. 2011). Considering these data, together with those regarding sugars, no clear influence of genotype on the sugar-to-acid ratio was evidenced (Fig. 1).

AA and DHAA were found to be generally higher in RDV than in ALP samples, even though only the latter parameter showed statistically significant differences (Table 3). Total vitamin C was also significantly higher in RDV than in ALP fruits. The determination of DHAA, together with AA, is very important since the oxidized form is almost completely converted into the reduced form in the human body (Gregory III 2007). Accordingly, RDV better contributes than ALP, as vitamin C supplier in the human diet. Similar conclusions were drawn by Doumett et al. (2011), who determined the two forms of Vitamin C in RDV and ALP berries grown in various Italian areas and under different rural practices.

TP and RSA were clearly affected by the genotype, being these parameters generally higher in ALP than RDV fruits (see Table 3).

PCA carried out on sensorial parameters did not allow any sample clustering in relation to the genotype (Fig. 2), thus suggesting no different appreciation by consumers of the two varieties. This finding was in general agreement with the lack of genotype effect observed for sugars and acids, which are responsible of an important part of fruit quality perception (Lobit et al. 2006).

Plant age effect

Plant age effect can be investigated by comparing the following sample pairs: ALP-2012-EMT vs. ALP-2013-EMT, ALP-2012-TFT vs. ALP-2013-TFT, RDV-2012-EMT vs. RDV-2013-EMT and RDV-2012-TFT vs. RDV-2013-TFT. The comparison of these sample pairs revealed a statistically significant effect ($p < 0.05$) of the plant age on all the physicochemical parameters reported in Table 2, with few exceptions for pH and RSR. Fruits collected in the

second year showed a much higher dimension and f. w., as well as higher colour indexes and acidity, together with lower pH, RSR and consequently higher RSR/TA ratio. It is interesting to underline that fruits harvested in 2013 were characterized by f.w. values higher than those determined in various *F. vesca* berries cultivated in open field (RDV, “Fragolina di Maletto” and “Fragolina di Ribera”) (Caracciolo et al. 2013) or in hydroponics (RDV) (Caruso et al. 2004b, Caruso et al. 2011). Moreover, the 2013 f.w. values were comparable with those found in RDV berries grown in open field in the same area (Doumett et al. 2011). Fruit yield was also found statistically affected by plant age ($p < 0.001$), being the 2012 and 2013 productions equal to 6.6 and 29.4 g/plant, respectively (data not shown). These findings can be explained on the basis of the much shorter crop cycle of fruits collected in late September 2012 in comparison to the longer one regarding fruits sampled in the last week of June 2013. A longer crop cycle was found to be beneficial for *F. vesca* berry production in hydroponics as well (Caruso et al. 2011). However, in our study, the third crop cycle showed a clear drop in fruit production (berry yield of 2.1 g/plant), thus evidencing the need of plant substitution after the second harvest, as elsewhere indicated also for *Fragaria x ananassa* (Moor et al. 2004, Conti et al. 2014).

Climatic variations may in principle contribute to changes of berry physicochemical properties, as well. However, in our study, only a lower T and PAR of about 2°C and 3 MJ m⁻² d⁻¹, and a 9.5% higher H, were found during the 2013 ripening season, compared to 2012 (Table 1). According to literature (Wang and Camp 2000, Krüger et al. 2008), these variations do not seem significant in order to interpret the strong physicochemical differences observed in the two crop cycles.

Higher sugar concentrations of about 10–15% were constantly determined in 2013 than in 2012 (Table 3), even though without evidencing statistically significant differences. This finding was quite in agreement with the results obtained in a trial on annual and biennial crop of *Fragaria x ananassa* fruits (Conti et al. 2014).

Citric and malic acids behaved similarly to sugars, being their concentrations generally higher in 2013 than in 2012. The variations observed for citric acid were in all cases statistically significant, whereas for malic acid, only the concentrations of RDV-TFT 2012 and 2013 samples were statistically different (Table 3). A similar trend, even though with much lower variations, was reported by Conti and co-workers on strawberry as a function of plant age (Conti et al. 2014). The ratio between TS and the sum of citric and malic acids reflected the stronger variations of organic acids and higher values of this ratio were consequently observed in 2012 than in 2013, with differences in most cases statistically significant (Fig. 1).

Interestingly, plant age did not exert any clear effect on secondary metabolism, as evaluated by vitamin C, TP and RSA.

Production year affected sensorial fruit quality, as well (Fig. 2). In fact, all samples collected in 2012 resulted essentially distributed into a same group located in the negative quarter of the PC1 vs. PC2 plane, whereas the samples harvested in 2013 (namely RDV-2013-TFT and ALP-2013-TFT) showed much higher values along the PC2, thus giving rise to another group, and were the most appreciated and sweetest.

Fertilization treatment effect

The effect that the fertilization treatment exerted on the investigated parameters was highlighted by the comparison of the following sample pairs: ALP-2012-TFT vs. ALP-2012-EMT, RDV-2012-TFT vs. RDV-2012-EMT, ALP-2013-TFT vs. ALP-2013-EMT and RDV-2013-TFT vs. RDV-2013-EMT. The different rural practice differently affected the physicochemical parameters, also depending on the plant age, thus giving rise to erratic occurrences of statistically significant differences within the aforementioned sample pairs. For instance, fruit f.w. and diameter were more influenced by the treatment in 2013 than in 2012. Furthermore, TA was found to be higher in EMT than in TFT samples in 2012, whereas an opposite prevalence was observed in ALP-2013 and identical values were found for RDV-2013 samples (Table 2). No effect of the rural practice was highlighted for berry yields, which were 19 and 17 g/plant in TFT and EMT plants, respectively (data not shown).

As regards nutritional and nutraceutical parameters, only citric acid, malic acid, TP and RSA showed a quite homogeneous influence of fertilization treatment, being the values found in berries produced by EMT generally higher than those obtained for fruits grown under TFT. A cross-effect between fertilization treatment and cultivar was therefore evidenced for TP and RSA data, being ALP-EMT samples those with the highest levels of these parameters. The higher TP and RSA values found in EMT than in TFT samples suggested the occurrence of a stress-like effect induced by EMT fertilization.

The differences in citric and especially malic acid concentrations, together with the consistent results found for sugars, caused sugar-to-acid ratios generally higher in TFT than in EMT samples (Fig. 1).

Sensorial parameters resulted from the panel taste performed during 2012 (the only one year of comparison of TFT and EMT treatments) were not significantly affected by the fertilization treatments (Fig. 2).

Conclusions

For the first time a very wide spectrum of physicochemical, nutritional, nutraceutical and sensorial parameters were simultaneously investigated on *F. vesca* berries in relation to three main factors (i.e. genotype, plant age and fertilization treatment), according to the fundamentals of a true multidisciplinary approach.

Genotype exerted a statistically significant effect on nutraceutical properties of *F. vesca* ALP and RDV berries, being the former 30% richer in TPs and providing a 25% higher antiradical activity, but a 20% lower total vitamin C. Conversely, no influence of cultivar was highlighted for physicochemical properties, as well as for investigated sugars and organic acids.

Data obtained in this study clearly evidenced the influence of plant age on physicochemical and nutritional berry parameters, being them generally higher in samples obtained during the second production year. Moreover, these samples were the most appreciated by panellists and the sensorial liking was strongly correlated with the RSR/TA index.

Fertilization treatment had a quite homogeneous influence only for organic acids, TPs and RSA; values found in berries cultivated by the EMT approach were generally higher than those obtained for fruits grown under TFT. An enhancing cross-effect between EMT fertilization treatment and ALP variety is therefore evidenced for TP and RSA data.

The CS evidenced values of nutraceutical parameters largely lower than those determined in cultivated ALP and RDV *F. vesca* cultivars (e.g. the lowest content of vitamin C) and had the worst appreciation score by panellists.

Further studies are needed to make clearer cause-effect mechanisms and synergies, namely for the nutraceutical parameters associated to secondary metabolic pathways, but also to reach a better understanding of the relationships between sensorial attributes and physical and biochemical characteristics of cultivated *Fragaria vesca* berries.

Acknowledgements

This research has been performed in the frame of PROFRAB-OIGA project, supported by the Ministry of Agriculture, Food and Forestry of Italy. The authors are grateful to Fabio Bizzarri of Azienda Agricola Il Sottobosco.

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Supplemental Figure

Suppl.Fig. 1. CIELAB colour chart providing the a-b coordinates of the investigated *Fragaria vesca* berry samples. 1) ALP-2012-TFT, 2) ALP-2012-EMT; 3) RDV-2012-TFT; 4) RDV-2012-EMT; 5) ALP-2013-TFT; 6) ALP-2013-EMT; 7) RDV-2013-TFT; 8) RDV-2013-EMT; 9) commercial sample

