

Antioxidants, Phenolic Compounds, and Nutritional Quality of Different Strawberry Genotypes

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Strawberry contains high levels of micronutrients and phytochemical compounds. These exhibit functional roles in plant growth and metabolism and are also essential for the nutritional and organoleptic qualities of the fruit. The aim of the present work was to better characterize the phytochemical and antioxidant profiles of the fruit of nine different genotypes of strawberry, by measuring the total flavonoid, anthocyanin, vitamin C, and folate contents. Cultivar effects on the total antioxidant capacities of strawberries were also tested. In addition, the individual contribution of the main antioxidant compounds was assessed by HPLC separation coupled to an online postcolumn antioxidant detection system. This study showed the important role played by the genetic background on the chemical and antioxidant profiles of strawberry fruits. Significant differences were found between genotypes for the total antioxidant capacity and for all tested classes of compounds. The HPLC analyses confirmed qualitative and quantitative variability in the antioxidant profiles. These studies show that differences exist among cultivars, applicable in dietary studies in human subjects.

KEYWORDS: Fruit; strawberry; nutritional quality; antioxidant capacity; phytochemical profiling

INTRODUCTION

The important role of diet in either promoting or preventing diseases has long been recognized. Global epidemiological studies confirm an inverse relationship between the consumption of fruit and the incidence of cardiovascular (1, 2), degenerative (3), and proliferative diseases (4, 5), and there is convincing evidence that the considerable health benefits of fruits are due to their specific chemical compositions, particularly to compounds of nutritional relevance.

Edible berries have been part of the human diet for centuries and represent a potentially important contribution to the intake of fresh fruit for populations in all those countries in which, as stated by WHO (<http://www.euro.who.int/nutrition/Security/>

20020630_1), there is a limited availability of fruits and vegetables, that is, mainly in northern latitudes.

Strawberry (*Fragaria × ananassa* Duch.) is one of the most commonly consumed berries. Together with other soft fruit, it is an important dietary source of fiber and bioactive compounds, both micronutrients and phytochemicals. In particular, strawberries are rich in vitamin C [a handful of strawberries is sufficient to cover the vitamin C recommended daily allowance (RDA)] (6) and are among the richest natural food sources of folate. For instance, 250–350 g of strawberries (~200 μg of folate on average) can supply 60–100% of the daily European folate intake recommendations (200–300 μg/day) (7).

Finally, strawberry is an important source of phytochemicals; in particular, the phenolic composition seems to strongly influence the quality of the fruits, contributing to both their sensorial–organoleptic attributes and their nutritional value (8–10). Anthocyanins are quantitatively the most important type of polyphenols in strawberry. The major anthocyanin representative compounds have already been identified [pelargonidin- (Pg) and cyanidin- (Cy) glycosides or acylated forms] (11–13), and the presence of the main derivatives seems to be constant in all varieties (i.e., Pg-3-gluc and in smaller proportion Cy-3-gluc). Nevertheless, new anthocyanin-related pigments (also called

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condensed pigments) keep being detected in small amounts (14, 15), and qualitative and quantitative variations on the anthocyanin profile have been observed among strawberry cultivars (16, 17) as well as within the same variety, depending on the genetic background, the degree of ripeness, postharvest storage of the fruits, and climatic factors.

Although their presence is minor in strawberry from a quantitative point of view, a relevant interest is focused on flavonols (mainly quercetin and kaempferol derivatives) due to their putative higher bioavailability, and the mechanisms of absorption and bioactivity of the main aglycones and glucosides derivatives have been widely studied (18, 19). Also, phenolic acids and derivatives contribute to the phenolic profile of the fruit and may play an important role in determining the genotype-to-genotype differences in the phytochemical composition. A range of hydroxycinnamates, mainly caffeoyl and ferulic esters and other classes of phenolic acids, are found in strawberry even if present at low concentrations (16, 20, 21). Furthermore, strawberries together with raspberries and blackberries are among the usually consumed foods with a relevant amount of ellagic acid (responsible for >30% of total phenolics in strawberries) (22). However, free ellagic acid levels are generally low, and their detection is the result of acid hydrolysis products of ellagitannin breakdown.

The potential health benefits of phytochemicals found in strawberries have received ample attention in the literature, but the mechanisms by which they exert their health-promoting effects are still unclear. Most of the past research has been focused on the antioxidant properties of flavonoids, although the marked bioactivities observed in mammalian cells could involve other biochemical and molecular mechanisms. Furthermore, there is an increasing awareness that the data obtained from *in vitro* studies are often conflicting, and extrapolation of *in vitro* results to the *in vivo* situation is uncertain. A rational procedure for the assessment of the nutritional quality of fruit should start from the phytochemical and antioxidant profiling and only subsequently pass to bioavailability and bioefficacy studies.

The influence of several pre- and postharvest factors on the phytochemical and nutritional composition of the strawberry is already known. The genetic background may play a pivotal role, although few genotypes have been well characterized for these important features.

The aim of the present work was to screen and compare nine different genotypes of strawberry, by measuring their contents of moisture, total phenolics, total flavonoids, total anthocyanins, vitamin C, and folates. In addition, the total antioxidant capacities of whole fruit extracts were measured by the Trolox equivalent antioxidant capacity (TEAC) assay with 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and the ferric reducing antioxidant power (FRAP) assays. Individual antioxidant metabolites were separated and characterized by high-performance liquid chromatography (HPLC) coupled to an online postcolumn antioxidant detection system. The chemical and antioxidant profiles of strawberries and the influence of genetic differences among cultivars will lead to a better understanding of the role of these substances in the physiology and organoleptic-sensorial quality of the fruit.

MATERIALS AND METHODS

Chemicals. All chemicals were of analytical grade. Folin-Ciocalteu's phenol reagent, ABTS diammonium salt, 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), and potassium chloride were purchased from Fluka Chemie (Buchs, Switzerland). 3,4,5-Trihydroxy-

benzoic acid (gallic acid), ascorbic acid (vitamin C), sodium carbonate anhydrous, potassium persulfate, sodium acetate trihydrate, ferrous ammonium sulfate, ferric chloride hexahydrate, and 2,4,6-tripyridyl-s-triazine (TPTZ) were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Pelargonidin-3-glucoside was obtained from Polyphenols AS (Sandnes, Norway).

Strawberry Material. Ripe strawberry fruits from five selected cultivars (Alba, Irma, Patty, Adria, and Sveva), two advanced selections of *F. × ananassa* (AN99.78.51, 95.617.1 × DARSELECT; AN03.338.51, Sveva × Patty), and F1 (AN94.414.52, Don × FVG 22) and F2 (AN00.239.55, AN94.414.52 × 91.143.5) advanced selections from interspecific crosses of *F. × ananassa* × *F. virginiana glauca* (FVG22) were harvested in May 2006 at the experimental field for genetic improvement of the Azienda Agraria Didattico Sperimentale (Marche Polytechnic University). Within 2 h after harvest, whole fruits were stored at -20 °C for further analysis. For the moisture content determination, HPLC analysis, and measurement of total folate content, frozen strawberries were ground to a fine powder in liquid nitrogen using a precooled chopper and grinder (IKA A11 basic). For each sample, a small amount of the frozen powder was freeze-dried, to check for differences in compound preservation, and both the wet and freeze-dried powders were stored at -80 °C until analysis.

Moisture Content Analysis. Moisture content was determined by weighing the powder samples in Petri dishes before and after drying in oven at 100 °C for at least 4 h and kept in an desiccator container during the measurements. The moisture content of randomly selected fruit samples was confirmed by weighing powder samples before and after freeze-drying overnight.

Folin-Ciocalteu Assay. Hydrophilic extraction was performed as previously described (8) with some modifications. Briefly, compounds were extracted by homogenizing (Ultraturrax T25, Janke & Kunkel, IKA Labortechnik) for 2 min exactly 10 g of fruit samples in 100 mL of extraction solution, consisting of methanol and Milli-Q water (80% v/v) and stirring for 2 h in the dark at room temperature. The mixture was centrifuged in two sequential times for 15 min at 3500 rpm, and supernatant was filtered through a 0.45 µm Minisart filter (PBI International) before analysis. The total phenolic content of the extracts was determined using the Folin-Ciocalteu colorimetric method as modified by Slinkard and Singleton (23).

Quantifications were calculated through a calibration curve daily prepared with known concentrations of gallic acid (GA) standards, and results are expressed as milligrams of GA equivalents per 100 g of fresh weight (FW) of strawberry. Data are reported as a mean value ± standard deviation (SD) for six measurements.

Determination of Total Flavonoid Content (FL). Total flavonoid content was determined by using a colorimetric method previously described (24). The results are expressed as micrograms of catechin equivalents (CE) per gram of FW of strawberries. Data are reported as a mean value ± SD for six measurements.

Determination of Total Anthocyanin Content (ACY). The ACY of the hydroalcoholic extracts was determined using a modified pH differential method previously described (25), with some modifications. Absorbance readings were converted to quantifications through a calibration curve, obtained by known concentrations of pelargonidin-3-glucoside (Pg-3-gluc) standards. Results are expressed as milligrams of Pg-3-gluc equivalents per gram of FW of strawberry. Data are reported as a mean value ± SD for six measurements.

Determination of Folate Content (Fo). The total Fo was quantified using a *Lactobacillus casei* microbiological assay, by far the most commonly used method and still considered to be the "gold standard" for this determination. The analysis was performed at NIZO (Ede, The Netherlands) as previously described (26). Before the assay was performed, a deconjugation pretreatment of polyglutamates contained in the strawberry extracts was required because microorganisms usually respond mainly to monoglutamates rather than to polyglutamates. For each genotype, the starting material for folate extraction and deconjugation consisted of randomly selected strawberries collected from different plants, to estimate the average content of folate in the germplasm. To assess the intracultivar biological variability, separate samplings of strawberries and individual extractions were conducted for two of the genotypes. Three of these separate extracts were subjected

to the microbiological assay, and analysis was repeated at least in four microtiter plates. Results are expressed as micrograms of folate per gram of FW. Data are reported as a mean value \pm SD for a minimum of four replicates.

Quantification of the Total Antioxidant Capacity (TAC). The TAC of the hydroalcoholic extracts was determined using in parallel the Trolox equivalent antioxidant capacity (TEAC) and the ferric reducing antioxidant power (FRAP) assays. The TEAC assay was carried out according to the recently modified method of Re and co-workers (27) and combined to a flow injection analysis (FIA) system as previously set up by our work group (28). This TEAC method, also called the FIA-ABTS decolorization assay, is based on the ability of antioxidant compounds to quench the ABTS radical cation (ABTS^{•+}) and reduce the radical to the colorless neutral form. The undiluted strawberry extract (10 μ L) is injected into a serpentine-knotted reaction coil and allowed to react with the ABTS^{•+} working solution pumped into the coil at a flow rate of 1.2 mL/min. The extent of decolorization, expressed as percentage of inhibition of absorbance, is then plotted as a function of concentrations of the antioxidants in the sample.

TEAC results are expressed as micromoles of Trolox equivalents per gram of FW of strawberry. Data are reported as a mean value \pm SD for four measurements.

The FRAP assay was carried out as described by Benzie and Strain (29) with some modifications, according to the procedure described earlier (13). Aqueous dilutions of ferrous ammonium sulfate were used for calibration, in parallel with Trolox standard dilutions. The ferric reducing antioxidant power of Trolox was approximately twice that of ferrous ammonium sulfate. Final results are expressed as micromoles of Trolox equivalents per gram of FW of strawberries. Data are reported as a mean value \pm SD for six measurements.

HPLC Determination of Vitamin C Content. Ascorbic acid was measured as described by Helsper et al. (30). Briefly, vitamin C was extracted by sonication of 0.5 g of wet frozen powder in 2 mL of ice-cold water with 5% metaphosphoric acid and 1 mM DTPA, followed by centrifugation at 2500 rpm for 10 min, filtering, and immediate analysis on an HPLC system.

Quantification was made through a standard calibration curve prepared by running standard concentrations of vitamin C prepared similarly and measured in duplicate at the beginning and end of the analysis. As for folate determination, the intracultivar biological variability was assessed by separate samplings of strawberries for two of the genotypes. Results are expressed as milligrams of vitamin C (vit C) per gram of FW \pm SD for biological variability.

HPLC Antioxidant Detection System. The HPLC analysis coupled to online antioxidant detection was essentially performed as described in ref 31, with a few modifications. Briefly, 0.5 g of wet frozen fruit powder was extracted with 2 mL of extraction solution (75% methanol and 0.1% formic acid). The mixture was centrifuged, filtered, and loaded on an HPLC system with a C18 250 mm \times 4.6 mm column. A gradient from 95% solution A (water with 0.1% TFA) and 5% solution B (acetonitrile acidified with 0.1% TFA) to 30% solution B in 50 min at a flow rate of 1 mL/min was used for separation. Compounds eluting passed first through the photodiode array detector (PDA) and subsequently through a postcolumn reaction loop, where they reacted for 30 s with a buffered solution of ABTS radicals. Then the mixture passed through a second detector, where the amount of ABTS cation radicals was measured at 412 nm. By integrating the main antioxidant peaks on the ABTS chromatogram, the contribution of each antioxidant compound to the TAC of the extracts was calculated.

Statistical Design and Methods. Statistical analysis was performed using STATISTICA software (Statsoft Inc., Tulsa, OK). Data were subjected to one-way analysis of variance for mean comparison, and intergenotype significant differences were calculated according to HSD Tukey's multiple-range test. Data are reported as mean \pm standard deviation (SD). Correlations were calculated on a genotype mean basis, according to Pearson's test. Differences at $p < 0.05$ were considered to be statistically significant.

RESULTS AND DISCUSSION

This study describes the role played by genetic background in the chemical and antioxidant profiles of strawberries. In this

Table 1. Antioxidant Capacity of Nine Selected Strawberry Genotypes

cultivar/selection	DM ^a (%)	TEAC ^b	FRAP ^b
Alba	7.2	11.27 \pm 0.40f	7.31 \pm 0.09e
Irma	5.3	11.90 \pm 0.38ef	9.16 \pm 0.28d
Patty	6.1	11.82 \pm 0.46ef	8.84 \pm 0.40d
Adria	6.0	13.30 \pm 0.30d	9.30 \pm 0.53cd
Sveva	7.2	15.87 \pm 0.69c	10.40 \pm 0.24b
AN99.78.51	6.8	13.08 \pm 0.29de	10.05 \pm 0.24bc
AN94.414.52	12.5	17.72 \pm 0.44b	13.99 \pm 0.56a
AN03.338.51	9.3	19.41 \pm 1.18a	14.22 \pm 0.72a
AN00.239.55	8.4	13.26 \pm 0.25d	9.56 \pm 0.26cd

^a DM, dry matter. ^b Micromoles of Trolox equivalents per gram of fresh weight. Means \pm standard deviation in the same column with the same letter are not significantly different at $p \leq 0.05$ using HSD Tukey's multiple-range test.

study we compared a number of phytochemical parameters of a selected number of genotypes from the Italian strawberry breeding program with those of a well-known genetic background. The genotypes used in this study were selected after a preliminary study (32) that allowed the differentiation of their fruits according to the total antioxidant capacity and the gallic acid equivalents they contained, measured through the Folin–Ciocalteu assay (TPC). Five of the selected genotypes are strawberry cultivars well-known in the Italian production system for their different ripening times and identified for their lower TAC and TPC (Alba > Adria > Irma) and higher TAC and TPC (Sveva > Patty). The other four genotypes are advanced selections of the Marche Polytechnic University strawberry breeding program, selected for further investigations because of the high TAC and TPC values previously shown. Among these, the selection AN94.414.52 in recent years was considered to be the most interesting genotype with the highest TAC and TPC. These findings are probably due to its genetic background, being an F1 cross between a *F. \times ananassa* cultivar and *F. virginiana glauca*, another wild octoploid strawberry species.

Significant differences among varieties and selections were outlined by using both more approximate general assays and HPLC fine separations.

The dry matter content of fruits differed strongly between genotypes (Table 1). For instance, the cultivar Irma has less than half the amount of dry matter compared to line AN94.414.52 (5.3 vs 12.5%, respectively). These findings should be taken into account when the other measurements are compared.

In Figure 1, the results of the Folin–Ciocalteu assay are shown. AN03.338.51 and AN94.414.52 selections had the highest gallic acid equivalents (3.13 and 3.12 mg of GAE/mg of FW, respectively), followed by Sveva (2.45 mg of GAE/mg of FW), whereas the lowest contents were measured in Irma, Patty, and Adria (1.73, 2.00, and 2.05 mg of GAE/g of FW, respectively). There was approximately a 1.8-fold difference between the highest and lowest values, which correlated with the different moisture contents calculated among the strawberry genotypes (Table 1). The results obtained confirmed previous measurements obtained in the past 2 years of harvest (32).

A significant correlation was observed between the total flavonoid content and the Folin–Ciocalteu assay ($r = 0.87$, $p = 0.0024$; Table 3) and with the total antioxidant capacity of the strawberry extracts, particularly when measured with the TEAC assay ($r = 0.94$, $p < 0.0001$; Table 3), confirming the findings previously observed for several species of berries (10). AN03.338.51, AN94.414.52, and Sveva were the strawberry genotypes richest in flavonoids [0.98, 0.67, and 0.67 mg of (+)CE/g of FW, respectively], whereas Patty showed the lowest content [0.40 mg of (+)CE/g of FW] (Figure 1). The lowest anthocyanin content was measured in Sveva (0.36 mg of Pg-

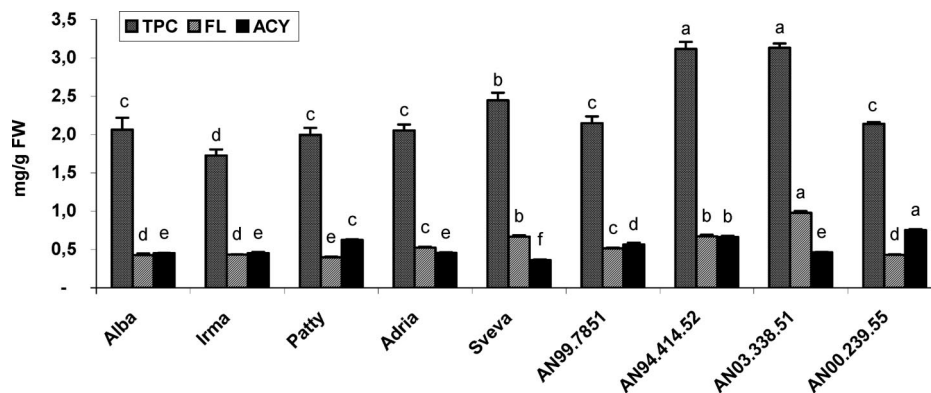


Figure 1. Total phenolic (mg of GAE/g of FW), flavonoid [mg of (+)CE/g of FW], anthocyanin (mg of Pg-3-gluc/g of FW) contents of the nine selected strawberry genotypes. Columns belonging to the same set of data with different letters are significantly different ($p < 0.05$, $n = 6$ analyses).

Table 2. Quantification of the Single Anthocyanins and Total Anthocyanin Content Based on HPLC Data^a

genotype	Cy-gluc	Pg-gluc	Pg-rutin	Pg-mal-gluc	ACY
Alba	6.60	128.82	7.26	20.30	162.97
Irma	1.61	95.80	0.71	0.87	99.00
Patty	1.06	125.76	4.94	23.64	155.39
Adria	2.31	160.41	13.80		176.52
Sveva	2.03	132.95	0.87	1.24	137.08
AN99.78.51		172.46	9.96	26.80	209.22
AN94.414.52	11.15	227.94	7.46	3.79	250.33
AN03.338.51	2.67	164.74			167.40
AN00.239.55	5.28	282.34	6.31	2.30	296.23

^a Cyanidin and pelargonidin derivatives were identified through their spectral and chromatographic characteristics. Results are expressed as $\mu\text{g/g}$ of FW.

Table 3. Pearson's Correlation Coefficients for Quantitative Determinations in the Nine Selected Strawberry Genotypes^a

variable	FRAP	TPC	ACY	FI	Vit C
TEAC	0.95**	0.95**	-0.07 ns	0.94**	0.66 ns
FRAP		0.92*	0.12 ns	0.85*	0.62 ns
TPC			0.07 ns	0.87*	0.77*
ACY				-0.30 ns	-0.18 ns
FI					0.73*

^a 95% confidence interval. ns, nonsignificant; *, significant at $p \leq 0.05$; **, significant at $p \leq 0.001$.

3-gluc/g of FW), and a >2-fold difference in the anthocyanin content was observed in comparison with the highest level (AN00.239.55, 0.75 mg of Pg-3-gluc/g of FW) (**Figure 1**). Despite the relevant importance of folate as a health-promoting compound, an actual and effective interest has only recently been raised, and few studies have been carried out, to date, to quantify the folate content in strawberries (33), to discuss folate stability (34), and to evaluate the effects of cultivar, ripeness, and other pre- and postharvest factors on its recovery (35). Recent observations (36) have emphasized that in many countries daily intakes of folic acid, and also the RDA, might have, for a long time, been inadequate and suboptimum for physiological functions, so that countries such as the United States and Canada fortify grain with folic acid to increase its daily intake. As confirmed by our work, strawberry can be considered among the most relevant natural food sources of folate. In this work, relevant differences among cultivars were observed when the folate content was compared (**Figure 2**). The highest folate contents were measured in the selections, whereas the cultivars were lower. Nearly a 7.5-fold difference was measured between the lowest (Adria, 0.128 μg of folic acid/g of FW) and the highest content (AN94.414.52, 0.96 μg

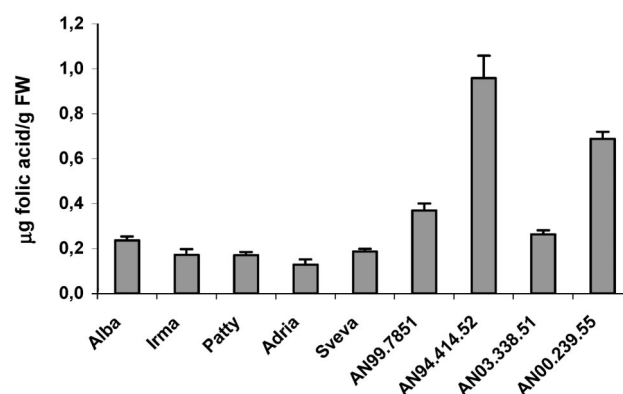


Figure 2. Total folate content of the nine strawberry cultivars and selections tested (minimum of four replicates). Error bars refer to the technical variability.

of folic acid/g of FW), if expressed on a FW basis. The same trend was found by calculating the folate content per gram of dry weight (DW), but the genotype-to-genotype differences were reduced. Folate contents shown in this work are in keeping with values reported in European food data tables, where folate in strawberries is indicated to range from 0.2 to 0.99 $\mu\text{g/g}$ of FW (37, 38). The high content in folate is even more relevant when considering that 50–95% of folate content in food is estimated to be lost during storage, preparation, or manufacturing processes, so that freshly consumed strawberries may represent a very high quality source of this class of essential dietary compounds. Furthermore, the data are interesting because previous studies do not indicate such relevant differences among genotypes and are even more remarkable if one considers that the strawberry plants in our study have all been grown in the same experimental field under the same environmental conditions.

The vitamin C content (**Figure 3**) strongly varied among the nine strawberry genotypes, and a 2-fold difference was found between genotypes with the lowest (Irma and AN00.239.55, averaging 0.23 and 0.24 mg of vitamin C/g of FW) and highest contents (AN03.338.51, averaging 0.47 mg of vitamin C/g of FW), with an intracultivar variability lower than 6%. In addition, to check vitamin C stability against oxidation, Alba, Adria, Sveva, and AN03.338.51 genotypes were randomly selected and subjected to HPLC analysis of vitamin C content both as wet (FW) and freeze-dried (DW) materials. Vitamin C concentrations in all dried samples analyzed were lower than the theoretical values deduced from the FW samples (data not shown), suggesting that sample preparation and storage are crucial factors for an accurate determination of the vitamin C content.

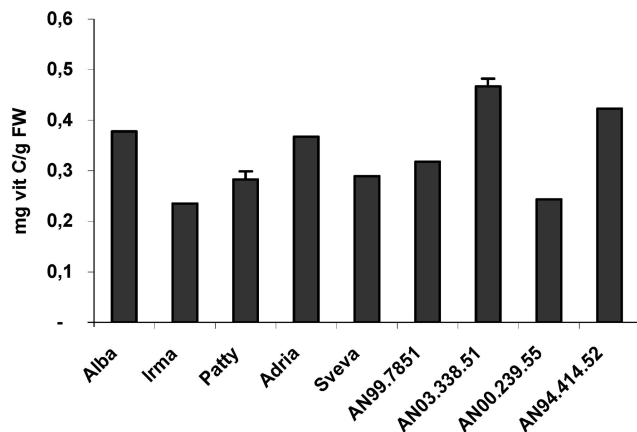


Figure 3. Vitamin C content of the nine selected strawberry genotypes, expressed as milligrams of vitamin C per gram of fresh weight. Error bars refer to biological variability.

The TAC of whole fruit extracts was quantified. In past decades, strong attention has been given to the antioxidant power of fruit as an eligible parameter for quality. This parameter is strictly correlated to the presence of efficient oxygen radical scavengers, such as vitamin C and phenolic compounds. For example, highly reactive species such as polyphenols act in plants as antioxidants and protective agents against several sources of damage (UV, pathogens, etc.) (39). Therefore, they may play an important role in controlling oxidative reactions also in the human body and exhibit their anticarcinogenic and antiatherogenic activities by direct, indirect, and mediated effects (40–42). Obviously, the mechanisms of action of polyphenols and other strawberry compounds go beyond their intrinsic reducing capabilities, being able to exert other additional effects that are as yet poorly understood. The antioxidant capacity per se is not in all cases correlated to the observed beneficial health effects (43), but many of the biological actions of phytochemicals have been attributed to this property. Therefore, the antioxidant capacity of fruit or individual components represents a useful parameter to couple with other measurements. The results of the TEAC and FRAP assays for total antioxidant activity were closely similar, suggesting that the two assays are almost comparable and interchangeable in the case of strawberry. FRAP values were in each case slightly lower than the corresponding TEAC values (Table 1), and TEAC values tended to highlight more intergenotype significant differences than FRAP values once they have undergone statistical analysis. Nevertheless, a strongly significant correlation between the two methods was observed ($r = 0.95$, $p < 0.0001$; Table 3). Selection AN03.338.51 showed the highest TAC (averaging 19.41 and 14.22 μmol of TE/g of FW, respectively), whereas the lowest TAC values were found in Alba (11.27 and 7.31 μmol of TE/g of FW, respectively), according to both TEAC and FRAP assays. A significant correlation was found between TAC of strawberry and Folin–Ciocalteu assay, often referred to as the phenolic content ($r = 0.95$, $p < 0.0001$; TAC measured using TEAC method, as shown in Table 3; $r = 0.92$, $p = 0.0004$; TAC measured by FRAP assay); no statistically significant correlation was observed between TAC values and vitamin C content, although the p value was very close to the limit of significance ($r = 0.66$, $p = 0.0511$; $r = 0.62$, $p = 0.0754$, when correlating vit C content with TEAC and FRAP results, respectively), and this finding indicated that vitamin C makes in any case an important contribution to the TAC.

The Folin–Ciocalteu reagent is a mixture of phosphomolybdate and phosphotungstate; the basic mechanism is an oxidation/reduction reaction and, as such, the method suffers from a number of interfering substances. In particular, the presence of reducing sugars, such as sucrose and fructose, ascorbic acid, aromatic amines, and amino acids, such as tyrosine, tryptophan, and cysteine, may influence the results of the Folin–Ciocalteu assay. For this reason, it is important to consider that the significant correlation also observed between the Folin–Ciocalteu assay (total phenolic) and the vitamin C content ($r = 0.77$, $p = 0.0156$; Table 3) could be the effect of the reactivity of compounds such as vitamin C and sugars in the assay. Correction for the interfering substances should be made, or alternatively the effect of such compounds could be minimized by using solid-phase extraction before analysis (44). We did not include these steps, in order to follow the actual trend to still use the Folin–Ciocalteu assay as a quick method for screening purposes when large numbers of samples are being assessed, for instance, in breeding strategies. A comparison between the value of total polyphenols obtained by the Folin–Ciocalteu assay and phenolic quantifications calculated on the basis of HPLC data is not warranted, because information about the exact reactivity in the Folin–Ciocalteu assay of each single polyphenolic compound and the contribution it gives to the total value is very incomplete.

Anthocyanins did not seem to be the only important polyphenols to influence the TAC of the fruits ($r = -0.07$, $p = 0.8675$, when correlating with TEAC data). These findings are in keeping with previous observations (10, 45) and suggested that associations between the antioxidant properties and the proportion of phenolics present as anthocyanins are generally not very evident in strawberry. Furthermore, as previously observed, no significant relationships were found between the total anthocyanin content and the Folin–Ciocalteu assay ($r = -0.07$, $p = 0.8595$; Table 3) and between anthocyanins and flavonoids ($r = -0.3014$, $p = 0.4307$). These results should be linked to the different principles, chemical reactions, and reference standard compounds that the methods are based on. In any case, the HPLC and antioxidant profiles that we observed suggested that the main anthocyanin derivatives play an important role in the antioxidant properties of strawberries (see below) and also confirmed that several other phenolic compounds give an important cumulative contribution to the TAC of the fruits. The observed variation of the non-anthocyanin antioxidant phenolics among genotypes, on a qualitative and quantitative basis, could explain why the total antioxidant capacity of the extracts is not strongly correlated to the anthocyanin content.

Furthermore, the easier identification of anthocyanins with respect to the other phenolic compounds, through the typical absorption at 500 nm, led us to conduct quantitative analyses on the main pelargonidin and cyanidin derivatives detected during HPLC runs (Table 2). A significant correlation was found ($r = 0.82$, $p = 0.0066$; data not shown) between the total anthocyanin content measured using the pH differential method and the sum of each clearly identified derivative (concentrations expressed as milligrams of anthocyanins per gram of FW).

Contribution of Single Compounds to the Total Antioxidant Capacity of Strawberry Extracts. The online antioxidant system shows which compounds contribute to the TAC of strawberry fruit extracts. The compounds of interest were putatively identified on the basis of their retention times and spectral characteristics. Attention was focused on ellagic acid derivatives ($\lambda_{\text{max}} = 254$ nm), *p*-coumaric acid derivatives ($\lambda_{\text{max}} = 312$ nm), flavonols ($\lambda_{\text{max}} = 360$ nm), and anthocyanins ($\lambda_{\text{max}} = 512$ nm). An example of the antioxidant analysis is shown

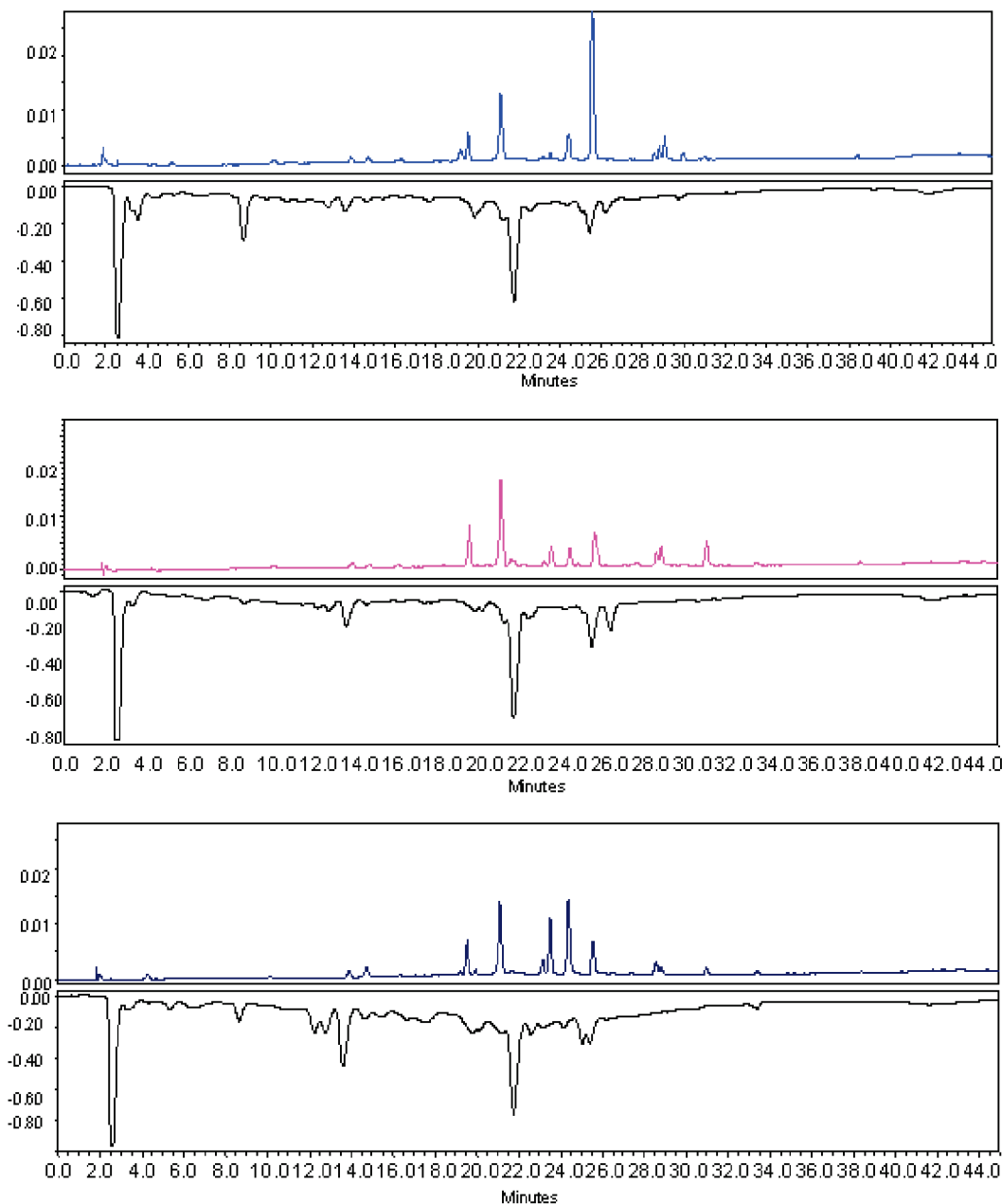


Figure 4. HPLC analysis of antioxidant compounds of different genotypes of strawberries. HPLC chromatograms were recorded at 360 nm and simultaneously at 412 nm after reaction with ABTS, from AN94.414.52 (upper panel), AN99.78.51 (middle panel) and Sveva (lower panel) extracts.

in **Figure 4**, where the antioxidant profiles of three strawberry extracts are compared: AN99.78.51, AN94.414.52, and Sveva, respectively. In **Figure 4**, the upper part of each box is the HPLC chromatogram of antioxidant compounds and represents the chromatograms recorded at 360 nm by the photodiode array detector (PDA), whereas the lower part represents the negative peaks, corresponding to ABTS radical quenching activities of the eluting compounds.

Four main regions containing the most antioxidant capacity were resolved. In all of the strawberry extracts analyzed, the first region consisted of a first sharp peak sometimes followed by smaller unresolved peaks (eluting between 2 and 3 min after sample injection) and represented the main contribution to the TAC of extracts (in all cultivars and selections the first-eluting compounds contributed >30% to the TAC). According to the elution patterns of pure compounds previously analyzed, this region contains vitamin C (RT = 2 min) and other highly polar antioxidants (31).

The second region included antioxidant compounds eluting between 5 and 9 min after sample injection and showed the presence of polar antioxidants, likely belonging to the class of phenolic acids, with an absorbance maximum at 280 nm. Relevant intergenotype differences in this region were evident in the HPLC and antioxidant profiles (see **Figure 5A**). Specific identification of these compounds, for example, by means of LC-MS/MS analysis, is required for further annotation. These compounds were found to contribute significantly to the TAC of, for instance, AN94.414.52 (**Figure 4**).

The third antioxidant region of the chromatograms contained compounds eluting between 11 and 15 min after sample injection. The most prominent compounds present in this region were hydroxycinnamic acids, mainly *p*-coumaric acid derivatives, with absorbance maximum at 312 nm. These compounds were present in all tested strawberry genotypes, but differed strongly in relative quantity (the highest content in Adria and

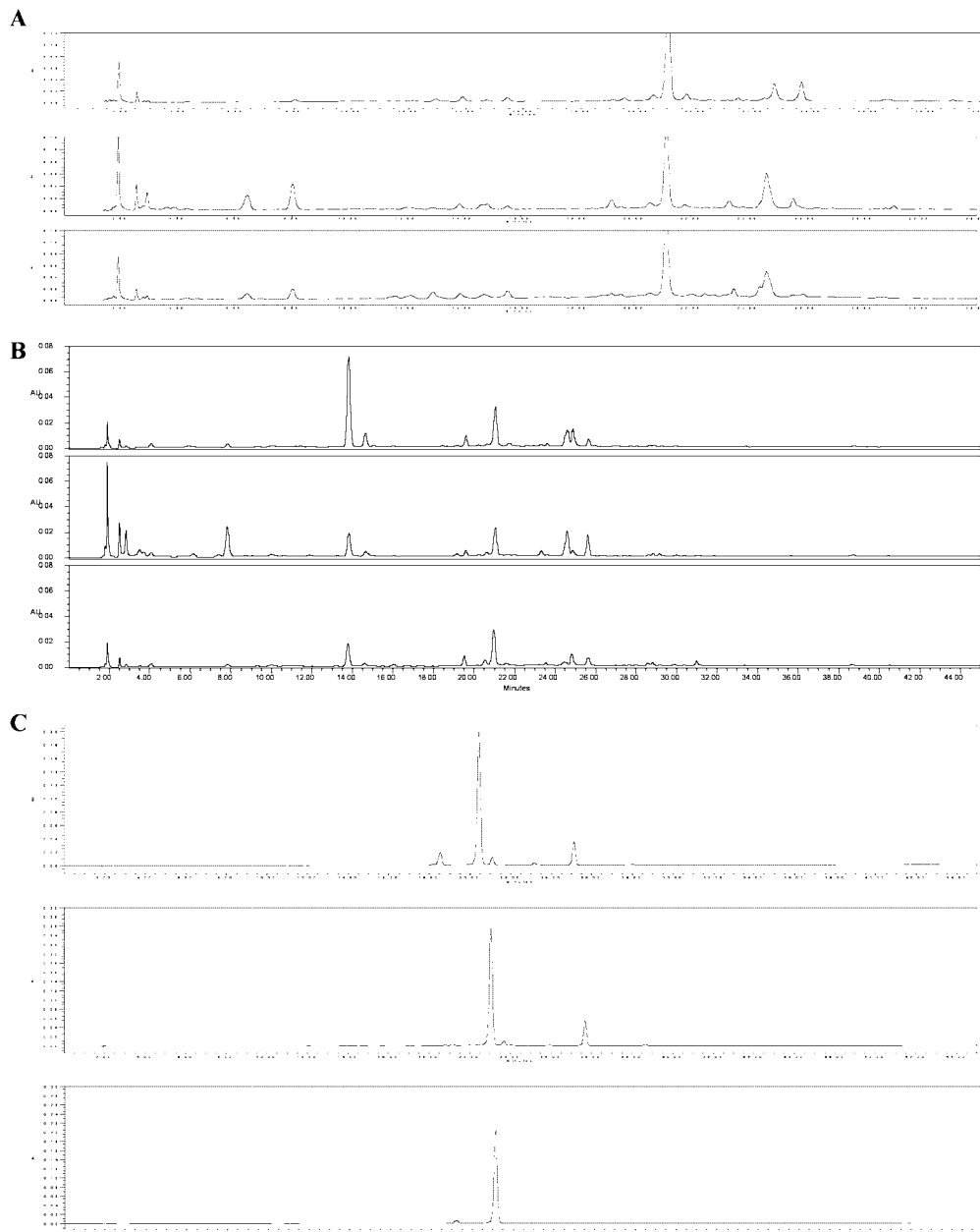


Figure 5. (A) HPLC chromatograms recorded at 280 nm of AN99.7851 (upper panel), Sveva (middle panel), and AN94.414.52 (lower panel). (B) HPLC chromatograms recorded at 312 nm of Adria (upper panel), AN94.414.52 (middle panel), and AN99.7851. (C) HPLC chromatograms recorded at 512 nm of Alba (upper panel), AN99.7851 (middle panel), and AN03.338.51 (lower panel).

Irma, the lowest in Sveva, AN99.78.51, and AN94.414.52) (Figure 5B).

p-Coumaric acid has been described as an abundant phenolic acid in strawberries, raspberries, and cloudberrries (16, 21). However, matching of the hydroxycinnamate peak with the antioxidant chromatogram indicated that this compound did not contribute significantly to the TAC of the extracts. In contrast, other compounds with absorbance maxima at 280 nm, probably flavanols (RT between 11.5 and 13.5 min), gave relevant antioxidant peaks (Figure 4). Further identification of these compounds is required for a better insight in their chemical nature.

The fourth region comprised antioxidant peaks eluting between 18 and 31 min after sample injection. The spectral analyses showed that compounds with RT = 19.2, 21.1, 21.7, and 25.7 min all have an absorbance maximum at around 510 nm, which is indicative of anthocyanins. Derivatives of both pelargonidin (absorbance maximum at 500 nm) and cyanidin

(absorbance maximum at 514 nm) were found in these extracts. Qualitative and quantitative differences were observed among strawberry genotypes (Figure 5C). Pelargonidin derivatives were the most represented anthocyanins in all of the samples, giving the main contribution to the TAC of the extracts outside the vitamin C region. For example, the anthocyanin compound identified as pelargonidin-3-glucoside according to the retention time and spectral absorption (see Figure 4) gave alone a contribution to the total antioxidant capacity of the eluting compounds (excluding the hydrophilic vitamin C-like compounds) ranging from approximately 25% (in Adria > AN03.338.51 > Sveva) to nearly 40% (in AN99.78.51 > Irma > AN94.414.52).

Spectral analyses and chromatographic patterns indicated that the antioxidant compounds later (RT between 23 and 31 min) eluting in the fourth region of the chromatograms mainly

comprised ellagic acid derivatives and flavonols (absorbance maximum at around 254 and 360 nm, respectively).

Ellagic acid derivatives (RT = 23.1, 23.4, and 24.3 min) are highly represented in Sveva, Alba, and AN03.338.51, where the antioxidant contribution to the TAC is evident, whereas a lower content is observed in AN94.414.52. Significant differences were also found on the flavonol content of the different strawberry genotypes. Two quercetin derivatives (RT = 24.4 and min) were identified in almost all of extracts, but the highest peaks were observed in AN94.414.52, Alba, and AN03.338.51. The kaempferol derivatives (RT = 28.6, 28.8, and 31.1 min) were present to a lower extent and less homogeneously represented among cultivars and selections. Hardly any antioxidant activity associated with these substances was observed, confirming that flavonols did not significantly contribute to the TAC of strawberries.

In contrast to what was previously observed in raspberry extracts (31), no relevant antioxidant peaks were present in the last part of the chromatograms. In this work we did not detect peaks clearly identified as ellagitannins. These findings may support previous studies (46), confirming that ellagitannin content in strawberry is significantly lower than that in raspberry. However, the results may also suggest that ellagitannins present in strawberries could be more easily decomposed and converted to free ellagic acid than in raspberry fruits (22).

Consumers are now aware that consumption of fruits rich in health-promoting compounds is an appropriate strategy to enjoy their benefits, and the nutritional quality of fruit today is becoming an attribute as important as the organoleptic—sensorial quality, even if it is still an extremely complex parameter. Breeding and biotechnological approaches are currently used to increase the content of specific bioactive compounds in plants, because higher levels of micronutrients and phytochemicals in fruit may be an important tool to support a higher antioxidant intake even in the case of low consumption of fruit.

Strawberry represents one of the most important sources of bioactive compounds with antioxidant activity, together with other berries. Several genetic and environmental factors affect the production and accumulation of bioactive compounds on strawberry fruits, and the effect of cultivar in affecting the nutritional quality of strawberry is well-known (47), even if still few genotypes are well characterized for these important features.

Results obtained in this study can be considered of particular interest to better define the varieties and breeding evaluation strategies and confirm the importance of the genetic background on the availability of specific compounds in strawberry fruits. Results on *F. × ananassa* cultivars and advanced selections show a good variability among genotypes of the cultivated species, whereas results from the *F. × ananassa × F. virginiana glauca* F1 and F2 show how a wild species can contribute to the introgression of interesting nutritional features in cultivated strawberries.

Nowadays these aspects are considered to be highly valuable for the commercial valorization of new varieties but mostly to the selection of new genotypes with high fruit nutritional quality combined with yield efficiency.

In any case, an accurate assessment of the fruit nutritional quality of new genotypes needs several years of evaluation, because year-to-year variability in quality and nutritional features of each genotype should be expected.

ABBREVIATIONS USED

ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); DW, dried weight; DM, dry matter; FVG, *Fragaria virginiana*

glauca; FW, fresh weight; RDA, recommended daily allowance; TAC, total antioxidant capacity; TE, Trolox equivalents; TPC, total phenolic content; GAE, gallic acid equivalents; ACY, total anthocyanin content; Pg-3-gluc, pelargonidin-3-glucoside; FL, total flavonoid content; (+)CE, catechin equivalents; Fo, total folate content; TEAC, Trolox equivalent antioxidant capacity assay; FRAP, ferric reducing antioxidant power assay; TPTZ, 2,4,6-tripyridyl-*s*-triazine; DTPA, diethylenetriaminepentaacetic acid; TFA, trifluoroacetic acid.

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