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The Nutritional Quality of Strawberries (*Fragaria x ananassa*) after Short-refrigeration: Genetic Influences

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ABSTRACT

The strawberry (*Fragaria* x *ananassa*, Duch.) has recently received great commercial development and represents the most commonly consumed berry. The strawberry is also a relevant source of micronutrients and phenolic substances, most of which are natural antioxidants and contribute to the high nutritional quality (NQ) of the fruit. In addition to genetic heritable differences, several pre- and post-harvest environmental conditions seem to strongly influence the NQ of strawberry. The short-refrigeration of the fruits is the most common method to control the decay of strawberries. However, little is known on the influence of cold storage on the antioxidant, phenolic and micronutrient contents in strawberries, and on the genetic influence on the storability of these fruits. In this work, five strawberry cultivars were analyzed for total antioxidant capacity, total phenolics, flavonoids and anthocyanins, and for micronutrient contents of both fresh and stored fruits. Three consecutive years of harvest were studied to assess the combined effect of genotype and pre-harvest environmental conditions on the NQ and storability of the fruits. Significant cultivar-to-cultivar differences were observed in the NQ parameters studied, confirming how the genetic background may significantly affect the nutritional value of strawberries. Short-refrigeration did not seem to affect either negatively or positively the main NQ attributes of the strawberries, with the exception of the folate content, which significantly increased after storage in all three years. These findings are particularly concerned with some varieties in this study, suggesting a genetic influence on fruit response to storage.

Keywords: antioxidant capacity, short-refrigeration, storability
Abbreviations: **CEq**, catechin equivalents; **FS**, fresh strawberries; **FW**, fresh weight; **GAEq**, gallic acid equivalents; $\mathbf{T_{min}}$, hourly minimum temperature; $\mathbf{T_{max}}$, hourly maximum temperature; **Hum**, humidity; **NQ**, nutritional quality; **Pg-glcEq**, pelargonidin-3-glucoside equivalents; **SS**, stored strawberries; $\mathbf{H_{T\geq 28}}$, thermic sums; **TAC**, total antioxidant capacity; **ACY**, total anthocyanin content; **FLAVO**, total flavonoid content; **Fol**, total folate content; **TPC**, total phenolic content; **Teq**, trolox equivalents; **vit C**, vitamin C

INTRODUCTION

Increasing epidemiological evidence suggests that the combination of micronutrients and non-essential phytochemicals such as phenolic compounds, contained in fruits and vegetables, may play a synergistic and cumulative role in health promotion. Particularly, fruits and vegetables contain many different antioxidant components which seem to have important protective properties against the development of several chronic diseases, due to the involvement of oxidative stress as a common denominator in their pathogenesis. Plant-derived antioxidants are also widely reported to have *in vivo* biological activities not necessarily related to their antioxidative properties, ranging from anticancer, anti-inflammatory to antiatherosclerotic activities (de Ruvo *et al*. 2000; Chu *et al*. 2002; Johnsen *et al*. 2003; Etminan *et al*. 2004; Hung *et al*. 2004). As a consequence, there is renewed interest in evaluating the micronutrient, phytochemical and antioxidant composition of plant foods. The improvement of the nutritional quality (NQ) of fruits and vegetables has become a new quality target of biotech and breeding strategies, and the evaluation of the pre- and post-harvest factors affecting the quality attributes are receiving ample attention.

Among the berry species, strawberry (*Fragaria* x *ananassa* Duch.) is one of the most commonly consumed fruit and has recently received the best commercial development. Moreover, strawberries are a good source of natural antioxidants (Wang *et al.* 1999; Wang and Lin 2000; Scalzo *et al.* 2005a, 2005b). In addition to the usual micronutrients such as vitamins and minerals, the total antioxidant capacity (TAC) of the fruit can largely be attributed to the phenolic compounds such as anthocyanins and other flavonoid and non-flavonoid compounds, which strongly contribute to both the sensorial-organoleptic and the nutritional quality of the fruits (Hannum 2004).

Together with heritable genetic differences, several pre- and post-harvest environmental conditions such as temperature and light seem to strongly influence the antioxidant and phytochemical composition of strawberry. In particular, post-harvest storage is a crucial factor for its NQ (Kalt *et al.* 1999; Pérez *et al* 1999; Mali *et al.* 2003), as the strawberry is a highly perishable fruit with a limited and rapidly compromised storage life. Short-refrigeration, consisting of storage of the fruits at 4°C for three days followed by one day at room temperature (Testoni *et al.* 1989; Faedi *et al.* 2004), in the dark, is the most common method to control decay and maintain their quality attributes until commercialization and consumption. However, little research has been conducted to evaluate the changes of TAC, micronutrient and phenolic contents in strawberries after short-refrigeration. Furthermore, little information is available on the potential influence of the genotype on the storability of the fruits.

In this work, five strawberry cultivars with well-known (Battino and Mezzetti 2006; Tulipani *et al.* 2008) genotype-dependent nutritional differences were analyzed for TAC, total phenolics (TPC), flavonoids (FLAVO) and anthocyanins (ACY), and the micronutrient content in both the fresh and the shortly-refrigerated fruits. Strawberries harvested in three consecutive fruiting seasons (2006, 2007,

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Table 1 General characteristics of plants and fruits of the genotypes in study

Genotype	Fruit	Ripening time	Productivity	Quality
ALBA	Large-sized, regular and well-shaped, firm, red glossy fruit	Early in the season	High, but inconstant	Average taste
IRMA	Large-sized, regular shaped, too soft and dark fruit	Intermediate in the season	Very high, with high amount of rottenness	Poor taste
PATTY	Medium-sized, very regular and well-shaped, medium firmness, red fruit	Intermediate in the season	High	Average taste
ADRIA	Large-sized, well-shaped, firm, orange-red glossy fruit. Deformed fruits depending on the season	Late in the season	High, with rottenness	Average taste
SVEVA	Large-sized, unregular-shaped, firm, dark fruit. Deformed fruits depending on the season	Very late in the season	High	Average taste

2008) were analysed, in order to assess the combined effect of genotype and pre-harvest environmental conditions on the NQ and the storability of the fruits.

MATERIALS AND METHODS

Strawberry material

For the study, we selected five commercial varieties of strawberry ('Alba', 'Irma', 'Patty', 'Adria', 'Sveva') cultivated in an experimental field for the genetic improvement of strawberry varieties in the Azienda Agraria Didattico Sperimentale "P. Rosati" of Marche Polytechnic University (Agugliano, Ancona, Italy) (**Table 1**). In May 2006, 2007 and 2008, the ripe fruits were hand-picked at the same time on different days, corresponding to the ripening times of the selected clones ('Alba': early ripening; 'Irma', 'Patty': intermediate ripening; 'Adria', 'Sveva': late ripening). For fresh strawberry (FS) analysis, half of the whole amount of fruits harvested was stored at –20°C within 2 h after harvest. For vitamin C and folate measurements, a part of these fruits were also snap-frozen in liquid nitrogen, ground to a fine powder by using a pre-cooled grinder (IKA A11 basic) and the frozen powders were stored at –80°C until analysis. The remaining half of the fruit, assigned to stored strawberry (SS) analysis, were subjected to short-refrigeration (3 d at 4°C + 1 d at room temperature, in the dark) prior to being stocked for further analysis.

Immediately prior to analysis, compound hydroalcoholic extraction was carried out via homogenization (Tulipani *et al.* 2008) or via sonication (see below Tulipani *et al.* 2009) as previously described, depending on the analysis.

TAC determination

The TAC of the hydroalcoholic extracts of the fruits was assessed by the Ferric Reducing Antioxidant Power (FRAP) assay, as previously described (Benzie and Strain 1996); results are expressed as micromoles of Trolox equivalents (TEq) per g of fresh weight of strawberries [μmoles TEq/g FW], and represent the mean value ± SD of eight measurements.

TPC determination

Total phenolic content of the extracts was determined using the Folin-Ciocalteu colorimetric method, as modified by Slinkard and Singleton (1977). Results are expressed as milligrams of gallic acid equivalents (GAEq) per g of fresh weight of strawberries [mg GAEq/g FW, mean value ± SD of eight measurements].

FLAVO determination

Total flavonoid value was determined according to a colorimetric method previously described (Dewanto *et al.* 2002) and catechin was used as a reference standard compound. Results are expressed as milligrams of catechin equivalents (CEq) per g of fresh weight of strawberries [mg CEq/g FW, mean value ± SD of eight measurements].

ACY determination

The total anthocyanins were measured using a modified pH differential method previously described (Giusti *et al.* 1999) with slight modifications, and absorbance readings were converted to quantifications through a calibration curve obtained by known concentrations of pelargonidin-3-glucoside (Pg-glc) standards. Results are expressed as milligrams of Pg-glc equivalents per g of fresh weight of strawberries [mg Pg-glcEq/g FW, mean value ± SD of eight measurements].

Vitamin C quantification

Immediately before analysis, 2 mL of ice-cold extraction solution, consisting of 5% meta-phosphoric acid and 1 mM DTPA in milliQ water, was added to 0.5 g FW strawberry frozen powder, and the mixture sonicated at 4°C for 15 min in the dark. After the ultrasound assisted extraction, the mixture was centrifuged at 2500 rpm for 10 min at 4°C to precipitate cell walls and proteins, the supernatant filtered (0.2 μm PTFE filters) and immediately analysed for vitamin C content by reverse-phase HPLC with a Photodiode array detector (CBM-20A Model, Shimadzu Italia S.r.l.) (Tulipani *et al.* 2008). Quantifications were carried out by running standard concentrations of pure vitamin C similarly prepared. Results are expressed as milligrams of vitamin C (vit C) per g of fresh weight of strawberries [mg vit C/g FW, mean value ± SD of three measurements].

Folate quantification

The total folate content (Fol) was quantified by using the microbiological assay (MA), utilizing a 96-well microplate technique, as previously described (Sysbema *et al.* 2003; Pandrangi and LaBorde 2004). The microbiological folate analysis was carried in the strawberry extracts once subjected to a deconjugation pretreatment, and the *Lactobacillus rhamnosus* growth was determined by measuring the increase in optical density at 580 nm using an automated microplate reader (Emax Molecular Devices Corporation, Sunnyvale, Calif.). Results were expressed as ng folate per gram of fresh weight of strawberry fruit [ng Fol/g FW] (mean value of four technical replicates) ± SD.

Climatic data

Climatic data were kindly provided by the agroweather resort located in Agugliano (Ancona, Italy) of the Marche Agency for Services in the Agroindustrial Sector (ASSAM). From the hourly minimum (T_{min}) and maximum (T_{max}) temperatures (°C) recorded by the agroweather resort we calculated the average daily temperatures (T_{day}) and we compared the T_{day} from the beginning of April until the end of May, for the three years of study (2006, 2007, 2008). To outline any relevant differences in the climatic stress conditions during the pre-ripening and ripening times of each studied years, we calculated the sum of the hours where temperature $T \geq 28$°C (called Thermic Sums, $H_{T \geq 28}$) both during the month preceding ripening (April), and during May. To distinguish the temperature stress conditions according to strawberry ripening and harvest times, we calculated the $H_{T \geq 28}$ until the 10th May (early-ripening clones), 20th May (intermediate-ripening clones), 30th May (late-very late-ripening clones), separately.

Also the humidity (Hum %) and the extent of rainfall (mm) in the months of April and May 2006, 2007 and 2008 were calculated, and the year-to-year differences observed.

Statistical analysis

Statistical analyses were performed using STATISTICA software (Statsoft Inc., Tulsa, OK, USA). Data were subjected to one-way analysis of variance for mean comparison, and inter-genotype significant differences were calculated according to HSD Tukey's multiple range test. Data are reported as mean ± standard deviation (SD). Differences at $p < 0.05$ and $p < 0.001$ were respectively considered statistically significant and highly significant.

RESULTS

Table 2 summarizes the TAC values, the TPC, FLAVO and ACY contents, and the Vit C and Fol concentrations of the FS and the SS from the five selected cultivars. Data corresponding to the three fruiting seasons in study are reported separately. **Fig. 1** graphically shows the FRAP values (**Fig. 1A**), the phenolic (**Fig. 1B-D**) and micronutrient (**Fig. 1E-F**) concentrations of the strawberry genotypes tested, when expressed as mean values over the three years in study. In each of the six graphs of the figure, significant genotype-to-genotype differences are expressed by different superscript letters above the FS columns; in addition, significant differences between the FS and the SS within cultivars are reported for each quality parameter analysed. The total phenolic content (TPC) reported in **Fig. 1B** has been measured through one of the most commonly used spectrophotometric methods (Slinkard and Singleton 1977). However, it is important to outline that 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 (Georgé *et al.* 2005). Particularly, the presence of reducing sugars such as sucrose and fructose, ascorbic acid, aromatic

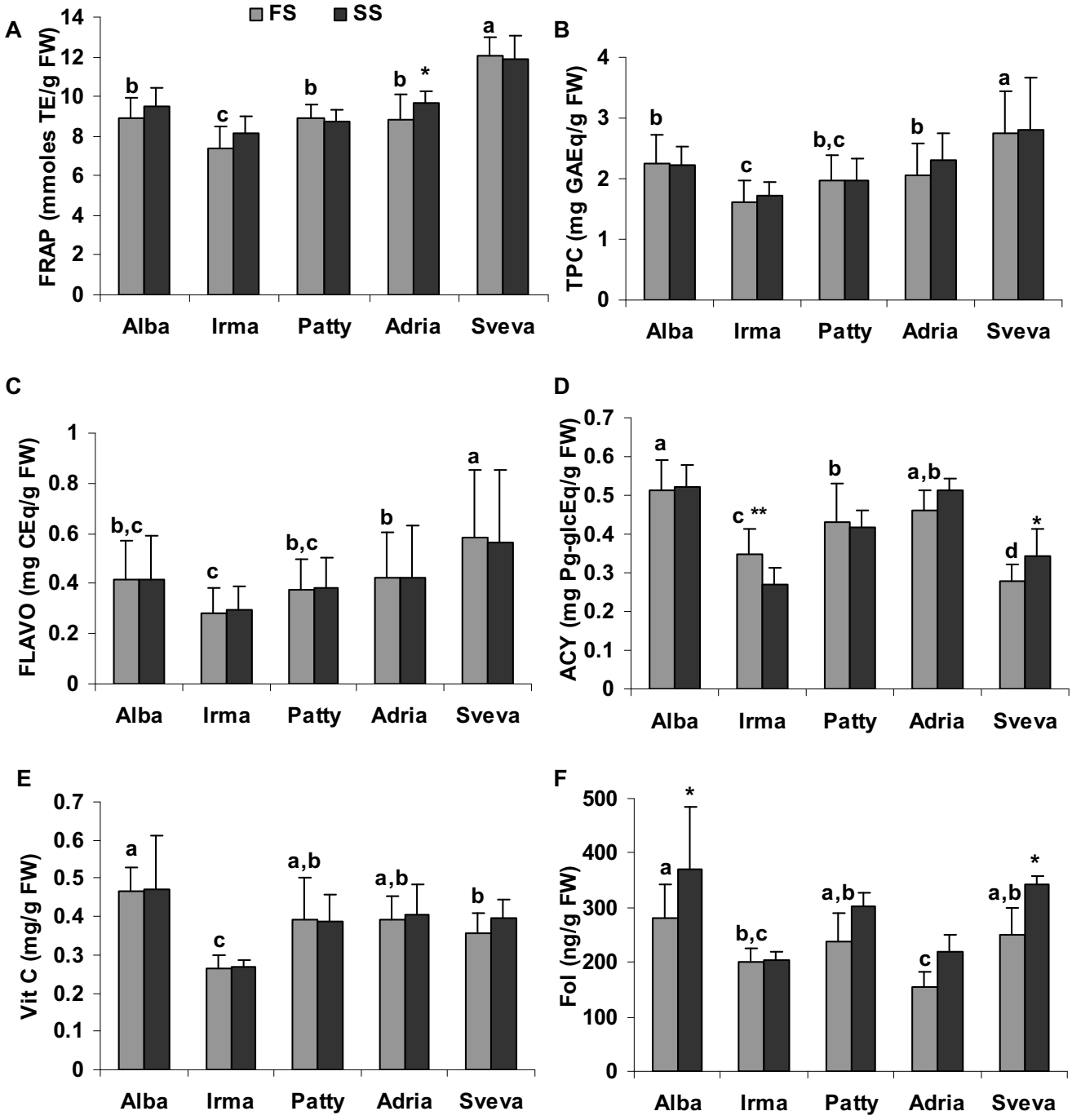

Fig. 1 TAC values (**A**), phenolic (**B-D**) and micronutrient (**E-F**) concentrations of the five selected strawberry genotypes, when expressed as mean values among the three fruiting seasons in study. In each of the six graphs, genotype-to-genotype significant differences ($p < 0.05$) are expressed by different superscript letters above the FS columns. Significant and highly significant differences between the FS and the SS within cultivars are expressed by a single ($p < 0.05$) or double ($p < 0.001$) asterisk above the column showing the higher value.

Table 2 TAC values (FRAP assay), total phenol (TPC), flavonoid (FLAVO) and anthocyanin (ACY) contents, vitamin C (Vit C) and folate (Fol) concentrations of the 5 selected cultivars in the three fruiting seasons in study. Values corresponding to the fresh (FS) and stored (SS) fruits are separately presented. Data are reported as mean ± SD. *The Fol data of 'Sveva' strawberries harvested in 2007 are missing. DM = dry matter (%).

Genotype	Year	DM (%)		TAC (FRAP assay) (µmoles TE/g FW)		TPC (mg GAE/g FW)		FLAVO (mg CE/g FW)		ACY (mg PgE/g FW)		Vit C (mg/g FW)		Fol (ng/g FW)	
		FS	SS	FS	SS	FS	SS	FS	SS	FS	SS	FS	SS	FS	SS
Alba	2006	6.8	5.9	9.0±1.3	10.4±0.3	2.1±0.1	2.5±0.1	0.5±0.1	0.6±0.0	0.5±0.0	0.6±0.0	0.4±0.0	0.4±0.0	236.6±17	329.8±13
	2007			9.5±0.9	8.3±0.3	2.8±0.1	2.3±0.1	0.5±0.0	0.4±0.0	0.6±0.0	0.5±0.0	0.5±0.0	0.4±0.0	246.0±11	263.5±27
	2008			8.4±0.2	9.7±0.2	1.8±0.1	1.9±0.1	0.2±0.0	0.2±0.0	0.4±0.0	0.6±0.0	0.5±0.0	0.7±0.0	359.2±27	514.3±42
Irma	2006	5.3	6.1	8.4±0.5	8.3±0.1	1.5±0.2	1.6±0.1	0.4±0.1	0.4±0.0	0.4±0.1	0.3±0.0	0.3±0.1	0.3±0.0	172.0±15	193.4±19
	2007			7.1±0.5	7.3±0.6	2.0±0.1	2.0±0.1	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.2±0.0	0.3±0.0	209.7±12	209.5±11
	2008			6.7±1.3	8.8±0.6	1.2±0.3	1.6±0.1	0.1±0.0	0.2±0.0	0.4±0.0	0.2±0.0	0.3±0.0	0.3±0.0	219.4±4	212.0±9
Patty	2006	6.0	6.8	8.3±0.6	8.1±0.2	1.8±0.2	1.8±0.1	0.4±0.0	0.4±0.0	0.5±0.2	0.4±0.1	0.3±0.0	0.3±0.0	170.6±24	295.8±13
	2007			9.3±0.6	8.9±0.6	2.5±0.1	2.5±0.1	0.5±0.0	0.5±0.0	0.5±0.0	0.4±0.0	0.3±0.0	0.4±0.0	270.0±32	277.9±14
	2008			9.1±0.4	9.2±0.5	1.6±0.0	1.7±0.1	0.2±0.0	0.2±0.0	0.4±0.0	0.4±0.0	0.5±0.0	0.5±0.0	268.8±28	332.1±12
Adria	2006	6.1	6.5	9.8±0.3	10.0±0.5	2.1±0.1	2.3±0.3	0.5±0.0	0.5±0.1	0.4±0.0	0.5±0.0	0.3±0.0	0.3±0.0	127.9±11	203.4±17
	2007			9.3±1.0	9.7±0.6	2.6±0.1	2.8±0.2	0.5±0.0	0.6±0.0	0.4±0.0	0.5±0.0	0.5±0.0	0.5±0.0	178.4±21	213.8±26
	2008			7.3±0.3	9.3±0.3	1.4±0.1	1.8±0.1	0.2±0.0	0.1±0.0	0.5±0.0	0.5±0.0	0.3±0.0	0.4±0.0	161.3±4	241.0±41
Sveva	2006	6.8	7.1	11.0±0.5	13.0±0.8	2.6±0.1	3.1±0.1	0.7±0.0	0.8±0.1	0.3±0.0	0.4±0.0	0.3±0.0	0.4±0.0	187.2±32	355.0±12
	2007			12.1±0.8	12.0±0.8	3.6±0.1	3.7±0.2	0.8±0.0	0.7±0.0	0.3±0.0	0.4±0.0	0.3±0.0	0.4±0.0	291.7±9	*
	2008			13.0±0.4	10.6±0.3	2.0±0.1	1.7±0.1	0.2±0.0	0.2±0.0	0.2±0.0	0.3±0.0	0.4±0.0	0.5±0.0	267.5±6	330.2±9

Table 3 TAC values (FRAP assay), total phenol (TPC), flavonoid (FLAVO) and anthocyanin (ACY) contents, vitamin C (Vit C) and folate (Fol) concentrations of the 5 selected genotypes, subdivided in Early-, Intermediate- and Late-ripening cultivars. Values corresponding to the fresh (FS) and stored (SS) fruits are separately presented. Data are reported as mean values of the three fruiting seasons ± SD. Different letters in the same row indicate a significant difference among ripening groups ($p < 0.05$).

		Ripening Time		
		Early	Intermediate	Late
FRAP^	FS	8.94 ± 1.0 b	8.15 ± 1.2 b	10.41 ± 2.0 a
	SS	9.47 ± 1.0 b	8.44 ± 0.8 c	10.77 ± 1.4 a
TPC°	FS	2.26 ± 0.5 a	1.80 ± 0.4 b	2.40 ± 0.7 a
	SS	2.23 ± 0.3 b	1.85 ± 0.3 c	2.55 ± 0.7 a
FLAVO°	FS	0.41 ± 0.2 ab	0.33 ± 0.1 b	0.50 ± 0.2 a
	SS	0.42 ± 0.2 ab	0.34 ± 0.1 b	0.47 ± 0.2 a
ACY°	FS	0.51 ± 0.1 a	0.39 ± 0.1 b	0.37 ± 0.2 b
	SS	0.52 ± 0.1 a	0.34 ± 0.1 c	0.43 ± 0.1 b
vit C°	FS	0.47 ± 0.1 a	0.33 ± 0.1 b	0.37 ± 0.1 b
	SS	0.47 ± 0.1 a	0.33 ± 0.1 b	0.40 ± 0.1 a
Fol"	FS	280.6 ± 61 a	218.4 ± 45 b	202.3 ± 61 b
	SS	369.2 ± 114 a	253.5 ± 54 b	268.7 ± 67 b

^ µmoles TE/g FW ; ° mg/g FW; " ng/g FW.

amines and ammnoacids such as tyrosine, tryptophan and cysteine may influence the results of the Folin-Ciocalteu assay giving aspecific data. Correction for the major interfering substances should be made, or alternatively the effect of such compounds may be minimised by using solid-phase extraction before analysis (Georgé *et al.* 2005). However, we did not include these steps in order to follow the actual trend and still use the Folin-Ciocalteu assay as a quick method for screening purposes when large numbers of samples are being assessed (i.e. in breeding strategies).

In **Table 3**, the influence of the ripening time on the NQ of the strawberry fruits was outlined by grouping and averaging the data related to the early- ('Alba'), intermediate- ('Irma' and 'Patty') and late-ripening ('Adria' and 'Sveva') clones, respectively. Data on the FS and SS of the three ripening groups are reported separately, and represent the mean values over the three years of study.

The main climatic indicators recorded during the months of April and May of the three fruiting seasons in study are shown in **Table 5**. In **Fig. 2**, the sum of daily hours where the temperature was above 28°C (Thermic Sums, $H_{T\geq28}$) are progressively reported for May 2006, 2007 and 2008, in order to identify the temperature stress conditions to which the plants were subjected during the three years. During the last ten days of April, $H_{T\geq28} = 0$ in all the three years. The $H_{T\geq28}$ recorded until the 10th May (early clones), the 20th May (intermediate clones) and 30th May (late-very late clones), are outlined in the graph.

DISCUSSION

The five strawberry genotypes selected for this study are commercial cultivars of Italian production, characterized by different ripening times, plant productivity, and organoleptic-sensorial quality of their fruits (**Table 1**) (Faedi *et al.* 2002). In previous years (Battino and Mezzetti 2006) these varieties had already been screened for the total antioxidant capacity and total phenolic contents of their strawberry extracts, and distinguished for their lower ('Alba' > 'Adria' > 'Irma') and higher ('Sveva' > 'Patty') TAC and TPC values. However, most of the information available concerns fresh fruit immediately analysed after harvest, or eventually frozen within a few hours for further analysis. Little research has been focused until now on the effects of cold storage on the NQ of these fruits, and on the possible influence of the genotype on their storability. In order to evaluate the potential impact of storage on the antioxidant, phenolic and micronutrient content of strawberries, we compared the nutritional quality of fresh strawberries and strawberries subjected to short-refrigeration, for each of the genotypes in study.

As outlined in **Table 2** and **Fig. 1**, significant cultivar-to-cultivar differences were observed in the NQ parameters studied, confirming how the genetic background may significantly affect all the nutritional quality attributes of the five strawberry clones (Battino and Mezzetti 2006; Tulipani *et al.* 2008). During the three fruiting seasons studied, the variety 'Irma' showed the lowest values in terms of both the TAC of the fruits and the phenolic (TPC, FLAVO and ACY) and micronutrient (vit C and Fol) contents, confirming previous reports (Battino and Mezzetti 2006; Tulipani *et al.* 2008). The varieties 'Sveva' and 'Alba' resulted as being the cultivars with the highest NQ attributes, while the other varieties 'Patty' and 'Adria' showed intermediate values. The only exception was the ACY content, which did not seem to follow the same trend, and variety 'Sveva' showed the lowest anthocyanin content in all three harvests evaluated.

When evaluating the varieties all together, the short-refrigeration did not seem to affect either negatively or positively the main NQ attributes of the strawberries, since no significant differences were observed between the average values of FS and SS. The only relevant exception was the Fol content which was found to be positively affected by the cold storage, since Fol concentration of the fruits significantly increased after their short-refrigeration, in all

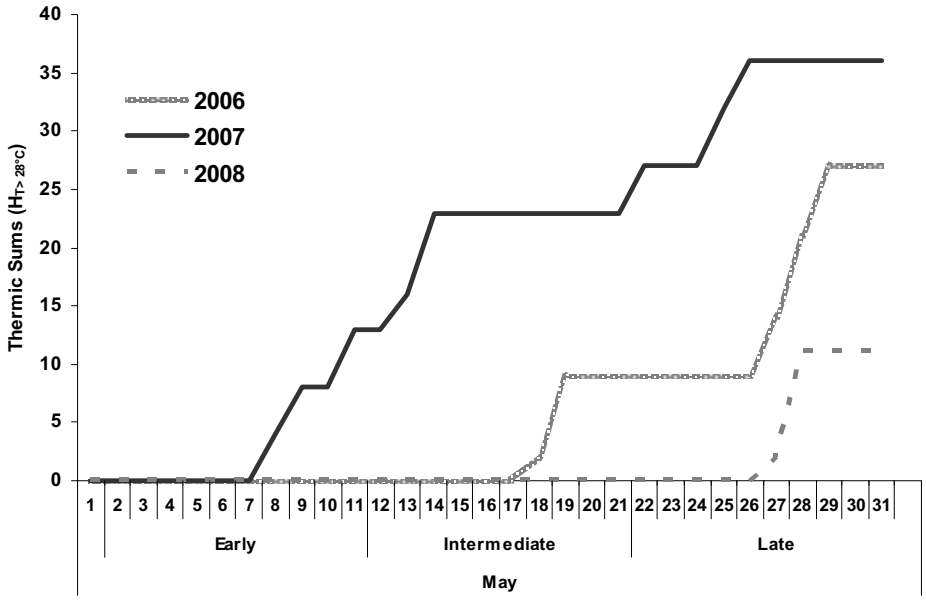

Fig. 2 Progressive sums of the daily hours where temperature was above 28°C (Thermic Sums, $H_{T\geq28}$) recorded during the months of May 2006, 2007 and 2008. $H_{T\geq28}$ = 0 for the last ten days of April, in all the three years. The $H_{T\geq28}$ at the 10th of May (early clones), at the 20th of May (intermediate clones) and at the 30th of May (late-very late clones) are reported in the graph.

the three fruiting seasons studied. This increase does not appear to be attributable to water loss and micronutrient concentration in the fruits, also when considering that the average dry matter of the fruits did not effectively change (DM_{FS} = 6.2%; DM_{SS} = 6.4%).

In the past, other treatments (i.e. ozone treatment) showed detrimental effects on strawberry NQ and shelf life (Pérez *et al.* 1999). On the contrary, our data confirmed that strawberry NQ does not suffer chilling injury when the storage temperature is kept at 4°C (Olsson *et al.* 2004; Strålsjö *et al.* 2003; Rivera-López et al. 2004). In addition, in respect to effective but more complex treatments such as the controlled atmosphere storage technology (Almenar *et al.* 2006)), our data confirmed the cold storage as one of the most simple and suitable methods to control strawberry decay and maintain its quality attributes.

When dividing the data of the individual genotypes studied (**Fig. 1**), however, significant increases in the short-refrigerated fruits were only observed for 'Alba' and 'Sveva', while 'Irma' did not appear to be affected by post-harvest storage. This finding seems to confirm the already observed genotype-to-genotype NQ differences and broadens this concept to a different storability of the fruits. In addition, when evaluating the cultivars separately, significant and highly significant changes in the antioxidant (**Fig. 1A**) and anthocyanin (**Fig. 1D**) contents of the fruits were outlined in individual varieties, suggesting the need for further evaluations in the coming years.

To evaluate the influence of the ripening time on the NQ of the strawberries studied, the data related respectively to the early-, intermediate- and late-ripening clones were separately grouped and the three ripening groups of cultivars were compared for their antioxidant, micronutrient and phenolic contents, both in fresh and in stored fruits (**Table 3**). When evaluating the three strawberry ripening groups without any distinction of the year of harvest, significant differences were found regarding all the NQ parameters studied. In particular, the late-ripening and early-ripening varieties showed the highest TAC, TPC and FLAVO contents (late clones > early clones > intermediate clones) and the highest micronutrient concentrations (early clones > late clones > intermediate clones). On the other hand, the later the ripening occurred, the lower the ACY contents appeared (early clones > intermediate clones > late clones), confirming how this parameter does not correlate with the other NQ attributes studied. The observed differences persisted among the short-refrigerated fruits, where the difference between the poorest strawberries (intermediate clones) and the remaining varieties was exacerbated (**Table 3**).

Finally, when the FS of the three fruiting seasons in study (2006, 2007, 2008) were analysed separately (**Table 4**), significant ripening-dependent differences in the TAC values persisted in all three years, with the intermediate clones showing the lowest values. However, no significant variations in the TPC and FLAVO contents were observed among the early-, intermediate- and late-ripening varieties in year 2008 (**Table 4**). Moreover, significant ripening time-dependent differences in the ACY and vit C contents were only found in 2007 (**Table 4**).

The more evident intra-year differences observed in 2007 should probably be imputed to the sharp changes in temperatures, observed during the month of May in that year. In fact, when comparing the $\mathbf{H_{T\geq28}}$ relative to May 2006, 2007 and 2008 (**Fig. 2**), it emerges that in May 2007

Table 4 TAC values (FRAP assay), total phenol (TPC), flavonoid (FLAVO) and anthocyanin (ACY) contents, vitamin C (Vit C) and folate (Fol) concentrations of the Early-, Intermediate- and Late-ripening cultivars, in the three fruiting seasons studied (2006, 2007 and 2008). Data refer to FF, and are reported as mean values ± SD. Different letters in the same row indicate a significant difference among ripening groups (p < 0.05).

	Year	Ripening Time		
		Early	Intermediate	Late
FRAP^	2006	9.00 ± 1.3 ab	8.37 ± 0.6 b	10.43 ± 0.7 a
	2007	9.46 ± 0.9 ab	8.21 ± 1.3 b	10.70 ± 1.7 a
	2008	8.37 ± 0.2 ab	7.89 ± 1.6 b	10.11 ± 3.0 a
TPC°	2006	2.15 ± 0.1 a	1.70 ± 0.2 b	2.39 ± 0.3 a
	2007	2.85 ± 0.1 a	2.24 ± 0.3 b	3.12 ± 0.5 a
	2008	1.77 ± 0.1	1.47 ± 0.2	1.70 ± 0.4
FLAVO°	2006	0.48 ± 0.1 b	0.37 ± 0.1 c	0.63 ± 0.1 a
	2007	0.55 ± 0.0 b	0.42 ± 0.1 c	0.69 ± 0.1 a
	2008	0.21 ± 0.0	0.20 ± 0.0	0.19 ± 0.0
ACY°	2006	0.48 ± 0.0	0.41 ± 0.1	0.38 ± 0.0
	2007	0.60 ± 0.0 a	0.39 ± 0.1 b	0.35 ± 0.1 b
	2008	0.44 ± 0.0	0.38 ± 0.0	0.37 ± 0.2
VIT C°	2006	0.40 ± 0.0	0.28 ± 0.0	0.33 ± 0.0
	2007	0.46 ± 0.0 a	0.29 ± 0.1 b	0.41 ± 0.1 ab
	2008	0.54 ± 0.0	0.41 ± 0.1	0.38 ± 0.0
FOL"	2006	236.6 ± 17	171.3 ± 18	157.5 ± 39
	2007	246.0 ± 11	239.8 ± 39	235.0 ± 62
	2008	359.2 ± 27 a	244.1 ± 32 b	214.4 ± 57 b

^ µmoles TE/g FW ; ° mg/g FW; " ng/g FW.

Table 5 The hourly minimum (Tmin) and maximum (Tmax) temperatures (°C), the percentage humidity (Hum %) and the extent of rainfall (mm) during the months from January to May, are presented for the three strawberry harvest years in study. data represent the mean values for each month.

	Year	April	May
T_{min} (°C)	2006	13.3	17.7
	2007	15.3	19.2
	2008	13.7	18.0
T_{max} (°C)	2006	14.3	18.8
	2007	16.4	20.4
	2008	14.8	19.1
$T_{day} \geq 6$°C (days)	2006	30	31
	2007	30	31
	2008	30	31
Hum (%)	2006	69.4	68.1
	2007	65.9	63.5
	2008	65.1	71.3
Rainfall ≥ 0.2 mm (days)	2006	5	1
	2007	1	7
	2008	6	2

there was a more acute increase in the thermic sum already from the beginning of the month, compared to the other two years. Particularly, during the last ten days of May 2007 the number of daily hours where the temperature was above 28°C (up to $H_{T\geq28°C}$ = 36) was much higher than in 2006 and 2008 **Fig. 2**. On the other hand, the milder and more homogeneous climatic conditions registered during April-May 2006 and particularly 2008 (**Fig. 2, Table 5**) probably accounted for the less relevant ripening-dependent differences in the NQ of the fruits, the only exception being that in 2008 the Fol concentrations differed among the ripening clones (**Table 4**). Certainly, other environmental factors may have played a crucial role in the intra- and inter-year variability of the NQ of the FS and in fruit response to cold storage and deeper investigations will be addressed in the future.

CONCLUSION

In keeping with previous results, significant genotype-to-genotype differences were found on TAC and on the micronutrient and phytochemical composition of the fresh strawberries in study. In particular, a relevant variation on the Fol content was observed among genotypes; the folate content also showed a significant increase after storage, not attributable to water losses and fruit concentration, and was the parameter most affected by short-refrigeration. In addition, the results led to suppose that the genetic background may influence the capacity of quality preservation in the strawberry. However, the evaluation of consecutive years of harvest characterized by different climatic conditions outlined a year-to-year variability in the NQ of the studied strawberries and in their response to cold storage. As a consequence, the accurate assessment of the keeping quality of strawberries would require the monitoring of several years of harvest and the response of the fruits to more selective storage conditions.

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