Food Chemistry 111 (2008) 872–878

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Combining quality and antioxidant attributes in the strawberry: The role of genotype

Franco Capocasa [a], Jessica Scalzo [a], Bruno Mezzetti [a], Maurizio Battino [b,*]

[a] Dipartimento di Scienze Ambientali e delle Produzioni Vegetali, Università Politecnica delle Marche, Italy
[b] Istituto di Biochimica, Facoltà di Medicina, Università Politecnica delle Marche, Via Ranieri, 65, 60131 Ancona, Italy

ARTICLE INFO

Article history:
Received 7 December 2007
Received in revised form 17 March 2008
Accepted 29 April 2008

Keywords:
Total antioxidant capacity
Fruit quality
Total phenolic content
Nutritional quality

ABSTRACT

The nutritional value of fruit has been widely studied and is demanded by consumers, especially for protection against cardiovascular disorder, cancer and other diseases, as well as for general health benefits. These benefits can also be ascribed to the total antioxidant capacity (TAC) of fruit.
Fruit nutritional quality can be described by a standard quality parameter and the analyses of nutritional parameters, such as antioxidant capacity (and specific related compounds). In this study, firmness, colour, soluble solids content and titratable acidity were considered as quality parameters and TAC and total phenolic content as nutritional parameters. All these attributes were screened in 20 strawberry genotypes (cultivars and selections) for the selection of new improved genetic material (offspring) originating from different cross combinations, including an F1 *Fragaria virginiana* spp. *glauca* among parents.
Results indicate that the effect of the genotype on strawberry nutritional quality is stronger than that of the cultivation conditions. However, commercial cultivation did not show a high range of variation of fruit nutritional quality, particularly for the nutritional parameters.
The study of offspring originating from different cross combinations showed that fruit nutritional quality can be considered an inheritable trait and that the variability of fruit nutritional quality among commercial cultivars can be improved by breeding.
Finally, results demonstrate the role of *F. virginiana* spp. *glauca* as an important genetic source of the fruit nutritional quality.

1. Introduction

In recent decades, agronomic research has set priorities to obtain high yield, better resistance to disease and transportation and longer shelf-life of fruit. Thus, the breeding programme of fruit has been aimed at improving the yield and fruit size, the resistance to diseases and pests, the adaptation to particular growing systems and the harvesting speeds (i.e. reducing harvesting costs). Recently, research has been focused on the quality of fruit (sensorial and nutritional).

Fruit have long been regarded as having considerable health benefits due to their nutritional attributes, and in particular their antioxidant activity against cellular oxidation reactions. The positive effects of fruits may depend on the high amounts of several antioxidants (Ames, Shigens, & Hagen, 1993; Cohen, Kristal, & Stanford, 2000; Cook & Samman, 1996; Halvorsen et al., 2002; Steinberg, 1989). These benefits have stimulated research to investigate the total antioxidant capacity (TAC) of fruit and vegetables and definitely contribute to preventing or suppressing disease-like states *in vitro* (McDougall, Dobson, Smith, Blake, & Stewart, 2005) and *in vivo* (Ramirez-Tortosa et al., 2001). TAC is strongly affected by the type of fruit, the species and the variety within species. Among fruit species, strawberries have more TAC (from 2- to 11-fold) than have apples, peaches, pears, grapes, tomatoes, oranges or kiwifruit (Scalzo, Politi, Pellegrini, Mezzetti, & Battino, 2005a). Genotype-variety is the major factor in determining fruit nutritional quality, but it is also affected by crop conditions (environmental and cultivation techniques), ripening season, pre-harvest and post-harvest conditions, shelf-life and processing (Cao, Verdon, Wu, Wang, & Prior, 1995; Connor, Luby, Tong, Finn, & Hancock, 2002; Prior et al., 1998; Proteggente et al., 2002; Wang, Cao, & Prior, 1996).

TAC of the strawberry and its by-products depends mainly on the high vitamin C content (Guo et al., 2003), but also on contents of polyphenols, flavonoids and anthocyanins (Proteggente et al., 2002).

Abbreviations: TAC, Total antioxidant capacity; SS, Soluble solid content; TA, Titratable acidity; FW, Fresh weight; TPH, Total phenolic content; TEAC, Trolox equivalent antioxidant capacity assay; FRAP, Ferric reducing antioxidant power assay.

* Corresponding author. Tel.: +39 071 2204646; fax: +39 071 2204398.
E-mail addresses: m.a.battino@univpm.it, mauriziobattino@yahoo.it (M. Battino).

0308-8146/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2008.04.068

A greater consumption of, and vegetables is considered as one way of increasing the intake of antioxidants, and strawberries, like other berries, represent the most important source of bioactive compounds with antioxidant activity (Deighton, Brennan, Finn, & Davies, 2000; Prior, 1998; Proteggente et al., 2002; Scalzo et al., 2005a). Accordingly, the increase of consumption of berries richer in "healthy compounds" is seen as an appropriate strategy for improving human health.

The increase of the level of antioxidants in fruit, through breeding and/or biotechnology, is an important option to support a higher antioxidant intake, even when the consumption of fruit is low. If nutritional components are also combined with high sensorial fruit quality the consumer health can be further improved by increased consumption.

The breeding approach can succeed if the variability and heritability of the TAC trait indicate the possibility of achieving breeding progress. The availability of genetic diversity within compatible species of any given crop will enhance improvement (Connor, Stephens, Hall, & Alspach, 2005). The biotechnological approach is now an integrative option to extend this improvement, but it is related to the knowledge of the molecular tools able to promote more general increases in several metabolites through the modification of specific biosynthetic pathways (Della Penna, 2001). However, the success of both breeding and biotechnological approaches is related to knowledge of the most useful wild and cultivated genetic diversity.

The effect on the nutritional quality of the strawberry is well known (Azodanlou, Darbellay, Luisier, Villettaz, & Amadò, 2003; Meyers, Watkins, Pritts, & Liu, 2003; Olsson et al., 2004; Wang, Zheng, & Galletta, 2002), but few genotypes are well characterised for these important features. Furthermore, only limited knowledge is available on the possibility of improving strawberry nutritional traits by breeding. In berries there are some results, and moderate heritabilities for TAC, total phenolic content (TPH) and anthocyanin content were demonstrated in blueberries and raspberries (Finley, 2005; Hancock et al., 2002; Heinonen, Meyer, & Frankel, 1998).

In this work strawberry nutritional quality was studied by considering firmness, colour, soluble solids content (SS) and titratable acidity (TA) as quality attribute parameters, and total antioxidant capacity (TAC) and total polyphenols (TPH) as nutritional parameters. The above mentioned parameters were used for screening 20 strawberry genotypes and selecting new genetic material derived from a breeding programme including six families derived from cross combination performed with parents selected for their highest nutritional quality.

2. Materials and methods

2.1. Chemicals

Bromothymol blue, sodium hydroxide, ethanol, hydrochloric acid, glacial acetic acid, Folin–Ciocalteu phenol reagent and anhydrous sodium carbonate were purchased from Fluka Chemie GmbH (Buchs, Switzerland). 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (trolox), potassium persulfate, sodium acetate trihydrate, ferric chloride hexahydrate, 2,4,6-tripyridyl-*s*-triazine (TPTZ), and ferrous sulphate heptahydrate, 3,4,5-trihydroxybenzoic acid (gallic acid) were purchased from Sigma-Aldrich (Sigma–Aldrich s.r.l., Milan, Italy).

2.2. Plant genetic material

Strawberry fruit nutritional quality was analysed in plants cultivated in open field experimental trials, including plots for cultivars, advanced selection and plots for seedling selection.

For two harvest seasons (2003 and 2004) 16 cultivars (of international and national commercial interest), three advanced selections of *Fragaria x ananassa* and a F1 advanced selection from the inter-specific cross *F. x ananassa x F. virginiana* spp. *glauca*, were evaluated. All genotypes were grown in a complete randomised block, with four replicates of 10 plants for each plot. Furthermore, a 2 year evaluation (2003 and 2004) was carried out on seedlings (single plant) from six families from different cross combinations (nine seedlings from each family) (Table 1).

2.3. Quality parameters

Quality parameters were studied on undamaged fruit samples (300–600 g), including pooled fruit of the 3rd, 4th and 5th main harvests, from each repetition of the variety plots, selections and from seedlings. Fruit colour firmness, soluble sugar and titratable acidity were measured on the same day of each harvest. Colour was determined for two sides of 10 ripe undamaged and uniform fruit by using the Minolta-Chromameter reflect II, that includes three parameters: L* (Luminance) a* (red tone) and b* (yellow tone). Data on colour were reported as L* and chroma index $[(a^{*2} + b^{*2})]½$. High chroma index means pale fruit and low chroma index dark strawberries. Firmness (g) was measured by using a hand-held penetrometer with an 8 mm piston. SS were determined using a hand-held refractometer and results are reported as °Brix. TA was determined from 10 ml of juice diluted with distilled water (1:2 v/v) and titrated with 0.1 N NaOH, to pH 8.2, and expressed in mEq of NaOH per 100 g of fruit.

2.4. Nutritional parameters

2.4.1. General

Nutritional parameters (TAC and TPH) were studied on undamaged fruit samples (300–600 g), including pooled fruit of the 3rd, 4th and 5th main harvests. These fruit samples were collected for each replicate of the variety plots, selections and from single seedlings, then placed in polyethylene bags and frozen at −20 °C prior to extraction under reduced-light conditions. Frozen fruit samples were homogenized (with a T25 Ultraturrax blender) with solvent solution (ethanol/water, 80:20 v/v) and extracts (Scalzo et al.,

Table 1
Cross combination of each family

Family	Maternal (M)		×	Paternal (P)	
	Genotype	Species		Genotype	Species
1	Paros	*F. x ananassa*	×	Queen Elisa	*F. x ananassa*
2	Onda	*F. x ananassa*	×	AN93.371.53	*F. x ananassa*
3	Queen Elisa	*F. x ananassa*	×	Sveva	*F. x ananassa*
4	AN 94.414.52	*F. x ananassa x F. virginiana glauca*	×	91.143.5	*F. x ananassa*
5	Sveva	*F. x ananassa*	×	Patty	*F. x ananassa*
6	AN 94.414.52	*F. x ananassa x F. virginiana glauca*	×	Onda	*F. x ananassa*

2005a) were utilized to assay the nutritional parameters by the following methods.

2.4.2. TEAC (trolox equivalent antioxidant capacity) assay

This was applied according to Bompadre, Leone, Politi, and Battino (2004), to study the antioxidant activity of the strawberry. After addition of 1.0 ml of $ABTS^{\bullet+}$ working solution (A_{734nm} = 0.700 ± 0.02) to 10 μl of trolox standards (0–1.5 mM), or extracts, the absorbance was read exactly 1 min after initial mixing. Final result was expressed as trolox equivalents (μmoles/g fresh weight (FW)).

2.4.3. FRAP (ferric reducing antioxidant power) assay

This was performed according to Benzie and Strain (1996, 1999), to study the antioxidant activity of the strawberry. Freshly prepared working FRAP solution (900 μl) was added to 100 μl of standards or extracts. Standards curves using trolox (0–0.25 mM) and ferrous sulphate (0–0.5 mM) were run with each set of extracts and were linear within these ranges. Final result was expressed as ferrous sulphate heptahydrate (μmoles/g fruit) or as trolox equivalents (μmoles/g FW).

2.4.4. Folin–ciocalteu assay

This was performed according to Slinkard and Singleton (1997) to study strawberry TPH. Fruit extracts were quantified by comparison with a standard curve of gallic acid and final results were expressed as gallic acid equivalents (GAE (mg/g FW)).

2.5. Experimental trial and statistical analysis

Nutritional quality analyses were performed during two harvesting seasons (2003 and 2004), in triplicate for each harvest and for each fruit sample. All the data were analysed using a one-way ANOVA test for mean comparisons, with standard errors. Analyses of variance (ANOVA) of strawberry cultivars and selections were performed with genotype and year as random effects. The ANOVA of families was performed using the year as random effect and offspring nested within family. Data on fruit quality and nutritional analyses are referred to as means of the three harvest times and are reported as means ± standard error medium (SEM). The differences were calculated according to the Student Newman Keuls (SNK) test, and were considered significant at $p < 0.05$. Correlations were calculated on a genotype mean basis. All analyses were performed using STATISTICA (Statsoft Inc., Tulsa).

3. Results

3.1. Nutritional quality of cultivars and advanced selections

The effect of cultivar conditions (years) and genotype on fruit nutritional quality was tested by comparing the standard quality and nutritional parameters of 16 commercial cultivars and four advanced selections, grown for two consecutive open field production cycles.

The overall result, evidences a significant effect (ANOVA) of both factors (genotype × years) and TA, firmness and L^*, while SS and chroma index remained stable in the two cultivation cycles (Table 2). The interaction between the two main factors (years × cultivars) was also significant for all variables.

Regarding the nutritional parameters, TAC (TEAC and FRAP) and TPH showed that all main effects were significant with the exception of FRAP. The interaction between the two main factors (years × cultivars) was also significant for all the antioxidant parameters.

Comparing varieties and standard quality parameters (Table 3), the advanced selection AN94.414.52 from an F1 inter-specific cross of *F. x ananassa x F. virginiana* spp. *glauca*, showed the highest TA (15.1 mEq NaOH/100 g); Onda had the lowest TA (7.4 mEq NaOH/100 g).

High variability among cultivars was also found for fruit SS. AN94.414.52 again had the highest SS content (10.7 °Brix). Queen Elisa showed the highest fruit firmness (514 g), followed by Adria (456 g), Camarosa and Alba (452 g and 447 g, respectively).

Idea was the shiniest fruit (higher L^*). This advanced selection also had the palest fruit (higher chroma index). Camarosa, Cifrance, Cilady, Madeleine, Roxana and AN94.414.52 were the dullest and Cilady the darkest.

Differences were found for TAC (Table 3) in the genotypes and results slightly differed according to the method of analyses. Using FRAP, the highest TAC was from Maya fruit (17.0 μmoles trolox eq/g FW) and the lowest from Adria and Irma. Conversely, when using TEAC, the highest TAC was from Sveva (18.4 μmoles trolox eq/g FW), followed by AN94.414.52, Cilady and Cifrance. Cifrance was again followed by AN94.414.52, AN93.371.53 and Sveva also showed the highest TPH (3.2, 3.0, 2.9 and 2.8 mg GAE/g FW, respectively). Adria, Idea and Irma displayed the lowest TPH values.

The correlations among quality and nutritional parameters are displayed in Table 4. The chroma index was highly correlated with L^* ($r = 0.77$ $p \leqslant 0.001$). Significant correlation existed between TA and SS ($r = 0.43$ $p \leqslant 0.001$), TA and firmness ($r = -0.19$ $p \leqslant 0.05$), and L^* with both SS and firmness ($r = 0.18$, $p < 0.05$, $r = -0.17$, $p < 0.05$, respectively). No correlation was found among the other standard quality parameters. Regarding nutritional parameters, the best correlations were found for TEAC vs TPH ($r = 0.52$, $p < 0.001$) and FRAP vs TPH ($r = 0.51$, $p < 0.001$). FRAP and TEAC results were also correlated ($r = 0.43$, $p < 0.001$). Finally, considering all quality and nutritional parameters of interest, positive correlations for TPH vs TA, and SS as well as negative correlations for TPH vs colour (L^* and chroma index) and firmness were found. A negative correlation was also found between colour and TAC (FRAP method).

Table 2
Analysis of variance (ANOVA) between the main factors (years and genotype), calculated for each of the quality and nutritional parameters

Sources	df	TA			SS			Firmness			L^*		
		MS	F	*p*-level	MS	F	*p*-level	MS	F	*p*-level	MS	F	*p*-level
Year	1	40.6	11.0^{*}	0.004	1.9	0.6^{ns}	0.4	40,0746	58.8^{**}	0.000	157.2	11.6^{*}	0.003
Genotype	19	58.3	15.8^{**}	0.000	26.8	8.7^{**}	0.000	39,888	5.8^{**}	0.000	105.4	7.8^{**}	0.000
Year × genotype	19	3.7	4.7^{**}	0.000	3.0	3.4^{**}	0.000	6809	4.2^{**}	0.000	13.5	4.9^{**}	0.000
Sources	df	Chroma index			TPH			FRAP			TEAC		
		MS	F	*p*-level	MS	F	*p*-level	MS	F	*p*-level	MS	F	*p*-level
Year	1	59.3	1.3^{ns}	0.3	5.7	7.6^{*}	0.01	23.0	0.4^{ns}	0.5	282.0	15.8^{**}	0.000
Genotype	19	159.9	3.4^{*}	0.005	3.1	4.1^{**}	0.001	61.5	0.9^{ns}	0.5	69.3	3.9^{*}	0.002
Year × genotype	19	47.3	8.5^{**}	0.000	0.7	31.8^{**}	0.000	63.0	75.6^{**}	0.000	17.8	6.6^{**}	0.000

$^{ns, *, **}$ Nonsignificant or significant at $p \leqslant 0.05$ or 0.001.TA: titratable acidity; SS: soluble solids; L^*: brightness; TPH: total polyphenol content; FRAP and TEAC: total antioxidant content.

Table 3
Mean values of 20 genotypes (cultivars and advanced selections) for all the standard quality and nutritional parameters

Cultivar/selection	Standard quality parameters					Nutritional parameters		
	TA[A]	SS (°Brix)	F (g)	L^*	Chroma index	FRAP[B]	TEAC[B]	TPH[C]
Adria	9.6 ± 0.3 fg	6.3 ± 0.4 def	456 ± 9 b	37.4 ± 0.4 cd	44.6 ± 0.5 def	9.5 ± 0.3 g	12.9 ± 0.5 def	1.8 ± 0.0 h
Alba	12.5 ± 0.2 bc	7.3 ± 0.3 d	447 ± 14 b	37.3 ± 0.3 cd	47.2 ± 0.5 bcd	11.8 ± 0.3 cdef	14.2 ± 0.4 d	2.0 ± 0.0 fgh
Camarosa	10.4 ± 0.1 e	7.2 ± 0.2 d	452 ± 18 b	32.9 ± 0.4 g	42.0 ± 0.6 gh	12.0 ± 0.4 cdef	14.5 ± 0.4 cd	2.6 ± 0.1 cd
Cifrance	12.3 ± 0.3 c	9.7 ± 0.1 b	387 ± 14 cde	34.1 ± 0.4 fg	41.6 ± 0.8 h	13.3 ± 0.5 bc	16.5 ± 0.6 bc	3.2 ± 0.1 a
Cilady	13.2 ± 0.3 b	6.5 ± 0.2 def	355 ± 10 e	33.2 ± 0.4 g	38.7 ± 0.7 i	13.4 ± 0.2 bcd	16.6 ± 0.4 bc	2.6 ± 0.1 cd
Don	11.0 ± 0.1 de	5.8 ± 0.3 f	380 ± 12 ce	35.5 ± 0.5 def	43.8 ± 0.8 efgh	12.0 ± 0.6 cdef	11.8 ± 0.5 ef	2.0 ± 0.1 gh
Idea	11.3 ± 0.1 de	6.9 ± 0.1 def	362 ± 10 e	42.1 ± 0.6 a	49.5 ± 0.4 ab	10.0 ± 0.2 fg	12.7 ± 0.4 ef	1.9 ± 0.01 h
Irma	8.5 ± 0.0.1 h	6.0 ± 0.2 ef	356 ± 14 e	37.2 ± 0.3 cd	47.3 ± 0.5 bcd	9.7 ± 0.5 g	11.2 ± 0.5 f	1.8 ± 0.01 h
Madeleine	10.6 ± 0.2 e	7.3 ± 0.3 d	391 ± 13 cd	34.4 ± 0.3 efg	43.5 ± 0.4 fgh	11.8 ± 0.1 cdef	14.8 ± 0.5 cd	2.2 ± 0.0 efg
Maya	11.1 ± 0.1 de	6.9 ± 0.3 de	356 ± 7 e	37.1 ± 0.4 cd	46.6 ± 0.5 cde	17.0 ± 1.3 a	15.5 ± 0.5 bcd	2.2 ± 0.0 fg
Onda	7.4 ± 0.2 i	6.8 ± 0.2 def	404 ± 9 bcd	36.2 ± 0.4 cde	45.2 ± 0.3 cdef	11.2 ± 0.2 def	13.5 ± 0.5 de	2.0 ± 0.0 gh
Paros	9.4 ± 0.3 g	6.5 ± 0.3 def	429 ± 17 bcd	37.3 ± 0.3 cd	47.7 ± 0.4 bc	11.9 ± 0.4 cdef	15.0 ± 0.2 cd	2.6 ± 0.2 cd
Patty	9.3 ± 0.3 g	7.2 ± 0.3 d	332 ± 13 f	35.5 ± 0.3 def	44.7 ± 0.5 def	12.1 ± 0.4 cdef	12.8 ± 0.4 ef	2.6 ± 0.0 cd
Queen Elisa	10.4 ± 0.2 ef	8.6 ± 0.2 c	514 ± 16 a	38.2 ± 0.2 c	49.3 ± 0.5 ab	11.6 ± 0.4 cdef	12.6 ± 0.4 ef	2.0 ± 0.1 gh
Roxana	9.2 ± 0.3 g	6.3 ± 0.2 def	366 ± 11 e	33.8 ± 0.7 fg	41.8 ± 1.0 h	11.9 ± 0.6 cdef	12.6 ± 0.5 ef	2.0 ± 0.1 gh
Sveva	10.8 ± 0.4 de	6.4 ± 0.2 def	427 ± 15 bc	35.2 ± 0.4 ef	45.3 ± 0.7 cdef	15.0 ± 0.2 b	18.4 ± 0.7 a	2.8 ± 0.1 bc
91.143.5	13.1 ± 0.3 b	7.3 ± 0.2 d	392 ± 15 d	37.4 ± 0.3 cd	47.6 ± 0.6 bc	10.9 ± 0.2 fg	15.1 ± 0.9 cd	2.4 ± 0.1 de
AN93.371.53	11.7 ± 0.2 cd	6.6 ± 0.1 def	403 ± 10 bc	36.1 ± 0.9 de	44.5 ± 1.5 def	13.7 ± 0.2 bc	12.4 ± 0.2 ef	2.9 ± 0.0 b
AN94.268.51	11.4 ± 0.2 de	6.6 ± 0.2 def	423 ± 15 bcd	40.7 ± 0.4 b	50.5 ± 0.6 a	11.1 ± 0.3 fg	15.6 ± 0.7 bcd	2.3 ± 0.0 efg
AN94.414.52	15.1 ± 0.3 a	10.7 ± 0.4 a	328 ± 12 f	33.1 ± 0.6 g	46.3 ± 0.7 cdef	14.8 ± 0.8 b	17.3 ± 0.4 ab	3.0 ± 0.0 ab

Values in the same column that are followed by different letters are significantly different ($p \leqslant 0.01$) using the Student Newman Keuls (SNK).
Analyses were performed in two consecutive production years (2003 and 2004) and on fruit sampled in the three main harvests for each genotype; the extracts were analyzed in triplicate. (TA: titratable acidity; SS: soluble solids; F: firmness; L^*: brightness; TPH: total polyphenol content; FRAP and TEAC: total antioxidant content).
[A] mEq NaOH/100 g FW.
[B] μmoles troloxEq/g FW.
[C] mg GAE/g FW.

Table 4
Pearson's correlation and significance based an genotype means for the standard quality and nutritional parameters: titratable acidity (TA), soluble solids (SS), firmness (F), brightness (L^*), total polyphenol content (TPH) and total antioxidant content (FRAP and TEAC:)

Variable	Standard quality parameters				Nutritional parameters		
	SS	F	L^*	Chroma Index	TPH	FRAP	TEAC
TA	0.43^{**}	-0.19^{*}	-0.12^{ns}	-0.02^{ns}	0.43^{**}	0.25^{*}	0.38^{**}
SS	–	-0.07^{ns}	-0.18^{s}	-0.02^{ns}	0.38^{**}	0.20^{*}	0.23^{**}
F	–	–	0.17^{*}	0.10^{ns}	-0.23^{**}	-0.08^{ns}	-0.14^{*}
L^*	–	–	–	0.77^{**}	-0.39^{**}	-0.26^{**}	-0.21^{*}
Chroma index	–	–	–	–	-0.28^{**}	-0.24^{**}	-0.13^{ns}
TPH	–	–	–	–	–	0.51	0.52^{**}
FRAP	–	–	–	–	–	–	0.43^{**}

$^{ns, *, **}$ Nonsignificant or significant at $p < 0.05$ or 0.001.

The outcome of this study underlines the predominant effect of the genotype rather than the influence of the cultivation cycle on strawberry nutritional quality. Results from commercial cultivars did not show a high range of variability of fruit quality, particularly for nutritional parameters. On the contrary, the advanced selections showed improved results which better characterise the fruit nutritional parameters.

3.2. Nutritional quality of the breeding material

The study on offspring obtained by different cross combinations showed that the variation of strawberry nutritional parameters can be improved, with a significant variability among the main effects (years, families and offspring) and their interactions (Table 5).

All nutritional parameters, tested on progenies from different families in the 2 years of evaluation, were significant. Only titratable acidity was not affected by the year of cultivation.

The parental lines were characterised by different fruit nutritional qualities. The selection AN94.414.52, from a F1 inter-specific cross of *F. x ananassa x F. virginiana* spp. *glauca*, was the parental line with fruit having the highest values for all fruit nutritional parameters (Table 6). Onda, Queen Elisa, Patty and AN94.414.52 were characterised by the most equilibrated fruit quality, with SS/TA ranging between 0.7 and 0.9. High TAC, measured as FRAP, was also shown by AN93.371.53 and Sveva (two sister genotypes selected by the same offspring), and Sveva also had the highest TEAC. AN94.414.52, AN93.371.53 and Sveva were characterised by the highest TPH values.

The study of fruit nutritional quality in the nine offspring from each of the six families confirmed the variability of these traits in strawberries and the importance of the parent combinations for their improvement. Mean comparison among families showed that the highest fruit TA was found for Family 4 (Table 6), followed by fruit of Family 6; both families had the F1 selection AN94.414.52 as common parent. The lowest TA was from fruit of Family 2. The highest SS content was found in fruits of Family 6, followed again by Family 4.

Families 4 and 6 were also characterised by very high (often the highest) FRAP, TEAC and TPH values (Table 6).

This study was also effective for the applied breeding programme, in fact it allowed the identification and selection of new genotypes producing fruits with much higher nutritional values than the corresponding parents.

Some genotypes with improved fruit nutritional quality were selected from each family, in some cases also with an improved balance among the quality and nutritional parameters (e.g. from Family 1, seedlings 01.70.53 and 01.70.54). New genotypes with

Table 5
Analysis of variance (ANOVA) between the main factors (years, family and offspring), and their interactions calculated for titratable acidity (TA), soluble solids (SS), FRAP and TEAC for total antioxidant capacity and total polyphenol content (TPH)

Sources	df	TA			SS			FRAP			TEAC			TPH		
		MS	F	*p*-level	MS	F	*p*-level	MS	F	*p*-level	MS	F	*p*-level	MS	F	*p*-level
Year	1	3.6	2.6^{ns}	0.11	86.3	87.9**	0.000	453.0	662.9**	0.000	2264.4	1850.3**	0.000	13.9	458.9**	0.000
Family	5	448.7	317.5**	0.000	166.2	169.1**	0.000	372.7	545.5**	0.000	141.7	115.8**	0.000	6.6	218.7**	0.000
Offspring (family)	42	24.4	17.3**	0.000	18.2	18.6**	0.000	131.5	192.4**	0.000	107.8	88.1**	0.000	3.7	123.7**	0.000
Year × Family	5	7.8	5.5**	0.000	3.2	3.3*	0.006	328.8	481.3**	0.000	84.9	69.4**	0.000	9.5	313.9**	0.000
Year × Offspring (Family)	42	5.7	4.0**	0.000	4.0	4.1**	0.000	28.0	41.0**	0.000	34.1	27.8**	0.000	1.0	34.2**	0.000

$^{ns, *, **}$ Nonsignificant or significant at $p < 0.05$ or 0.001.

Table 6
Mean values of parents and offspring from six families for titratable acidity (TA), soluble solids (SS), FRAP and TEAC for total antioxidant capacity and total polyphenol content (TPH)

Assay	Family	Parent		Offspring	
		Maternal	Paternal	Average ± SEM	Range (min–max)
TA[A]	1 (Paros × Queen Elisa)	9.4 ± 0.3	10.4 ± 0.3	11.1 ± 0.4 c	9.3–12.7
	2 (Onda × AN93.371.53)	7.4 ± 0.2	11.7 ± 0.2	8.9 ± 0.5 f	7.8–9.9
	3 (Queen Elisa × Sveva)	10.4 ± 0.3	10.9 ± 0.5	9.4 ± 0.4 e	6.6–12.0
	4 (AN 94.414.52 × 91.143.5)	15.1 ± 0.5	13.1 ± 0.2	13.4 ± 0.4 a	12.4–16.0
	5 (Sveva × Patty)	10.9 ± 0.5	9.3 ± 0.3	11.8 ± 0.3 d	9.9–10.8
	6 (AN 94.414.52 × Onda)	15.1 ± 0.5	7.4 ± 0.2	12.0 ± 0.3 b	11.2–13.1
SS	1 (Paros × Queen Elisa)	6.4 ± 0.2	8.6 ± 0.3	9.1 ± 0.4 b	7.9–12.1
	2 (Onda × AN93.371.53)	6.8 ± 0.4	6.6 ± 0.1	7.1 ± 0.4 d	6.0–8.1
	3 (Queen Elisa × Sveva)	8.6 ± 0.3	6.5 ± 0.2	7.9 ± 0.3 c	6.6–9.3
	4 (AN 94.414.52 × 91.143.5)	10.7 ± 0.5	7.3 ± 0.2	9.3 ± 0.2 b	8.2–10.6
	5 (Sveva × Patty)	6.6 ± 0.2	7.2 ± 0.4	8.4 ± 0.2 c	6.9–9.0
	6 (AN 94.414.52 × Onda)	10.7 ± 0.5	6.8 ± 0.4	9.7 ± 0.2 a	9.0–10.5
FRAP[B]	1 (Paros × Queen Elisa)	11.9 ± 0.3	11.7 ± 0.2	13.7 ± 0.1 b	12.3–15.2
	2 (Onda × AN93.371.53)	11.2 ± 0.3	13.7 ± 0.1	12.3 ± 0.1 c	10.7–14.1
	3 (Queen Elisa × Sveva)	11.7 ± 0.2	14.9 ± 0.2	13.3 ± 0.3 b	11.1–16.5
	4 (AN 94.414.52 × 91.143.5)	14.8 ± 0.4	10.9 ± 0.2	16.0 ± 0.3 a	8.7–23.4
	5 (Sveva x Patty)	14.9 ± 0.2	12.1 ± 0.2	11.8 ± 0.2 c	9.4–13.2
	6 (AN 94.414.52 × Onda)	14.8 ± 0.4	11.2 ± 0.3	15.5 ± 0.5 a	11.2–21.3
TEAC[B]	1 (Paros × Queen Elisa)	15.0 ± 0.3	12.6 ± 0.4	14.3 ± 0.3 b	11.6–15.9
	2 (Onda × AN93.371.53)	13.5 ± 0.6	12.4 ± 0.2	13.6 ± 0.2 bc	12.2–16.2
	3 (Queen Elisa × Sveva)	12.6 ± 0.4	18.3 ± 0.6	13.2 ± 0.3 c	11.2–15.1
	4 (AN 94.414.52 × 91.143.5)	17.3 ± 0.4	15.1 ± 0.8	15.9 ± 0.2 a	9.5–19.0
	5 (Sveva × Patty)	18.3 ± 0.6	13.0 ± 0.1	14.5 ± 0.5 b	9.5–18.9
	6 (AN 94.414.52 × Onda)	17.3 ± 0.4	13.5 ± 0.6	14.9 ± 0.3 b	10.9–19.8
TPH[C]	1 (Paros × Queen Elisa)	2.6 ± 0.05	2.0 ± 0.04	2.7 ± 0.1 b	2.4–2.9
	2 (Onda × AN93.371.53)	2.0 ± 0.04	2.9 ± 0.01	2.3 ± 0.1 c	2.1–2.7
	3 (Queen Elisa × Sveva)	2.0 ± 0.04	2.7 ± 0.1	2.6 ± 0.1 b	2.2–3.2
	4 (AN 94.414.52 × 91.143.5)	3.0 ± 0.05	2.4 ± 0.05	2.8 ± 0.04 a	2.2–3.5
	5 (Sveva × Patty)	2.7 ± 0.1	2.6 ± 0.06	2.9 ± 0.04 a	2.4–3.1
	6 (AN 94.414.52 × Onda)	3.0 ± 0.05	2.0 ± 0.04	2.9 ± 0.1 a	2.1–4.6

For each parameter values in the same column followed by different letters are significantly different ($p \leqslant 0.01$) using the Student Newman Keuls (SNK).
Analyses were performed in two consecutive production years (2003 and 2004) on fruits sampled in the three main harvest for each genotype; the extracts were analysed in triplicate.
[A] mEq NaOH/100 g FW.
[B] μmoles troloxEq/g FW.
[C] mg GAE/g FW.

more equilibrated fruit quality, combined with high levels of TEAC and TPH, were selected in Family 2 (01.186.51 and 01.186.58) and one in Family 3 (01.187.54). Genotypes with fruits having the highest nutritional value were selected from Family 4: AN00.239.55 had the highest FRAP and TEAC values, combined also with high TPH, SS and TA. In this family, four more selections were identified for their high excellent nutritional attributes (AN00.239.53, AN00.239.56, AN00.239.57 and AN00.239.58). A similar situation was found in Family 6 having AN 94.414.52 as common maternal parent; in fact, three other selections

Table 7
Pearson's correlation and significance and means of genotype for the six families for titratable acidity (TA), soluble solids (SS), total polyphenol content (TPH), FRAP and TEAC (total antioxidant content)

Variable	SS	TPH	FRAP	TEAC
TA	0.45**	0.24*	0.35**	0.28**
SS	–	0.37**	0.38**	0.37**
TPH	–	–	0.75**	0.57**
FRAP	–	–	–	0.66**

$^{ns, *, **}$Nonsignificant or significant at $p < 0.05$ or 0.001.

(AN00.240.52, AN00.240.57 and AN00.240.58) were chosen because of fruit combining high values of nutritional parameters and SS content. Parents used in Family 5 (Sveva × Patty), even with high TAC, did not produce high improvement in offspring fruit nutritional quality, and only one selection (AN00.235.54) was considered of interest for the high values of nutritional parameters and SS (Table 7).

4. Discussion

The increase of fruit consumption is strictly related to several factors, including its price, but mostly to the consumer quality acceptance. As a consequence, the increase of consumer health is also strictly related to new released varieties with improved fruit nutritional quality.

The evaluation of strawberry fruit nutritional quality represents an important task to better identify the commercial exploitation of new cultivar commercially released. This study demonstrates that, among the major strawberry cultivars cultivated inside and outside of Europe, a high variation in fruit nutritional quality does not exist. Among these cultivars, very few are characterised by fruit with a good combination of standard quality and nutritional parameters. Unfortunately, the quality of fruit is often associated with negative agronomic characteristics and, in this study (data not shown), a negative correlation was found between fruit size and most of the nutritional quality parameters. In particular, strawberry fruit size negatively correlated with the commercial quality (TA and SS) and nutritional parameters (TPH, TEAC and FRAP). This latter result was partially confirmed in a blackberry study (Connor et al., 2005).

From this study, it emerged that the genotypes rarely associate production efficiency and sensorial quality with the nutritional values of fruit, probably because the most recent breeding programmes consider nutritional characters as a minor priority. Cifrance and Cilady are the only cultivars showing a sufficient combination of standard quality and nutritional parameters (sufficiently high SS, TA and antioxidant features), but both lack in fruit firmness. New Italian varieties, e.g. Queen Elisa, Adria, Alba and Sveva and the selection AN94.268.51, differed by the high firmness, but only in Sveva was this feature also combined with a high nutritional parameter.

Regarding the methods of analyses of the fruit antioxidant capacity, the TAC values resulting from TEAC and FRAP methods showed a weak correlation ($r = 0.43$, $p < 0.001$). This behaviour depends on the specific features of the methods employed (Cao & Prior, 1998; Scalzo, Mezzetti, & Battino, 2005b): in the TEAC assay, the radical cation used is pre-formed prior to addition of antioxidants while, on the other hand, the FRAP assay depends upon the reduction of the ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex to Fe^{2+}-TPTZ by a reductant at low pH, and what is really measured is the ability of a compound to reduce Fe^{3+} to Fe^{2+}. Some antioxidants, such as ascorbic acid and uric acid, can reduce Fe^{3+} and their ability in reducing Fe^{3+} may reflect their capacity to reduce reactive species, but not all reductants that are able to reduce Fe^{3+} are antioxidants, and an antioxidant that can effectively reduce a pro-oxidant may not be able to efficiently reduce Fe^{3+}. Therefore, a concomitant employment of both methods may be useful in research trials because the data that they give are often complementary.

Furthermore, our cultivar/genotype study showed a high correlation of TAC vs TPH, which is in agreement with previous reports (Heinonen et al., 1998; Meyers et al., 2003; Proteggente et al., 2002).

In this study, the negative correlations between chroma index and nutritional parameters confirmed their importance in determining the type of fruit colour, as already observed by Connor et al. (2005). In fact, in strawberry, the pale shiny fruit (e.g. fruit of Idea) had a lower TAC, while the dark dull fruit (e.g. fruit of AN94.414.52 and Sveva) had the highest TAC values.

Taking into account the year-to-year variability of fruit nutritional quality, this research demonstrated that the accurate assessment of new strawberry genotypes needs several years of evaluation, possibly in different cultivation conditions (Anttonen, Hoppula, Nestby, Verheul, & Karjalainen, 2006; Davik, Bakken, Holte, & Blomhoff, 2006).

The study of nutritional quality in the nine offspring from each of six families showed a higher variation of these traits in comparison with the commercial cultivars; the extent of this variation was specifically related to the parent combinations. Results from Families 4 and 6 show, for the first time, the importance of the *F. virginiana* spp. *glauca* genetic base for improving the nutritional quality of commercial strawberries, as already observed also for other characteristics, such as male fertility, fruit size and disease resistance (Hancock et al., 2002). In fact, the plant materials showing the best fruit nutritional quality were selected from Families 4 and 6 which reflect the maternal parent (the F1 selection AN94.414.52) behaviour. Among the other cross combinations, only families having Sveva as a parent contained offspring with increased values of antioxidant parameters (mainly TPH), but combined with lower standard quality parameters. These results underlined that: (i) the loss of these attributes occurred during domestication, (ii) the fruit nutritional quality can be considered an inheritable trait and (iii) the genetic background available in *F. x ananassa* cultivars can be improved by using wild strawberry species, such as *F. virginiana* spp. *glauca*.

The correlation analysis among the fruit nutritional quality parameters indicates interesting results on the type of association among standard quality (SS and TA) and nutritional parameters (TAC and TPH). The positive correlations resulting for TA and SS vs TPH, for TA vs FRAP/TEAC and for TPH vs FRAP/TEAC are of interest.

The genetic base of the different parents used in different cross combinations, has determined an improvement of fruit TAC of the new populations that should probably be ascribed to the increase of several compounds rather than only to TPH.

5. Conclusion

Breeding and biotechnological approaches are currently used to increase the content of specific bioactive components of plants, but the manipulation of plant metabolism is still not easy to address. There is an increasing awareness that multiple genetic and environmental factors affect production and accumulation of bioactive compounds, but these factors are rarely taken into account when fruit is marketed. The assumption underlying 'functional fruit' is that bioactive compounds (in fruit) are efficacious for the improvement of health (Finley, 2005). Rigorous and unprejudiced evaluation of scientific evidence requires a defined set of criteria and methods of evaluation, particularly when breeding and biotechnology programmes are aimed to produce new varieties with improved nutritional values combined with high plant production efficiency and fruit quality.

Results obtained in this study can be considered of particular interest to better define the varieties and breeding evaluation strategies. Nowadays these aspects are considered highly useful for the commercialisation of new varieties, but mostly to select new genotypes with high fruit nutritional quality, combined with yield efficiency, which now corresponds to plant adaptability, production efficiency and fruit size. Furthermore, this work also demonstrated year-to-year variability in quality attribute parameters and antioxidant features of each genotype; therefore, the accurate assessment of the fruit nutritional quality of new genotypes needs several years of evaluation.

Commercial cultivars and the availability of new sources of fruit nutrition quality should be explored in order to develop new genotypes. Wild species as *F. virginiana* spp. *glauca* and *F. vesca* are good sources of bioactive compounds (Scalzo et al., 2005a; Tulipani et al., 2008) but, in other berry species, the introduction of the wild germplasm did not improve the nutritional quality of fruit (Connor et al., 2005; Deighton et al., 2000). Our results demonstrate the role of *F. virginiana* spp. *glauca* as an important genetic source of the fruit nutritional quality trait, as was also demonstrated for other unique traits, such as photo-insensitivity plant habitus (day neutral plant) and disease resistance (Hancock et al., 2002). The new positive transgressive segregants, identified as those offspring, whose 2-year mean exceeded the higher parental mean of fruit nutritional quality parameters, can really open new perspectives in breeding strawberries for the achievement of new commercial varieties with improved nutritional quality.

The availability of high quality fruit (antioxidant-enriched), at inexpensive and competitive price, will be a useful tool in the planning of healthy diets, especially when patients do not eat enough vegetables and there is the need to use an attractive and tasty alternative, as strawberries usually are.

Acknowledgments

This study was partially supported by Marche Polytechnic University and by the finalized research project "Fruit Variety Network", supported by Italian Minister of Agriculture and Forestry (Rome, Italy), paper number 265. The valuable help of Ms M. Glebocki in editing the manuscript is gratefully acknowledged.

References

Ames, B. M., Shigens, M. K., & Hagen, T. M. (1993). Oxidants, antioxidants and the degenerative diseases of aging. *Proceedings of the National Academy of Sciences, 90*, 7915–7922.

Anttonen, M., Hoppula, K. I., Nestby, R., Verheul, M. J., & Karjalainen, R. O. (2006). Influence of fertilization, mulch colour, early forcing, fruit order, planting date, shading, growing environment and genotype on the content of selected phenolics in strawberry (*Fragaria x ananassa* Duch) fruits. *Journal of Agricultural and Food Chemistry, 2006*, 2614–2620.

Azodanlou, R., Darbellay, C., Luisier, J., Villettaz, J., & Amadò, R. (2003). Quality assessment of strawberry (*Fragaria* species). *Journal of Agricultural and Food Chemistry, 51*, 715–721.

Benzie, I. F. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical Biochemistry, 239*, 70–76.

Benzie, I. F. F., & Strain, J. J. (1999). Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods in Enzymology, 299*, 15–27.

Bompadre, S., Leone, L., Politi, A., & Battino, M. (2004). Improved FIA-ABTS method for antioxidant capacity determination in different biological samples. *Free Radical Research, 38*, 831–838.

Cao, G., & Prior, R. L. (1998). Comparison of different analytical methods for assessing total antioxidant capacity of human serum. *Clinical Chemistry, 44*, 1309–1315.

Cao, G., Verdon, C. P., Wu, A. H. B., Wang, H., & Prior, R. L. (1995). Automated-assay of oxygen radical absorbance capacity with the COBAS FARA-II. *Clinical Chemistry, 41*, 1738–1744.

Cohen, J. H., Kristal, A. R., & Stanford, J. L. (2000). Fruit and vegetable intakes and prostate cancer risk. *Journal of the National Cancer Institute, 92*, 61–68.

Connor, A. M., Luby, J. J., Tong, C. B. S., Finn, C. E., & Hancock, J. F. (2002). Variation and heritability estimates for antioxidant activity total phenolic content and anthocyanin content in blueberry progenies. *Journal of the American Society for Horticultural Science, 1*, 82–88.

Connor, A. M., Stephens, M. J., Hall, H. K., & Alspach, P. A. (2005). Variation and heritabilities of antioxidant activity and total phenolic content estimated from a red raspberry factorial experiment. *Journal of the American Society for Horticultural Science, 130*, 3.

Cook, N. C., & Samman, S. (1996). Flavonoids – Chemistry, metabolism, cardioprotective effects, and dietary sources. *Journal of Nutrition and Biochemistry, 7*, 66–76.

Davik, J., Bakken, A. K., Holte, K., & Blomhoff, R. (2006). Effects on genotype and environment on total anti-oxidant capacity and the content of sugars and acids in strawberries (*Fragaria x ananassa* Duch. *Journal of Horticulture Science and Biotechnology, 81*, 1057–1063.

Deighton, N., Brennan, R., Finn, C., & Davies, H. V. (2000). Antioxidant properties of domesticated and wild Rubus species. *Journal of the Science of Food and Agriculture, 80*, 1307–1313.

Della Penna, D. (2001). Plant metabolic engineering. *Plant Physiology, 125*, 160–163.

Finley, J. W. (2005). Proposed criteria for assessing the efficacy of cancer reduction by plant foods enriched in carotenoids, glucosinolates, polyphenols and selenocompounds. *Annals of Botany, 95*, 1075–1096.

Guo, C., Yang, J., Wie, J., Li, Y., Xu, J., & Jiang, Y. (2003). Antioxidant activities of peel, pulp and seed fraction of common fruits as determined by FRAP assay. *Nutrition Research, 23*, 1719–1726.

Halvorsen, B. L., Holte, K., Myhrstad, M. C. W., Barikmo, I., Hvattum, E., Remberg, S. F., et al. (2002). A systematic screening of total antioxidants in dietary plants. *Journal of Nutrition, 132*, 461–471.

Hancock, J. F., Luby, J. J., Dale, A., Callow, P. W., Serce, S., & El-Shiek, A. (2002). Utilizing wild *Fragaria virginiana* in strawberry cultivar development: inheritance of photoperiod sensitivity, fruit size, gender, female fertility and disease resistance. *Euphytica, 126*, 177–184.

Heinonen, I. M., Meyer, A. S., & Frankel, E. N. (1998). Antioxidant activity of berry phenolics on human low-density lipoprotein and liposome oxidation. *Journal of Agricultural and Food Chemistry, 46*, 4107–4112.

McDougall, G. J., Dobson, P., Smith, P., Blake, A., & Stewart, D. (2005). Assessing potential bioavailability of raspberry anthocyanins using an in vitro digestion system. *Journal of Agricultural and Food Chemistry, 53*, 5896–5904.

Meyers, K. J., Watkins, C. B., Pritts, M. P., & Liu, R. H. (2003). Antioxidant and antiproliferative activities of strawberries. *Journal of Agricultural and Food Chemistry, 51*, 6887–6892.

Olsson, M. E., Ekvall, J., Gustavsson, K., Nilsson, J., Pillai, D., Sjoholm, I., et al. (2004). Antioxidants, low molecular weight carbohydrates, and total antioxidant capacity in strawberry (*Fragaria x ananassa*): Effects of cultivar, ripening, and storage. *Journal of Agricultural and Food Chemistry, 52*, 2490–2498.

Prior, R. L., Cao, G., Martin, A., Sofic McEwen, J., O'Brien, C., et al. (1998). Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity and variety of *Vaccinium species*. *Journal of Agricultural and Food Chemistry, 46*, 2686–2693.

Proteggente, A. R., Pannala, A. S., Pagana, G., Van Buren, L., Wagner, E., Wiswman, S., et al. (2002). The antioxidant activity of regular consumed fruit and vegetables reflects their phenolic and vitamin C composition. *Free Radical Research, 36*, 217–233.

Ramirez-Tortosa, C., Andersen, O. M., Gardner, P. T., Morrice, P. C., Wood, S. G., Duthie, S. J., et al. (2001). Anthocyanin-rich extract decreases indices of lipid peroxidation and DNA damage in vitamin E-depleted rats. *Free Radical Biology and Medicine, 31*, 1033–1037.

Scalzo, J., Politi, A., Pellegrini, N., Mezzetti, B., & Battino, M. (2005a). Plant genotype affects total antioxidant capacity and phenolic contents in fruit. *Nutrition, 21*, 207–213.

Scalzo, J., Mezzetti, B., & Battino, M. (2005b). Total antioxidant capacity evaluation: critical steps for assaying berry antioxidant features. *BioFactor, 23*, 221–227.

Slinkard, K., & Singleton, V. L. (1997). Total phenol analysis: automation and comparison with manual methods. *American Journal of Enology and Viticulture, 28*, 49–55.

Steinberg, D. (1989). Beyond cholesterol: modifications of low-density lipoprotein that increase its atherogenicity. *New England Journal of Medicine, 320*, 915–924.

Tulipani, S., Mezzetti, B., Capocasa, F., Bompadre, S., Beekwilder, J., de Vos, C. H., et al. (2008). Antioxidants, phenolic compounds, and nutritional quality of different strawberry genotypes. *Journal of Agricultural and Food Chemistry, 56*, 696–704.

Wang, H., Cao, G., & Prior, R. L. (1996). Total antioxidant capacity of fruits. *Journal of Agricultural and Food Chemistry, 44*, 701–705.

Wang, S. Y., Zheng, W., & Galletta, G. J. (2002). Cultural System Affects Fruit Quality and Antioxidant Capacity in Strawberries. *Journal of Agricultural and Food Chemistry, 50*, 6534–6542.