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Comparative characterization of fruit quality, phenols and antioxidant activity of de-pigmented "Ghiaccio" and white flesh peaches

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Abstract: Quality traits and nutraceutical potential of new de-pigmented peaches ("Ghiaccio") were investigated and compared with those of four white flesh peaches. Total soluble solids (TSS) were measured by digital refractometer, and titratable acidity (TA) by volumetric titration. Total phenolic (TPC) and anthocyanin content (TAC) were analysed spectrophotometrically and the antioxidant capacity (AC) evaluated by DPPH• assay. A strong influence of genotype on quality traits and phytochemical profile of "Ghiaccio" peaches was observed. "Ghiaccio" series showed, on average, a higher TPC content than that of white flesh peaches, both in flesh and peels (+129% and +14%, respectively). The peels of all peaches analysed were significantly richer in TPC than the flesh. TAC was not detectable in de-pigmented genotypes; on the contrary, in the white flesh peaches, it was higher in the peels than in the flesh. AC correlated well with TPC. Data confirm, for all peaches analysed, the influence of genotype and fruit tissue on the nutraceutical properties. Among genotypes, the best candidates for "Ghiaccio" peaches with enriched nutraceutical properties are the advanced selections GØ and GX. Observed differences in the nutraceutical potential among "Ghiaccio" series may open new opportunities for breeding de-pigmented peach varieties with a higher nutritional value.

1. Introduction

Peach (*Prunus persica*, L.) is the second most important temperate fruit crop after apple. The top producer of peaches is China, followed by the EU with Italy, Greece and Spain being the biggest European producers (Faostat, 2015). At present, the demand from the market to develop and introduce new varieties with different characteristics, which could make possible to expand cultivation areas and production calendars and improve technology with regards to production and post-harvest handling of these delicate fruits, is increasing. However, breeders have traditionally selected new cultivars mainly for external fruit traits (i.e. size and appearance), with organoleptic and nutritional characteristics being a secondary goal. In spite of this, fruit quality is fundamental for the acceptance of different cultivars by consumers, due to the high competition in the markets with the presence of numerous new varieties, other fruits and other foods (Iglesias and Echeverria, 2009).

Abbot (1999) indicates that food quality is a concept, which includes sensory, mechanical and functional properties as well as chemical composition and nutritional values. The latter is a key point as fruit has long been promoted for its health benefits in preventing various cancer and age-related diseases (Bazzano *et al*., 2002; Liu, 2003; Casacchia and Sofo, 2013). This is due to the presence of high added value bioactive compounds, named phytochemicals (Iriti and Faoro, 2006). These compounds have strong antioxidant properties that enable them to scavenge free radicals, donate hydrogen, chelate metals, break radical chain reactions, and quench singlet oxygen *in vitro* and *in vivo* (Dai and Mumper, 2010). All these properties enable them to act in the prevention of oxidative stress-related diseases (Pandey and Rizvi, 2009). Among phytochemicals, the most abundant class present in fruit is that of polyphenols (Manach *et al*., 2004). Phenolic rings have the capacity to scavenge free radicals, first of all hydroxyl ones, by virtue

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of the aromatic hydroxylation at the ortho-position (Xia *et al*., 2010).

These compounds are distributed in every part of the fruit in different ratios, focusing more in the peels (rich in anthocyanins, hydroxycinnamic acids and flavans) and in the seeds (rich in proanthocyanidins and flavans) (Lachman *et al.*, 2009). Peaches, even though having a lower antiradical capacity than other fruits, are ones of the most important commodities consumed worldwide, both as fresh and processed product (i.e. fruit juice, jam or canned) (Cantin *et al*., 2009). As a consequence, breeding programs aimed to increase peach nutraceutical value is desirable. “Ghiaccio” peach series is a new type of peach variety, resulting from a breeding program conducted in the past years at the Fruit Tree Research Centre of Rome with the aim to obtain varieties with enhanced postharvest fruit characteristics and an improved resistance to disease and pests (Nicotra *et al*., 2001). Their progenitor is a stony hard-type peach cultivar, the Korean “Yumyeong” (Kim *et al*., 1978), from which “Ghiaccio” selections have been obtained by self-pollination (Nicotra *et al*., 2001). Peaches of “Ghiaccio” series have different ripening times but similar pomological traits (Nicotra *et al*., 2001). To the best of our knowledge, there is no phytochemical and nutritional characterisation of “Ghiaccio” peaches in literature, making this study quite relevant, providing breeders and consumers with experimental data on this emerging varieties.

Hence, the aim of the present study was to characterise the quality and nutraceutical properties of these new genotypes by measuring their total polyphenolic and total anthocyanin content, and their relative antioxidant capacity. A comparison with four commercial white flesh peach cultivars was also performed. The ultimate goal was to select “Ghiaccio” peach genotypes with enhanced nutraceutical traits, to provide breeders with new varieties having more healthful properties, making them competitive with other fruits known for their healthy properties.

2. Materials and Methods

Chemicals

All used reagents were of analytical spectrophotometric grade (Carlo Erba, Rome, Italy). Cyanidin chloride was purchased from Extrasyntese (Genay Cedex, France). Folin-Ciocalteu reagent, malic acid, chlorogenic acid, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•), and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich (Milan, Italy).

Plant materials

Peach fruits from five early-to-late ripening “Ghiaccio” genotypes (Table 1) and four white flesh peach cultivars were collected in the experimental orchards of the Fruit Tree Research Centre of Rome (CREA-FRU, Italy), at fully ripening phase. All plants (six years old) were grafted on the same rootstock (GF677), spaced at 4.5x2.5 m and standard pruning, drip irrigation and cultural practices were performed. For each genotype, 15 undamaged and disease-free fruits were collected in order to have three replications of five fruits each for the analysis.

Table 1 - Pomological and phenological traits of “Ghiaccio” series [z]

Traits	Cultivar/Selection				
	Ghiaccio Ø (G Ø)	Ghiaccio 1® (G1)	Ghiaccio X (GX)	Ghiaccio 2® (G2)	Ghiaccio 3® (G3)
Size (g)	large (168)	very large (178)	very large (217)	very large (205)	very large (200)
Shape	oblate	oblate	oblate	oblate	oblate
Shape of pistil end	weakly depressed	weakly depressed	weakly depressed	weakly depressed	weakly depressed
Symmetry	symmetric	symmetric	asymmetric	symmetric	symmetric
Prominence of suture	weak	weak	weak	weak	weak
Ground colour	cream white	cream white	cream white	cream white	cream white
Over colour	present	absent	absent	present	absent
Hue of over colour	pink			pink	
Density of pubescence	medium	sparse	medium	very sparse	very sparse
Firmness of flesh	very firm	very firm	very firm	very firm	very firm
Ground colour of flesh	cream white	cream white	cream white	cream white	cream white
Sweetness	high	high	high	high	high
Acidity	low	low	low	low	low
Ripening time [y]	-20	-8	-5	11	25

[z] Data were detected as Community of Plant Varity Office (CPVO) descriptors.
[y] The reference cv. Rome Star ripens in Center Italy between 25th-30th of July.

Fruit quality attributes

After harvesting, fruits were washed, stoned and homogenized, and the homogenate samples were analysed for total soluble solid (TSS) content using a digital refractometer (Refracto 30 PX, Mettler Toledo, Milan, Italy); data are given as °Brix. The method for analysis of titratable acidity (TA) was based on titration of the acids present in the fruit juice with sodium hydroxide (0.1 N). Data are given as g malic acid L^{-1}, since this is the dominant organic acid in peach (David *et al*., 1956). The pH value was measured using a digital pH-meter (785 DMP, Methrom, Milan, Italy). Every analysis was replicated three times.

Extraction of bioactive compounds

Fruits from the different genotypes were carefully separated in two different tissues: peel and flesh. The peel fraction was removed from the whole fruit with a sharp knife and immediately frozen with liquid nitrogen, placed in a plastic freezer bag, and stored at -80°C until evaluation. The flesh fraction consisted of a peeled wedge, which was chopped into small pieces, frozen in liquid nitrogen, and also stored at -80°C. Samples (5 g) of the different fractions were extracted with a 25 mL hydro alcoholic solution (methanol:water= 70:30, v/v) acidified with HCl (0.005 N) and homogenized with an Ultra-Turrax blender (Ultra Turrax T25, IKA, Milan, Italy) at 9000 rpm. Then, the homogenates were allowed to stand for 2 hours at 37°C under magnetic stirring to rich a complete solvent extraction. Extracts were centrifuged at 8400 rpm for 15 min at 5°C and then the obtained supernatants were analysed as follows. Extractions were repeated on three independent samples of the initial homogenate to give triplicate readings.

Determination of total polyphenol content (TPC)

TPC of both flesh and peel samples was determined using the Folin-Ciocalteu (F-C) method (Waterhouse, 2002). TPC was calculated from a calibration curve, using chlorogenic acid as a standard. Results were expressed as milligrams of chlorogenic acid equivalents (CAE) *per* 100 g fresh weight (FW). Every analysis was replicated three times.

Determination of total anthocyanin content (TAC)

TAC of both flesh and peel fractions was estimated according to the method of Mondello *et al.* (2000). TAC was calculated from a calibration curve, using cyanidin chloride (CC) as a standard. Results were expressed on a fresh weight basis as milligrams of CC equivalents (CCE) x 100 g FW. Every analysis was replicated three times.

Measurement of antioxidant capacity (AC)

AC was assessed by measuring the effect of the bioactive extracts on the content of 2,2-diphenyl-1, picrylhydrazyl radical (DPPH•) according to Brand-Williams *et al.* (1995). All the measurements were made in triplicate. AC was expressed as micrograms of Trolox equivalents (TE) x mg FW.

Statistical analysis

Data analysis was performed with SPSS 17.0 software (SPSS, Inc., Chicago, Illinois). All measurements were performed at least in triplicate and data were reported, where not specified differently, as means ± standard error of the mean (SE). An exploratory data analysis was made to check the data normal distribution (Shapiro-Wilkinson test) and the equality of variances (Levene's test). When these conditions were met (TSS, TA, AC), data were subjected to one-way analysis of variance (ANOVA) and comparisons between means were determined according to Tukey's HSD test. Significant differences were accepted at $p<0.05$ and represented by different letters.

When ANOVA assumptions were violated (TPC), even after mathematical transformation of data, a non-parametric data analysis was carried out (Kruskal-Wallis non parametric test) and significant mean differences were established using the Mann-Whitney test for independent and non-parametric procedures and a Bonferroni's correction to set the critical value for significance for each test. Box-plots were used to display the range, median and distribution density of phytochemicals and AC in the peel and flesh of genotypes analysed. Spearman's correlation coefficient (ρ) was used to determine the correlation among variables in the non-parametric analysis ($p<0.01$).

3. Results and Discussion

Fruit quality attributes

Table 1 shows the main pomological and phenological traits of "Ghiaccio" series analysed.

Ghiaccio means ice in Italian to remind consumers that the colour of the fruit is white or pale cream. They are very different from the common peach type grown in Europe and the U.S. These peaches are characterized by totally white cream skin and flesh, very firm flesh, high sugar content (up to 17°Brix), 20-25 days longevity on the tree, a great productivity and a noteworthy shelf life. Moreover, this variety shows a great resistance against diseases, rottenness and pathogenic agents making it particularly suitable for organic practice (Nicotra *et al*., 2001). The "Ghiaccio" series includes "Ghiaccio 1" (G1), "Ghiaccio 2" (G2) and "Ghiaccio 3" (G3) genotypes, which have been already licensed, while two advanced selections, "Ghiaccio Ø" (GØ) and "Ghiaccio X" (GX) are still under evaluation. All genotypes analysed were harvested between early July and late August and, generally, significant differences in quality traits were detected (Table 2).

In "Ghiaccio" series, the highest TSS level (15.2 °Brix) was observed in G1 samples, while the lowest one in GØ ones (9.1°Brix). All samples analysed showed a TSS content greater than 8 °Brix, which represents the minimum TSS content established by the EU to market peaches and nectarines [Commission Regulation (EC) No. 1861/2004, 10/28/2004]. Moreover, "Ghiaccio" genotypes showed TSS similar to the white flesh peaches analysed. TA values ranged from 3.18 (G3) to 4.23 (G1) g malic acid L^{-1}, with significant differences among selections (Table 2). On average, these values are lower than that of the progenitor and "Ghiaccio" series can be collocated in sub acidic peach varieties (Crisosto *et al*., 2001). These results are relevant since the acceptance of new cultivars by consumers, which is the ultimate goal of breeders, is linked to the fruit quality. In fact, acid levels, expressed in terms of pH and TA, and sugar concentrations, reported as TSS, affect flavour perception of the fruit influencing peach sensory profile and consumer acceptance of peach fruits (Crisosto *et al*., 2001; Crisosto and Crisosto, 2005).

In the present study, we also reported the TSS/TA ratio as the relationship between these parameters has an important role in fruit consumer acceptance (Diaz-Mula *et al*., 2009). Among "Ghiaccio" peaches, TSS/TA values ranged from 3.62 for G3 to 3.16 for G2, being the latter significant different from the other genotypes of the series. In the fresh market, consumers desire large shaped and flavourful fruit with a high sugar content and low to moderate acidity and these new genotypes appear to respond adequately to such requests. In this regard, "Ghiaccio" series showed, on average, a TA content lower than that of the white flesh cultivars analysed, which is reflected in a higher value of the TSS/TA ratio.

Bioactive compounds distribution within the peach genotypes and relative antioxidant capacity

Fruit antioxidant potential varies in relation to the phytochemical moieties present, and variations can occur among genotypes within a single species (Van der Sluis *et al*., 2001; Cantin *et al.*, 2009). Moreover, it is well known that the content of phytochemicals can vary within different tissues (Carbone *et al*., 2011). In figure 1, box-plots showed the distribution of polyphenols, and the antiradical activity between the peel and the flesh of "Ghiaccio" peaches analysed, independently from the genotype. TPC was significantly higher in the peach peel extracts than in the flesh ones (+103%). These data are consistent with those reported in the literature about the influence of the type of fruit tissue on the accumulation of nutraceutical substances (Carbone *et al*., 2011;

Table 2 - Fruit quality attributes of genotype analysed (mean±SE)

Genotype	Harvest date	TSS (°Brix)	TA (g L^{-1} malic acid)	TSS/TA ratio
Ghiaccio 1	July, 22nd	15.20±0.03 e	4.23±0.06 b	3.58±0.04 d
Ghiaccio 2	August, 11th	13.00±0.03 d	4.13±0.01 b	3.16±0.02 c
Ghiaccio 3	August, 25th	11.5±0.1 b	3.18±0.02 a	3.62±0.05 d
Ghiaccio Ø	July, 7th	9.10±0.07 a	ND	ND
Ghiaccio X	July, 21st	12.10±0.07 c	3.43±0.04 a	3.54±0.06 d
Crizia	June, 28th	9.6±0.4 a	7.96±0.03 c	1.21±0.05 a
Maria Anna	August, 4th	13.60±0.03 d	12.0±0.2 e	1.13±0.02 a
Redhaven Bianca	July, 21st	12.2±0.1 c	8.74±0.01 d	1.39±0.01 b
Silver Late	September, 8th	13.20±0.06 d	12.61±0.02 f	1.05±0.00 a
"Ghiaccio" Series		12.2±0.5 a	3.7±0.1a	3.47±0.06 b
White Flesh cvs.		12.1±0.5 a	10.3±0.6 b	1.19±0.04 a

TSS= Total soluble solids.
TA= Titratable acidity.
ND = Not determined.
Significant differences were accepted at p<0.05 and represented by different letters on the column, within the cultivars or within the groups ("Ghiaccio" series and white flesh cultivars).

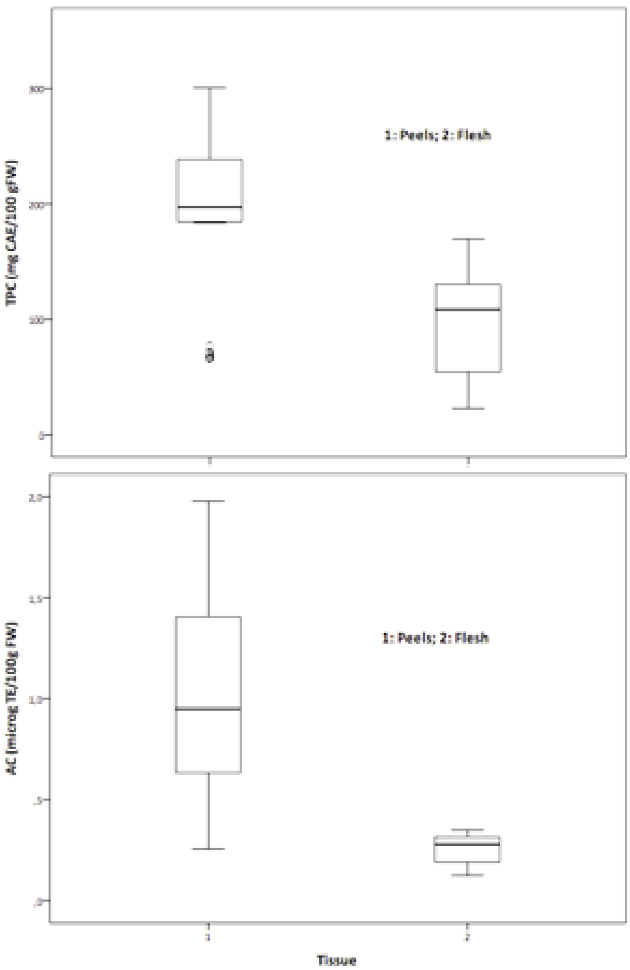

Fig. 1 - Box-plots for the phytochemicals and antioxidant capacity of different peach tissues. The line in the box indicates the median value of the data; the right and the left edges of the box respectively indicates the 75th and the 25th percentiles of the data set, the ends of the horizontal lines indicate the minimum and maximun data values; the point outside the box are outliers or suspected outliers. A) TPC= Total polyphenol content (mg CAE 100 g^{-1} FW). B) AC = Antioxidant capacity (µg TE mg^{-1} FW). Outlier: value more than 1.5 and less than 3 box-lengths from end of box.

Table 3 - Phytochemicals and antioxidant capacity of the peel of genotypes analyzed (mean±SE)

Genotype	AC	TPC	TAC
Ghiaccio 1	1.0±0.1 bc	232±6 e	ND
Ghiaccio 2	0.53±0.08 a	70±2 a	ND
Ghiaccio 3	0.7±0.2 ab	197±4 d	ND
Ghiaccio Ø	1.3 ±0.1 cd	260±4 f	ND
Ghiaccio X	1.5±0.1 d	144±4 c	ND
Crizia	1.34±0.03 cd	102±3 b	26.53±0.03 c
Maria Anna	1.42±0.06 cd	260.9±0.7 f	34.55±0.06 d
Redhaven Bianca	1.13±0.04 cd	100±3 b	19.70±0.05 a
Silver Late	1.50±0.08 d	151±2 c	23.40±0.03 b
“Ghiaccio” series	1.00±0.08 a	175±14 a	ND
White flesh cvs.	1.35±0.04 b	154±14 a	26±2

AC= Antioxidant capacity.
TPC= Total polyphenol content.
TAC= Total anthocyanin content.
ND = Not determined.
Significant differences were accepted at p<0.05 and represented by different letters on the column, within the cultivars or within the groups (“Ghiaccio” series and white flesh cultivars).

Table 4 - Phytochemicals and antioxidant capacity of the flesh of genotypes analyzed (mean±SE)

Genotype	AC	TPC	TAC
Ghiaccio 1	0.286±0.007 cd	59±8 c	ND
Ghiaccio 2	0.16±0.01 a	28±2 ab	ND
Ghiaccio 3	0.325±0.007 d	126±2 d	ND
Ghiaccio Ø	0.308±0.007 d	134±4 d	ND
Ghiaccio X	0.20±0.02 b	71±3 c	ND
Crizia	0.256±0.005 c	23±2 a	7.54±0.01 c
Maria Anna	0.157±0.003 a	38±2 b	ND
Redhaven Bianca	0.263±0.004 c	28±2 ab	1.67±0.01 a
Silver Late	0.412±0.005 e	62±2 c	6.45±0.01 b
“Ghiaccio” series	0.25±0.01 a	86±8 b	ND
White flesh cvs.	0.27±0.02 a	38±3 a	4.1±0.9

AC= Antioxidant capacity.
TPC= Total polyphenol content.
TAC= Total anthocyanin content.
ND = Not determined.
Significant differences were accepted at p<0.05 and represented by different letters on the column, within the cultivars or within the groups (“Ghiaccio” series and white flesh cultivars).

Tomás-Barberán *et al*., 2001).

Tables 3 and 4 show the flesh and peel phytochemical content and AC of different cultivars analysed. Among “Ghiaccio” genotypes, GØ showed the highest TPC both in the peel and flesh (260 and 134 mg CAE 100 g^{-1} FW, respectively), while G2 the lowest one (70 and 28 mg CAE 100 g^{-1} FW, respectively) (Tables 3 and 4). These findings highlight that TPC of “Ghiaccio” series is related to the genotype, in agreement with those reported in literature, not only for other peaches but also for other fruit species (Tomás-Barberán *et al*., 2001; Ceccarelli *et al*., 2016). Interestingly, the average peel polyphenol content of “Ghiaccio” series (175 mg CAE 100 g^{-1} FW) was not significantly different from that of the white flesh cultivars analysed (Table 3), while the average TPC of the flesh was significantly higher (Table 4). Obtained results emphasize the healthy properties of the flesh of these new de-pigmented peaches.

As regards total anthocyanins, their content was not detectable in “Ghiaccio” peaches both in the peel and the flesh. Besides, white flesh peaches revealed the presence of these compounds in all tissue analysed.

In agreement with the phytochemical data, genotype also influenced the AC of peaches analysed. Among “Ghiaccio” genotypes, the highest value of AC was found in GØ (1.3 µg TE mg^{-1} FW) and GX (1.5 µg

TE mg^{-1} FW) for what concern the peel and in GØ (0.308 µg TE mg^{-1} FW) and G3 (0.325 µg TE mg^{-1} FW) for the flesh. On average, the antioxidant capacity of the peel was lower in "Ghiaccio" series than in white flesh cultivars (Table 3), probably due to the absence of anthocyanins in the peel of "Ghiaccio" series. Moreover, correlation analysis pointed out that the scavenging capacity against $DPPH^{\bullet}$ of peach extracts and TPC were significantly and positively correlated (ρ= 0.796; p<0.01). This result confirms previous reports on commercial white and yellow flesh cultivars, showing that phenolic compounds can be considered the main phytochemicals contributing to AC in peaches (Vizzotto *et al*., 2007).

4. Conclusions

Peach breeding has supplied a large number of improved cultivars each year to satisfy different market demands. Nevertheless, there are some critical issues not yet fully addressed through breeding such as the increasing competition between peach and a broad range of other fruits, as well as the consumer eating habits, which are changed in the last years, with a growing emphasis on nutrition and health properties of food. To meet these challenges, it is necessary to explore new germplasm for the production of new cultivars with improved quality and nutritional characteristics as well as to provide fruits to the market for long periods of time. In this context, the "Ghiaccio" series could be a right answer for breeders. The present study describes for the first time the quality traits, phytochemical composition and AC of five genotypes (three cultivars and two advanced selections) belonging to the "Ghiaccio" peaches. Data point out the key role played by the genotype also within the "Ghiaccio" series, underling the importance of the varietal selection. Among the genotypes analysed, the best candidates for a "Ghiaccio" peach with enriched nutraceutical properties are the advanced selections GØ and GX. In addition, data pointed out a higher nutraceutical potential of "Ghiaccio" series than that of the commercial white flesh peaches analysed. Finally, taking into account that the fruit characteristics (i.e. shape, colour and size) are very similar to each other, but with different ripening periods, "Ghiaccio" genotypes, if grown together, would allow the producers to supply the markets with the same type of fruit for a long period of time (i.e. 75 days).

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