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# Phenotypic and biochemical diversity in peach [*Prunus persica* (L.) Batsch] cultivars

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**Abstract –** Peach (*Prunus persica* L. Batsch) is the third most abundant fruit worldwide, after the apple and pear. This study was conducted in six peach and nectarine cultivar. The main objective of this work was to search for the phenotypic variability in agronomical and biochemical fruit quality traits. Agronomical traits such as fruit weight (g) and the analysis of fruit quality parameters [firmness, soluble solids content (SSC), pH, titratable acidity (TA) and ripening index (RI)] were determined. Biochemical fruit quality traits such as vitamin C, total phenolics, flavonoids, anthocyanins and the relative antioxidant capacity were evaluated. Such phonemics analysis is an obligatory requirement for identification of molecular markers for distinct fruit quality traits and for selection of appropriate parents for future breeding programs. Results showed that the cultivars exhibited wide phenotypic variation in agronomic and biochemical traits. In addition, principal component analysis (PCA) revealed several relationships among quality traits in the evaluated cultivars. This relationship should be considered in the current breeding programs to select cultivars with high health-enhancing properties and good postharvest performance.

**Keywords:** peach, fruit quality, phenolic compounds

## 1. Introduction

Peach tree (*Prunus persica*) is a species of *Prunus*, a genus that also includes nectarine, plum, apricot, cherry, and almond belonging to the subfamily *Prunoideae* of the Rosaceae family. Nowadays there are more than 3000 peach cultivars in the world, which can be variously classified as melting and non-melting flesh, or hairy and smooth skin, or clingstone and freestone, *etc.* (Zhao et al. 2015). Peach is considered one of the genetically most characterized species in the Rosaceae, and it has distinct advantages that make it suitable as a model genome species for *Prunus* as well as for other species in the Rosaceae (Abbot 2002).

Peaches are economically and nutritionally important because they can be a significant component of the diet during spring and summer because they are consumed in large quantities (Remorini et al. 2008). Different studies have reported a significant variability among the various cultivars of the peaches that might be due to the geographical zones, climatic conditions and crop genetics. Important sensorial qualitative traits such as sweetness, juiciness and flavour vary from cultivar to cultivar in peaches and nectarines (Cano-Salazar et al. 2013). Therefore, individual fruit must be monitored for changes during ripening, because fruit ripening pattern in one cultivar may not be applicable to other cultivars within the same species (Goulao and Oliveira 2008). More recently, the biochemical components of peach as well as several other fruits have received greater attention because of their potential health benefits (Prior and Cao 2000).The major soluble sugars in peach are sucrose followed by glucose and fructose, with lower levels of sorbitol (Brooks et al. 1993). In ripe fruit, these sugars comprise about 60% of the soluble sugar content (SSC) (Cantín et al. 2009). Glucose and fructose concentrations show a continuous increase during fruit development, while sucrose accumulates primarily during maturation (Hancock 1999). It is increasingly challenging for peach producers to select the ideal scion cultivar that satisfies market requirements and maximizes their profits (Yue et al. 2014). The improvement of fruit quality traits can be achieved through breeding new cultivars using genetic and phenotypic information to develop trees with more desirable fruit characteristics.

The objective of the present study was to characterize the agronomic and phytochemical fruit quality in six peach cultivars, and to evaluate the phenotypic variability among cultivars. Such results may

help to select new genotypes rich in phenolic content and enhanced nutritional properties and to provide useful information for the utilization of peach genetic resources.

## 2. Material and methods

### 2.1. Plant material

Six peach and nectarine cultivars obtained from the peach germplasm collection at the 'Experimental Station of Aula Dei' (CSIC) have been evaluated (Table 1). All cultivars were budded on the 'Pollizo' plum rootstock 'Adesoto' (Moreno et al. 1995) and established in an experimental orchard (three trees per genotype). Most accessions are non-melting, clingstone and yellow flesh peach cultivars. Harvest season ranges from June to October. The orchard was located in the Ebro Valley (Northeast Spain, Zaragoza), and grown under a Mediterranean climate, on a heavy and calcareous soil, with 27% total calcium carbonate, 8% active lime, pH 8.3, and a clay-loam texture. Trees were grown under standard conditions of fertilization, irrigation, pest and disease control, spring thinning and winter pruning. Trees were hand-thinned at 45–50 days after full bloom (DAFB) leaving approximately 20 cm between fruits. Open vase trees were pruned to strengthen existing scaffold branches and eliminate vigorous shoots, inside and outside the vase, that would compete with selected scaffolds or shade fruiting wood. Most vegetative and fruit quality traits have been evaluated over two consecutive years (2008-2009).

**Table 1.** Peach and nectarine commercial cultivars fruit type (round or flat, peach or nectarine), flesh colour (yellow or white) and stone adhesion (cling or free)

| Cultivar | Fruit type | Flesh color | Stone | Flesh type |
|---|---|---|---|---|
| **Andross** | Round peach | yellow | clingstone | Non-melting |
| **Babygold 9** | Round peach | yellow | clingstone | Non-melting |
| **Big top** | Round nectarine | yellow | clingstone | melting |
| **Calante** | Round peach | yellow | clingstone | Non-melting |
| **Rich lady** | Round peach | yellow | semi-freestone | melting |
| **Venus** | Round nectarine | yellow | freestone | melting |

### 2.2. Agronomic fruit quality traits

Fruits were hand-picked at commercial maturity, assessed by peel fruit color and firmness. Total yield (kg/tree) was determined for each individual tree recording also the total number of fruits. For the evaluation of fruit quality parameters, a representative sample of twenty fruits per tree was selected. Flesh firmness was determined with a penetrometer fitted with a 8-mm diameter probe. The soluble solids content (SSC) of the juice was measured with a digital refractometer (model ATC-1, Atago Co., Tokyo, Japan); and data are given as ºBrix. The titratable acidity (TA) was measured by titration of 5 ml of juice with NaOH 0.1N to pH 8.1 (AOAC 1984). Ripening index (RI) was calculated as the ratio of SSC to TA. Then, 5 g flesh samples were prepared for each tree, frozen in liquid nitrogen and stored at -20ºC for analyses.

### 2.3. Phytochemical extraction

For all analyses only fruit flesh was used, as it is usually consumed. Ten representative fruits were peeled, flesh was weighted, immediately frozen in liquid nitrogen, and stored at -20 ºC for analysis. For vitamin C determination, samples at harvest were kept in 5 mL of 5 % metaphosphoric acid for preservation of ascorbic acid, frozen in liquid nitrogen and stored at -20 ºC. Samples were homogenized with a polytron in 5 mL of 5 % metaphosphoric acid and centrifuged at 16,000 rpm for 20 min at 4 ºC, filtered with Miracloth and the supernatant was used for vitamin C analysis. For phenolic compounds, frozen fruit material (5 g) was homogenized in a polytron with 10 mL of extraction solution, consisting of 0.5 N HCl in methanol/Milli-Q water (80% v/v). The mixture was then centrifuged for 20 min at 4 ºC and 16,000 rpm. Supernatant was recovered and the volume measured. This hydroalcoholic extract was used for total phenolics, flavonoids, anthocyanins and antioxidant capacity assays. For the determination of sugars, the frozen fruit material (5 g) was homogenised in a Polytron with 10 mL of extraction solution consisting of ethanol/Milli-Q water (80% v/v). The mixture was centrifuged at 16,000 rpm for 20 min at 4 ºC. The supernatant was

recovered and processed to be assayed by high-performance liquid chromatography (HPLC) as described by Cantín et al. (2009).

#### 2.3.1. Vitamin C

To measure vitamin C, the procedures used were as described by Law et al. (1983), adapted from Okamuraet al. (1980) for the spectrophotometric determination of ascorbic acid. Absorbance was measured at 525 nm using a spectrophotometer (Beckman Coulter DU 800). The standard calibration curve was daily prepared using ascorbic acid as standard. Vitamin C was expressed as mg ascorbic acid (AA) per 100 g FW.

#### 2.3.2. Total phenolics

The total phenol content in fruits was evaluated according to Swain and Hills method (1959), the assay consists of a colorimetric method based on the chemical reduction of the Folin-Ciocalteau reagent. Absorbance was measured at 725 nm using a spectrophotometer (Beckman Coulter DU 800). The phenolic content was expressed in milligrams of gallic acid equivalents (GAE) per 100 g fresh weight (FW).

#### 2.3.3. Flavonoids

Total flavonoids content was determined using a colorimetric assay based on the method of Zhishen et al. (1999). Absorbance was measured at 510 nm against a blank with a spectrophotometer (Beckman Coulter DU 800). Results were expressed as milligrams of catechin equivalents (CE) per 100 g of FW based on a standard curve using catechin as standard.

#### 2.3.4. Anthocyanins

Total anthocyanin content was evaluated using the method of Fuleki and Francis (1968) adapted to peach tissue. Absorbance of the extract was measured at 535 and 700 nm. The anthocyanins concentration in the original sample was calculated in mg cyaniding 3-glucoside equivalent /kg of FW using the molar extinction absorptivity coefficient $\varepsilon = 25{,}965\ cm^{-1}\ M^{-1}$.

#### 2.3.5. Relative antioxidant capacity (RAC)

Antioxidant capacity of peach extracts was measured using the 1, 1-diphenyl-2-picrylhydrazyl (DPPH), as described by Brand-Williams et al. (1995). The absorbance of samples was measured at 515 nm after 10 min of reaction. The antioxidant activity was expressed as µg Trolox/g of FW.

### 2.4. Statistical Analyses

All traits were measured for each genotype separately. All statistical analyses were performed using SPSS 20.0 (SPSS Inc., Chicago, IL). To obtain basic statistics for the entire plant material studied, minimum and maximum values, mean and mean standard error (SE), were calculated for each trait. Principal component analysis (PCA) was applied on the sugar content, antioxidant compounds and basic agronomical traits as an attempt to identify superior genotypes based on their agronomic and biochemical attributes. The component matrix was evaluated and orthogonal factors were rotated using variance maximizing (Varimax).

## 3. Results and discussion

### 3.1. Agronomic fruit quality traits

Traits were evaluated in each cultivar separately over two years (2008–2009) of study (Table 2). Mean values of fruit weight, firmness, soluble solids content (SSC), pH, titratable acidity (TA) and the ripening index ratio (RI= SSC/TA) were calculated. Results showed high variability among cultivars in different agronomic fruit traits.

The fruit weight showed differences between cultivars as a consequence of the variability in tree production and fruits number for each cultivar. In this way Dirlewanger et al. (1999) reported that fruit weight is a major quantitative inherited factor determining yield, fruit quality and consumer acceptability.

Regarding flesh fruit firmness, our analysis revealed that the cultivar Calante showed the highest value of firmness (43.3 N) which is lower than the maximum level of fruit firmness for marketing fresh

peaches and nectarines, set by the EU at a 6.5 kg/0.5 $cm^2$ (= 63.7 N), using a 8 mm diameter probe (Commission Regulation EC, No.1861/2004 of 28 October 2004).

Regarding soluble solids content (SSC), the cultivars showed values from 10.5 to 16.4 ºBrix, which is greater than the minimum (8 ºBrix) established by the EU to market peaches and nectarines (R-CE No.1861/2004). Kader (1999) considered mean values of SSC over 10 ºBrix as the minimum value for consumer acceptance for yellow-flesh nectarines. The variability found in SSC among cultivars can be explained by the quantitative performance of this quality trait (Quilot et al. 2004).

The pH values varied from 3.53 to 4.0, which are values of normal acidity fruits. Monet (1979) reported that fruit with a pH higher than 4.0 at maturity are considered as non-acid.

TA showed significant differences between cultivars. Significantly higher TA (TA= 0.97 %) was observed in Venus cultivar.

The ripening index (RI= SSC/TA) varied greatly among genotypes, depending on their SSC and TA values. It was found that cv. Andross and Calante could have good consumer acceptability, due to their highest TSS/TA. In peaches, the RI is a major organoleptic quality trait of the mature fruit and is commonly used as a quality index (Bassi and Selli 1990). The relationship between TA and SSC has an important role in consumer acceptance of some apricot, peach, nectarine and plum cultivars. Crisosto et al. (2004) reported that in the case of cultivars with TA >0.9 % and SSC <12.0 ºBrix, consumer acceptance was controlled by the interaction between TA and SSC rather than SSC alone.

**Table 2.** Mean values and standard error (SE) of the quality attributes in peach and nectarine cultivars.

| Cultivar | Fruit weight | Firmness | SSC | pH | TA | RI |
|---|---|---|---|---|---|---|
| **Andross** | $214^{b}$ | $32.8^{a}$ | $15.9^{bd}$ | $3.93^{a}$ | $0.61^{a}$ | $26.1^{a}$ |
| **Babygold-9** | $159^{a}$ | $39.2^{b}$ | $16.4^{d}$ | $3.97^{a}$ | $0.82^{ac}$ | $20.0^{b}$ |
| **Big Top** | $154^{a}$ | $40.2^{b}$ | $15.0^{bd}$ | $4.00^{a}$ | $0.85^{ac}$ | $17.6^{c}$ |
| **Calante** | $291^{c}$ | $43.3^{c}$ | $12.9^{c}$ | $3.80^{a}$ | $0.50^{a}$ | $25.7^{d}$ |
| **Rich Lady** | $267^{d}$ | $35.8^{a}$ | $10.5^{a}$ | $3.65^{a}$ | $0.89^{c}$ | $11.8^{e}$ |
| **Venus** | $251^{d}$ | $36.3^{a}$ | $14.1^{bc}$ | $3.53^{a}$ | $0.97^{c}$ | $14.5^{f}$ |

Units and abbreviations: Fruit weight (g); Firmness (N); N = Newtons; SSC = Soluble solids content (°Brix); TA = Titratable acidity (g malic acid/100 g FW); RI = Ripening index (SSC/TA).

### 3.2. Biochemical fruit quality traits

The antioxidant compounds content showed a high variability among cultivars (Table 3). The ascorbic acid content ranged from 3.3 to 7.0 mg of AsA kg-1 of FW. The cultivars differed significantly according to vitamin C content. Our results indicate that peach is a good source of vitamin C and highlight the fact that ascorbic acid content is an important part of the overall evaluation of peach fruit quality. Cantín et al. (2009) reported that total ascorbic acid content in 218 peach genotypes from different progenies varied greatly from approximately 1 to 9 mg of AsA/100 g of FW, with a mean value of 3.7 mg of AsA/100 g of FW.

There were significant differences between cultivars in total phenolics content. The amount of total phenolics in cultivars fell within the range reported in the literature for peach fruits, namely 14-77 mg GAE kg-1 of FW (Proteggente et al. 2002). Regarding flavonoids, the results, revealed high flavonoids content in cv. Babygold 9 with an average of 53.1 mg of CE kg-1 of FW. This fact could be very interesting in the peach cultivars selection process, mainly selecting fruits rich in flavonoids, since the consumption of flavonoid-rich foods holds the potential to limit neurodegeneration preventing age-dependent loses in cognitive performance (Vauzour et al. 2008).

The anthocyanins content ranged from 1.2 to 6.3 mg C3Geq kg-1 of FW, showing high variability among genotypes. Cantín et al. (2009) reported that in some progenies total anthocyanins greatly varied among genotypes [0.1-26.7 mg of C3Geq kg-1 of FW] depending on the red pigmentation of the flesh.

The relative antioxidant capacity (RAC) ranged from 418 to 986 (mg Trolox kg-1 of FW) showing a high variability among genotypes. This could be explained by the fact that the antioxidant capacity of fruits varies in relation to the antioxidant molecules present in the different species but variations can also occur within the genotypes of a single species (Gil et al., 2002). Cantín et al. (2009) reported that values of RAC (227.3 to 629.9 mg of Trolox kg-1 of FW, with an average of 405 mg of Trolox kg-1 of FW) in the same range of these results even slightly lower.

**Table 3.** Variation in the content of antioxidant compounds

| Cultivars | Vitamin C | Total phenolics | Flavonoids | Anthocyanins | RAC |
|---|---|---|---|---|---|
| **Andross** | $7.0 \pm 0.1^{c}$ | $42.1 \pm 2^{a}$ | $34.1 \pm 2^{b}$ | $1.2 \pm 0.2^{a}$ | $952.7 \pm 10^{a}$ |
| **Babygold9** | $6.0 \pm 0.7^{c}$ | $52.0 \pm 5^{c}$ | $53.1 \pm 4^{a}$ | $2.4 \pm 0.5^{b}$ | $986.1 \pm 14^{b}$ |
| **Big Top** | $5.6 \pm 0.5^{a}$ | $46.6 \pm 4^{b}$ | $16.8 \pm 2^{c}$ | $1.4 \pm 0.3^{a}$ | $926.2 \pm 12^{c}$ |
| **Calante** | $3.8 \pm 0.5^{ab}$ | $45.4 \pm 2^{b}$ | $15.4 \pm 1^{c}$ | $1.3 \pm 0.2^{a}$ | $418.9 \pm 6^{d}$ |
| **Rich lady** | $5.3 \pm 0.3^{a}$ | $18.4 \pm 2^{d}$ | $3.5 \pm 1^{d}$ | $6.3 \pm 1^{c}$ | $549.4 \pm 9^{e}$ |
| **Venus** | $3.3 \pm 0.3^{b}$ | $36.4 \pm 3^{e}$ | $13.7 \pm 1^{e}$ | $1.8 \pm 0.5^{ab}$ | $422.8 \pm 7^{f}$ |

Units and abbreviations: Vitamin C (mg AsA/100 g of FW); Total phenolics (mg GAE/100 g of FW); Flavonoids (mg CE/100 g of FW); RAC; anthocyanin (mg C3Geq $kg^{-1}$ of FW ); Relative Antioxidant Capacity (µg TE/g of FW). Abbreviations: AsA = Ascorbic acid; C3GE = Cyanidin-3-glucoside equivalents; CE = Catechin equivalents; FW = Fresh weight; GAE = Gallic acid equivalents; TE = Trolox equivalent.

### 3.3. Sugar content

The sucrose, glucose, fructose and sorbitol contents in the cultivars were analysed separately (Figure 1), as they play an important role in peach flavour quality (Robertson and Meredith 1988).The studied cultivars exhibited considerable phenotypic variation in sugar contents. Sucrose was the major sugar present in the evaluated cultivars, followed by fructose, glucose and sorbitol. Wu et al. (2005) reported that sucrose in peaches is dominant at maturity, followed by the reducing sugars (glucose and fructose) and then sorbitol. Identifying cultivars with low glucose/fructose ratio might be of particular interest, since the relative concentrations of these sugars influence sweetness, as fructose is 2.3 times and 1.7 times sweeter than glucose and sucrose, respectively (Kulp et al. 1991). Total sugars (the sum of sucrose, glucose, fructose and sorbitol contents) in peeled fruit ranged from 63.5 to 103.6g $kg^{-1}$ FW among cultivars. Total sugar content is an important quality trait in fruit breeding programmes, since it has been reported to be highly related to the aroma and taste of peaches and nectarines (Colaric et al. 2005). Quilot et al. (2004) reported that for total sugars content, variation among trees, among fruits of the same tree, and among years are not negligible in comparison with the variation among genotypes.

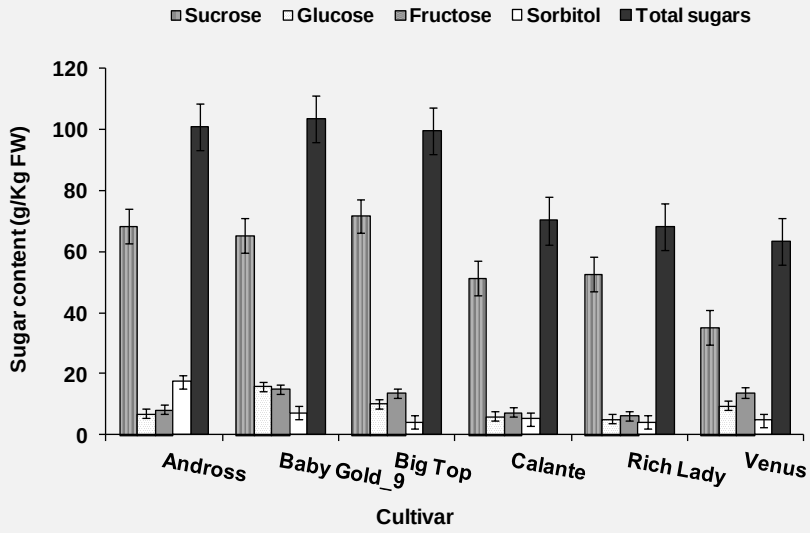


**Figure 1.** Sugar content in peach and nectarine cultivars (g per kg FW) for 2008 and 2009.

### 3.4. Principal component analysis

A PCA was performed on agronomical and biochemical data (Figure 2). A four component model accounted for more than 77% of total variance, with the first two components, PC1 and PC2, explaining 56.9% and 20.7% of total variance, respectively. Cultivars displayed a great variability

(Figure3.2a). An examination of PC1 loadings (Figure3.2a) suggested that this separation was mainly due to the fruit weight and the biochemical traits (TA, flavonoids, total phenolics and RAC). Genotypes on the positive side of PC1 showed higher phenolics content and accumulated more sugars and less anthocyanin than individuals on the negative side. The results obtained in this progeny were coherent and reflected the known correlations between bioactive and agronomical traits as described in others studies (Cantín et al.2009).

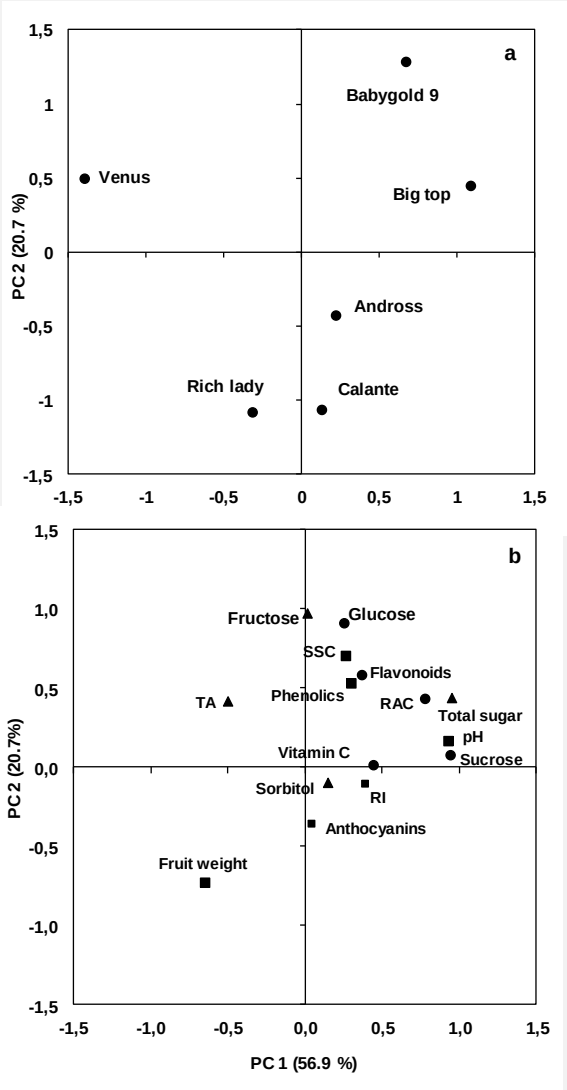


**Figure 2.** Principal component analysis of agronomical and biochemical traits in peach cultivars for 2008 and 2009. Abbreviations: RAC, relative antioxidant capacity; RI, ripening index (SSC/TA); SSC, soluble solids content; TA, titratable acidity.

## 4. Conclusion

The cultivars showed a great phenotypic variance for agronomical, pomological, sugar profile and phytochemical traits. This variability found in fruit weight, TA, total phenolics, flavonoids, antioxidant capacity, and total sugars indicating their genetic diversity and their diverse genetic origins. Our results lead us to the conclusion that the antioxidant capacity of peach is characterized by huge levels of variations, much explained by the genotype but harvest conditions and season may also be significant factors. This fact could be of importance because there is an important genetic potential to select new peach cultivars with high fruit quality.

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