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Influence of antioxidant compounds, total sugars and genetic background on the chilling injury susceptibility of a non-melting peach [*Prunus persica* (L.) Batsch] progeny

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Running title: Influence of antioxidant and sugar contents on postharvest performance of non-melting peaches

Abstract

BACKGROUND: To identify genotypes with good organoleptic properties, antioxidant-rich content and low susceptibility to chilling injury (CI), fruit from 130 peach cultivars were studied over three years. Pomological traits and L-ascorbic acid, flavonoids, total phenolics, relative antioxidant capacity (RAC), and sugars were determined. Major symptoms of CI developed at 5ºC, such as leatheriness, flesh browning, bleeding and loss of flavor, were evaluated.

RESULTS: The population exhibited wide phenotypic variation in agronomic and biochemical traits. Six genotypes with high total phenolics, RAC, flavonoids and total sugars were selected. The progeny also showed variability for all the evaluated CI symptoms and 16 genotypes showed considerable lower susceptibility to CI. After 2 weeks of cold storage, leatheriness and bleeding were the main CI symptoms observed, whereas flesh browning was predominant after 4 weeks.

CONCLUSIONS: It was possible to find varieties with high phenolics concentration and relatively low or intermediate CI susceptibility (22, 33, 68, 80, 81, 96 and 120). However, the correlations observed between CI and phenol contents highlight their potential influence on susceptibility to internal browning. This relationship should be considered in the current breeding programs to select cultivars with high bioactive compound contents, health-enhancing properties and good post-harvest performance.

Keywords: antioxidant capacity, chilling injury, postharvest disorders, sugars, total phenolics, vitamin C.

INTRODUCTION

The commercialization of peach, one of the most important fruit crops in Spain[1], is limited due to its short post-harvest life because of the rapid ripening and microbial decay.[2] Traits, such as, flesh firmness, acidity, texture, aroma, and content of total sugars and antioxidant compounds, are influenced by pre- and post-harvest factors and might negatively or positively affect consumer acceptance.[3]

Cold storage, a widely used industrial procedure, remains the main method to inhibit fruit decay and extend shelf life. However, the fruit are very sensitive to low temperature and exhibit chilling injury (CI) after long periods of cold storage.[3] If susceptible varieties of peach, nectarine and other stone fruits are held too long at a low temperature, they will not ripen properly when re-warmed and they will suffer physiological disorders, collectively known as chilling injury.[4, 5] This manifests itself as dry, mealy, woolly (lack of juice) or hard-textured fruit with no juice (leatheriness), flesh or pit cavity browning, and flesh bleeding or internal reddening.[3] Browning is often seen in mealy or leathery fruit, although it may occur in the absence of mealiness, when enzymes such as polyphenol oxidase act on phenolic substrates.[4, 6] Flesh bleeding has been also reported as a spread of red pigment, presumably anthocyanins, through the fruit flesh during cold storage or after subsequent ripening.[6] Mealiness and leatheriness are fruit flesh textural disorders, where affected ripe fruit has a dry grainy feel when chewed.[6] In simple terms, mealy fruits are dry and soft when ripe, whereas leathery fruits have dry and firm texture when ripe.[6, 7] Leathery fruit show a high degree of cell wall thickening compared with mealy or juicy fruit.[8] Leatheriness has been described as similar but with even less free juice than in mealy fruit, and the texture of the flesh firm rather than grainy.[9] Although clingstone non-melting flesh (CNMF) cultivars do not become mealy, some may have a short market life due to their high susceptibility to leatheriness or browning.[6] In contrast, freestone melting flesh (FMF) and clingstone melting flesh (CMF)

cultivars have the potential to develop mealiness in their fruit, depending on whether they carry further genes for susceptibility.[6] The genetic control of CI in peach has been studied and it has been demonstrated that mealiness, leatheriness, browning and bleeding are probably controlled by major genes.[5, 6, 10] Moreover, one major quantitative trait locus (QTL) has been detected for each of these symptoms (mealiness, bleeding, browning) on linkage groups (LG) 4 and 5.[6, 10, 11]

An important field of research in human nutrition nowadays is the control of 'redox' status by consuming foods with high polyphenolic contents with beneficial effect on human health.[12] However, few studies have put emphasis on the effects of antioxidant components and their activity on the susceptibility of fruit to CI. Lee *et al.*,[13] reported that CI development has been associated with enzymatic activity of polyphenol oxidase (PPO) which leads to oxidative degradation of mono-phenolic compounds to produce the polyphenolic polymers that impart a brown color to fruit flesh. Also, the development of CI symptoms has been associated with decreased total phenolics, flavonoids and total antioxidant concentrations and reduced juiciness during storage.[14] On the other hand, pre-treatment of chilling sensitive peaches with salicylic acid (SA, a natural phenolic compound) alleviated browning symptoms by promoting the ascorbate–glutathione cycle.[15] Post-harvest treatments with methyl jasmonate (MeJA) have been shown to reduce browning by increasing peroxidase activity (POD) and thus decreasing phenolic concentrations.[16] Thus, varieties that have a balance of high phenolic compound content and low PPO activity can be very attractive.[17] Additionally, sugar metabolism of soluble carbohydrates is closely related to CI susceptibility during cold storage in several fruits, including peach.[18]

In this context, we still do not fully understand how fruit antioxidant-related variables or sugars evaluated at harvest affect CI symptoms in non-melting flesh peach genotypes. The main objectives of this work were (1) to evaluate the existing phenotypic diversity in

antioxidant compounds and total sugar content of a CNMF population; (2) to quantify the expression of different CI symptoms in the entire population after two different periods of cold storage and (3) to study the possible relationships of pomological fruit quality attributes, antioxidant compounds and total sugars content at harvest, with the expression of CI symptoms.

MATERIALS AND METHODS

Plant material

The progeny assayed was a segregant F1 population of 130 seedlings obtained from a controlled cross, between *Prunus persica* (L.) Batsch cultivars 'Babygold 9' × 'VAC-9510'. Both parents and the entire segregant population are CNMF. The resulting seedlings were budded on the same rootstock (GF 677) and 1-year-old trees were established in an experimental orchard at the Experimental Station of Aula Dei-CSIC (Zaragoza, northern Spain) in the winter 2001-2002. Trees were trained to the standard open vase and grown under standard conditions of irrigation, fertilization and pest and disease control. Fruit quality traits were evaluated over three consecutive years (2009-2011) on 8 to 10-year-old trees, depending on the year of evaluation.

Quality parameters

Agronomic and fruit quality traits were measured individually in each seedling tree for three years. Fruits were hand-picked from early July to mid August at commercial maturity as assessed by peel fruit color and flesh firmness. Yield (kg per tree) was weighed and a representative fruit sample (40 pieces of fruit) was taken for fruit quality evaluations as described by Cantín *et al*,.[19]. Flesh firmness (N) was measured with a penetrometer equipped with an 8-mm diameter flat tip probe. Two measurements were made on opposite sides of each fruit after the removal of a 1 mm thick slice of skin. Soluble Solids Content (SSC in %)

were measured on the juice using a digital hand-held refractometer (Atago, Tokyo, Japan). The initial pH and titratable acidity (TA, g malic acid per 100 g fresh weight) were measured on the same juice samples by automatic titration using NaOH 0.1 mol L^{-1} and results expressed as (862 Compact Titrosampler, Methrom, Herisau, Switzerland). Ripening Index (RI) was calculated as the ratio between SSC and TA (SSC/TA).

Phytochemical extraction

For all the assays, 5 g of peeled fruit flesh from 20 representative fruits were immediately frozen in liquid nitrogen, and stored at -20 ºC until analysis, except for vitamin C where samples were kept in 5 mL of 50 mL L^{-1} metaphosphoric acid for preservation of ascorbic acid. Samples were homogenized with a polytron for 2 min with 10 mL of extraction solution of 0.5 mol L^{-1} HCl in 800 mL L^{-1} methanol for phenolics, 800 mL L^{-1} of ethanol for sugars and 50 mL L^{-1} of metaphosphoric for vitamin C and processed as reported in Abidi *et al.*[1] The supernatant was recovered and processed to be assayed as described elsewhere.[19] All chemicals were of analytical grade and purchased from Sigma-Aldrich (St Louis, MO, USA).

Antioxidant and total sugar determinations

Specific details for quantification are widely defined by Cantín *et al.*,[19] and methods therein. Vitamin C was measured at 525 nm and expressed as mg of ascorbic acid (AsA) per 100 g fresh weight (FW). For total phenolic content, a colorimetric method based on the chemical reduction of the Folin-Ciocalteau reagent was used. The absorbance at 725 nm was measured and the content was expressed in mg of gallic acid equivalent (GAE) per 100 g FW. Total flavonoid content was determined measuring absorbance at 510 nm expressed as mg catechin equivalent (CE) per 100 g FW. Relative antioxidant capacity (RAC) was determined using DPPH (2,2-diphenyl-1-picrylhydrazyl). The absorbance at 515 nm was measured after 10 min of reaction and RAC was expressed as mg Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) equivalent (TE) kg^{-1} FW.

For sugar analysis, 250 µL of the fruit homogenized extract was incubated at 80 °C for 20 min in 200 µL of 800 mL L^{-1} ethanol. Mannitol (5 g L^{C}) was added as internal standard. The ethanol was then evaporated and samples were purified using ion exchange resins (Bio-Rad Barcelona, Spain). To estimate the variation in sugar profile among genotypes, 20 µL were injected into the HPLC system (Aminex HPX-87C column, 300 mm × 7.8 mm; Bio-Rad, Barcelona, Spain) with a refractive index detector (Waters 2410, Milford, Mass, US). The solvent was deionized water at 85 °C at a flow rate of 0.6 mL min^{-1}. Sugar quantification was performed with with Empower Login software (Waters) using standards of analytical grade (Panreac Quimica SA, Barcelona, Spain). Sugar concentrations were expressed as g kg^{-1} FW according to Cantín *et al.*[20], with few modifications in Abidi *et al.*[1]

Chilling injury symptoms evaluation

Chilling injury susceptibility was evaluated in the progeny for two consecutive years (2010 and 2011) after storage of samples of 20 fruits per seedling at 5 ºC and 95% RH (relative humidity) according to Crisosto *et al.*,[4] during 2 or 4 weeks and subsequent ripening at room temperature during 2-3 days. Fruits were then evaluated as described elsewhere[5] for symptoms of CI such as hard texture with no juice (leatheriness), flesh browning and flesh bleeding (internal reddening). Mealiness was not evaluated since this population is CNMF and dry fruit cannot be considered as mealy. Observations were made on the mesocarp and the area around the pit immediately after fruits were cut into two halves through the suture plane. Fruits which had a dry appearance and little or no juice after hand squeezing were considered leathery. Leatheriness and off-flavor were scored as the proportion of fruit affected with these symptoms in the sample. Internal browning was visually scored on a scale of 1 (no browning) to 6 (severe browning). Bleeding was visually scored on a scale of 1 (no bleeding) to 3 (more than 50% of the flesh with bleeding). Then the percentage of progenies of the population within the proportion/score was calculated for every CI symptom. Finally, the

degree of CI (CI index) was visually assessed according to the global fruit appearance of each genotype, from healthy fruit with no symptoms (1) to severe CI symptoms (6) when the fruit was extremely injured with CI symptoms.

Statistical analysis

All agronomic and biochemical traits were measured or scored for each genotype separately over the three year period. Minimum and maximum values, mean and average standard error (SE), were calculated for each studied trait in the progeny during the three years using SPSS 19.0 (SPSS Inc., Chicago, IL). Data were also analyzed by year. When analysis of variances showed statistical differences among years ($P \leq 0.05$), means were separated by Duncan's multiple test. CI symptoms analysis was carried out by ANOVA, followed by Tukey's test ($P \leq 0.1$), considering genotype, year and storage duration as fixed factors). The contribution of each factor to the phenotypic variance of CI symptoms, which describes the proportion of total variability attributable to a factor, was estimated by the eta-squared statistic by using Maximum Likelihood methods using the PROC VARCOMP option in the SPSS 19.0 statistical package program. The proportion of phenotypic variance attributable to genotype is the broad sense heritability (H^2) and represent the relation between genetic variance (additive and dominant) and total phenotypic variance. Data for each genotype were averaged, and mean values were used as estimated genotypic values. Principal component analysis (PCA) of agronomic and biochemical traits and CI symptoms was carried out using SPSS 19.0. The component matrix (correlated matrix) was evaluated and orthogonal factors were rotated using variance maximizing (varimax). Finally, correlations were calculated with raw data of two years (2010-2011), according to Pearson's test at $P \leq 0.05$.

RESULTS AND DISCUSSION

Agronomic and basic fruit quality traits

Agronomic and basic biochemical fruit quality traits were evaluated separately in each seedling over the three years of the study (2009-2011). Mean values of yield, fruit weight, firmness, SSC, pH, TA and RI were calculated from the 130 seedlings. All tested parameters were found to show considerable variability among genotypes **(Table 1)**. Regarding flesh firmness, values were below the maximum level of fruit firmness for commercial peaches fixed by the EU (63.7 N). Mean firmness (32.5 N) for all the progenies during the three years of study were in the standard commercial firmness range, and more precisely in the range considered as ´ready to buy´ (18-35 N).[21] Regarding SSC, all the progeny showed values over the minimum (8 ºBrix) established by the EU to market peaches and nectarines. The progeny showed variability of TA among genotypes with a mean value of 0.59 g malic acid/100 g FW. All genotypes showed lower TA than the maximum limit (0.9 g malic acid/100 g FW) for high-acidity peaches.[22] Also, in this progeny the SSC/TA ratio (RI) showed a broad variability among genotypes (13.8-33.6). RI is an important quality index and is commonly used as a maturity index. In peach, these traits are very important to consider since consumer acceptance is correlated with SSC and TA. The eating quality of peach depends on the composition of individual sugars and organic acids and the ratio between them. The sugar/acid ratio and other sensory quality attributes such as aroma, peach or nectarine flavor, aroma intensity and texture may also be contributing to their consumer acceptance.[22]

Significant year-to-year variations in agronomic and basic biochemical traits were also observed in the progeny (**Table 2**). However, the first year of study (2009) differed significantly from the last two years (2010 and 2011) for yield, fruit weight, firmness, pH, TA and RI. As observed in **Supplementary Figure 1**, the first year of the study (2009) had significantly lower precipitation during the summer period than the following two years, which could partially explain the differences found in the studied traits. Temperatures did not really change during the three years of study. However, yield depends on other factors such as

density of flower buds and flowers, fruit set, fruit size, winter and late spring freeze damage, precipitation amount, and orchard management.[23]

Antioxidant compounds content

Vitamin C content ranged in this progeny from 2.8 to 10.0 mg of AsA/100 g FW **(Table 1)** representing a more than 3.5-fold variation. Values were in the same range as previously reported for vitamin C contents in peach flesh (1-14 mg of AsA/100 g FW).[1, 19, 24] The mean amount of total phenolics in our progeny fell within the range reported in the literature for peach (14-77 mg GAE/100 g FW).[1, 19, 25, 26] It has been reported that climatic conditions and agricultural factors can affect the fruit nutritional composition including bioactive compounds.[27] However, in this work, all genotypes were grown under the same environmental conditions and cultural practices and thus the differences in fruit quality parameters noted above as well as in the content in bioactive compounds may be due to differences in the genotype and year of study. In accordance with this statement, Hegedús *et al,.*[28] have found 21- to 35-fold differences in total phenolic content and antioxidant activity among a wide range of apricot genotypes grown under the same environmental conditions. Also, Cantín *et al.*,[19] found a big influence of genotype on antioxidants among 15 different peach populations. Regarding flavonoids, the mean value (6.4 mg of CE/100 g FW) was comparable to that obtained by the same authors in other progenies (8.8 mg of CE/100 g FW). The RAC also showed a high variability among genotypes (395 mg of Trolox equivalent kg^{-1} FW). Similar contents and variations were showed in other peach populations by Cantín *et al.*,[19] (average of 405 mg of TE kg^{-1} FW) and Abidi *et al.*,[1] (average of 464.2 mg of TE kg^{-1} FW). The antioxidant capacity of fruit- varies in relation to antioxidant moieties present in the different species, although variations may occur among cultivars within a single species.[19, 29] Vizzotto *et al.*,[26] reported that the total phenolic content had the most consistent and highest correlation with antioxidant activity indicating that it is more important in determining the

antioxidant activity of peach and plum fruit than are the anthocyanin or carotenoid contents. Phenolic compounds are responsible for antioxidant, anti-inflammatory and immune-stimulating functions that benefit human health. In particular, fractions containing polyphenols from peach extracts showed potential growth inhibition in colon cancer cells.[30, 31]

Regarding the annual variation of antioxidant compounds content, results showed changes in the contents of flavonoids and RAC, whereas vitamin C and total phenolics showed similar results among years of study **(Table 2)**. Manach *et al.*,[12] reported that pedoclimatic and agronomic factors have a major effect on polyphenol content. Exposure to light has a considerable effect on most flavonoids content and the degree of ripeness considerably affects the concentrations and proportions of the various polyphenols.[12] Our results allowed us the preselection of six genotypes with high RAC and enhanced concentrations of antioxidant compounds and total sugars (Table S1). All values were over the mean for most of the evaluated traits.

Regarding heritability of antioxidant compounds (data not shown), values are relatively low ($H^2 \leq 0.32$). Mratinić *et al.*,[32] reported that the values of heritability coefficients for yield and other morphological traits studied were higher (H^2=0.88-0.93) than those observed in our progeny (0.01-0.32). Much lower values for peach fruit weight (H^2=0.32) SSC (H^2=0.33) and TA (H^2=0.31) were obtained by De Souza *et al.*,[33]. These differences might be partially explained considering that heritability coefficient value is a function of variability of a specific character in the studied population as well as a function of environmental variance that the trees are growing in.[32] In addition, in most of the previous work, heritability coefficients were used in a narrow sense (h^2), which represents the relation between additive genetic variance and phenotypic variance. However, in the present work, heritability coefficients have been used in a broader sense and they represent the relation between genetic variance (additive and dominant), and total phenotypic variance.

Total sugar content

The studied population exhibited wide phenotypic variations in sugar contents among genotypes **(Table 3)**. Total sugars (the sum of sucrose, glucose, fructose and sorbitol contents) in peeled fruit ranged from 46.5 to 102.4 g kg^{-1} FW with an average of 70.3 g kg^{-1} FW. Cantín *et al.*,[20] studying 205 peach genotypes from fifteen different progenies reported an average content of total sugars of 72.1 g kg^{-1} FW. Sucrose was the major sugar present in the evaluated genotypes (75.1% of total sugar), followed by fructose, glucose and sorbitol. Sorbitol content varied greatly among genotypes, ranging from 1.0 to 7.5 g kg^{-1} FW. Consequently, the percentage of sorbitol in the sugar composition was significantly different among genotypes (1.9-8.3%).

Sorbitol is the sugar that was significantly most affected by year (**Table 2**). Colaric *et al.*,[34] reported that sorbitol was the attribute most related to peach aroma and taste among carbohydrates and organic acids. Results showed different distribution of sugar compounds throughout years with slight variability of sucrose and total sugar content among years **(Table 2)**, which could be due to the influence of weather conditions **(Figure S1)**. Sorbitol showed significant differences among the three years of study, and sucrose, sorbitol and total sugars showed a slight superiority in 2010. Brooks *et al.*,[35] also reported a year-to-year variation in sugar and acid content in fruit of four clingstone peach seedling populations. Year-to-year variation in the percentage of sugar may be explained by differences in climate and crop load among years[35], and also by differences in maturity at harvest. The transgressive segregation that was found for all the sugars also indicates that it is possible to select for high sugar content or specific sugar content in most segregating peach seedling populations.[20] The content of total sugars in the six preselected genotypes (see Table S1) was also considered for the pre-selection process.

Quantitative variation for chilling injury symptoms

The F1 progeny showed a high variability for all the evaluated CI symptoms. The variation of the symptoms was studied using the mean of two-year data (2010-2011)**.** Continuous distribution was shown for leatheriness, browning, bleeding, and off-flavor, suggesting polygenic control of these symptoms as was reported in other non-related peach progeny populations.[5, 6, 11] Leatheriness, bleeding and browning were the major CI symptoms observed in this CNMF progeny. As expected, the duration of storage (2 or 4 weeks at 5 ºC) increased the severity of CI symptoms.

After 2 weeks of cold storage, the main CI symptoms observed were leatheriness and bleeding **(Figure 1)**. Although 15% of the progeny showed bleeding, its manifestation was less severe compared to other studied peach progenies.[6, 10] However, the flesh red color observed in the fruit flesh could be due to the characteristic pigmentation of fruit flesh in this progeny and may have hindered the evaluation of CI-related red coloration. On the other hand, Lurie and Crisosto[3] reported that flesh bleeding could be associated with fruit senescence and not with CI disorders which could be an explanation to the low impact of storage duration on this CI symptom in this study. For leatheriness, this progeny showed higher susceptibility to this CI symptom (41.2%) when compared to FMF progeny[5], and similar results were observed for flesh leatheriness when other authors analyzed it only within a CNMF progeny.[6] It should be noted that the population evaluated in this study was entirely CNMF.

After 4 weeks of cold storage, a considerable higher proportion of fruit was significantly affected by CI symptoms (with the exception of bleeding), showing that these disorders are triggered by the cold storage duration, as previously reported.[3] The major CI symptom observed after 4 weeks of storage was flesh browning. On the other hand, it is worth noting that browning scoring might be underestimated in the population since the visual scoring of this trait in the area surrounding the stone is more difficult to accomplish in the

clingstone individuals due to the adhesion of the flesh tissue to the stone. Leatheriness expression in the fruit also increased after 4 weeks of cold storage, compared to the shorter cold storage period studied. A decrease in bleeding was scored after 4 weeks of cold storage, which could be explained by the oxidation of the red pigments, and the difficulty of accurately scoring bleeding in this population (at 2 weeks) due to its natural red pigmentation. Similar results were found by other authors for bleeding when analyzed only within a FMF peach progeny and for mealiness when analyzed only within a CNMF progeny.[6] These authors reported that mealiness was almost non-existent in a CNMF progeny, while bleeding incidence was higher in the CNMF progeny after 2-3 weeks of cold storage.[6, 10] Similarly, Martínez-García *et al.*,[36] reported that flesh bleeding occurs primarily in non-melting-flesh fruit, and particularly when the fruit is white-fleshed. Interestingly, the results of this work showed at least seventeen genotypes (20, 22, 33, 44, 58, 68, 70, 72, 76, 80, 81, 96, 106, 118, 120, 124 and 129) with low susceptibility to CI symptoms and with several good organoleptic traits (values for fruit weight, vitamin C, RAC, total phenolics, flavonoids or sugars over the average).

Genotype was the main factor contributing to phenotypic variation for all the CI symptoms **(Table 4)**, showing a contribution between 32.2% for leatheriness and 95.1% for bleeding. These results agree with others authors[6, 36]who reported that susceptibility of stone fruit to CI is highly influenced by the genetic background of the cultivar. Off-flavor and bleeding showed the higher proportion of phenotypic variance attributed to year and storage duration, which agrees with reported variations between years in CI symptoms.[36, 37] Cantín *et al.*,[5] reported that genotype was the main factor contributing to phenotypic variation for all the CI symptoms measured in the cross 'Venus' × 'Big Top', showing a contribution of 29%-65% to total variability.

PCA analysis and correlations between CI symptoms and pomological traits

A principal component (PC) analysis of the main agronomic and biochemical traits and CI symptoms was performed to evaluate the ripening related variables during harvest and storage (**Figure 2**). PC1 and PC2 accounted for 15.2 and 12.2% of total variance, respectively. On one hand, an examination of PC1 loadings suggested that genotypes containing high antioxidant capacity (high levels of total phenolics and flavonoids and high RAC), high sugar content (SSC and total sugars), and high fruit weight (right side of the axis) presented low bleeding after 2 and 4 weeks of storage (left side of the axis). These results can be explained by the negative correlation found between bleeding after 2 weeks of storage with SSC (r=-0.188, $P \leq 0.05$) and fruit weight (r=-0.287, $P \leq 0.01$). Moreover, comparable results obtained by other authors in peach fruits stored at 5 ºC support these data.[18] These authors confirmed that high contents in sugars (sucrose and glucose) alleviate CI symptoms in peach fruit because carbohydrates may serve as osmoregulators and cryoprotectants contributing to membrane stability. Besides carbohydrates may act as scavengers of reactive oxygen species[38], and sugar metabolism might provide reducing power to the ascorbate glutathione cycle protecting cells against chilling stress. On the other hand, an examination of PC2 loadings suggested that genotypes with high CI symptoms after both 2 weeks (leatheriness, bleeding and off-flavor) and 4 weeks (browning, off-flavor and CI index) of storage where those with higher ripening index (RI) and lower yield, firmness and titratable acidity (TA). These results are also sustained with some of the correlations found. Firmness was negatively correlated with leatheriness and off-flavor after 2 weeks of storage (r=-0.253, $P \leq 0.01$; r=-0.202, $P \leq 0.05$) and browning after 4 weeks of storage (r=-0.189, $P \leq 0.05$). Similarly, other authors found in apricot more proportions of disordered fruit (CI affected) when were harvested at low firmness.[39] Furthermore, the relationship between CI symptoms and RI could be explained since the severity of CI damage depends on the ripening stage at harvest.[40] Higher incidence of injury was reported for cultivars picked immature or at too advanced

ripening stage.[36] As in our study, González-Buesa *et al.*,[41] found positive correlations between browning and RI when studying CNMF peach genotypes.

In addition, CI index after 2 weeks of storage and off-flavor after 4 weeks showed significant correlations with total phenolics (r=0.203 and r=0.219), off-flavor after 4 weeks of storage showed significant correlations with vitamin C (r=0.184), and browning after 2 weeks of storage showed a significant correlation with RAC (r=0.198) ($P \leq 0.05$). These results point out the important role of antioxidant compound content in the development of CI symptoms after cold storage. Prohens *et al.*,[42] observed significant positive correlations (r=0.388, $P \leq 0.01$) between phenolics and the degree of browning and color difference in hybrids of eggplant. Other authors reported that storage may also affect the content of polyphenols that are easily oxidized.[12] However, no significant correlation was found between flavonoids and CI symptoms in our progeny.

Finally, at each period of storage, significant correlations ($P \leq 0.01$) between chilling injury symptoms were found, i.e. leatheriness with off-flavor (r=0.496 and r=0.313), after 2 and 4 weeks of cold storage, respectively) and browning with CI index (r=0.298 and r=0.276, after 2 and 4 weeks of cold storage, respectively). These data indicated that all CI symptoms were related to the process of fruit being damaged by cold. It is interesting to note the correlation between leatheriness after 2 weeks of cold storage and browning after 4 weeks of storage (r=0.239, $P \leq 0.05$), implying that with time, leatheriness could progress into browning. Oxidation reactions after cold storage of fruit result in the formation of more or less polymerized substances, which lead to changes in the quality of fruit, particularly in color and organoleptic characteristics and such changes may be harmful (browning) to consumer acceptability. The selection for reduced degree of browning in commercial varieties has resulted probably in the indirect selection of materials with lower concentrations of phenolics.[42]

Nowadays, the development of new varieties with higher content of phenolics is of interest for the improvement of the nutritional quality of peaches. However, the oxidation of phenolic compounds causes browning of the fruit and reduces its acceptability. The correlations observed between antioxidant traits and CI symptoms suggests the possible role of these compounds in the development of texture disorders and internal flesh browning of peach fruit after a long cold storage period. On the other hand, the positive interaction demonstrated between antioxidant compounds and carbohydrates may alleviate CI symptoms in peach. The results found here could be of great interest in future breeding programs in order to develop peach varieties with higher concentrations of phenolics and sugars and reduced CI susceptibility.

It should be noted that there have been few studies in which CI susceptibility has been examined together with antioxidant, sugar compounds and other agronomic traits in an entirely CNMF peach progeny. This study demonstrates that it is possible to find varieties with a high concentration of phenolics, antioxidants or sugars and relatively low or intermediate CI susceptibility (22, 33, 68, 80, 81, 96 and 120). However, further research has to be done in order to elucidate the specific role of the antioxidant and sugar compounds, and other agronomic variables in the development of CI disorder in peach fruit.

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534 hybrids of eggplant: implications for breeding for higher nutritional quality and reduced
535 browning. *J Am Soc Hortic Sci* **132**:638-646 (2007).
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Table 1. Range, mean and standard error (SE) of agronomic and biochemical fruit quality traits in the 'Babygold 9' × 'VAC-9510' progeny. For the progeny (n = 130 genotypes), data are mean of three years of study (2009-2011).

Traits	Range	Mean	SE
Yield	2.0-24.4	11.4	0.3
Fruit weight	153-308	218	3
Firmness	21.4-53.9	32.5	0.4
SSC	9.1-14.4	11.0	0.1
pH	3.50-4.16	3.73	0.01
TA	0.37-0.84	0.59	0.01
RI	13.8-33.6	19.7	0.3
Vitamin C	2.8-10.0	5.5	0.1
Total phenolics	11.3-41.7	25.1	0.6
Flavonoids	2.3-18.0	6.4	0.3
RAC	238-610	395	6

Units and abbreviations: Yield (kg per tree); 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); Vitamin C (mg AsA/100 g of FW); Total phenolics (mg GAE/100 g of FW); Flavonoids (mg CE/100 g of FW); RAC; Relative Antioxidant Capacity (mg TE kg^{-1} FW). Abbreviations: AsA = Ascorbic acid; CE = Catechin equivalents; FW = Fresh weight; GAE = Gallic acid equivalent; SE = Standard error; TE = Trolox equivalent.

549 **Table 2.** Annual variability of agronomical and biochemical fruit quality traits in
550 'Babygold 9' × 'VAC-9510' progeny. Data are mean (n = 130 genotypes) ± SE
551 (Duncan's test at $P \leq 0.05$).

Traits	2009	2010	2011
Yield	6.4 ± 0.3^{a}	13.8 ± 0.4^{b}	14.6 ± 0.5^{b}
Fruit weight	199.6 ± 2.5^{a}	221.3 ± 3.1^{b}	228.0 ± 5.5^{b}
Firmness	37.1 ± 0.6^{b}	29.7 ± 0.6^{a}	31.1 ± 0.7^{a}
SSC	11.2 ± 0.1^{b}	10.8 ± 0.1^{a}	11.0 ± 0.2^{ab}
pH	3.85 ± 0.01^{b}	3.66 ± 0.01^{a}	3.68 ± 0.02^{a}
TA	0.51 ± 0.01^{a}	0.69 ± 0.01^{c}	0.58 ± 0.01^{b}
RI	22.6 ± 0.5^{c}	16.2 ± 0.4^{a}	19.9 ± 0.4^{b}
Vitamin C	5.2 ± 0.2^{a}	5.4 ± 0.2^{a}	5.7 ± 0.2^{a}
Total phenolics	26.1 ± 1.0^{a}	23.8 ± 0.8^{a}	24.1 ± 1.0^{a}
Flavonoids	5.8 ± 0.3^{a}	5.4 ± 0.2^{a}	7.6 ± 0.4^{b}
RAC	396.2 ± 6.0^{b}	363.9 ± 13.1^{a}	415.7 ± 9.6^{b}
Sucrose	54.1 ± 1.0^{a}	52.6 ± 1.0^{a}	51.6 ± 1.1^{a}
Glucose	6.3 ± 0.2^{a}	7.2 ± 0.1^{b}	6.5 ± 0.1^{a}
Fructose	8.0 ± 0.3^{b}	8.0 ± 0.1^{b}	7.5 ± 0.1^{a}
Sorbitol	3.2 ± 0.1^{c}	2.6 ± 0.1^{b}	2.0 ± 0.1^{a}
Sucrose/glucose	8.8 ± 0.3^{b}	7.5 ± 0.1^{a}	8.2 ± 0.2^{b}
Glucose/fructose	0.79 ± 0.01^{a}	0.90 ± 0.00^{c}	0.87 ± 0.00^{b}
% of Sucrose	75.4 ± 0.4^{ab}	74.2 ± 0.5^{a}	75.9 ± 0.5^{b}
% of Glucose	8.9 ± 0.1^{a}	10.5 ± 0.2^{c}	9.8 ± 0.2^{b}
% of Fructose	11.3 ± 0.2^{a}	11.7 ± 0.3^{a}	11.4 ± 0.3^{a}
% of Sorbitol	4.4 ± 0.2^{c}	3.6 ± 0.1^{b}	2.8 ± 0.1^{a}
Total sugars	71.6 ± 1.3^{b}	70.4 ± 1.1^{ab}	67.5 ± 1.2^{a}

552 Units and abbreviations: Yield (kg per tree); Fruit weight (g); Firmness (N); N = Newtons; SSC = Soluble
553 solids content (°Brix); TA = Titratable acidity (g malic acid/100 g FW); RI = Ripening index (SSC/TA);
554 Vitamin C (mg AsA/100 g of FW); Total phenolics (mg GAE/100 g of FW); Flavonoids (mg CE/100 g of
555 FW); RAC; Relative Antioxidant Capacity (mg Trolox Equivalents kg^{-1} FW); sucrose, glucose, fructose,
556 sorbitol and total sugars (g kg^{-1} FW). AsA = Ascorbic acid; CE = Catechin equivalent; FW = Fresh
557 weight; GAE = Gallic acid equivalent; SE = Standard error; TE = Trolox equivalent.
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561 **Table 3.** Sugar content in 'Babygold 9' × 'VAC-9510' progeny. For the progeny (n =

562 130 genotypes), data are mean of three years of study (2009-2011).

Sugar content	Range	Mean	SE
Sucrose	29.9-70.6	53.0	0.6
Glucose	3.8-9.6	6.7	0.1
Fructose	4.5-10.8	7.9	0.1
Sorbitol	1.0-7.5	2.6	0.1
% of Sucrose	64.0-84.7	75.1	0.3
% of Glucose	6.1-15.1	9.8	0.1
% of Fructose	7.3-18.6	11.5	0.2
% of Sorbitol	1.9-8.3	3.6	0.1
Total sugars	46.5-102.4	70.3	0.8

563 Units and abbreviations: Sucrose, glucose, fructose, sorbitol and total sugars (g kg-1 FW). FW = Fresh weight;
564 SE = Standard error
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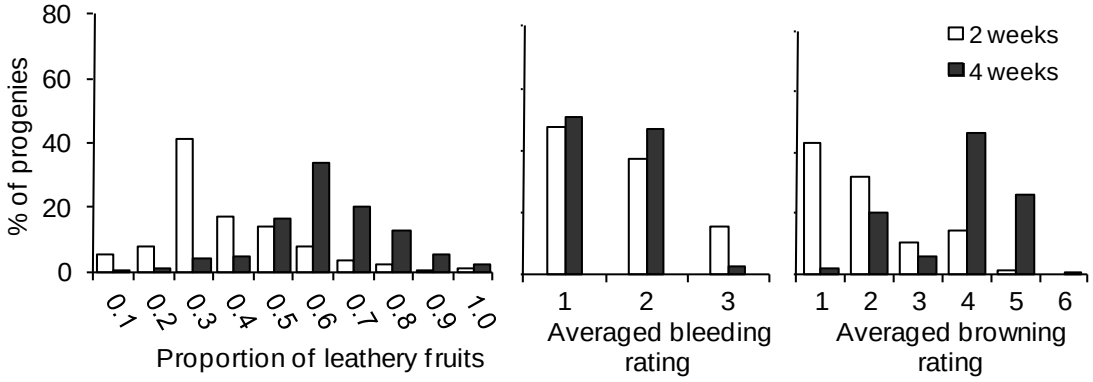
Table 4. Contribution (%) of factors (genotype, year and storage duration) to phenotypic variance that affect chilling injury symptoms, observed for 2 years (2010-2011) in 'Babygold 9' × 'VAC-9510' progeny.

CI symptoms	Genotype[a]	Year	Storage duration[b]
Off flavor	53.4	35.8	35.0
Leatheriness	57.6	39.5	39.5
Bleeding	95.1	59.5	61.0
Browning	40.8	29.4	27.8
CI index	42.4	31.8	30.5

[a] This proportion of phenotypic variance attributed to Genotype is the broad sense heritability (H^2).

[b] Two or four weeks of storage



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574 **Figure 1.** Distribution of internal breakdown symptoms in 'Babygold 9' × 'VAC-9510'
575 progeny averaged over 2 years of study (2010-2011) after storage at 5 ºC for 2 and 4 weeks
576 and then ripened at 20 ºC during 2 or 3 days. Leatheriness was scored as the proportion of
577 fruit affected with these symptoms in the sample (0-1). Bleeding was scored on a scale of 1
578 (no bleeding) to 3 (more than 50% of the flesh with bleeding). Browning scored on a scale of
579 1 (no browning) to 6 (severe browning).

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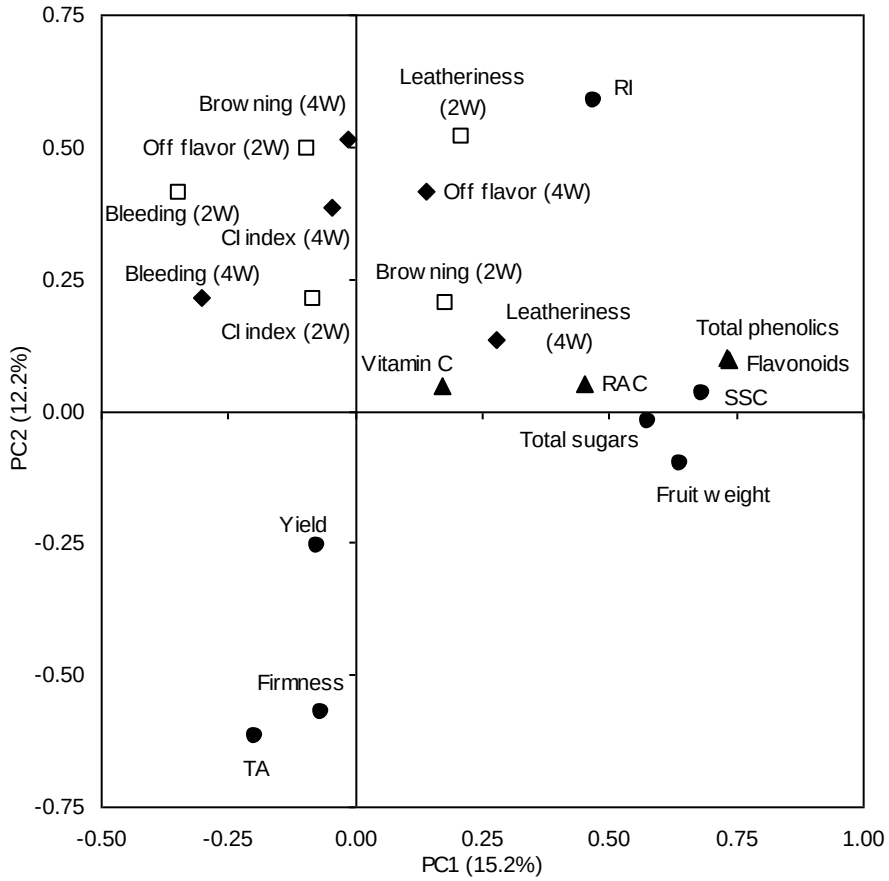

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588 **Figure 2.** Principal component analysis of the main agronomic and biochemical traits and
589 chilling injury symptoms in 'Babygold 9' × 'VAC-9510' progeny for 2010 and 2011.
590 Symbols: (●) pomological traits, (▲) antioxidant traits, (□) chilling injury symptoms after
591 storage at 5ºC for 2 weeks and then ripening at 20ºC during 2 or 3 days, (♦) chilling injury
592 symptoms after storage at 5ºC for 4 weeks and then ripening at 20ºC during 2 or 3 days.
593 Abbreviations: CI = Chilling Injury, RAC = Relative Antioxidant Capacity, RI = Ripening
594 Index (SSC/TA); SSC = Soluble Solids Content; TA = Titratable Acidity.

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599 **Figure S1.** Monthly precipitation and mean, maximum and minimum temperature during
600 2009-2011 at the Experimental Station of Aula Dei-CSIC in Zaragoza, Spain.

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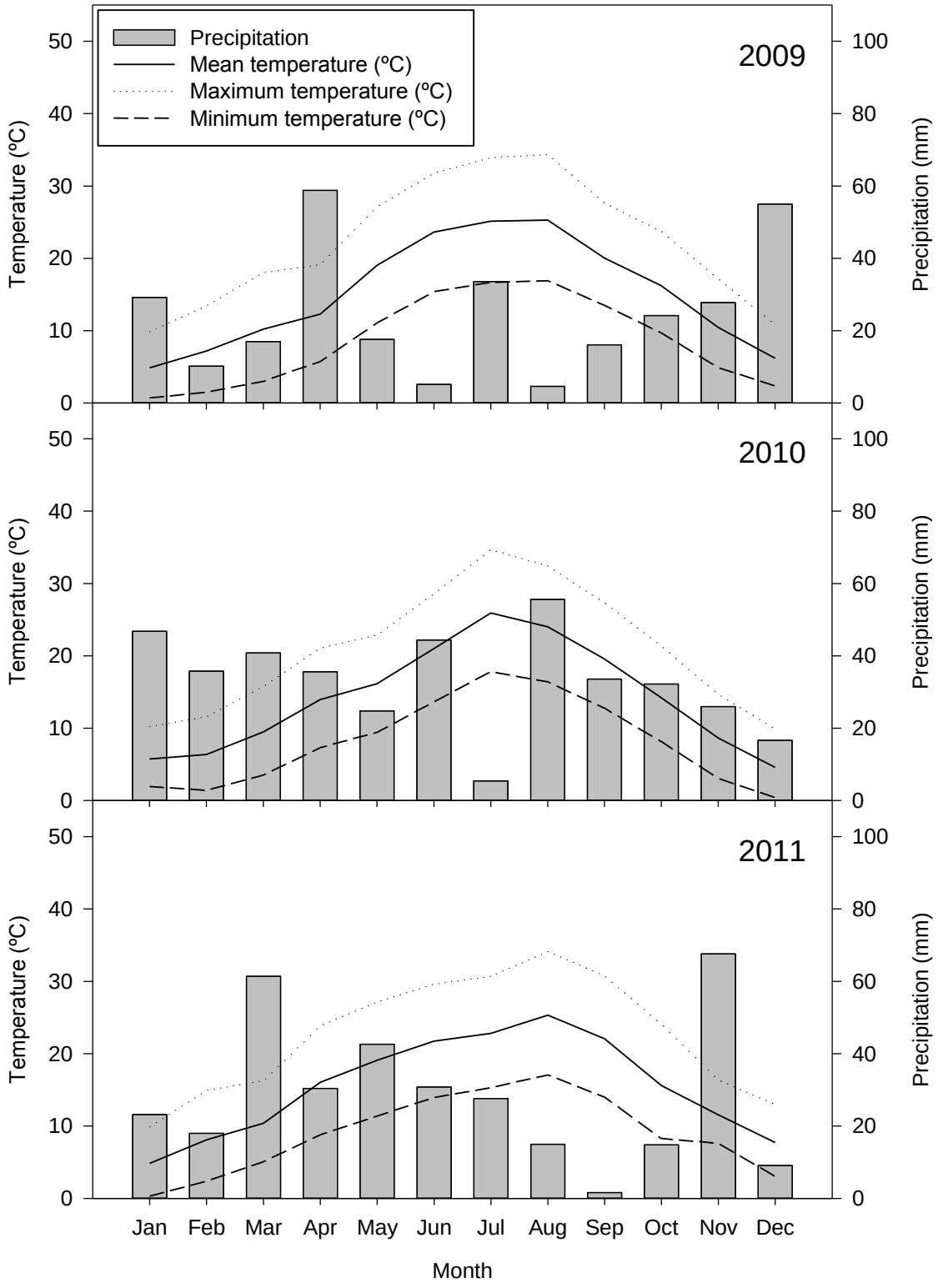

603 **Table S1.** Content of biochemical fruit quality traits in the preselected genotypes in
604 'Babygold9' × 'VAC-9510'progeny. Data are the mean of three years of study ±
605 standard error.

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Genotypes	Vitamin C	Total phenolics	Flavonoids	RAC	Total sugars
10	7.6 ± 2.4	33.5 ± 3.0	9.8 ± 1.7	487.4 ± 28.1	75.8 ± 5.5
54	9.5 ± 0.2	35.3 ± 0.1	8.1 ± 0.2	476.4 ± 0.7	80.1 ± 1.1
65	5.9 ± 0.8	33.0 ± 2.7	8.1 ± 1.9	473.6 ± 61.1	74.6 ± 1.2
73	5.7 ± 2.0	36.5 ± 4.4	10.0 ± 0.5	467.5 ± 57.2	63.8 ± 19.5
91	4.2 ± 1.1	27.6 ± 8.7	9.9 ± 3.9	453.7 ± 50.0	75.0 ± 10.2
120	7.5 ± 0.1	32.1 ± 0.5	7.7 ± 0.1	529.8 ± 2.4	84.6 ± 0.2

607 Units and abbreviations: vitamin C (mg AsA per 100 g FW); total phenolics (mg GAE per 100 g FW);
608 flavonoids (mg CE per 100 g FW); RAC, relative antioxidant capacity (mg TE kg^{-1} FW); total sugars (g
609 kg^{-1} FW); AsA, ascorbic acid; CE, catechin equivalent; FW, fresh weight; GAE, gallic acid equivalent; t
610 TE, trolox equivalent.
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