

**INFLUENCE OF FRUIT MATURITY ON ANTIOXIDANT POTENTIAL AND  
CHILLING INJURY RESISTANCE OF PEACH FRUIT (*PRUNUS PERSICA*)  
DURING COLD STORAGE**

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## ABSTRACT

Postharvest handling of peach (*Prunus persica*) fruits is challenging as they deteriorate quickly under ambient conditions. Cold storage slows deterioration but causes chilling injury (CI), reducing quality of fruits. To overcome this challenge the influence of fruit maturity on antioxidant capacity and CI development in “Ryan sun” peach fruits was investigated. Fruits were harvested from commercial farms in Lleida in Spain. Optimum harvest date (OHD) was determined on-tree visually by ground skin colour when 70% of skin turned reddish using colour discs. Fruits were harvested 7 days before OHD (OHD-7) and seven days after OHD (OHD+7) with OHD fruits serving as control. The fruits were stored at 5 °C for 15, 30 and 45 days. Fruits were evaluated for CI manifestations such as lack of juiciness (wooliness) and flesh bleeding. The lack of free juice released upon crushing fruit flesh through cheese cloth reveals symptoms of wooliness. Percentage CI resistance was calculated (100% - %CI incidence) for each group. Antioxidants were extracted and analysed using the Ferric Reducing Antioxidant Property (FRAP) method. Fruits harvested earlier (OHD-7) recorded the highest antioxidant capacity of 1.080 mgTE/g followed by control fruits (OHD) with antioxidant capacity of 0.976 mgTE/g. Fruits harvested late (OHD+7) recorded the lowest antioxidant capacity of 0.471 mgTE/g. After 15 days of cold storage, OHD resisted CI by 70% followed by OHD+7 (60%) and OHD-7 fruits (55%). After 30 days of storage, OHD-7 fruits recorded 0 resistance to CI but OHD and OHD+7 fruits resisted by 20% each. Fruits of all harvest dates showed no resistance to CI after 45 days of storage. Fruit maturity and cold storage length were found to significantly ( $P < 0.05$ ) influence CI resistance. For good keeping quality, “Ryan sun” peach fruits should be harvested mature for long keeping but harvested earlier when intended for best antioxidant property.

**Key words:** chilling injury, antioxidants, fruit maturity

## INTRODUCTION

Fruit consumption is well known for its health benefits. Epidemiological studies show that the consumption of fruits and vegetables may be important for good health and the prevention of many chronic diseases, including cardiovascular disease, type II diabetes, dementia, macular degeneration and some cancers [1,2]. Besides the health benefits associated with fruit consumption, the production and sale of fruits is a source of employment to many worldwide. Postharvest handling of fruits through appropriate methods to preserve and enhance their sensory and nutritional quality cannot be overemphasized. Fruit quality standards should nonetheless be encouraged to support increased consumption. Research shows that postharvest handling and storage conditions such as refrigeration, ambient temperature storage and freezing among others affect fruit quality in different ways physically and biochemically. Cold storage (5°C) has been associated with chilling injury (CI) development in peach fruits and this has been a major postharvest concern to both producers and consumers [3].

Chilling injury (CI) development is a physiological and structural deteriorative phenomenon induced or triggered by cold storage causing important losses to peach growers worldwide [3]. The main symptoms of CI include mealiness, graininess, internal flesh browning, off-flavour development and red pigmentation (bleeding) in peach fruits [4]. However, within and between cultivars variations exist in fruits regarding their susceptibility to CI. Ryan sun peach has been known to undergo CI when stored at temperature ranges, 2.5 - 5 °C [3,5]. The method of cooling can also induce CI as slow cooling conditions favour CI development than fast cooling conditions. This is because the rate of cooling determines the sizes of ice crystals formed within fruits and the extent of their physical damage to cell walls [6-9]. When cooling is not appropriately carried out on fruits, it becomes difficult to consider CI as a direct evidence of oxidative stress or improper handling [6]. Several factors can be linked to CI susceptibility in fruits such as pectin, calcium contents and genetic factors [10,11]. Fruits with high pectin contents may be less susceptible than those with low pectin contents [11,12].

Studies have also shown that the reduction in protective antioxidants during the prolonged stress of cold storage may be one of the major causes of CI in pawpaw [13]. In another study, it was found that the effect of 1-methylcyclopropene on alleviating CI may be due to its capability to enhance antioxidant enzyme activities and to reduce oxidative damage [14]. This means when antioxidant systems are enhanced, CI may be alleviated. Antioxidant systems are enhanced when the amounts of antioxidant enzymes such as ascorbate peroxidase, catalase and peroxidase as well as antioxidant compounds like ascorbic acid and phenols among others are high. Another study recorded 50% decrease in juiciness and reduced antioxidant activity for peach fruits stored at 4°C [15]. However, mention was not made of the particular peach variety used. It was also suggested by Cao *et al.* [16] that the reduction in chilling injury by methyl jasmonate (MeJA) may be due to enhanced antioxidant enzyme activity in Loquat fruits stored at 1°C. Lee [17] also reported the possibility of high levels of antioxidant capacity inducing CI resistance in carbon dioxide (CO<sub>2</sub>) treated peach fruits stored at 7 °C for four weeks.

In relation to susceptibility to CI, fruit maturity has been shown by studies to play crucial roles [11,18]. Maturity of fruits may simply be defined as the stage of development that gives minimum acceptable quality [19,20]. However, studies have showed that harvesting fruits at different dates (early or late harvest dates) have significant influence on their postharvest quality, composition and handling [18,21]. Research on other peach cultivars such as the “Flavorcrest” also showed how fruit maturity affects the quality characteristics and nutritional value as well as total antioxidant capacity of the fruits [21]. Fruit maturity has also been found to influence fruit antioxidant capacity based on the ability of the tissues to scavenge free radicals (FR) by the action of antioxidants. In apples, less mature fruits were associated with lower levels of lipophilic antioxidants, anthocyanins and phenolics [6]. Over-mature fruits in general at certain shelf-life might have spent more antioxidants in scavenging reactive oxygen species (ROS) and FR such that they are in the state of oxidative stress resulting in lower antioxidant capacities than less mature fruits [6]. Also, less matured fruits have been found to be more susceptible to CI during cold storage [3,22]. It was found that during prolonged cold storage, maintaining the ability of the fruits to produce ethylene, or the addition of exogenous ethylene to the storage atmosphere may help prevent CI in peach fruits. Nectarines that developed CI were found to be deficient in their ability to produce ethylene during cold storage [5]. This may mean mature fruits which have the ability to produce ethylene may be able to resist CI.

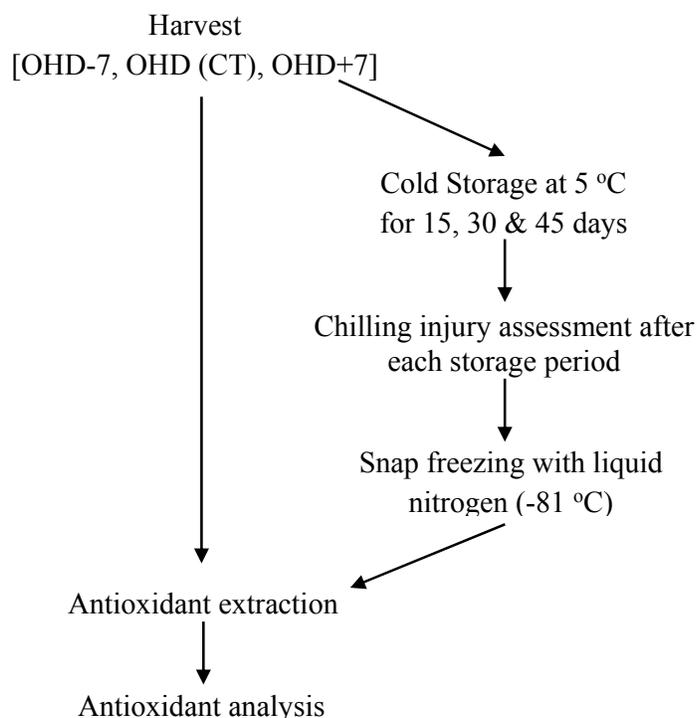
Cold storage is a common method of preserving fruit quality [23]. From literature, however, it contributes to CI development. There is, therefore, the need to understand factors inherent in fruits that may help resist CI development during cold storage. There is the need for more research on the effects of the maturity at harvest and cold storage on the biochemical composition of fruits (antioxidant capacity) as these may influence changes in fruit quality and CI development [3,18,21]. This study, therefore, sought to determine the maturity at which peach fruits pose the greatest antioxidant potential during cold storage and to assess the effects of fruit maturity on antioxidant capacity and chilling injury resistance during cold storage.

## MATERIALS AND METHODS

### *Peach fruit sampling*

Peach fruits (*Prunus persica* cv. ‘Ryan sun’) were harvested from a commercial farm in Catalunya, in the region of Lleida in Spain. Trees were systematically selected at the farm to allow even spreading of samples across the entire farm. It started with a random selection of the first tree by use of a random table and then proceeded with the selection of every  $k^{th}$  tree across the entire farm as described by Bellhouse [24]. On each selected tree, optimum harvest date (OHD) was visually determined by fruit skin ground colour when 70% of skin turned red and was the same as that used by local commercial farmers [25]. Ryan Sun peach fruits are generally harvested in Mid-September when they are about 168 days old [26]. The fruits were harvested on three different harvest dates (fruits harvested 7 days before optimum harvest date, OHD-7, fruits harvested on optimum harvest date, OHD and fruits harvested 7 days after optimum harvest date,

OHD+7) and aggregated into three different lots according to their harvest dates. Control (CT) sample was OHD fruits because they represented optimum harvest date used by commercial farmers conventionally. Immediately after harvesting the fruits were transported in plastic crates at ambient temperature (25 °C) to the storage unit (20 °C) where they were air-cooled. A sample consisted of 20 fruits. Fresh fruits were analyzed directly for antioxidant capacity while those meant for cold storage were stored at 5 °C for 15, 30 and 45 days. At the end of each cold storage period CI was assessed [27] and pulps quickly frozen at -81°C in sealed, labeled plastic containers to prevent biochemical and physiological changes prior to antioxidant extraction and analysis as shown in Figure 1.



**Figure 1: Flow diagram of methodology.**

Legend: OHD-7: Fruits harvested 7 days before optimum harvest date, OHD: fruits harvested on optimum harvest date and control sample; OHD+7-fruits harvested 7 days after optimum harvest date. CT means control

### *Chilling injury assessment*

Chilling injury (CI) was evaluated by the manifestation of wooliness and flesh bleeding [27]. To further confirm by complementary observations, pulps that did not release free juice when crushed in cheese cloth by the fingers or homogenized fruits that resulted in turbid juices and did not set reveal CI [22,27,28]. The results were expressed as a percentage of affected fruits per treatment and percentage CI resistance was calculated by the formula, 100% - %CI incidence. Each treatment group contained 20 fruits.

## Antioxidant extraction

### *Hydrophilic antioxidant extraction*

Three grams (3 g) of frozen samples were weighed into centrifuge tubes and 10 mL (79.5: 0.5:20) CH<sub>3</sub>OH: HCl: H<sub>2</sub>O immediately added and vortexed to mix. They were then shaken for 2 hours and intermittently vortexed every 30 minutes. Samples were then centrifuged at 20000 rpm at 4 °C for 20 minutes. Supernatants were collected and stored in eppendorf tubes at -20 °C for further analysis. Pellets were kept for immediate lipophilic antioxidant extraction.

## Antioxidant capacity determination

### *Ferric Reducing Antioxidant Property (FRAP)*

The FRAP assay was used with some modification [29]. Three hundred milli Moles (300 mM) acetate buffer, 10 mM TPTZ (2, 4, 6- tripyridyl-s-triazine) solution in 40 mM HCl, and 10 mM FeCl<sub>3</sub> x 6H<sub>2</sub>O solution were used. The fresh working FRAP solution was prepared by mixing acetate buffer, TPTZ solution and FeCl<sub>3</sub> x 6H<sub>2</sub>O solution in a 10:1:1 ratio and then mixed well with magnetic stirrer while warmed to enhance dissolution. Hydrophilic sample extracts were diluted (1:10 ratio of sample to acetate buffer) and 50 µL of diluted extracts allowed to react with 3.5 mL of the FRAP solution for 10 minutes at 37°C. Readings of the coloured product (ferrous tripyridyltriazine complex) were then taken at 593 nm by spectrophotometry.

## Statistical analysis

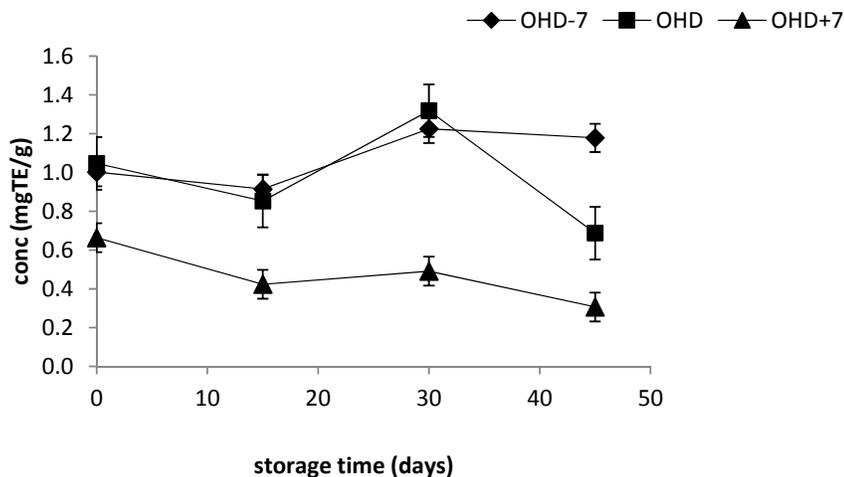
All analyses were in triplicate, and results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using GenStat statistical software for windows (Version 14.2) and Excel 2007. Single factor analysis of variance (ANOVA) was performed to assess inter-treatment differences between OHD+7, OHD and OHD-7 separately for antioxidant capacities, storage period and resistance to CI. OHD fruits were the control group and  $P < 0.05$  was considered to be statistically significant. Multifactorial ANOVA was also done to see if there were any interactions between treatments on CI resistance. Also, a multiple correlation on the three variables (fruit maturity, antioxidant capacity and chilling injury resistance) was performed and correlation coefficients used to determine the relationships between each variable.

## RESULTS

### *Antioxidant capacity*

Antioxidant capacities for the different levels of fruit maturity (Figure 2) showed that OHD+7 significantly ( $P < 0.05$ ) had the lowest average record (0.471 mgTE/g) throughout storage compared to control (OHD) (0.976 mgTE/g) and OHD-7 (1.080 mgTE/g). No significant difference was observed between the control (OHD) and OHD-7 fruits in terms of antioxidant capacity. However, significant differences were observed ( $P < 0.05$ ) for all samples after 45 days of cold storage with OHD-7 recording the highest antioxidant capacity (1.178), followed by OHD (0.687) and OHD+7 (0.306 mgTE/g). Generally, antioxidant capacity recorded higher values for fresh samples compared values after cold storage for 15 days. Antioxidant capacities for all samples increased

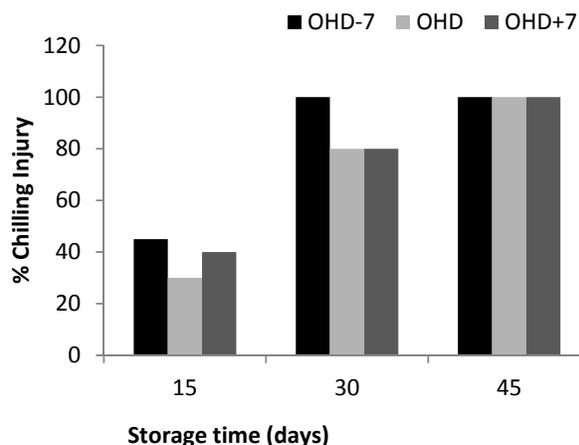
generally after 30 days of cold storage and this was significant ( $P < 0.05$ ) for control (OHD) and OHD-7 fruits. After 45 days of cold storage, antioxidant capacity for OHD fruits decreased significantly and sharply from 1.319 to 0.687 mgTE/g. However, decreases recorded in antioxidant capacities for OHD+7 and OHD-7 fruits after 45 days of cold storage were not significantly different from those after 30 days of storage.



**Figure 2: Antioxidant capacity for fruits stored over a 45-days period at 5 °C. Values are means of 3 replicates and error bars show standard deviation**

### *Chilling injury*

Comparing CI development between the different groups (OHD+7, OHD [CT] and OHD-7), a significant level of resistance was observed for all groups after 15 days of cold storage (Figure 3) compared to 30 and 45 days. On day 15, control fruits (OHD) had the highest observed resistance to CI (70%) compared to OHD+7 and OHD-7 fruits which recorded resistance levels of 60 and 55% respectively. After 30 days of cold storage, it was observed that 80% of fruits from control group (OHD) and OHD+7 developed CI with only 20% resistance. On the other hand, the less mature fruits (OHD-7) had no observed resistance to CI after 30 days of cold storage. All groups fully developed CI after 45 days of cold storage at 5°C (Figure 3). Although no significant differences were observed for treatments, storage length significantly influenced CI resistance in fruits. Fifteen days of cold storage significantly had the lowest CI incidence than 30 and 45 days of storage (Figure 3).



**Figure 3: Percentage chilling injury (CI) incidence over a 45 day storage period for different fruit maturity periods at 5°C**

## DISCUSSION

The results for antioxidant capacity for OHD+7 (0.471 mgTE/g) which was the least in Figure 2 is supported by literature as mature peach fruits under stress induced by cold storage may have reduced antioxidant activities [14,15]. This can also be explained by the fact that mature fruits (OHD+7) had utilized most of their antioxidant compounds in eradicating FR and ROS resulting in their lowest antioxidant capacity [6,21]. The least mature fruits (OHD-7) recording the highest antioxidant capacity confirms the same point. Length of storage also had a significant influence on the evolution of antioxidant capacity such that 30 days recorded the highest value (1.012 mgTE/g) as compared to 15 and 45 days of cold storage which recorded 0.730 and 0.724 mgTE/g respectively. It was said by Jin *et al.* [14] that cold storage can induce stress hence it is expected that fruits encountered a relatively higher stress after 30 days than after 15 days of cold storage. A study by Davies (1995) ascertained that organisms are able to adapt to fluctuating stresses by inducing the synthesis of antioxidant enzymes. Probably due to the relatively higher stress after 30 days of storage, fruits had more antioxidant compounds released or synthesized to combat FRs and ROS.

It is evident from results that CI resistance could not be attributed to antioxidant capacity as results from Figure 3 did not significantly correlate with those of Figure 2. In other words, higher antioxidant capacities observed did not confer higher resistance to CI. Fruits harvested earliest (OHD-7) with the highest antioxidant capacity (Figure 2) were the least resistant to CI development during cold storage. This is supported by a study of three harvests, early, commercial and late, of 'Huangjin' peaches and it was found that the early harvested fruit were those that developed symptoms of CI [11]. Fruits harvested earlier (OHD-7) had the highest CI incidence of 45%, followed by OHD+7 (40%), both higher than the control (OHD) which had 30% after 15 days of cold storage. When cold storage lasted for 30 days, only OHD-7 had a 100% CI incidence. Thus all fruits harvested earlier than control fruits (OHD) had developed CI after 30 days of cold storage at 5°C. This is confirmed by literature that immaturity of fruits exposes them to

CI development [3]. On the contrary, fruits harvested later (OHD+7) and control (OHD) still had 20% of fruits which had not developed CI after 30 days of cold storage. This observation is confirmed by literature that mature fruits which produce more ethylene may resist CI [5]. However, CI was observed in all fruits for all harvest dates after 45 days of cold storage including control fruits (OHD).

It could then be said that storage length significantly influenced CI development in fruits. Also, allowing fruits to mature (OHD and OHD+7) enabled them to develop tougher cell walls and produce more ethylene which might support their resistance to CI although some tradeoffs may have been made for relatively lower antioxidant levels (Figures 2 and 3) [5,6]. These tradeoffs can be explained by the fact that mature fruits which may resist CI as reported by Crisosto [5] also have reduced antioxidant levels. Other tradeoffs that may occur with maturity are softening and off flavour development. As living organs, fruits are able to modify their physiology to enhance protection depending on factors in the environment. For example, intermittently warmed fruit get toughened against CI [5,30]. Oxidative stress also related to higher ethylene production is used as a signal to trigger other defence mechanisms in plants, sometimes protecting against CI development [5,6,31]. Therefore it may be argued that the more mature (OHD and OHD+7) fruits had toughened against CI due to earlier exposure to oxidative stress and concomitant ethylene production evident in their relatively lower antioxidant capacities.

As CI symptoms render fruits unacceptable to consumers, it is advised that commercial producers or marketers of fresh fruits apply research findings to minimize losses and enhance keeping quality and nutritional value. From this study, fruit maturity was found to influence fruit resistance to CI development and as such, fruits should be allowed to mature properly but within acceptable commercial parameters such that firmness is assured. This means that as much as postharvest handling and storage are important, pre-harvest factors such as maturity are also crucial. Maturity standards should therefore be developed and used especially in developing countries to guide farmers and to assist in enhancing fruit quality in the short term after harvest. For good keeping quality and storability, fruits should be harvested mature before cold storage if intended to be kept for long periods but could be harvested earlier for best firmness and antioxidant property when intended for immediate use. Multiple correlation on the three variables (fruit maturity, antioxidant capacity and chilling injury resistance) was carried out and correlation coefficients represented in Table 1. Fruit maturity positively correlated with CI resistance ( $R^2 = 0.6933$ ) as more mature fruits (OHD and OHD+7) resisted CI better than less mature fruits (OHD-7) but negatively correlated with antioxidant capacity (Table 1) [3,22]. Antioxidant capacity also corelated negatively with CI resistance as fruits with the highest antioxidant capacity (OHD-7) on the contrary had the least resistance to CI (Table 1).

## CONCLUSION

Antioxidant capacity was not found to support fruits against the development of CI during cold storage as on the contrary fruits with highest observed antioxidant capacity (OHD-7) were the most affected by CI during cold storage according to observation. However, maturity level of fruits was related to CI resistance as the more mature fruit (OHD+7) had the highest resistance to CI but which lasted only up to 30 days of cold storage. Length of storage also significantly influenced CI development with longer storage periods resulting in higher incidence of CI in fruits. Notwithstanding, more work has to be done to find out if the low resistance of less mature (OHD-7) fruits was due to its maturity stage or its high antioxidant capacity.

**Table 1: Correlation coefficients for data**

	<i>Chilling Injury</i>	<i>Antioxidant capacity</i>	<i>Fruit maturity</i>
Chilling Injury	1		
Antioxidant capacity	-0.3920	1	
Fruit maturity	0.6933	-0.9347	1

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