Original article

Peel pitting of Encore mandarin fruits: etiology, control and implications in fruit quality

Rute Filipe Vitor[a], Fernando Cebola Lidon[a*], Maria da Graça Barreiro[b], Maria Isabel Maia[b], Maria Clara Medeira[b], António Guerreiro[c]

[a] Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2825 Monte de Caparica, Portugal (fjl@mail.fct.unl.pt)

[b] Plant Physiology Department, Estação Agronómica Nacional, 2780 Oeiras, Portugal (ean@mail.telepac.pt)

[c] Direcção Regional de Agricultura do Algarve, Patacão, Faro, Portugal

** Correspondence and reprints*

Received 19 June 2000
Accepted 17 October 2000

Fruits, 2001, vol. 56, p. 27–35

***Resumen Español**, p. 35*

Peel pitting of Encore mandarin fruit: etiology, control and implications in fruit quality.

Abstract — **Introduction**. The fruit of 'Encore' mandarin (*Citrus deliciosa* × *Citrus nobilis)* usually shows chlorotic spots in the epicarp from the first stages of development. This peel disorder triggers off, thereafter, the occurrence of a large quantity of dark stains (pre-harvest 'peel pitting'). **Materials and methods**. A biochemical and ultra-structural study of the flavedo, using epicarp with and without stains, was carried out. Quality assays were assessed on the edible portion of the fruit or on juice. **Results**. The cell structure of pitted and unpitted epicarp analysed by electron microscopy revealed that dark spots were associated with the degradation of cellular membranes. The peroxidase system that shields the epicarp against photooxidative stress showed an inhibition of catalase activity and an increase of superoxide dismutase functioning. Moreover, the activities of ascorbate peroxidase and glutathione reductase remained unaffected in stained tissues. In sunshade trees, it was also found that the number of dark stains per fruit decreased but the internal quality of the endocarp was not significantly affected. The weight and the caliber of the fruit also slightly decreased. **Discussion**. High light intensities induce an oxidative stress in the epicarp, which is characterized by an increased peroxidation and degradation of biological membranes. On the other hand, protection against solar radiation alleviates the stress, but, although not affecting the internal quality of the fruit, slightly decreases the fruit weight and size.

Portugal / *Citrus reticulata* / mandarins / cell structure / ultrastructure / epidermis / fruit / quality

Taches sur l'écorce de la mandarine Encore : étiologie, contrôle et implications dans la qualité du fruit.

Résumé — **Introduction**. Le fruit de la mandarine « Encore » (*Citrus deliciosa* × *Citrus nobilis*) montre fréquemment des taches chlorotiques de l'épicarpe dès les premiers stades de développement. Cette anomalie de l'écorce déclenche, ensuite, avant la récolte, l'apparition d'une grande quantité de taches sombres, ultrastructurales. **Matériel et méthodes**. Une étude biochimique du flavédo, utilisant des épicarpes avec ou sans taches, a été effectuée. Des mesures de qualité ont été effectuées sur la partie comestible du fruit ou sur le jus. **Résultats**. La structure des cellules d'épicarpes maculés ou non, analysée par microscopie électronique, a révélé que les taches sombres étaient associées à la dégradation de membranes cellulaires. Le système peroxydase qui protège l'épicarpe contre le stress photooxydatif a montré une inhibition de l'activité de la catalase et une augmentation du fonctionnement de la superoxyde dismutase. Par ailleurs, les activités de l'ascorbate peroxydase et de la glutathione reductase sont restées inchangées dans des tissus affectés. Le nombre de taches sombres par fruit récolté sur les arbres sous ombrage a diminué, mais la qualité interne de l'endocarpe n'a pas été significativement affectée. Le poids et le calibre du fruit ont aussi légèrement diminué. **Discussion**. De fortes intensités lumineuses favorisent un stress oxydatif de l'épicarpe du fruit, qui se caractérise par une peroxydation accrue et une dégradation de membranes biologiques. Une protection contre les rayons solaires limite le stress, mais, sans cependant affecter la qualité interne du fruit, il en diminue légèrement le poids et la taille.

Portugal / *Citrus reticulata* / mandarine / structure cellulaire / ultrastructure / épiderme / fruit / qualité

1. Introduction

During fruit development, 'Encore' mandarin is affected by pre-harvest peel pitting [1]. This disorder is characterized by cellular damage [2] related to specific senescence processes [3, 4]. In the first stages of fruit growth, the observations of flavedo spot sections reveal parenchymal cells flattened and collapsed, increasing in parallel with the epidermis. In these cell layers, the cytoplasm is plasmolysed, being visible as a dense and shrunken mass, resulting from the destruction of the cytoplasmic membranes and from the accumulation of large amounts of osmiophilic material [2]. In this context, the production and control of oxy radicals play a fundamental role. To living cells, dioxygen is both useful and toxic [5]. Throughout excitation and univalent reductions, dioxygen produces strongly reactive chemical entities such as superoxide and hydroxyl radicals, as well as hydrogen peroxide. These chemical species oxidise acyl lipids and proteins namely in cellular membranes, increasing the rate of senescence. Even through such chain reactions can seriously amplify the damage caused by the initiating radical, the cells contain antioxidant systems which interrupt free radical chain reactions, decreasing the damage done per radical initiation [6, 7]. Nevertheless, during senescence, these chemical entities increase throughout inactivation of the antioxidant systems [5].

Following the general assumption that the defence mechanisms against oxygen toxicity are impaired in the dark stained tissues, the main objective of this work was to study in detail the cellular degradation and to characterize the alterations of the antioxidant defence mechanisms in order to identify the limiting steps of the amphibolic pathway. Additionally, through a minimisation of the photooxidative stress, using as a modelling system cutting nets of solar radiance (to 50%) applied over whole the trees, the implications of a decreasing light supply to the internal quality of the fruits during the harvest / market period was also evaluated.

2. Materials and methods

2.1. Plant material

In the beginning of the development of fruit of Encore mandarin (July 11, 1998) a cutting net of 50% solar radiance was installed over four trees of Encore mandarin in an orchard located in the South of Portugal (Almansil, Algarve). Two trees were shaded until the end of November, 1998 (E1), and the other two remained protected until fruit maturity (end of April, 1999) (E2). Between September, 1998, and April, 1999, fruits were picked.

2.2. Transmission electron microscopy

Between September and November of 1998, fragments of healthy flavedo with encircled spots were fixed overnight at 4 °C in 3% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4. The specimens were washed three times for 30 min in the same buffer, stained in block in 1% buffered uranyl acetate, dehydrated in an ethanol gradient to absolute ethanol and embedded in Spurr's resin [8]. Sections 80–100 nm thick were cut in an LKB-ultramicrotome IV using glass knives. Thin sections for transmission electron microscopy (TEM) were stained in an aqueous saturated solution of uranyl acetate for 30 min and post-stained in a 3% aqueous solution of lead citrate at room temperature [2]. Ultrastructural observation was made with a Philips 300 Transmission Electron Microscope at 80 kV.

2.3. Enzyme activities

On February of 1999, the maximum activities of superoxide dismutase (E.C. 1.15.1.1), glutathione reductase (E.C. 1.6.4.2), ascorbate peroxidase (E.C. 1.11.1.7) and catalase (E.C. 1.11.1.6) were measured following McCord and Fridovich [9], Dalton et al. [10], Nakano and Asada [11] and Patra et al. [12], respectively. Superoxide dismutase was extracted from pieces of flavedo homogenized in 0.1 M Tris – HCl (pH 8), 0.1 mM EDTA and 0.3% Triton X-100. A 50–80% ammonium persulphate precipitation was

therefore applied to the supernatant, being the extracted protein homogenized in the extraction buffer (2 min, 4–6 °C). After filtration and centrifugation at 2000 × *g* (4 min, 4–6 °C), the total activity of superoxide dismutase was measured at 550 nm. The enzyme activity is expressed as units mg protein, being one unit defined as the amount of enzyme required to inhibit the reduction rate of cytochrome *c* by 50% under the assay conditions. The activity of glutathione reductase was measured indirectly through the oxidation of nicotinamide adenine dinucleotide phosphate (NADPH). Samples of flavedo were homogenized in 75 mM phosphate buffer (pH 7.5) with polyvinylpirrolidone (PVP, 15% weight / volume) at 4–7 °C. The solution was filtered and centrifuged at 2000 × *g* (5 min, 6–8 °C), and the activity measured in the supernatant at 25 °C, using 200 µL of supernatant in a reaction mixture having 0.5 mM GSSG (oxidised glutathione), 0.25 mM NADPH, 75 mM Tricina (pH 7.8) and 0.5 mM EDTA (ethylene diamine tetraacetic acid). The results were corrected considering the independent rate of NADPH oxidation. The measurement of ascorbate peroxidase activity was carried out using pieces of flavedo homogenized in 0.35 M sorbitol, 25 mM HEPES[1] (pH 7.6), 1 mM EDTA (1 min, 4–6 °C). After filtration and centrifugation at 200 × g (2 min, 2–4 °C), the activity was determined at 290 nm, using the absorption coefficient of 2.8 $mM \cdot cm^{-1}$ in a reactive mixture containing the supernatant fraction and 0.5 mM ascorbate. To measure the activity of catalase, samples of flavedo were homogenized in 100 mM phosphate buffer (pH 7.8) during 1 min at 4–6 °C. After filtration and centrifugation at 10000 × *g* (45 min, 4–6 °C), the activity was measured using the supernatant fraction in a reaction mixture having 0.035% H_2O_2 and 100 mM phosphate buffer (pH 7.8). The consumption of H_2O_2 was followed at 540 nm. All the enzymatic activities were measured using two disks of the epicarp tissue (1.86 cm^2 each) from three fruits.

The concentrations of ascorbate and dehydroascorbate in stained and unstained epicarp were measured using two disks (1.86 cm^2 each) from three fruits. The disks were frozen in liquid nitrogen and processed as described by Kampfenkel et al. [13]. Freezing 6% (w/v) TCA was added to the tissues. After grinding to a fine powder, the mixture was continuously homogenized until completely thawed and, then, was kept on ice for 15 min. The homogenate was quantitatively transferred to a 2-mL reaction vessel on ice, and adjusted to a volume of 2 mL with 6% (w/v) TCA and then centrifuged for 5 min at 15600 × *g* (4 °C). The supernatant was immediately used for measuring ascorbate and dehydroascorbate concentrations.

2.4. Effects of solar radiance protection on fruit quality

On February 22, March 7, March 21, April 11 and April 25, fruits were collected from trees: without any protection against fotooxidative damage (T); submitted to solar radiance protection until the end of November (E1); submitted to solar radiance protection until the end of April (E2).

In the fruits collected until April 11, the following parameters were analysed: number of dark stains per fruit; caliber (diameter); weight; colour of the epicarp (using a Minolta Chromamiter CR-300, Japan); soluble solid concentration (using a Euromex digital hand refractometer) and titratable acidity, as described by Alavoine et al. [14]; sensorial quality according to the visual appearance, texture and flavour of the fruits (evaluated by a panel of 8–10 juries). Sensorial quality parameters were evaluated using a scale varying from zero (unacceptable) to twenty (high quality fruits).

In the fruits collected on April 25, the following characteristics were analysed: ascorbic acid concentration [13]; starch content following Nielson [15]; sucrose, total and reducing sugars according to Sumner [16]. The reducing sugar concentration measurement was carried out using a standard calibration curve of glucose. After addition of invertase to the sample, the total sugars were measured using the same curve.

The number of dark stains per fruit, caliber, weight and colour of the epicarp were measured using one hundred fruits of each

[1] HEPES: N–(2–hydroxyethyl) piperazine N'– (2–ethanesulfonic acid)

treatment (T, E1 and E2). The soluble solid concentration, titratable acidity, starch content, sucrose, total and reducing sugars were determined using samples of twenty-five fruits with four replicates. Ascorbic acid was measured using an intimate mixture of four replicates of five fruits each.

2.5. Statistics

Statistical analysis was performed using a one way anova (F-ratio test, for $p = 0.05$). Based on the anova results, a Tuckey's test was performed for mean comparison (for a 95% confidence level).

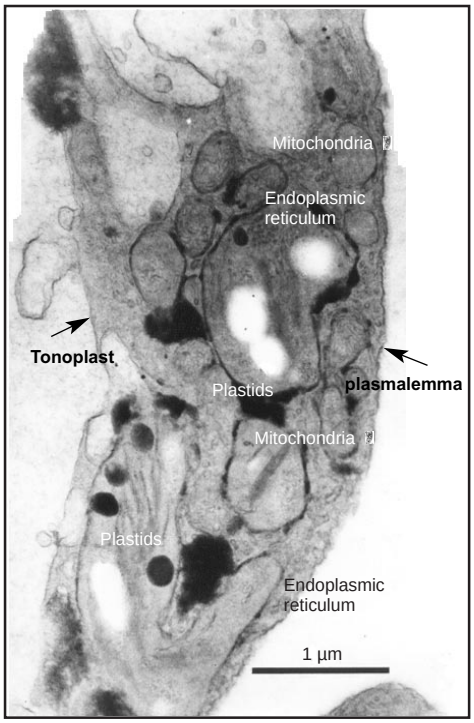

Figure 1.
Healthy sub-epidermal cell (Encore mandarin fruit peel). Cytoplasm with numerous organelles: plastids, mitochondria and cisternae of endoplasmic reticulum. There were no signs of injured cell membranes (electron mycroscopy).

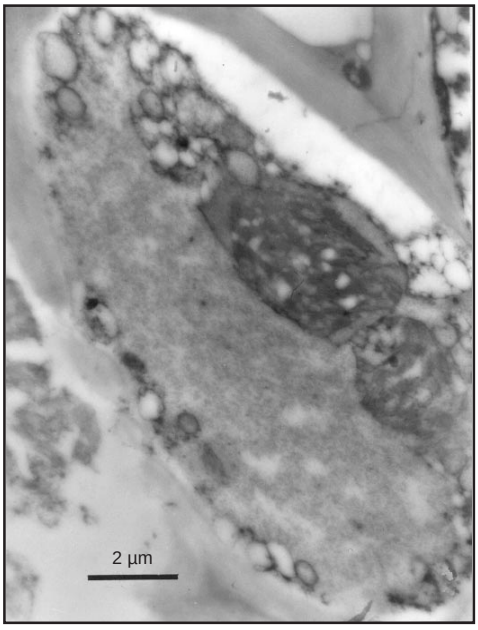

Figure 2.
A disorganised sub-epidermal cell of parenchyma (Encore mandarin fruit peel). The plasmalemma, the tonoplast and plastidial membranes lost their integrity (electron mycroscopy).

3. Results

In the healthy flavedo of Encore mandarin the sub-epidermal cells have an abundant cytoplasm and a large number of cellular organelles. Large number of plastids, abundant mitochondria and abundant endoplasmic reticulum cisternae can be seen (*figure 1*). The plasmalemma, the tonoplast, the plastid's envelope and the others cellular membranes still maintain their integrity.

The damaged flavedo cells revealed a great intracellular disorganisation due to the degradation of cell membranes (*figure 2*). This degradation process leads to the formation of amorphous and electron-dense masses (*figure 3*). The senescence process was confirmed by the development of inhibitory steps in the amphibolic system coupled to oxy radicals production and control. Indeed, in pitted tissue the activity of superoxide dismutase increased (280%), that of catalase decreased significantly by 42%, while ascorbate peroxidase and glutathione reductase did not vary significantly (*table I*). Additionally, in close association with these enzymatic changes, lower levels of ascorbate and dehydroascorbate were also found in the pitted tissues (*figure 4*).

Due to the application of the tree shading, the fruits showed a significant decrease in the number of dark stains over the epicarp (*figure 5*) with the related area apparently not being changed. This beneficial effect increased with time during the harvest / market period, since the decreasing number of the dark stains changed from 50% (in the first picking) to 70% (in the last picking). The weight of the fruits belonging

to the protected trees was significantly reduced at all sampling dates (*table II*). Titratable acidity was increased while soluble solid concentration decreased in these fruits. However, this tendency was not evident in the last harvest. The sensorial quality of the fruits did not seem to be affected in any sampling date. The analyses of the epicarp colour of the fruits of the protected trees revealed that the patterns of the L^* increased whereas that of a^* decreased (*table III*). However, in the last picking of E2, there were not any significant differences concerning to the colour of the fruit. In the fruits of the trees protected against solar radiance occurred a significant decrease of the content of reducing sugars. Until the end of April, these fruits also showed a significant decrease in the total sugars (*table IV*). Starch and sucrose content did not show any significant variation while the level of ascorbate sharply increased in the fruits of the protected trees against solar radiation until the end of November.

4. Discussion

At present time, the causes of the lesions associated to the peel pitting are not known, but, as confirmed by TEM, they seem to be coupled with unrepaired damage of cell membranes in the flavedo. The breakdown of the tonoplast in injured cells would accelerate the oxidation of phenolic substances and the oxidation products might prevent the normal function of organelles and membranes, as Abe [17] observed in dehydrated chilled tissues. Water loss of parenchymal cells of the flavedo was observed by cuticular cracking in *Citrus* [18]. Solar radiation may be involved in the alteration of the cuticle and high rind temperatures over a large period may induce localised dehydration in epidermal and sub-epidermal cells leading to membrane collapse [2].

Following the alterations on the regulatory steps of the peroxidase system coupled to the control of oxy radicals, this peel disorder indicates a major accumulation of high levels of H_2O_2. This assumption is closely related with the decreasing activity of catalase (*table I*) and, furthermore, these

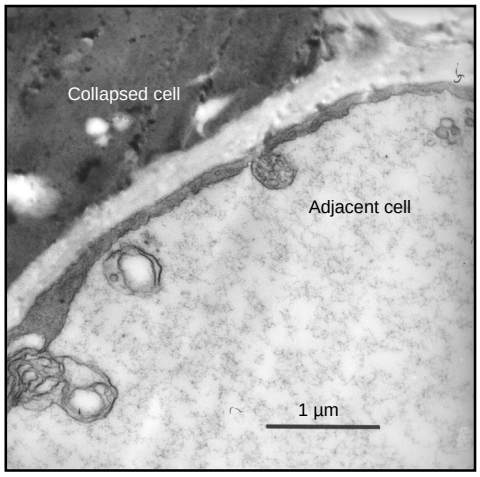

Figure 3.
A collapsed cell was completely disorganised being visible as a electron dense mass. The adjacent cell still maintained preserved the cell membranes (Encore mandarin fruit peel, electron mycroscopy).

Figure 4.
Ascorbate and dehydroascorbate content of pitted and unpitted tissue of the epicarp (Encore mandarin fruit peel). Each value is the mean ± standard error, based on three independent series. There are not significant differences.

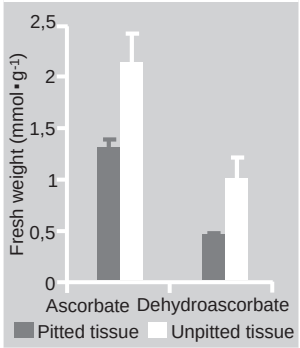

Table I.
Performance of superoxide dismutase, ascorbate peroxidase, glutathione reductase and catalase total activities in pitted and unpitted tissues of the Encore mandarin fruit epicarp. Each value is the mean ± standard error, based on three independent series.

Type of fruit tissue	Superoxide dismutase (units·µg^{-1} protein)	Ascorbate peroxidase (mmol·mg^{-1} protein·min^{-1})	Glutathione reductase (nmol·mg^{-1} protein·min^{-1})	Catalase (µmol·mg^{-1} protein·min^{-1})
Pitted	131.7 ± 30.60	1.78 ± 0.199 3	4.84 ± 1.471	62.6 ± 9.10
Unpitted	47.0 ± 7.556	1.86 ± 0.100	33.09 ± 2.975	150.9 ± 12.84
Significance	ns	ns	ns	**

Significance at $p \leq 0.05$: ns, not significant; ** significant, according to the Tuckey's test.

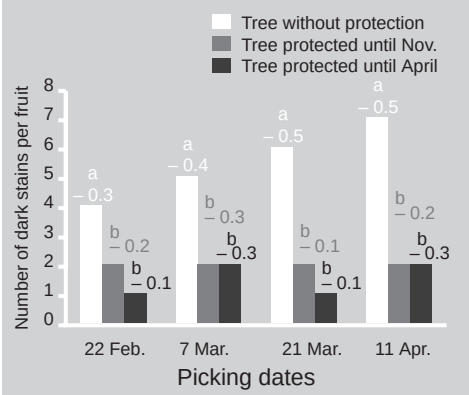

Figure 5.
Number of dark stains on the peel of Encore mandarin fruits (mean value) collected at different picking dates from trees: without any protection against solar radiance, protected until the end of November, or until the end of April. Each value is the mean ± standard error (n = 100). Different letters for a same picking date indicate significant differences between the trees at this date.

data are in agreement with previous findings [3] stating that the accumulation of H_2O_2 seems to be further reduced to the hydroxyl radical. Indeed, this oxy radical is known to be highly responsible for the peroxidation of the acyl lipids in Encore mandarin [3, 6], mediating senescence [19]. The ascorbic – glutathione cycle regulates the accumulation of H_2O_2, yet, in pitted tissue, the levels of ascorbate and dehydroascorbate tending to be lower than in the unpitted tissue and, furthermore, the combined total activity of ascorbate peroxidase also

Table II.
Physiological parameters of Encore mandarin fruits collected at different picking dates according to different treatments: trees without any protection against solar radiance, protected until the end of November or until the end of April.
Each value is the mean ± standard error (n = 100). Different letters in a same row indicate the means with significant differences ($p \leq 5\%$).

Harvest / market period	Trees without protection	Trees protected	
		until end of November	until end of April
a) Weight (g)			
February 22	96.3 ± 1.70 a	73.2 ± 1.30 b	71.9 ± 1.31 b
March 7	97.8 ± 1.75 a	79.6 ± 1.50 b	83.3 ± 1.30 b
March 21	104.3 ± 1.68 a	83.1 ± 1.24 b	84.1 ± 1.22 b
April 11	118.5 ± 1.31 a	86.8 ± 1.74 b	100.4 ± 1.21 b
b) Diameter (cm)			
February 22	62.9 ± 0.38	56.3 ± 0.38	55.8 ± 0.37
March 7	63.0 ± 0.42	58.0 ± 0.43	59.6 ± 0.36
March 21	64.9 ± 0.49	60.0 ± 0.44	60.0 ± 0.41
April 11	68.4 ± 0.41 a	61.2 ± 0.44 b	64.2 ± 0.40 a
c) Acidity (g citric acid·L^{-1})			
February 22	22.77 ± 0.405 a	31.66 ± 0.515 b	31.26 ± 1.194 b
March 7	23.21 ± 0.713	27.52 ± 0.923	26.16 ± 0.562
March 21	19.81 ± 0.388 a	26.59 ± 0.523 b	22.63 ± 0.459 a
April 11	14.95 ± 0.394	19.55 ± 1.139	16.71 ± 0.401
d) Total soluble solids (°Brix)			
February 22	12.6 ± 0.16 a	11.2 ± 0.18 b	9.6 ± 0.21 a
March 7	12.2 ± 0.17 a	11.0 ± 0.16 b	9.5 ± 0.20 a
March 21	11.9 ± 0.21 a	11.2 ± 0.16 a	9.6 ± 0.20 b
April 11	11.8 ± 0.21 a	11.3 ± 0.18 a	9.7 ± 0.21 b
e) Score of sensorial quality (painel test) ranged from zero (unacceptable) to twenty (high quality fruit)			
February 22	14.1 ± 0.46	12.9 ± 0.77	13.7 ± 0.69
March 7	16.3 ± 0.55	15.1 ± 0.48	14.1 ± 0.48
March 21	15.0 ± 0.84	16.0 ± 0.85	15.4 ± 0.64
April 11	15.5 ± 0.76	15.4 ± 0.45	15.7 ± 0.49

Table III.
Colour of the epicarp of Encore mandarin fruits collected at different picking dates according to different treatments: trees without any solar protection against solar radiance, or with protection until the end of November or the end of April.
Each value is the mean ± standard error (n = 100). Different letters in a same row indicate the means with significant differences ($p \leq 5\%$).

Harvest / market period	Trees without protection	Trees protected	
		until end of November	until end of April
*L**			
February 22	61.0 ± 0.11 a	63.7 ± 0.45 b	62.2 ± 0.34 ab
March 7	60.7 + 0.51	62.0 + 0.42	61.3 + 0.37
March 21	60.3 + 0.20 a	62.2 + 0.39 b	61.2 + 0.35 ab
April 11	60.2 + 0.87	61.0 + 0.35	61.5 + 0.44
*a**			
February 22	34.4 ± 0.40 b	30.5 ± 0.96 a	32.5 ± 0.53 ab
March 7	34.5 + 0.76	33.2 + 0.67	33.6 + 0.55
March 21	34.7 + 0.47 a	31.7 + 0.39 b	32.6 + 0.62 ab
April 11	30.1 + 1.35	32.3 + 0.40	30.8 + 0.77
*b**			
February 22	55.1 ± 0.22 a	57.3 ± 0.47 b	56.1 ± 0.39 ab
March 7	54.5 + 0.46	55.1 + 0.36	55.0 + 0.47
March 21	53.4 + 0.28	54.2 + 0.38	54.0 + 0.59
April 11	53.0 + 1.14	53.2 + 0.54	54.3 + 0.72

The CIE (Commission internationale de l’éclairage) 1976 *L a b* colour system was used: *L** indicates the luminosity variation, *a** and *b** are the chromatic variables. *a** indicates the variation from green (–) to red (+) and *b** from blue (–) to yellow (+).

Table IV.
Chemical composition of the endocarp of Encore mandarin fruits collected on April 25, at the commercial harvest date, according to different treatments: trees remained without any protection against solar radiance, or trees protected until the end of November or until the fruit harvest in April. Each value is the mean ± standard error (n = 100).

Tree protection	Total sugar	Reducing sugar	Sucrose	Starch	Vitamin C [mg·100 g^{-1})
	(g·100 g^{-1})				
Without protection	13.45 ± 0.603 a	4.62 ± 0.087 a	8.84 ± 0.51	13.66 ± 0.854	23.36 ± 1.643 a
With protection until November	10.84 ± 0.534 b	3.75 ± 0.149 a	7.10 ± 0.39	13.97 ± 0.498	39.31 ± 1.471 b
With protection until harvest	10.29 ± 0.393 b	2.84 ± 0.116 b	7.44 ± 0.28	13.14 ± 0.849	23.64 ± 1.727 a
Significance	**	**	ns	ns	**

Significance at $p \leq 5\%$: ns, not significant; ** significant. Different letters in a same column indicate the means with significant differences.

decreased. These alterations indicate that the higher activity of glutathione reductase is limited in vivo because an additional consumption of dehydroascorbate to recycle the GSH is not possible. Thus, this becomes a metabolic limitation to the antioxidant capacity of that enzymatic system.

The application of a cutting net (to 50%) over the trees decreased the number of dark stains per fruit limiting the photooxidative stress and, therefore, the concurrent senescence of flavedo. Although the decreased light intensity probably limited the photosynthetic rates in the leaves and, therefore, the mobilisation of photoassimilates to the fruit, the sensorial evaluation of the protected fruit was not significantly affected. Indeed, only weight and size in the trees receiving protection against solar radiation were decreased. As a result of the minor mobilisation of photoassimilates to the fruit, the soluble solid contents decreased with the increasing time protection of the trees. Additionally, total sugar and sucrose in the fruits of the trees protected against solar radiation decreased. Coupled to these alterations, the decreasing concentration of reducing sugars is a definitive indication of the catabolism of the complex carbohydrates. Moreover, the level of acidity was higher in the fruit of the trees covered until November, which represents an accumulation of organic acids in the cells. The consumed sugar might act as immediate precursors of the accumulation of this organic acids, being the respiration rate of these fruits restricted probably because the energetic needs were lower (which is surely associated to their minor caliber and weight). Indeed, the minor levels of the proportion ATP/ADP activate the catabolism of the reducing sugar, eventually triggering the accumulation of the organic acids and a less efficient production of ATP. The unavoided decreasing production of the fruits was however counterbalanced by an increasing nutritional value due to the increase of vitamin C in the trees covered until November. Indeed, when the trees become unprotected against solar radiance, the fruits have to adapt to a high photooxidative stress. Consequently, the levels of antioxidants in the fruit endocarp sharply increase during the harvest / market period which represents an additional nutritional value.

The measurement of the epicarp colour of these fruits show a decrease in a^* value and an increase in b^* value. These variations reveal that fruits present a tendency to the yellow, colour that indicates an increasing accumulation of carotenoids. The conclusion is that the protection of the trees until the end of November triggers off a significant reduction of the number of dark stains without affecting the internal quality. A putative explanation of this tendency seems difficult since that pattern could be attributed to a great amount of physiological parameters. However the time of exposure and the light intensity is a determinant external factor.

Note

This work was supported by the project PRAXIS XXI – 3 / 3.2 / Hort / 2156 / 95 / from Ministry of Science and Technology (Portugal).

References

[1] Vitor R.F., A tangerina 'Encore'. Fisiologia da Planta, Histologia e Bioquímica do Flavedo, Qualidade do Fruto, Fac. Ciênc. Univ. Lisboa, Portugal, 1998.

[2] Medeira M.C., Maia M.I., Vitor R.F., The first stages of pre-harvest 'peel pitting' development in 'Encore' mandarin. An histological and ultrastrutural study, Ann. Bot. 83 (1999) 667–673.

[3] Vitor R.F., Lidon, F.C., Carvalho, C.S., Dark stained tissue of the epicarp of Encore mandarin: interactions with the production of hydroxyl radicals, Free Rad. Res. 31 (2000) 163–169.

[4] Vitor R.F., Lidon F.C., Carvalho C.S., Barreiro M.G., Pre-harvest dark stains of the flavedo of 'Encore' mandarin: tissue chemical composition and implications to the fruit internal quality, Fruits 54 (1999) 393–402.

[5] Winston G.W., Physiochemical basis for free radical formation in cells: production and defences, in: Stress responses in plants: adaptation and acclimation mechanisms, Wiley-Liss, inc., New York, 1990, pp. 57–86.

[6] Lidon F.C., Henriques F.S., Oxygen metabolism in higher plant chloroplasts, Photosynth. 29 (2) (1993) 249–279.

[7] Polle A., Defence against photooxidative damage in plants. Oxidative stress and the molecular biology of antioxidant defences, Cold Spring Harbour Lab. Press, 1997, pp. 623–665.

[8] Spurr A.R., A low viscosity epoxy resin embedding medium for electron microscopy, J. Ultrastructural Res. 26 (1969) 31–43.

[9] McCord J.M., Fridovich I., Superoxide dismutase: an enzymic function for crythrocuprein (hemocuprein), J. Biol. Chem. 244 (1969) 6049–6055.

[10] Dalton D.A., Russell S.A., Hanus F.J., Pascoe G.A., Evans H.J., Enzymatic reactions of ascorbate and glutathione that prevent peroxide damage in soybean root nobules, Proc. Nat. Acad. Sci. 83 (1986) 3811–3815.

[11] Nakano Y., Asada K., Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts, Plant Cell Physiol. 22 (1981) 867–880.

[12] Patra H. K., Kar M., Mishra D., Catalase activity in leaves and cotyledons during plant development and senescence, Biochem. Physiol. Pflanzenph. 172 (1978) 385–390.

[13] Kampfenkel K., Montagu M. V., Inzé D., Extraction and determination of ascorbate and dehydroascorbate from plant tissue, Anal. Biochem. 225 (1995) 165–167.

[14] Alavoine F., Crochon M., Fady C., Fallot J., Moras P., Pech J., La qualité gustative des fruits, Cemagref, Mission Qualité alimentaire, DGAL, Min. Agric., Paris, 1988.

[15] Nielson J.P., Rapid determination of starch. An index to maturity in starch and vegetables, Ind. Eng. Chem. 15 (1943) 176–179.

[16] Sumner J.B., A more specific reagent for the determination of sugar in urine, J. Biol. Chem. 65 (1925) 393–396.

[17] Abe K., Ultrastructural changes during chilling stress, in: Chilling injury of horticultural crops, CRC. Press, Inc., Boca Raton, USA, 1990, pp. 71–84.

[18] Cohen E., Shapiro B., Shalom Y., Klein J.D., Water loss: a non-destructive indicator of enhanced cell membrane permeability of chilling-injured *Citrus* fruit, J. Am. Soc. Hortic. Sci. 119 (1994) 983–986.

[19] Robinowitch H.D., Fridovich I., Superoxide radicals, superoxide dismutases and oxygen toxicity in plants, Photochem. Photobiol. 37 (1983) 679–690.

Podredumbre negra de la corteza de la mandarina: etiología, control y consecuencias en la calidad del fruto.

Resumen — **Introducción**. El fruto de la mandarina (*Citrus deliciosa* × *Citrus nobilis*) presenta frecuentemente unas manchas cloróticas en el epicarpo desde las primeras fases de su desarrollo. Esta anomalía de la corteza provoca, posteriormente, la aparición de una gran cantidad de manchas oscuras que dan un aspecto alveolado a la cáscara del fruto antes de la cosecha. **Material y métodos**. Se efectuó un estudio bioquímico de la clorosis, utilizando epicarpos con y sin manchas. Se realizaron mediciones de la calidad en la parte comestible del fruto o en el jugo. **Resultados**. La estructura de las células de epicarpo manchadas o no, analizada con microscopio electrónico, reveló que las manchas oscuras estaban asociadas a la degradación de las membranas celulares. El sistema peroxidasa que protege el epicarpo contra el estrés fotooxidativo mostró inhibición de la actividad de la catalasa y aumento del funcionamiento de la superóxido dismutasa. Por otra parte, las actividades del ascorbato peroxidasa y de la glutación reductasa permanecieron inalterables en los tejidos afectados. El número de manchas oscuras por fruto cosechado en los árboles con sombreado disminuyó pero la calidad interna del endocarpo no se vio afectada de forma significativa. El peso y calibre del fruto también disminuyeron ligeramente. **Discusión**. La fuerte intensidad luminosa favorece el estrés oxidativo del epicarpo del fruto, que se caracteriza por una peroxidación incrementada y la degradación de las membranas biológicas. Una protección contra los rayos solares disminuye el estrés pero sin que la calidad interna del fruto se vea afectada; el peso y el calibre disminuyen ligeramente.

Portugal / *Citrus reticulata* / mandarina / estructura celular / ultraestructura / epidermis / fruto / calidad