Preharvest dark stains of the flavedo of 'Encore' mandarin: tissue chemical composition and implications to the fruit internal quality

Rute Maria Filipe Vitor[a]*
Fernando Cebola Lidon[a]
Carlos Santiago Carvalho[b]
Maria da Graça Barreiro[b]

[a] Plant Biology Unit,
Faculdade de Ciências
e Tecnologia,
Universidade Nova de Lisboa,
2780 Monte da Caparica,
Portugal

[b] Plant Physiology Department,
Estação Agronómica
Nacional,
2784-505 Oeiras,
Portugal

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Abstract — Introduction. The epicarp tissue of Encore mandarin usually shows some dark stains. This disorder is characterized by an alteration of cell structure. The disorder of the pitted tissues has been fully characterized from biochemical parameter studies, and it was also analysed whether these alterations affect the chemical composition and quality of the endocarp or not. **Materials and methods**. A biochemical study of the flavedo, using epicarp with and without dark stains, was carried out. Quality assays were assessed on the edible portion of the fruit or on juice. **Results**. A significant change of the electrolytic conductance was associated to the stained tissues. A significant variation of the polypeptide patterns as well as on the concentration of aminoacids was also found. The concentrations of hydroxyl radicals, carotenoids and acyl lipoperoxides showed a significant increase, whereas the patterns for ethylene production revealed different linear regression types. Quality parameters of the fruits having a pitted epicarp did not show significant alterations. **Discussion**. The physiological disorder of the flavedo of Encore mandarin was characterised by a sharp increase of the senescence rate in localised epicarp cells. The stained tissue show an increase degradation of cellular membranes in a metabolism that seems to be modulated by the oxidative modifications that occur in cells components. However, this epicarp disorder does not affect significantly the chemical quality of the fruit. © Éditions scientifiques et médicales Elsevier SAS

Portugal / *Citrus* / plant physiology / degradation / pericarp / browning

Taches sombres dans le flavedo de la mandarine « Encore » en verger : composition chimique des tissus et implications sur la qualité interne du fruit.

Résumé — Introduction. Des taches sombres affectent fréquemment l'épicarpe de la mandarine Encore. Cette anomalie est caractérisée par une altération de la structure des cellules. Les tissus concernés par ce problème ont été étudiés à partir de l'analyse de paramètres biochimiques et les altérations observées ont été reliées à la qualité interne des fruits atteints. **Matériel et méthodes**. La composition biochimique de tissus du flavedo a été étudiée à partir de l'analyse d'épicarpes avec ou sans taches sombres. La qualité de la partie comestible du fruit et de son jus a été évaluée. **Résultats**. Les tissus altérés ont présenté une conductance electrolytique significativement plus élevée que celle des tissus normaux. De même, le comportement de certains polypeptides et la concentration en acides aminés ont varié de façon significative. La concentration en radicaux hydroxyles, en caroténoïdes et en lipoperoxydes acylés a augmenté significativement, alors que la production d'éthylène suivait différents types de régressions linéaires. La qualité des fruits présentant un épicarpe anormal n'a pas été significativement altérée. **Discussion**. Le désordre physiologique du flavedo de la mandarine Encore a été cartactérisé par une brusque augmentation de la vitesse de sénescence de certaines cellules de l'épicarpe. Les tissus tachés présentent une dégradation accrue de la membrane cellulaire du fait d'un métabolisme qui semble être affecté par des modifications oxydatives qui se produisent dans certains composants de la cellule. Cependant, cette anomalie de l'épicarpe n'a pas d'effet significatif sur la qualité interne du fruit. © Éditions scientifiques et médicales Elsevier SAS

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* *Correspondence and reprints*

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1. introduction

The 'Encore' mandarin is a hybrid of the *Citrus deliciosa* and *Citrus nobilis*. It was introduced in Portugal toward the end of the 70's and has been considered a good quality product due to its sensorial quality, easiness of peeling and reasonable size. An additional benefit for growers is its long harvest period (from March until July). Yet, the epicarp usually shows a great amount of dark stains which decrease the consumer acceptance and, therefore, the marketable value. These stains correspond to parenchymal flattening and collapse of a variable number of sub-epidermal cell layers [1]. This unusual layer increases in parallel with the epiderme, extending between apparently healthy zones. The first signs of cellular damage are associated with internal membrane disorganisation of the plastids and with the occurrence of a great vesiculation of the cytoplasm, followed by an accumulation of abundant osmiophilic material [1, 2].

In the tree of Encore mandarin, the browning or peel pitting appears since the early stages of the development of the fruits [1]. This symptom also occurs in Valencia orange [3, 4] and in Fortuna mandarin [5, 6]. The epicarp disorder of Valencia orange has been related with a physical damage [3], but the stress factor implicated in this process remains unknown. Indeed, inadequate humidity or temperature, excess of radiation and all natural senescence processes might decrease the internal or external quality of citrus fruits. Almela et al. [6] suggested that cold winds might increase the dehydration of the epicarp tissue of Fortuna mandarin, promoting some microscopic damages in the cuticula (thus, favouring the lost of water). The author referred that low temperatures and dryness are also involved in the development of pitting in Fortuna mandarin.

In citrus, as in the physiological disorder named Kohansho [7, 8], this physiological alteration might also appear during the storage period prior to sailing. This damage was namely "chilling injury" [7, 9–13] and occurs pre and/or post-harvest. It has been hardly studied, not only because it affects a great amount of varieties of fruits, but because it is influenced by the storage methods. Nevertheless, in Encore mandarin the pitting symptoms become evident with temperatures higher than 15 °C in the orchard, which indicates that the chilling is not the stress factor responsible for this damage. Moreover, possible photooxidative stress mediates this process [2].

A general characterization of the metabolism involved in the "confined" senescence process of the exocarp of Encore mandarin has prompted this study. A qualitative and quantitative characterization of the protein and lipid contents was performed, and possible oxidative modifications implicated in these molecules have been discussed; some possible qualitative alterations of the carbohydrates were also investigated. Following a chemical characterization of the edible portion of the fruit, the implication of this disorder in the fruit quality was further investigated.

2. materials and methods

Studies were performed using fruits collected from a commercial orchard located in the South of Portugal (Almansil-Algarve). They were harvested in 1998, on March 18th, April 12th, April 21st and May 16th.

2.1. tissue chemical composition analysis

The chemical components of the flavedo were analysed using stained and unstained epicarp disks (1.86 cm^2 each) from fruits collected in only one of the mentioned days. Acyl lipids from the pitted and unpitted tissue of the epicarp were extracted and quantitatively spotted onto Silicagel 60 plates to separate the polar lipids in acetone/benzene/water (91:30:8 v/v/v) as solvent. The plates were then sprayed with 1% 8-anilinonaphtalene sulphonic acid in methanol and viewed under UV light at 254 and 366 nm [14]. Ten disks (1.86 cm^2 each) of the epicarp from four fruits were used.

The phospholipids were scrapped from the thin layer chromatography (TLC) plates and quantified with an 5000 atomic absorption spectrophotom attached to a UV-VIS Spectrophotometer Hitachi Perkin-Elmer 139 [15].

The concentration of soluble protein from the epicarp tissue was determined with the Folin reagent [16]. SDS– PAGE electrophoresis of extracted protein was carried out from the epicarp [17], plotted with a discontinuous buffer system 0.5 mm thin precast polyacrylamide 8–18 gradient gel (Excel-Gel SDS) and the ExcelGel SDS buffer strips.

Protein separation was carried out at 200 V, 50 mA, 30 W with a cooling temperature of 15 °C in a Pharmacia Multiphor II electrophoresis unit with an IEF electrode holder, using an Electrophoresis Power Supply EPS 3500 XL. Molecular weights were estimated using BSA (68 kDa), ovalbumin (43 kDa), trypsin inhibitor (20.1 kDa) and cytochrome c (12.4 kDa), as markers. After electrophoresis, the gel was silver stained and scanned in the phastimage (Pharmacia Fine Chemicals) with a 546 nm filter. Following Hayakawa and Oizumi [18], aminoacids were additionally quantified after isocratic separation by reversed phase of high pressure chromatography. A Nucleosil 5 C18 250 × 4.6 mm column and an eluent mixture containing acetonitrile:water (40:60 v/v) with 0.1% TFA (at a flow rate of 1.0 mL·min^{-1}) were used. Detection was set up at 269 nm. Electrolytic conductance was determined on epicarp disks using a Crison 522 conductimeter following the modified method of Ketchie [19]. Three disks were placed in an erlenmeyer flash with 20 mL deionized water incubated for circa 30 min and then the absolute conductance measured. The disks were then boiled for 7 min, the volume of deionized water brought back to 29 mL and the electrolytic yield obtained was taken as 100%. The results are expressed on a percentage basis.

Starch was quantified from the epicarp following McCready et al. [20] and several other soluble carbohydrates were quantified after isocratic separation by ionic change high pressure chromatography [21]. A Spherisorb NH2CARB 10 µm column and an eluent mixture containing acetonitrile:water (77:23 v/v) at a flow rate of 0.9 mL·min^{-1} were used.

Lipid peroxidation was measured by quantifying the malondialdehyde compounds (MDA) formed by thiobarbituric acid reaction [22]. Ethylene evolution was quantified by gas chromatography [23], using flasks of 33 mL, sealed with serum caps. Total carotenoids [24] and chlorophyll (Chl) [25] were extracted and quantified spectrophotometrically.

2.2. fruit internal quality analysis

The studies of the internal quality of the fruits were performed using stained and unstained fruits collected at all the mentioned dates. In the last three harvest dates, the colour of the epicarp of ten fruits was also evaluated with a Minolta CR-300 colorimeter. The measuring CIE 1976 L a b colour system was used. L indicates the luminosity variation; a and b, the chromatic variables, indicate, respectively, the variation from green (–) to red (+) and the variation from blue (–) to yellow (+).

Juiciness, titrable acidity and total soluble solids (TSS) of juice were determined as described by Alavoine et al. [26]. Starch of the edible portion of the fruit was quantified as described by Nielson [27]; total and reducing sugars were determined as described by Sumner [28]. Quantification of ascorbic acid from juice was measured by titration using 2,6-dichlorophenol-indophenol reaction [29]. Ten fruits were used for juiciness analysis. Samples of five fruits with two replicates were tested for titrable acidity, TSS, starch, total and reducing sugars and ascorbic acid. TSS were expressed by Brix degree, titrable acidity was expressed by g·L^{-1} citric acid and all other internal quality parameters are expressed in relation to edible portion weight.

Statistical analysis was performed using a one way Anova for all the data except ethylene data and internal quality parameters which were studied with a two way Anova.

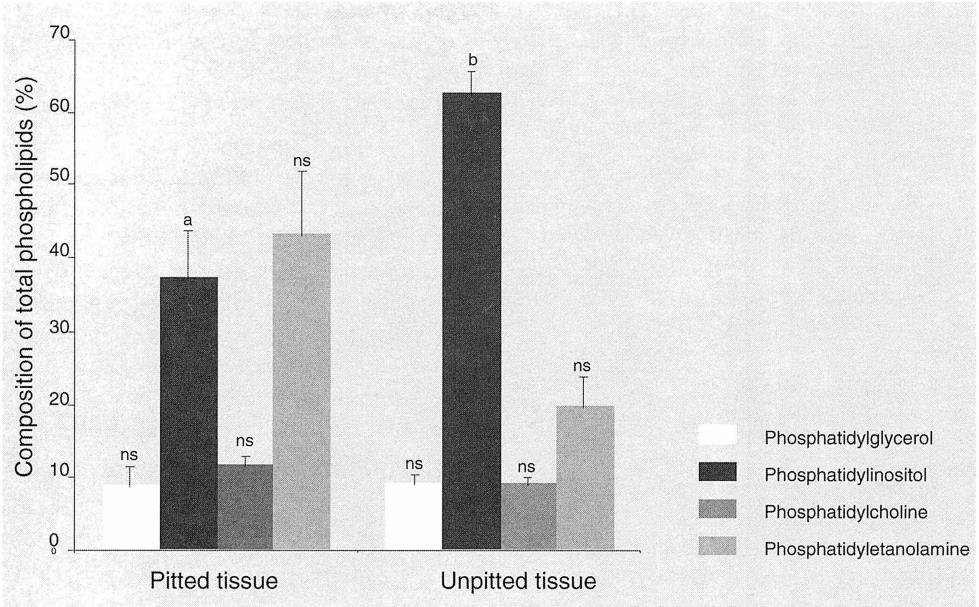

Figure 1.
Concentration of phospholipids of pitted and unpitted tissues of the epicarp of 'Encore' mandarin (ns: means not significantly different).

3. results

3.1. tissue chemical composition

The percentage of phosphatidylinositol was significantly lower in dark stained epicarp than in the tissue without pitting (*figure 1*). The percentage of phosphatidylglycerol and phosphatidylcholine did not vary significantly, neither did phosphatidylethanolamine in spite of apparent lower values in the unpitted epicarp (*figure 1*).

The level of soluble protein were significantly higher in the pitted tissue than in the unpitted one (*table I*). The study of the cellular polypeptides revealed quantitative and qualitative differences (*table II*). To make quantitative comparisons, the height of the 32.6/25.2 kDa peak was kept constant in the different tracings. The major quantitative changes in the pitted tissues were the appearance of a new polypeptide band with an apparent molecular mass of 50.4 kDa as well as the disappearance of another one having 10 kDa. In addition, a significant change of the aminoacid composition was also found (*table III*). On a percentage basis, Gln, Thr, Asp, Glu and Gly were significantly higher in the dark stained tissue, the opposite trend is observed with Ala and Tyr. The electrolytic conductance of the tissue associated to these stains increased significantly (*figure 2*). Starch content was significantly lower in the pitted tissue (*table I*), while the other soluble carbohydrates did not show any significant change (*table IV*). The malondialdehyde compounds which measure the lipid peroxidation were significantly higher in the pitted tissues, whereas the patterns for ethylene production revealed different linear regressions (*table I*). The level of carotenoids was significantly lower in the pitted tissues and chlorophyll contents also sharply decreased (*table I*).

3.2. fruit internal quality

On the studied period, the internal quality characteristics of stained and unstained fruits did not show any significant differences (*table V*).

By comparing the harvest dates with each other, it was found that only the titrable acidity showed significant differences. In March, both samples showed values close to 5 $g \cdot L^{-1}$ citric acid. On stained and unstained fruits, this parameter sharply increased in the beginning of April. This value (circa 9 $g \cdot L^{-1}$) decreased by the end of the month, yet, the differences with April

Table I.
General biochemical composition of the pitted and unpitted tissues of the epicarp of 'Encore' mandarin. Results are expressed by g of tissue dry weight.

Tissue type	Protein ($mg \cdot g^{-1}$)	Lipid peroxidation ($pmol \cdot g^{-1}$)	Ethylene production ($pmol \cdot g^{-1}$)		Starch ($\times 10^{-6}$ $mg \cdot g^{-1}$)	Pigments ($\mu g \cdot g^{-1}$)			
			6 h after incubation started	26 h after incubation started		Chlorophyll *a*	Chlorophyll *b*	Chlorophyll *a* + *b*	Total carotenoids
Pitted tissue	277 ± 10.0 a	11.79 ± 0.28 a	13.00 ± 0.18 a	350.0 ± 36.0a	35.73 ± 2.14 a	42.5 ± 18.8 ns	36.7 ± 29.8 ns	92 ± 30 ns	719 ± 10 a
Unpitted tissue	174 ± 6.3 b	10.25 ± 0.25 b	23.50 ± 2.45 b	63.5 ± 6.7 b	53.65 ± 2.34 b	75.0 ± 28.0 ns	89.4 ± 52.0 ns	138 ± 53 ns	1163 ± 32 b

Tissue type	Ethylene linear regression
Pitted tissue	$Y = 28.207 x + 3.067$, $R^2 = 0.9770$
Unpitted tissue	$Y = 51.808 x + 4.575$, $R^2 = 0.9764$

Each value represents the mean ± standard error.
a, b : significant differences between the pitted and unpitted tissues, in a multiple range analysis for 95% confidence level (Tukey test).
ns : means not significantly different.

were not significant. At the end of that month, the titrable acidity was also similar to that of March. The values obtained in May did not show any significant differences from the values previously measured.

Colour parameters found in April 4th showed significant differences from the next harvest dates (*table VI*). The last harvest measurement showed a new significant difference on b axis. Indeed, significant differences were observed on the colour of fruits on the harvest period, particularly in b axis which indicates a progressive increase on yellow colour.

Table II.
Polypeptide composition of stained and unstained tissues of the epicarp of 'Encore' mandarin.

Polypeptide molecular weights of the bands (kDa)	Optical density		Relative electrophoretic mobility
	Pitted tissue	Unpitted tissue	
105.2 – 96.0	1.805	1.856	0.376 – 0.399
50.4	0.805	Not detected	0.564
46.3 – 42.2	0.202	0.307	0.586 – 0.610
26.5 – 21.6	1.138	0.747	0.729 – 0.787
25.1 – 20.2	Below detection limits	0.702	0.742 – 0.799
23.7 – 18.5	Below detection limits	0.980	0.757 – 0.814
21.1 – 17.2	0.754	1.562	0.787 – 0.840
10.8 – 10.3	0.459	0.199	0.958 – 0.971
10	Not detected	0.180	0.975 – 0.985

Table III.
Aminoacid composition (%) of pitted and unpitted tissues of the epicarp of 'Encore' mandarin.

Aminoacid	Pitted tissue	Unpitted tissue
His	5.1 ± 0.41 ns	1.0 ± 0.04 ns
Arg	3.1 ± 1.59 ns	0.8 ± 0.15 ns
Asn	3.2 ± 1.13 ns	0.3 ± 0.06 ns
Gln	9.1 ± 0.74 a	0.3 ± 0.07 b
Ser	2.1 ± 0.29 ns	1.6 ± 0.39 ns
Thr	4.3 ± 0.16 a	0.2 ± 0.01 b
Asp	51.2 ± 1.51 a	0.8 ± 0.15 b
Glu	15.0 ± 1.26 a	0.5 ± 0.11 b
Gly	0.4 ± 0.01 a	0.1 ± 0.02 b
Ala	0.5 ± 0.12 a	15.8 ± 1.74 b
Tyr	6.0 ± 1.30 a	78.7 ± 2.51 b

Each value represents the mean ± standard error.
a, b : significant differences between the fruits with and without stains, in a multiple range analysis for 95% confidence level (Tukey test).
ns : means not significantly different.

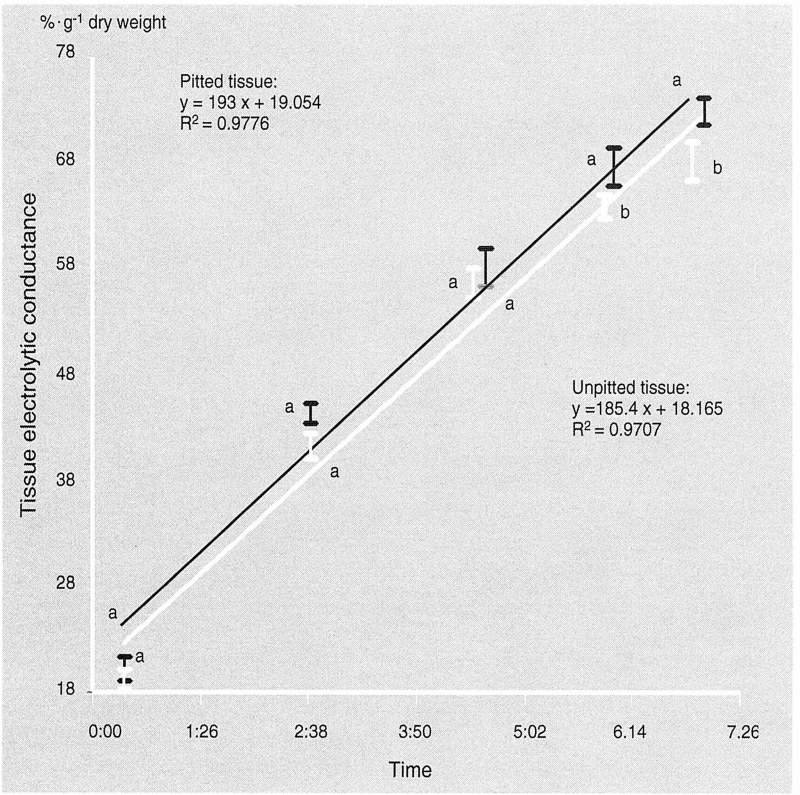

Figure 2.
Electrolytic conductance in pitted and unpitted tissues of the epicarp of 'Encore' mandarin.

4. discussion

The observed variations of membrane acyl lipids associated to a possible alteration of the unsaturation of the fatty acids may have affected the fluidity characteristics of the cellular membranes of the dark stained tissue [7]. This effect is a recognised symptom in Kiyomi tangor pitting, which has a quite similar epicarp physiological disorder [7]. Associated to these alterations, the polypeptides and aminoacids separation further indicated the occurrence of qualitative and quantitative variations of the protein patterns. The inhibition of the synthesis and/or degradation of a 10 kDa protein and the appearance of that with a 50.4 kDa was the most relevant change. The significant increase of the hydrophobic aminoacids concentration with uncharged polar R groups (namely, Gly, Thr and Glu) as well as the decrease of the Tyr levels could also be the result of mechanisms which trigger the cross-linking of proteins. Indeed, the new 50.4 kDa protein band could result directly from the mentioned cross-linking or, in spite of that, could be the result of a synthesis de novo. The variation of the membrane acyl lipids, protein patterns and aminoacids content might also be responsible for the alteration of the planar conformation of the lipid bilayer. This alteration may block the formation of concave curvatures in the membranes, which are absolutely necessary to stabilise the bilayer as a sealed unit. Thus, this is a main factor affecting membrane permeability [30]. In fact, as in Marsh grapefruit and Vila Franca lemon affected by chilling injury [31], the permeability of cellular membrane of the epicarp was altered in the cells closely associated to the dark stains. This membrane alteration in pitted epicarp was visible through the significant differences on the electrolytic conductivity detected 5 h after incubation. Purvis [32] suggests that membrane stabilisation can be associated to reducing sugar metabolization. However, the data of this work does not allow that conclusion since the differences on reducing sugar content between pitted and unpitted epicarp are not significant. Indeed, the analysis of sugar content only showed a sig-

Table IV.
Carbohydrates concentration (mg·g^{-1} tissue dry weight) of pitted and unpitted tissues of the epicarp of 'Encore' mandarin.

Tissue type	Ribulose	Ribose	Xylose	Fructose	Glucose + galactose	Sucrose
Pitted tissue	72.0 ± 5.1	51.2 ± 19.9	17.4 ± 6.4	21.7 ± 8.8	31.9 ± 13.5	10.5 ± 3.5
Unpitted tissue	63.8 ± 13.8	10.9 ± 4.02	5.5 ± 3.2	9.4 ± 4.6	21.2 ± 9.3	7.1 ± 2.7

Each value represents the mean ± standard error.
Means are not significantly different.

nificant variation of starch concentration. The sharp decrease of the pool of carbohydrates suggests an increase of energy consumption in pitted tissues. In fact, when plants are submitted to biotic or abiotic stresses, carbohydrates are mobilised, resulting in an increase of reducing sugars. These sugars are used as respiration substrate, stimulating glycolysis.

The variation of the permeability of cellular membranes is a physiological indicator associated with the mentioned alterations of membrane structure and, in addition, seems to be related to the variation of lipoperoxides. This last parameter is a determinant factor mediating senescence in pitted tissues. The increase in malondialdehyde compounds is the result of the lipoperoxidation of acyl lipids, which revealed a deregulation in the redox balance of the cell. When cells cannot perform the convenient condition to keep the redox steady-state, the lipoperoxidation starts. This cyclic process initiates by a reactive oxygen species, which sharply increases the oxidative damage in the presence of molecular oxygen. During the fruit ripening, the chlorophyll degradation parallels with carotenoids accumulation [13]. In the pitted

Table V.
Internal quality parameters measured in stained and unstained fruits (stained and unstained refers to fruits with stained or unstained epicarp, respectively).

Harvest date	Tissue type	Juiciness mL·100 g^{-1}	TSS º Brix	Titrable acidity g·L^{-1} citric acid	Reducing sugar g (glucose)·100 g^{-1}	Total sugar g (glucose)·100 g^{-1}	Starch g·100 g^{-1}	Vitamin C mg·100 g^{-1}
18 March	Stained	65.4 ± 3.32	10.8 ± 0.10	5.2 ± 0.00 b	4.1 ± 0.25	11.3 ± 0.53	36.0 ± 0.94	10.6 ± 1.58
	Unstained	60.9 ± 2.64	12.9 ± 0.10	5.2 ± 0.00 b	4.9 ± 0.14	13.1 ± 0.22	47.6 ± 1.43	8.6 ± 1.26
12 April	Stained	67.7 ± 1.65	12.9 ± 0.44	8.8 ± 0.25 a	5.0 ± 0.26	12.6 ± 0.30	54.1 ± 1.69	6.6 ± 0.21
	Unstained	62.3 ± 3.60	13.6 ± 0.68	8.9 ± 0.17 a	4.6 ± 0.30	12.9 ± 0.19	47.1 ± 1.27	8.4 ± 0.35
21 April	Stained	70.7 ± 3.26	10.9 ± 0.59	7.7 ± 0.09 a	3.4 ± 0.35	10.2 ± 0.60	50.6 ± 1.18	8.4 ± 0.86
	Unstained	71.9 ± 2.05	10.9 ± 0.03	6.7 ± 0.05 ab	2.7 ± 0.07	10.7 ± 0.11	48.3 ± 2.85	22.5 ± 1.10
16 May	Stained	70.2 ± 2.00	14.0 ± 0.78	7.2 ± 0.39 ab	4.3 ± 0.12	13.5 ± 0.06	62.0 ± 3.40	13.1 ± 0.54
	Unstained	74.5 ± 1.36	12.4 ± 0.43	7.4 ± 0.05 ab	3.8 ± 0.11	12.2 ± 0.16	58.8 ± 1.49	15.8 ± 0.78

Each value represents the mean ± standard error.
a, b : significant differences between the harvest data for the stained or the unstained tissues, in a multiple range analysis for 95% confidence level (Tukey test).
Means without letter a or b are not significantly different.

Table VI.
Epicarp colour of pitted and unpitted fruits. Stained and unstained refers to fruits with stained or unstained epicarp, respectively. The measuring CIE 1976 L a b colour system was used. L indicates the luminosity variation; a and b, the chromatic variables, indicate, respectively, the variation from green (–) to red (+) and the variation from blue (–) to yellow (+).

Harvest date	Epicarp type	L*	a*	b*
12 April	Stained	57.1 ± 0.43 a	36.1 ± 0.64 a	52.4 ± 0.63 a
	Unstained	56.8 ± 0.50 a	37.2 ± 0.61 a	52.1 ± 0.64 a
21 April	Stained	60.0 ± 0.42 b	32.3 ± 0.87 b	59.7 ± 0.64 b
	Unstained	60.0 ± 0.42 b	32.3 ± 0.87 b	59.7 ± 0.64 b
16 March	Stained	59.6 ± 0.64 b	30.7 ± 1.18 b	59.0 ± 0.78 c
	Unstained	59.1 ± 0.49 b	32.1 ± 0.85 b	58.6 ± 0.61 c

Each value represents the mean ± standard error.
a, b,c : significant differences between the harvest data for the stained or the unstained epicarps, in a multiple range analysis for 95% confidence level (Tukey test).

epicarp of Encore mandarin, the observed reduction of carotenoids probably triggered an increasing rate of the photooxidative stress [1]. With a minor protection, caused by a decrease on carotenoids content, the epicarp could not resist to some biotic or abiotic stress. Concomitantly to the degradation of the acil lipids, the integrity of cellular membranes was affected as well as, to a limit, the protein content. Nevertheless, in spite of the increased senescence rate of the pitted tissue, the ethylene evolution, as on pitted Kiyomi tangor [8], showed a decreasing rate. This process strongly suggests an incomplete degradation of fatty acids, and, therefore, probably an increasing formation of alkoxy or ethyl radicals [33].

According to the study of the quality parameter, it must be pointed that, as previously reported [13], the values found for reducing sugar in Encore mandarin varied between 2.7 and 5% (fructose and glucose being the most frequent reducing sugar in citrus fruits), whereas the values of vitamin C (circa 11.7 mg·100 g^{-1}) were lower than the values found by Wills et al. [13] or by Ferreira and Graça [34] for mandarin (40 and 23–40 mg·100 g^{-1}, respectively). However, the physiological alterations of the pitted epicarp did not show appreciable changes on internal fruit quality of citrus. The results show that, during the marketable period of the fruits, appreciable differences were not found on the physiological indexes associated with maturation, and fruits chemical and internal quality was not affected by the preharvest peel disorder, even if the fruit remained in the tree during the ripening period.

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Manchas sombrías en el flavedo de la mandarina Encore en vergel: composición química de los tejidos e implicaciones sobre la calidad interna de la fruta.

Resumen — Introducción. Manchas sombrías afectan frecuentemente el epicarpio de la mandarina Encore. Esta anomalía se caracteriza por una alteración de la estructura de las células. Se estudiaron los tejidos concernidos por este problema a partir del análisis de parámetros bioquímicos y se relacionaron las alteraciones observadas con la calidad interna de las frutas afectadas. **Material y métodos**. La composición bioquímica de tejidos del flavedo fue estudiada a partir del análisis de epicarpios con o sin manchas sombrías. Se evaluaron la calidad de la parte comestible de la fruta y su jugo. **Resultados**. Los tejidos alterados presentaron una conductancia electrolítica significativamente más elevada que la de los tejidos normales. Asimismo, el comportamiento de ciertos polipeptidos y la concentración de ácidos aminos variaron significativamente. La concentración en radicales hidroxilos, en carotinoides y en lipoperóxidos acilados aumentó significativamente, mientras que la producción de etileno seguía distintos tipos de regresión lineares. La calidad de las frutas presentando un epicarpio anormal no se alteró significativamente. **Discusión**. El desorden fisiológico del flavedo de la mandarina Encore fue caracterizado mediante un brusco aumento de la velocidad de senescencia de ciertas células del epicarpio. Los tejidos manchados presentan una degradación incrementada de la membrana celular debido a un metabolismo que parece ser afectado por modificaciones oxidativas que se producen en ciertos componentes de la célula. Sin embargo, esta anomalía del epicarpio no surte efecto significativo en la calidad interna de la fruta. © Éditions scientifiques et médicales Elsevier SAS

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