Journal of Applied Botany and Food Quality 82, 1 - 8 (2008)

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Quality of two table grape guard cultivars treated with single or dual-phase release SO_2 generators

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(Received September 3, 2007)

Summary

Berries of seeded table grapes (cultivars Napoleón and Aledo) were trimmed in commercial packing houses, inoculated with *Botrytis cinerea*, packed with single and dual-phase release SO_2 pads, and stored for up to four months at 0 ºC and 85±5 % relative humidity. Control grapes with or without inoculation, but without SO_2 pads, were stored for up to 2 months. *Botrytis cinerea* rot (grey mould) limited the shelf-life of Aledo grapes to one month, while a two month shelf-life was established for Napoleón grapes, which suffered from berry splitting, *Cladosporium herbarum* and *Botrytis cinerea* rots. Yeasts of the Candida genera and secondary fungi were also identified in fruit suffering sour rot after 2 months at 0 ºC. No differences in grey mould development in the treatments without SO_2 pads with or without inoculation. The native grey mould of these grapes was purified and included as the *B. cinerea* strain 20248 in the Spanish Type Culture Collection. Storage time, but not SO_2 pads, slightly affected fruit quality. During the first month at 0 ºC, total soluble solids decreased by 1 ºBrix in 'Áledo' and berry hardness temporarily increased by around 30% in both cultivars. The pads provoked an SO_2 taste acceptable for consumption, which was slightly higher in Napoleón than in Aledo grapes. The dual-phase release SO_2 pads showed better performance for the long-term storage of grapes than single-release pads (both as regards berry sensory attributes and stem appearance, with lower stem browning). The dual-phase release SO_2 pads extended the shelf-life of grapes by around 1 month, depending on the cultivar. Napoleón grapes showed a better potential for long-term storage than Aledo grapes due to thicker and more compact epidermis, thicker cell walls, and different epidermal microstructure including the transition cells between epidermis and the parenchyma.

Introduction

Botrytis rot (grey mould) caused by *Botrytis cinerea* Pers (ex Fr.) is the main decay suffered by table grapes during cold storage (CRISOSTO et al., 2002a; NELSON, 1985; SNOWDON, 1990). Other fungi, yeasts and bacteria may also cause decay particularly in wounded berries. However, they develop more slowly than Botrytis rot (CRISOSTO and MITCHELL, 2002; FRANCK et al., 2005; SNOWDON, 1990; ZAHAVI et al., 2000).

Storage of table grapes at 0 ºC requires the use of a coadjutant to extend their shelf-life; for example grape guard pads, with or without previous SO_2 fumigation (5000-7000 $\mu L \cdot L^{-1}$ for 1 min in each single package, or cyclical applications every 10 d), with or without controlled or modified atmospheres (ARTÉS-HERNÁNDEZ et al., 2006; CRISOSTO et al., 2002a; PRETEL et al., 2006). The application of sodium metabisulphite ($Na_2S_2O_5$) in the form of granular or powdered salts releases fungistatic SO_2 in contact with moist air. This may kill some spores (including *B. cinerea)* and have a fungistatic effect on the grape bunch, but some fungi and yeasts are resistant to SO_2 (ADAMS and MOSS, 2000; CRISOSTO and MITCHELL, 2002; CRISOSTO et al., 2002b; PALOU et al., 2002).

SO_2 absorption and possible damage depend on the grape cultivars (LAGUNAS-SOLAR et al., 1992; PALOU et al., 2002; ZHANG et al., 2003). The generation of H_2SO_3 and H_2SO_4 acids by the pads may induce fruit and stem bleaching (CRISOSTO and MITCHELL, 2002; DEL SOLAR et al., 2000; SNOWDON, 1990), particularly when fast-releasing SO_2 pads are used (ZOFFOLI et al., 2001). Because of the impact of sulphites may have on human health, the EU and US regulations permit a maximum total SO_2 residue value for table grapes of 10 mg $SO_2 \cdot kg^{-1}$ on a fresh weight basis (EPA, 1989; EU DIRECTIVE 2006/52/CE). The use of physical or chemical SO_2 alternatives alone or as refrigeration coadjutants have been proposed (ARTÉS-HERNÁNDEZ et al., 2006; CRISOSTO et al., 2002a; NIGRO et al., 1998). However, SO_2 pads are still used due to their efficacy, easy of use and affordable cost, particularly for white table grapes consumed on New Year's Eve in Spain (PRETEL et al., 2006). Traditional SO_2 pads have been replaced by dual-phase pads to ensure a regular SO_2 supply to avoid the spread of *B. cinerea* without increasing sulphite residues, and without compromising fruit quality or shelf-life.

The goal of this research was to evaluate berry quality (particularly decay and other quality losses) in two cultivars using single and dual-phase release pads to enable us to recommend the best option for commercial purposes. The link between grape epidermis microstructure and cultivar response were also studied.

Material and methods

Plant material and experimental design

Bunches of red and white skin seeded table guard grapes (*Vitis vinifera* L. cultivars Napoleón and Aledo, respectively) attached to the plant were covered with paper to prolong their shelf-life. Both cultivars were harvested on November 4th (Napoleón grapes in Abarán, Murcia, Spain; Aledo grapes in Novelda, Alicante, Spain). In the commercial packing house, berry bunches (extra class quality according to EU regulation 2789/1999 and amendments) were trimmed, classified and bunch packed (IMAL, 2001) as follows: the bottom and sides of a cardboard box were lined with a perforated polyethylene (PE) bag (Plásticos del Segura S.L., Abarán, Murcia, Spain), in such a way that it could be later folded over grapes. To absorb humidity inside this bag, the bottom of the box contained a paper board covered by corrugated cardboard, and on both sides of the box tissue paper. Each box contained eight to ten bunches of grapes individually packed in carry bags (around 4.5 kg grapes in total). Folded tissue paper was placed on top of the carry bags, and a generator with the printed side up, followed by another corrugated carton.

Both cultivars were treated with paper/PE pads containing $Na_2S_2O_5$ (40/15 for traditional –T- and 40/20 for long-life- LL- "Uvas Quality" pads; IMAL, Santiago de Chile). The T pads consisted of 20 fast SO_2 releasing cells. LL pads are considered as dual-phase generators because had 20 slow SO_2 releasing cells and 4 fast SO_2 releasing cells, manufactured with natural kraft paper and bleached kraft paper extruded with low density PE, respectively (IMAL, 2001). Napoleón grapes were also treated with a Sys dual-phase pad (J. Pego SL, Aspe, Alicante, Spain). This pad contained 12 slow SO_2 releasing pockets (97.5 % $Na_2S_2O_5$ and 2.5 % inert) and is considered a mid-life

generator. Aledo table grapes were also treated with a Jumbo-Euro (Osku-Vid ©, Osku Spain S.L., Abarán) dual-phase pad (98 % $Na_2S_2O_5$, 32 cells containing a total of 0.6 g fast SO_2 releasing $Na_2S_2O_5$ and the rest of slow SO_2 releasing $Na_2S_2O_5$). Average $Na_2S_2O_5$ active determined (in g per pad) was 5.32±0.1 for J, 4.10±0.02 for T, 6.53±0.08 for LL, and 6.45±0.08 for Sys.

Inoculation with *Botrytis cinerea* and storage

The aqueous sterilized water for inoculation contained 10^5 conidia·mL^{-1} of *Botrytis cinerea* Pers (ex Fr.) strain 2100 obtained from the Spanish Type Culture Collection (CECT, Valencia, Spain). Five bags per box were inoculated by labeling the inoculated area on the berry according to PALOU et al. (2002).

The boxes with $Na_2S_2O_5$ pads were inoculated with *B. cinerea* as described below and labeled with the *i* letter after the letter that indicated the treatment, but a control without inoculation (C) was prepared for both cultivars. Both treatments (C and Ci) were inspected after one or two months of storage, the maximum shelf-life expected (ARTÉS-HERNÁNDEZ et al., 2003; PRETEL et al., 2006). Then boxes were randomly distributed in the pallet and stored at 0±1.5 ºC and 80±5 % relative humidity (RH) for up to 4 months.

Quality trait analysis

The grape bunch stems, sensory quality, weight loss, preliminary decay classification and splitting were assessed every month (n=4 replications) by the same people to standardize quality evaluation. In every inspection date, digital photographs of each treatment were obtained. Samples of grape berries free from defects were transported using top and bottom ice to the laboratory by car (1 hour). The grapes were stored at 1 ºC for analysis the next day.

The overall visual quality of the bunch of each box (including rachis browning lignified or not, deliquescent rachis, dehydration, overall appearance, etc.) was evaluated on a four-point scale (1=poor; 2= limit of marketability; 3=good; 4=excellent) (adapted from ARTÉS-HERNÁNDEZ et al., 2006).

The overall stem appearance included dehydration and stem green color, which was classified on a five-degree scale (adapted from CRISOSTO et al., 2002a), considering 1=healthy, 4=limit of acceptability for stem quality with absence of decay, and 5=unacceptable (stem completely dry and sometimes colonized by yeasts). Stem browning was classified on a similar scale, but with two as the limit of acceptability. The intensity of residual SO_2 detected by tasting the apex of at least three berries was evaluated on a four-degree scale (1=very slight; 2= slight; 3=moderate, limit of acceptability; 4= severe).

Berry taste was classified on a five-degree scale, two being the limit of acceptability (1=fermentation, off flavor, taste of decay; 2=insipid and obvious SO_2 taste; 3=acceptable, but variable, astringent with slight taste of SO_2; 4=acceptable, with slight taste of SO_2; 5=sweet, balanced with the corresponding acidity, absence of SO_2 taste).

Weight loss was evaluated with a balance with ±2 g precision and the results were recorded by reference to fresh weight at harvest. The same system was used for rotten berries with or without splitting that were weighed with a BH-3000 (± 0.1 g precision) balance (Gram Precision S.L., Barcelona). The berries showing the same decay symptoms were weighed and stored in polypropylene bags to be later examined. The fruit inoculated with the 2100 strain was checked after the first month to compare its development with that of native strains. The decay provoked by other fungus genera were identified and classified as reported elsewhere (MARTÍNEZ and FERNÁNDEZ-TRUJILLO, 2007). Sour rot was considered to be due to yeasts. Cracked berries (usually at the peduncle) were classified as splitting (NELSON, 1985), which also included skin bruises, and, rarely, others damages (e.g. in Napoleón grapes SO_2-damage). Total loss included the percentage of fruit affected by either decay and/or splitting. Berry quality trait analysis (total soluble solids or TSS, pH, titratable acidity (TA), color – measured in the skin, flesh or juice –, and the TSS/TA ration) was performed according to ARTÉS-HERNÁNDEZ et al. (2006) and PRETEL et al. (2006).

Cryoscanning electron microscopy

Cross epidermal sections of the fresh berries, skin (with or without ground tissue) side up, were examined by cryoscanning electron microscopy according to FERNÁNDEZ-TRUJILLO and MARTÍNEZ (2006).

Statistical analysis

Two types of analysis of variance were conducted separately for each cultivar. In the first case, the analyses included the control and the model was: $Y_{(j\,k)\ell} = \mu + T_j + t_k + T_j * t_k + \varepsilon_{(j\,k)\ell}$, where $Y_{(j\,k)\ell}$ is the ℓ^{th} replicate observed in the variable under study (ℓ = 1, 2, 3, 4) of the *j* treatment (j = C, Ci, Ti, LLi, Sysi or Ji, the last two only assayed in Napoleón and Aledo grapes, respectively) during *k* storage time t (k=1 or 2 months). The parameter μ is the mean effect and T and t are the mean effects of treatment or time; ε is the error of the model estimated. A similar model was performed excluding the controls to compare exclusively the three pads used per cultivar and in this case t (k=1, 2, 3 or 4 months, the first two the same data as those used by the former model). Data which did not follow a normal distribution were first transformed to their log (sometimes by adding at least 0.01 when 0 values were found), arcsine, or λ root (usually λ=0.5). When the interactions and/or effects of the first or second model (I or II) were significant at $P \leq 0.05$, pooled LSD (P=0.01) was calculated.

Results and discussion

Total losses

Weight loss was greater in Napoleón than in Aledo grapes, with the greatest losses occurring during the first month of storage, with a subsequent linear trend to increase thereafter. Weight loss and splitting followed the same tendency in Napoleón grapes, in contrast with the onset of Botrytis rot (Tab. 1).

Splitting were clearly cultivar dependent. In Napoleón grapes the pads increased splitting during the first month at 0 ºC compared with harvest levels, particularly the Ti single-phase SO_2 release pads (Tab. 1), in agreement with the higher weight loss observed (Tab. 1). Therefore SO_2 does not seem to be responsible for most of the cracks because in this case the damage would accelerate the weight losses (CRISOSTO and MITCHELL, 2002). It is possible that splitting was caused by preharvest environmental factors or excessive pressure on the fruit during the cooling and storage at 0 ºC (NELSON, 1985), as a result of the condensation when RH increased within the box (ZOFFOLI et al., 2001), or due to a negative interaction among SO_2 uptake, skin thickness, and environmental factors. In fact, berry rehardening was observed during the first month of storage in both cultivars (see below). Increased splitting in other grape cultivars under cold storage had no apparent explanation (DEL SOLAR et al., 2000). In the cultivar Aledo, the low prevalence of splitting in Ti or Ji treatments was probably the result of a faster decay development than in Napoleón grapes in the damaged fruit (Tab. 1 and 2; interaction T x t had P=0.07).

Grapes stored with SO_2 pads showed less decay than the controls irrespective of the pad used, in agreement with MORRIS et al. (1992).

Tab. 1: Percentage of weight loss, splitting and decay loss on a fresh weight basis at harvest (w/w) in table grapes from cultivar Napoleón (n=4 boxes) stored up to four months at 0 ºC and 85% relative humidity. Total decay included those reported and some other fungi. Treatments were: C=Control; Ci=Control inoculated with *B. cinerea* ; SYSi, Ti, LLi are different kinds of SO_2 pads. LSDI and LSDII (*P = 0.01*) were calculated for the highest significant interaction of the model containing all the treatments (first and second month) (I), or just the pads during storage (II).

Time	Treatment	Weight loss	Splitting	Total decay	*Botrytis cinerea*	*Cladosporium herbarum*	Sour rot
(months)		(% w/w)					
0	Harvest	-	3.4	-	-	-	-
	C	0.6	4.3	4.0	0.5	3.5	-
	Ci	0.5	3.6	4.7	0.9	3.8	-
1	SYSi	0.7	11.9	0.4	0.1	0.3	-
	Ti	0.6	13.9	1.5	0.4	1.2	-
	Lli	0.6	8.8	0.7	0.1	0.6	-
	C	0.8	4.6	14.8	3.8	11.0	-
	Ci	0.6	3.5	15.0	5.5	9.2	-
2	SYSi	0.7	10.7	3.2	2.0	1.2	0.0
	Ti	0.6	10.4	6.0	1.5	4.0	0.5
	Lli	0.7	9.8	5.0	1.6	2.7	0.7
	SYSi	1.5	12.6	4.5	0.7	2.8	1.0
3	Ti	1.6	12.9	11.5	2.0	6.7	2.8
	Lli	1.9	10.3	7.8	1.7	3.0	3.0
	SYSi	2.1	12.7	7.9	4.7	1.6	1.7
4	Ti	2.0	9.3	10.7	6.2	2.7	1.9
	Lli	2.2	9.8	10.9	6.5	1.9	2.5
LSD I	(*P = 0.01*)	0.2	3.9	0.9	0.3	0.8	0.2
LSD II	(*P = 0.01*)	0.4	1.3	0.7	0.8	0.5	0.5

Tab. 2: Percentage of weight loss, splitting and decay loss on a fresh weight basis at harvest (w/w) in table grapes from cultivar Aledo (n=4 boxes) stored up to four months at 0 ºC and 85 % relative humidity. Total decay included those reported and some other fungi. Treatments were: C=Control; Ci=Control inoculated with *B. cinerea*; Ji, Ti, LLi are different kinds of SO_2 pads. LSDI and LSDII (*P = 0.01*) were calculated for the highest significant interaction of the model containing all the treatments (first and second month) (I), or just the pads during storage (II).

Time	Treatment	Weight loss	Splitting	Total decay	*Botrytis cinerea*	*Cladosporium herbarum*	Sour rot
(months)		(% w/w)					
0	At harvest	-	10.3	-	-	-	-
	C	0.6	1.9	12.2	6.0	6.2	-
	Ci	0.5	1.0	6.3	4.4	1.8	-
1	Ji	0.7	10.5	1.2	0.7	0.4	-
	Ti	0.7	10.7	0.5	0.4	0.1	-
	Lli	0.9	7.6	0.7	0.5	0.2	-
	C	0.9	2.2	71.9	66.3	5.6	-
	Ci	1.0	1.2	68.0	63.6	4.4	-
2	Ji	1.0	11.2	7.9	7.6	0.3	-
	Ti	1.0	9.1	9.1	8.9	0.2	-
	Lli	0.8	6.7	8.8	8.3	0.5	-
	Ji	1.6	8.8	20.6	14.8	2.5	3.4
3	Ti	1.6	10.3	15.3	8.7	3.6	3.0
	Lli	1.4	7.6	18.5	13.9	2.6	2.0
	Ji	1.6	5.4	31.3	22.8	2.5	6.0
4	Ti	2.5	5.1	34.7	29.4	2.3	2.9
	Lli	1.6	7.8	24.3	19.9	2.3	2.1
LSD I	(*P = 0.01*)	0.2	2.9	0.8	1.0	0.4	ND
LSD II	(*P = 0.01*)	0.1	4.4	6.1	0.6	0.5	0.50

Botrytis cinerea was the main fungus found in both cultivars, but *Cladosporium herbarum* was also noticeable in Napoleón berries (Tab. 1 and 2). C or Ci stored for 2 months showed fruit developing *B. cinerea* and *C. herbarum* together (around 13 % in Aledo and 1 % in Napoleón grapes) (data not shown). Some of the controls of Napoleón table grapes were maintained at 0 ºC for 3 months and almost 100 % fruit became rotten (data not shown). Grey mould induced berry shatter after 3 or 4 months of storage (in the cultivars Aledo and Napoleón, respectively), resulting in sour rot developing in the stems, caused by yeasts and probably acetic acid bacteria, particularly in Ti and Sysi treatments (Tab. 1 and 2; data not shown). The native species of *Botrytis cinerea* was predominant over the inoculated one. In fact C and Ci showed similar Botrytis rot in both cultivars. The SO_2-pads delayed the onset of Botrytis rot by one month compared with the controls. The higher decay in Napoleón grapes compared with the results of experiments that used less $Na_2S_2O_5$ (3.4 g $Na_2S_2O_5$ per 4.5 kg berries, ARTÉS et al., 2001), was probably due to cross-contamination during commercial processing (the experiment took place in commercial packing houses). The native species of *Botrytis cinerea* isolated from natural grey mould of these grapes was later purified and included as the *B. cinerea* strain 20248 in the CECT.

Sour rot appeared in Napoleón and Aledo grapes treated fruit after 2 or 3 months, respectively (Tab. 1 and 2), sometimes associated to *B. cinerea* or *Cladosporium herbarum* (usually less than 1.4 % of total decay, data not shown). The incidence of sour rot was noticeable compared with *B. cinerea* rot only in Napoleón grapes. Filamentous yeasts were isolated on berries with sour rot and were classified as being of the *Candida* genera. *Candida guilliermondii* has been reported as an epiphytic microorganism on table and wine grapes and has been inoculated to control grape decay caused by *B. cinerea*, *Aspergillus niger* and *Rhizopus stolonifer* (ZAHAVI et al., 2000). However, the population of yeasts, probably including *Candida* sp., increased in our experiment with SO_2 pads as occurs in ultraviolet-C-treated grapes (NIGRO et al., 1998), a treatment used as an alternative to SO_2 because both effectively controls *B. cinerea*. The proliferation of yeasts could be associated with the relative SO_2 tolerance of yeasts (ADAMS and MOSS, 2000) and with competition among *B. cinerea* and other fungi.

Infection by *Botrytis* sp. opens up the way for necrotrophic microorganisms, such as *Penicillium expansum* or *Alternaria alternata*, alone or in combination with *B. cinerea*, *C. herbarum*, *Aspergillus niger*, or bacteria or yeasts, causing sour rot, which is not able to penetrate into the berry unless the skin is injured (MAGYAR and BENE, 2006).

Overall visual quality, stem browning, bunch appearance and organoleptic evaluation

The acceptable shelf-life of untreated Napoleón grapes using overall quality scores was 2 months, and two months more when treated with generators, which showed no significant differences among themselves (Tab. 3). The acceptable shelf-life for untreated Aledo grapes was 1-2 months and one month more using generators or two months more in the case of LLi, which showed the highest scores throughout the experiment (Tab. 4).

Berry astringency was more frequent in Aledo than in Napoleón grapes but varied greatly between bunches and treatments. This characteristic was noticeable in Napoleón grapes treated with Ti after

Tab. 3: Mean overall visual, stem and sensory quality in table grapes from cultivar Napoleón (n=4 boxes of 6-10 bags each) stored for up to four months at 0 ºC and 85 % relative humidity. Treatments were: C=Control; Ci=Control inoculated with *B. cinerea* ; SYSi, Ti, LLi are different kinds of SO_2 pads. The LSDI and LSDII were calculated for the interaction or the most unfavorable effect (P = 0.01).

Time (months)	Treatment[1]	Overall quality[1] (1-4)	Stem browning[2] (1-5)	Stem aspect[2] (1-5)	Sensory SO_2 residues[2] (1-4)	Taste[3] (1-5)
0	At harvest	4.0	1.5	1.3	1.0	5.0
	C	4.0	1.5	1.5	1.0	3.8
	Ci	1.3	1.8	1.3	1.0	3.8
1	SYSi	3.5	1.0	1.5	1.4	4.5
	Ti	3.3	1.3	1.8	1.0	5.0
	Lli	3.0	1.0	2.0	1.0	4.5
	C	3.5	3.4	4.4	1.8	2.5
	Ci	1.3	3.9	4.6	1.0	1.0
2	SYSi	2.0	2.5	3.5	2.6	3.5
	Ti	2.5	1.8	3.0	2.1	4.3
	Lli	3.0	2.0	2.8	1.3	4.3
	SYSi	2.0	2.0	3.8	1.8	4.3
3	Ti	2.0	2.1	4.6	3.6	2.8
	Lli	2.0	2.8	3.8	1.0	5.0
	SYSi	2.5	3.0	4.5	2.1	4.0
4	Ti	2.0	2.8	4.0	2.5	2.5
	Lli	2.5	1.8	3.5	1.8	2.8
LSDI	(*P = 0.01*)		0.8	0.9	1.3	0.3
LSDII	(*P = 0.01*)		0.8	1.0	0.7	0.6

[1] Overall quality scoring scale: 1=poor; 2=limit of marketability; 3=good; 4=excellent.

[2] Stem browning or aspect scores and sensory SO_2 residues scores: 1=very slight; 2=slight; 3=moderate, limit of acceptability; 4=severe.

[3] Taste scores: 1=fermentation, off flavor, taste of decay; 2=insipid and obvious SO_2 taste; 3=acceptable, but variable, astringent with slight taste of SO_2; 4=acceptable, with slight taste of SO_2; 5=sweet balanced with the corresponding acidity, absence of SO_2 taste.

Tab. 4: Mean overall visual, stem and sensory quality in table grapes from cultivar Aledo (n=4 boxes of 6-10 bags each) stored for up to four months at 0 ºC and 85 % relative humidity. Treatments were: C=Control; Ci=Control inoculated with *B. cinerea* ; SYSi, Ti, LLi are different kinds of SO_2 pads. The LSDI and LSDII were calculated for the interaction or the most unfavorable effect (P = 0.01).

Time (months)	Treatment[1]	Overall quality[1] (1-4)	Stem browning[2] (1-5)	Stem aspect[2] (1-5)	Sensory SO_2 residues[2] (1-4)	Taste[3] (1-5)
0	At harvest	4.0	1.8	2.0	1.0	5.0
	C	2.5	3.5	2.3	1.0	2.0
	Ci	3.5	2.9	2.0	1.0	2.0
1	Ji	3.0	3.0	2.0	1.6	3.0
	Ti	3.0	2.6	2.0	1.8	3.8
	Lli	3.3	1.9	2.0	1.0	4.5
	C	1.0	5.0	4.0	1.0	1.0
	Ci	1.0	4.8	4.5	1.0	1.0
2	Ji	1.8	2.5	3.3	1.4	3.0
	Ti	1.8	3.3	4.3	1.8	1.8
	Lli	2.0	2.4	2.8	1.0	4.5
	Ji	1.3	3.1	4.3	2.0	2.3
3	Ti	1.5	3.6	4.5	2.3	3.3
	Lli	1.8	2.6	3.5	1.0	5.0
	Ji	1.7	3.0	4.0	1.0	1.7
4	Ti	1.7	3.6	4.8	1.0	1.5
	Lli	1.0	2.6	2.5	2.0	3.0
LSDI	(*P = 0.01*)	0.9	0.8	0.6	0.7	1.3
LSDII	(*P = 0.01*)	0.8	0.7	1.0	0.6	0.4

[1] The overall quality scores scale was the following: 1=poor; 2=limit of marketability; 3=good; 4=excellent.

[2] The stem browning or aspect scores, and sensory SO_2 residues scores were the following: 1=very slight; 2=slight; 3=moderate, limit of acceptability; 4=severe.

[3] Taste scores: 1=fermentation, off flavor, taste of decay; 2=insipid and obvious SO_2 taste; 3=acceptable, but variable, astringent with slight taste of SO_2; 4=acceptable, with slight taste of SO_2; 5=sweet balanced with the corresponding acidity, absence of SO_2 taste.

4 months and in Aledo grapes treated with Ji after 3 or 4 months (data not shown).

The SO_2 taste was slightly higher in Napoleón than in Aledo grapes but still acceptable (Tab. 3 and 4). The opposite trend was found in the quantitative evaluation of total SO_2 with 3.7 $mg \cdot kg^{-1}$ and 9.5 $mg \cdot kg^{-1}$ in Napoleón and Aledo grapes, respectively, independent of storage time and the generator used (data not shown). This pattern could be due to slight differences in free SO_2 or the differential accumulation of SO_2 within the berry between cultivars, and to the more compact and thicker epidermal microstructure of Napoleón compared with Aledo grapes (Fig. 1). In fact, the SO_2 taken up by table grapes remains primarily as a deposit in the form of sulphite, and free SO_2 is usually very low compared with the total (LAGUNAS-SOLAR et al., 1992).

The inhibition of Botrytis rot by the pads (Tab. 1 and 2) was concomitant with the maintenance of stem appearance and delay in browning, particularly during the second month at 0 ºC (Tab. 3 and 4), in agreement with MORRIS et al., 1992. Though the results varied between storage times, the use of Lli pads generally led to the best stem appearance and lower browning among the pads tested within the same month, followed by Sysi treatment in Napoleón and Ji in Aledo grapes (Tab. 3 and 4). The overall appearance of the grape bunches treated with SO_2 pads was good during the first 2 months of storage, which agrees with the findings of other authors (MANSOUR et al., 1984; MORRIS et al., 1992). The intervarietal differences in SO_2 uptake detected by mouth were also found in other studies (DEL SOLAR et al., 1992), and may be due to individual differences between grape sample as regards size, degree of ripeness, skin lenticel density, etc. (LAGUNAS-SOLAR et al., 1992), as suggested by a comparison of the epidermal microstructure of Napoleón and Aledo grapes (Fig. 1; see below). Curiously, after freezing the berries at -80 ºC for other purposes, Napoleón grape berry resembled to have a typical SO_2 injury (SNOWDON, 1990), and perhaps was the area of the skin with higher SO_2 uptake. The skin discoloration after freezing in Napoleón was not systematically measured, but perhaps may reflect the higher SO_2 observed in some treatments of Napoleón compared with Aledo grapes as detected by sensory analysis (Tab. 3 and 4).

Fruit quality traits

The quality traits determined at harvest in Napoleón and Aledo grapes (mean±SE), respectively were: 7.60±0.4 and 6.75±0.3 g of fruit weight; 17.2±0.3 and 20.5±0.2 ºBrix of soluble solids; 3.44±0.05 and 3.71±0.01 pH units; 58±1 and 60±5 Durofel units of berry hardness; 346±4º and 107±1º of skin H* (D65 illuminant); 0.4± 0.05% tartaric acid of titratable acidity for both cultivars. Each bunch weighed around 400 g, 97 % of which representing the berries, with Aledo having a more compact bunch than Napoleón grapes. At harvest slight berry astringency but no significant differences in overall quality between both cultivars were found (data not shown).

Overall, storage time but not SO_2 pads, affected TSS, TA and pH in each cultivar, in agreement with MORRIS et al., 1992. Neither TA nor colour (skin, pulp or juice) changed during the first month of storage (data not shown). The pH increase of 0.2 units during the second month of storage in Napoleón grapes have been related with increased susceptibility to splitting (Tab. 1). The average TSS decreased by about 1 ºBrix during the first month of storage in Aledo grapes,

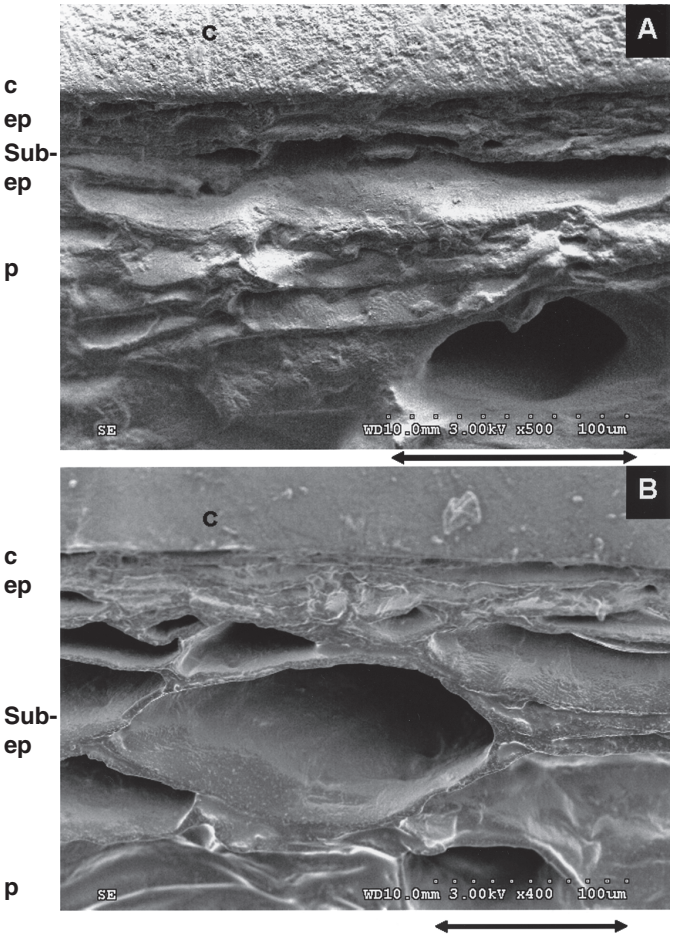

Fig. 1: Cross section of fresh sound epidermal berry tissue from table grape obtained with cryoscanning electron microscopy. Letters indicate cuticle (c), epidermal cells (ep), sub epidermal cells (sub-ep), and parenchyma (p). A. Cultivar Napoleón. B. Cultivar Aledo. Arrows are 100 µm.

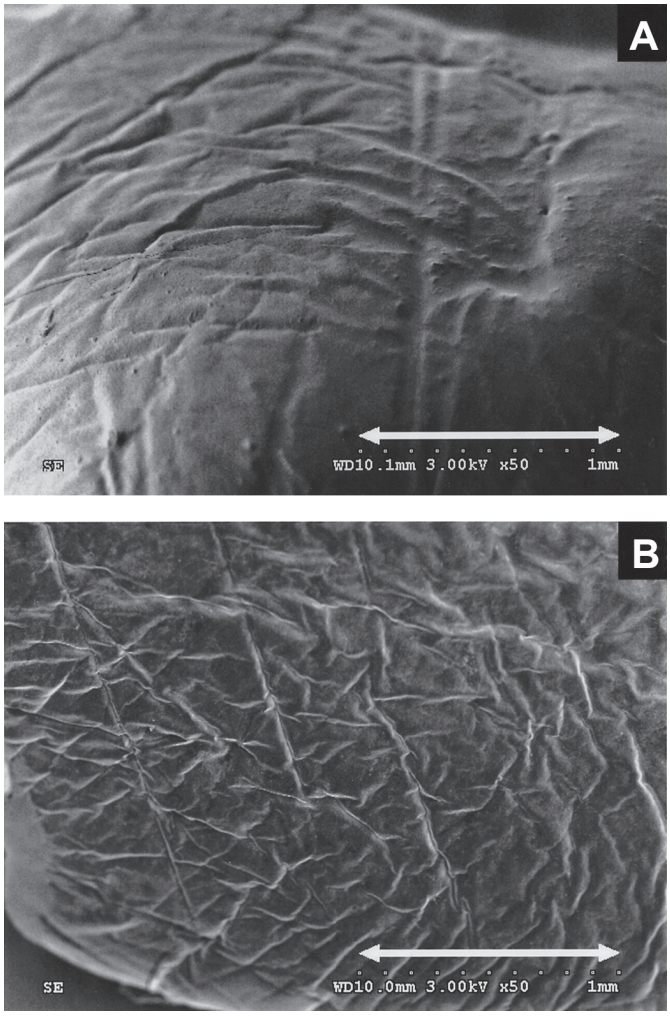

Fig. 2: Cuticle aspect of fresh sound berry from table grape obtained with cryoscanning electron microscopy. A. Cultivar Napoleón. B. Cultivar Aledo. Arrows are 1 mm.

and by 0.6 ^{o}Brix·month^{-1} in the third and fourth month of storage, in Aledo or Napoleón grapes, respectively. In Napoleón grapes, berry hardness increased by 34 % and 25 % during the first month in Napoleón and in Aledo grapes, respectively, to progressively decrease to harvest hardness after four months.

Epidermal microstructure

The cuticle showed a similar wrinkled structure in both cultivars, but slight microcracking was only detected in Aledo grapes (Fig. 2). The epidermal structure of Napoleón red grapes was different from that of the Aledo white grapes (Fig. 1). Splitting was higher in Napoleón grapes (Tab. 1), which had a thicker skin (25±5 µm) than in Aledo grapes (6.5±1 µm) and greater compactness of the epidermal and sub epidermal cells below the cuticle (Fig. 1 and 3). The epidermal thickness of Napoleón grapes was 60-125 µm, and in Aledo grapes was 30-95 µm (Fig. 1). The cell wall of the epidermal cells of Napoleón grapes (4 to 5 µm) was thicker than in Aledo grapes (3.5 to 4 µm). Napoleón grape had round shape of transition cells between the epidermis and the parenchyma (Fig. 1 and 3).

The variability in epidermal cell thickness and in epidermal thickness itself was also evident and could lead to greater local resistance to damages or to SO_2 uptake in different parts of the berry. It could also be responsible for the different degrees of susceptibility to damage and decay between cultivars. The differences in the epidermal layer and grey mould between both cultivars agree with the higher susceptibility of Aledo grapes to *B. cinerea* and the results obtained for other cultivars (Pezet et al., 2003). These authors established that a susceptible cultivar has a thin epidermis without the transition layers between the spongy parenchyma and the cuticle. This higher susceptibility of Aledo grapes could also be due to differences in SO_2 uptake between cultivars (Tab. 4) and sensitivity to SO_2 (Zhang et al., 2003).

Overall these results support the idea that cultivar breeding programs for table grapes should be adapted to local environmental conditions, and aimed at improving postharvest quality, rather than simply doing breeding and postharvest separately, or optimizing well-known postharvest treatments alone (Fernández-Trujillo et al., 2007).

Conclusions

Napoleón showed an approximately one month longer shelf-life than Aledo white grapes, even though Napoleón red berries were more affected by splitting.

The native species of *Botrytis cinerea* (later included as the 20248 strain in the CECT) was predominant over the 2100 strain inoculated.

Dual-phase release pads provided about one additional month of storage for both cultivars. The main fungus found was *Botrytis*

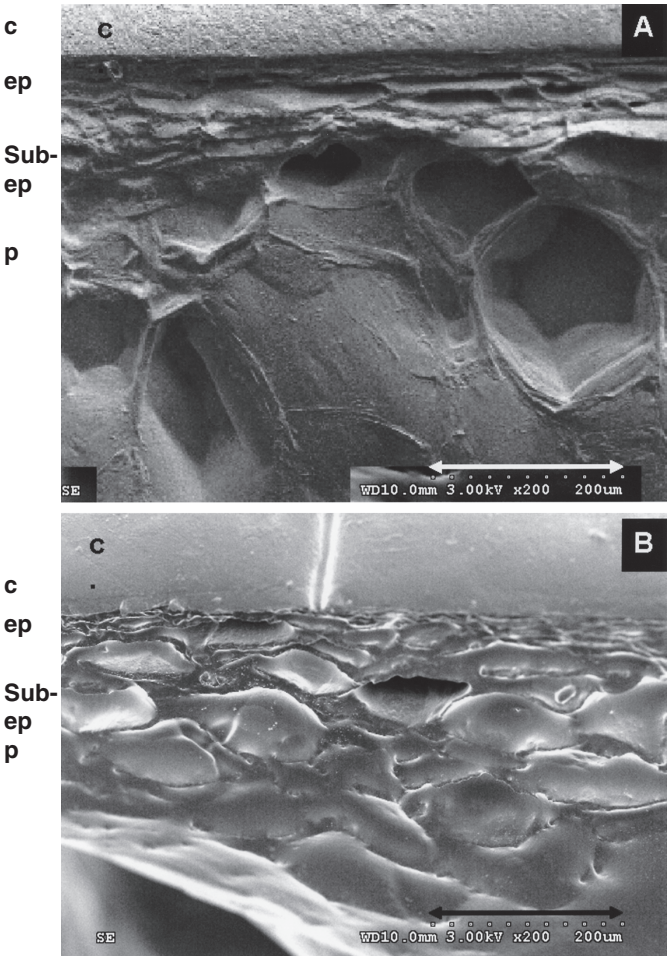

Fig. 3: Cross section of fresh sound berry tissue from table grape obtained with cryoscanning electron microscopy. Letters indicate cuticle (c), epidermal cells (ep), sub epidermal cells (sub-ep), and parenchyma (p). A. Cultivar Napoleón. B. Cultivar Aledo.

cinerea, followed by sour rot (*Candida* sp.) in Aledo grapes and *Cladosporium herbarum* in Napoleón grapes. In the dual-phase release pads, $Na_2S_2O_5$ remained active longer than in the Ti pad, and particularly reduced decay in Aledo grapes. The lower susceptibility to Botrytis rot of Napoleón grapes, but greater susceptibility to splitting, was associated with a thicker and more compact epidermal microstructure, the absence of microcracking, thicker epidermal cell walls, and the round shape of transition cells between the epidermis and the parenchyma. Dual-phase SO_2 release pads can be recommended for storage period of less than 3 months, especially in Aledo grapes. The Sys dual-phase pads can also be used to store Napoleón grapes for 3 months, but the benefits obtained in reducing total decay compared with LL pads are accompanied by other detrimental effects in berry taste, both as regards to overall loss of taste and increased SO_2 taste.

Acknowledgment

This research was funded by the contract 486/IAEA with Plásticos del Segura S.L. (Puente Tocinos, Murcia), and the project PB/22/FS/02 (Fundación Séneca de la Región de Murcia). Javier Obando is indebted to the Ministry of Foreign Affairs of Spain for a MAE-AECI fellowship. Thanks are due to Alberto Alcolea (SAIT-UPCT), Juan Carlos Lavanderos (Uvas Quality, Chile), José Antonio Carrasco and Rafael Cuquerella (Plásticos del Segura S.L.), Mari Carmen Pérez, Ana Vanesa Merlos and José Fermín Moreno for supplying information, materials, transportation, and technical assistance. Our gratitude to Frutas Esther S.A. and Antonio Carrillo e Hijos S.A. (Abarán, Murcia, Spain) to kindly provide the berries, the commercial infrastructure and the personnel to process and store the grapes.

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