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Effects of Preharvest Bagging on Quality of Black Table Grapes

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Abstract: The effects of bagging grapes bunches from a black variety of table grapes (Perla) with cellulose bags on their chemical and sensory properties were studied. This pre-harvest technique, bagging, provided a more uniform color of Perla grapes than non-bagged samples (lower values of the standard deviations of all color coordinates). Experimental data on soluble solids, titratable acidity, maturity index, sugars and organic acids compositions, CIEL*a*b* color coordinates and volatile aroma composition supported the fact that grape ripening was delayed by the bagging operation. Therefore, scientific data confirmed the statements of the Regulating Body of the Designation of Origin "Uva de Mesa Embolsada del Vinalopó" regarding the facts that the pre-harvest bagging technique will increase color uniformity and will delay the ripening process even in a black grape variety, Perla.

Key words: *Vitis vinifera* · ripening · color · aroma · volatile compounds

INTRODUCTION

One of the Protected Designations included within the category of fruits, vegetables and cereals in Spain is "*Uva de Mesa Embolsada del Vinalopó*" (Vinalopó Bagged Table Grape). The geographical area covered includes the middle valley of the river Vinalopó in west central Alicante (Spain).

Only vineyards located in the production area and registered with the Regulating Body may produce grapes to be protected by the Designation of Origin (D.O.). The Regulating Body is the organization which should provide proof of origin by approving production and marketing certificates and carrying out inspections. Only three varieties are included within the P.D.O. "*Uva de Mesa Embolsada del Vinalopó*" Aledo, Italia and Rosetti; all of these three varieties are white and seeded grapes [1]. The protected grapes come exclusively into the "Extra" and "1st Class" categories of European Community Regulation 1730/87.

The climate in Alicante is Mediterranean with a mean temperature of roughly 16°C, plenty of sunshine and less than 300 mm rainfall. The soils are limy deriving from Miocene marls and sandstones [2, 3].

The main production method is espalier vines. After ripening, grape bunches are covered with bags made of cellulose film which remain in place until harvesting. The practice of bagging grapes dates from 1919.

The distinctive fact of bagging grape bunches with cellulose bags provides table grapes with the following advantages, according to the "Regulating Body of this Designation of Origin" [1]:

- Uniform color of the whole bunch of grapes. A uniform color is reached because the grapes ripen protected against the direct incidence of the sunlight.
- Delay in the ripening process, which increases the time grapes may remain in the vine. For instance, if the "bagging" technique is used with the Aledo variety (the one with the late maturation period), bunches can reach New Year's Eve day without any loss of sensory quality. This is of tremendous importance for the Spanish market because each Spanish people eat 12 grapes to wish for happiness during the Coming New Year.
- Increase in the hygiene of the fruit. The grape bunches will be covered by the cellulose bags from July-August until the harvesting; in this way, no pesticides, fungicides, etc. will reach the grapes, at least in a direct way. The physic barrier of the cellulose bag also provides protection of the grapes

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against atmospheric events (e.g. hail) and/or the attack of insects or birds.

However, no scientific studies have been carried out to support these statements. Besides, this Protected Designation of Origin (PDO) is currently confronting some market problems:

- The main one is that only white and seeded varieties are protected.
- The Rosetti variety is not being cultivated by farmers anymore for its high sensitivity to *Botrytis cinerea*.
- Aledo grapes are characterized by a limited sensory quality; this is, its sweetness and the aroma intensities are low, resulting in a tasteless fruit.
- The Italia grapes are of a high sensory quality; however, their limitation are that they will not reach New Year's Eve because they are already ripen at the middle of September and it is quite sensitive to *Botrytis* infections.

Therefore, new varieties are being sought with a high sensory quality and that can be hold in the vines until New Year's Eve without a significant loss of quality. These new varieties should be red ones and if possible seedless. One of the varieties being tested by some farmers is the red but seeded Perla in both methods of production typical of this geographic area, espalier and arbour-trained vines. This variety has some advantages over the current varieties: a) it is a red variety, which will open some markets by answering some of the consumers' demands, b) it provides high production yields and c) it is more resistant to *Botrytis* infections than Italia.

Our objectives were to compare the physico-chemical and sensory quality of control (non-bagged) and bagged table grapes, cv. Perla, in order to find whether scientific data support the statements of the Regulating Body of the Designation of Origin "*Uva de Mesa Embolsada del Vinalopó*" about color uniformity and delay of the ripening process even in a black grape variety, Perla.

MATERIALS AND METHODS

Fruit material: Table grapes (*Vitis vinifera,* cultivar Perla) were kindly provided by the “Cooperativa del Campo Santa María Magdalena”, Novelda, Alicante (Eastern Spain). Twelve bunches per treatment (control samples, without cellulose bags and treated samples, with cellulose bags) were analyzed.

Bunches of Perla grapes were bagged on the first week of August (2003) with cellulose bags. The bags must remain in the vines for at least 70 days. Bags are made of virgin cellulose, satin in the outside, opened at both ends and had a thickness of approximately 5 μm. Each bag was secured by tieing it tightly around the fruit peduncle.

The main characteristics of the Perla grapes are: bunches with a mean weight of 705.5±44.7 g (±values stand for standard error); grapes with a mean weight of 6.12±0.31 g; seeded; dark red color; elliptic form; and high juiciness.

Chemical and sensory analyses: The chemical parameters studied were: a) CIEL*a*b* color using a Minolta CR-300 colorimeter (Minolta, Osaka, Japan); b) titratable acidity (expressed as % of malic acid, the main organic acid) by titration of 1 ml of the sample supernatant with 0.1 N NaOH using phenolphthalein until pH 8.1 [4]; c) soluble solids using a digital refractometer Comecta DR101 (Comecta, S.A., Barcelona, Spain).

Sugars and organic acids were analyzed according to Pérez *et al.* [5]. Briefly, 10 g of tissue were homogenized in a Polytron with 25 ml of cold 95% ethanol for 3-5 min. The sample was centrifuged at 12000 rpm for 20 min and vacuum filter through 2 layers of Whatman #1 paper. The solution was made up to 50 ml with 80% ethanol. Then, an aliquot of 10 ml was taken and dried under a nitrogen stream at 50°C. The residue was dissolved in 2 ml of 0.2 N H_2SO_4 with 0.05% EDTA. The sample was loaded onto an activated Sep-Pak C18 cartridge and the elute collected. The sample was washed through with further 4 ml of the solution. The elute was filtered through a 0.45 μm filter and analyzed by HPLC (Hewlett Packard model 1100 Series) connected to a Photodiode array detector, DAD (HP Model 1100 Series) with an autosampler (HP Model 1100 Series), operated by HP ChemStations software (Hewlett Packard, Waldbronn, Germany). Besides, a refractive index detector, RID (HP Model 1100 Series) was also connected in series with the DAD. Sugars and organic acids were separated by a stainless steel Supelcogel C-610H column (30.0 cm×7.8 mm id) with a Supelguard C-610H guard column (5.0 cm×4.6 mm id) using 0.0085 N H_2SO_4 at a flow rate of 0.4 ml min^{-1}. Detection was at 210 nm for organic acids (DAD) and 250 nm for sugars (RID). Authenticated standards of citric, tartaric, malic and succinic acids and sucrose, glucose and fructose were used for the quantification of the organic acids and sugars, respectively.

Instrumental color data are provided as CIEL*a*b* coordinates, which define the color in a three-dimensional space. L* indicates lightness and a* and b* are the chromaticity coordinates, green-red and blue-yellow coordinates, respectively. L* is an approximate measurement of luminosity, which is the property according to which each color can be considered as equivalent to a member of the gray scale, between black and white, taking values within the range 0-100; a* takes positives values for reddish colors and negative values for the greenish ones, whereas b* takes positive values for yellowish colors and negative values for the bluish ones [6].

For the color measurement, 10 grapes per bunch were selected from two opposite sides of the bunch and at 5 different heights. In this way, color data was the mean of 10 grapes per 12 different bunches per treatment; this is, a total of 120 measurements for each of the two different treatments.

Volatile aroma compounds. For the extraction of volatile compounds from the grapes the method of dynamic headspace described by López *et al.* [7] was used. Analyses were run in duplicate. Each grape sample (200 g of blended grapes) was placed in an Erlenmeyer vessel hermetically closed by a plastic tap. Then, 600 ml of ultrapure water and 150 ml of saturated calcium chloride were added to enable agitation of the samples and to avoid oxidative reactions [8, 9]. Chromatographic nitrogen (Carburos Metálicos, Barcelona, Spain) at a flow rate of 200 ml min^{-1} was passed through for 24 h and the effluent was passed through an ORBO-32 (Supelco, Bellefonte, PA, USA) tube, containing activated charcoal. The volatile compounds were recovered from the adsorbent material with 1 ml of carbon disulfide, CS_2, (Supelco). The solid-CS_2 phases were treated by ultrasonic waves for 5 min. Phases were separated by centrifugation for 5 min at 1000 rpm at 0° C and the supernatant was manually collected using a Pasteur pipette.

The isolation, identification and quantification of the volatile compounds were performed on a Shimadzu GC-17A coupled with a Shimadzu mass spectrometer detector GC-MS QP-5050A. The GC-MS system was equipped with a Supelcowax™-10 column (Supelco, Kyoto, Japan; 60 m × 0.25 mm × 0.25 µm film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 0.8 ml min^{-1} in a split ratio of 1:20 and a program of 70°C for 5 min, from 70 to 230°C at 10°C min^{-1}. Injector and detector were held at 250°C. Mass spectra were obtained by electron ionization (EI) at 70 eV and spectra range of 40 to 450 m/z was used. Identification of compounds was confirmed by comparison of collected mass spectra with those of authenticated reference standards and spectra in the Wiley 229 mass spectra library. In all analyses, a volume of 3 µl was injected.

Statistical analysis: Data from table grapes were examined by analysis of variance using STATGRAPHICS Plus 5.0 software (Manugistics, Inc., Rockville, MD). Wherever F values were significant, Duncan's Multiple Range Test was used to separate of mean effects. Significance was defined at $p = 0.05$.

RESULTS AND DISCUSSION

Soluble Solids (SS), Titratable Acidity (TA) and Maturity Index (MI). The use of cellulose bags significantly decreased both the soluble solids content of Perla grapes (20.6±0.2 and 21.8±0.1 °Brix, bagged and non-bagged grapes, respectively) and the maturity index, defined as SS/TA (29.4±0.5 and 32.7±0.5, bagged and non-bagged grapes, respectively) but significantly increased the titratable acidity (0.70±0.01 and 0.67±0.01% malic acid, bagged and non-bagged grapes, respectively) (Table 1).

The practice of preharvest bagging has been previously used in several fruit crops to delay ripening [10], to reduce splitting and mechanical damage [11] and to improve marketability [12]. However, several authors [11, 13] have reported contradictory results for the effects of preharvest bagging on maturity. For instance, Weaver

Table 1: Effect of bagging grape bunches, cv. Perla, on soluble solids content, titratable acidity and maturity index

Cellulose bag	Soluble solids (°Brix)	Titratable acidity (% malic acid, w/v)	Maturity index
Yes	20.6±0.2[ξ]	0.70±0.01	29.4±0.5
No	21.8±0.1	0.67±0.01	32.7±0.5
ANOVA test			
Cellulose bag	***[†]	***	***
Duncan's Multiple Range Test			
Cellulose bag			
Yes	20.6[‡] b[¥]	0.70 a	29.4 b
No	21.8 a	0.67 b	32.7 a

[ξ]±Values stand for the standard error of the mean. [†] N.S. = not significant F ratio ($p<0.05$); *, ** and ***, significant at $p<0.05$, 0.01 and 0.001, respectively. [‡] Treatment means of the ANOVA test; values are the mean value of 24 replications. [¥] Values followed by the same letter, within the same source of variation, are not significant different ($p<0.05$), Duncan's multiple-range test.

Table 2: Effect of bagging grape bunches, cv. Perla, on sugars concentrations

	Sugars (g kg^{-1})			
Cellulose bag	Sucrose	Glucose	Fructose	Total
Yes	4.1±0.7$^{\xi}$	87.2±2.2	88.1±2.3	179.4±4.8
No	3.8±1.0	90.4±1.6	91.1±1.5	185.4±2.9
	ANOVA Test			
Cellulose bag	NS†	NS	NS	NS
	Duncan's Multiple Range Test			
Cellulose bag				
Yes	4.1‡	87.2	88.1	179.4
No	3.8	90.4	91.1	185.4

$^{\xi}$±Values stand for the standard error of the mean. † N.S. = not significant F ratio ($p<0.05$). ‡ Treatment means of the ANOVA test; values are the mean value of 5 replications

Table 3: Effect of bagging grape bunches, cv. Perla, on organic acids concentrations

	Organic acids (g kg^{-1})				
Cellulose bag	Citric	Malic	Tartaric	Succinic	Total
Yes	0.5±0.1$^{\xi}$	6.7±0.2	6.8±0.3	0.25±0.03	14.2±0.1
No	0.4±0.1	6.9±0.1	6.3±0.1	0.21±0.01	13.9±0.2
	ANOVA Test				
Cellulose bag	NS†	NS	NS	NS	NS
	Duncan's Multiple Range Test				
Cellulose bag					
Yes	0.5‡	6.7	6.8	0.25	14.2
No	0.4	6.9	6.3	0.21	13.9

$^{\xi}$±Values stand for the standard error of the mean. † N.S. = not significant F ratio ($p<0.05$). ‡ Treatment means of the ANOVA test; values are the mean value of 5 replications

and Mccune [13] reported little or no effect on maturation of grapes from 40 varieties of *Vitis vinifera*. This may reflect differences in the type of bag used, fruit stage when it was bagged, duration of fruit exposure to natural light after bag removal (before harvesting) and/or fruit and cultivar specific responses [11].

These experimental data supported the fact that bagging table grapes causes a delay in the ripening process according to the reduction of the maturity index of bagged Perla grapes.

Sugar and organic acid compositions. As expected, the main two sugars found in both types of Perla grapes, bagged and non-bagged, were glucose and fructose (Table 2). However, no statistically significant differences were found between the sugars compositions of these two types of Perla grapes, even though slightly higher total sugars contents were found for the non-bagged samples. Glucose concentrations were 87.2±2.2 and 90.4±1.6 g kg^{-1} f.w. for bagged and non-bagged samples, respectively and fructose concentrations were 88.1±2.3 and 91.1±1.5 g kg^{-1} f.w. for bagged and non-bagged samples, respectively.

The importance of these two sugars, glucose and fructose, in table grape was previously reported by Soulis and Avgerinos [14], who studied the glucose:fructose ratio in table grapes, cv. Razaki and found that it ranged from 1.95 to 1.55. This ratio was significantly lower in the present experiment and had mean values of 0.99±0.01 for both bagged and non-bagged Perla grapes. These low values compared with the literature were indicative of the high sweetness of this cultivar because the sweetness intensity of sugars can be arranged in the following order: glucose< sucrose<fructose [15].

The main two organic acids found in both types of Perla grapes, bagged and non-bagged, were tartaric and malic, as expected. Similar results were reported by Wills *et al.* [16], who found that these two acids dominated in Australian grapes. However, no statistically significant differences were found between the organic acid compositions of these two types of Perla grapes, even though slightly higher total organic acids contents were found for the bagged samples (Table 3).

The slightly lower content of sugars and at the same time higher content of organic acids in bagged Perla grapes compared to non-bagged grapes seems to imply a slight delay in the ripening process caused by the practice of preharvest bagging.

Color. The L*a*b* color space is presently one of the most popular color space for measuring object color and is widely used in virtually all fields [6]. Table 4 shows the effect of using cellulose bags for covering grape bunches on the lightness, L*, redness, a* and yellowness, b*, chroma (C*) and Hue angle (h_{ab}) of black table grapes, cv. Perla.

All five color coordinates were statistically affected by the use of the cellulose bags. The bagged grapes presented higher values of lightness and Hue angle but lower values of redness, yellowness and chroma. Therefore, the non-bagged grapes were slightly darker (L* = 28.59±0.09), more red (a* = 0.97±0.03), with a higher intensity of color (C* = 1.32±0.02) but were less blue (b* =-0.85±0.02) than bagged samples (L*=28.81±0.06; a*=0.52±0.02; b*=-1.04±0.02; C*=1.19±0.02). These experimental results implied that the bagging technique slightly delayed the ripening process of this black grape cultivar, Perla.

Table 4: Effect of bagging grape bunches, cv. Perla, on the CIEL*a*b* color coordinates, chroma and hue angle

Cellulose bag	Color coordinates L*	a*	b*	C*	h_{ab}
Yes	28.81±0.06[ξ]	0.52±0.02	-1.04±0.02	1.19±0.02	91.07±0.02
No	28.59±0.09	0.97±0.03	-0.85±0.02	1.32±0.02	90.73±0.03
			ANOVA Test		
Cellulose bag	*[†]	***	***	***	***
			Duncan's Multiple Range Test		
Cellulose bag					
Yes	28.81[‡] a[¥]	0.52 b	-1.04 b	1.19 b	91.07 a
No	28.59 b	0.97 a	-0.85 a	1.32 a	90.73 b

[ξ]±Values stand for the standard error of the mean. [†] N.S. = not significant F ratio (p<0.05); *, ** and ***, significant at p<0.05, 0.01 and 0.001, respectively. [‡]Treatment means of the ANOVA test; values are the mean value of 120 replications (10 grapes per 12 bunches). [¥] Values followed by the same letter, within the same source of variation, are not significant different (p<0.05), Duncan multiple-range test.

Table 5: Effect of bagging grape bunches, cv. Perla, on the standard deviation (uniformity) of the CIEL*a*b* color coordinates

Standard deviation	Bagged table grape	Control table grape
L*	0.676[†]	1.040
a*	0.224	0.307
b*	0.192	0.258
C*	0.228	0.244
h_{ab}	0.200	0.318

[†] Values are the mean value of 120 replications (10 grapes per 12 bunches)

Several authors have also reported for bagged fruit increases in skin lightness [17, 18], with some results showing reduction in anthocyanin accumulation and red colour development of the skin [19], whereas others observed improvement in red colour development [20]. For instance, Hofman *et al.* [19] reported that in mangoes, the percentage of the skin with red colour and its intensity decreased with increasing duration of fruit bagging, which agreed with results found for Perla grapes. Reduced colour intensity (lower C* values) in Perla grapes bagged with non-perforated cellulose bags may be the result of modification of the internal atmosphere [21, 22] and/or elevated temperature inside the bag [21, 22], which retarded maturity and reduced anthocyanin accumulation [11, 23].

The standard deviation of the arithmetic mean was selected to study the uniformity of the color within a treatment (bagged or non-bagged samples) (Table 5). In all five coordinates, the values of this statistical parameter were significantly higher in control (non-bagged) grapes than in bagged samples. Therefore, the use of cellulose bags as a pre-harvest task proved to be useful in providing a more uniform color even in a black grape cultivar.

In this way, the statements of the Control Board of the Designation of Origin "*Uva de Mesa Embolsada del Vinalopó*", regarding the fact that covering the grape bunches will produce a more uniform color of the grapes within a bunch and a delay in the ripening process, that will help in keeping the grapes in the plant for a longer period of time without a decrease in the quality of the grapes [1], seemed to be true, if only color data were considered.

Volatile aroma compounds. The percentages of the five major aroma compounds found in the Perla grapes are compiled in Table 6, with 2-hexen-1-ol and ethyl acetate prevailing. The relative percentages of bagged and non-bagged samples were significantly different; in this way, 2-hexen-1-ol predominated in bagged grapes while ethyl acetate prevailed in non-bagged grapes.

Table 6: Effect of bagging grape bunches, cv. Perla, on the volatile aroma composition

Compound	Grape type: Bagged perla (%)	Non-bagged perla (%)	Descriptor[†]
Ethyl acetate	17.4[‡]±0.9[¥]	66.2±3.2	Pineapple, ethereal
Limonene	9.6±0.4	5.8±0.2	Mild, citrus, sweet, orange, lemon
Trans-2-Hexenal	2.2±0.1	0.9±0.1	Sweet, fragrant, almond, fruity, green, leafy, apple, plum, vegetable
3-Hexanol	2.4±0.1	0.3±0.1	Alcoholic, ethereal, medicinal
2-Hexen-1-ol	59.4±3.3	20.9±1.5	Powerful, leafy, green, wine-like, fruity

[†][24]. [‡] Values are the mean value of 2 replications. [¥]Standard error values

According to the sensory descriptors provided by Sigma-Aldrich [24] for these two chemicals, the ripening process could have been delayed in those grapes in which 2-hexen-1-ol (powerful, leafy and green) predominated, this is bagged Perla grapes, as compared to those in which ethyl acetate predominated (pineapple, ethereal), non-bagged Perla grapes. There were statistical significant differences among the other three main compounds but their sensory descriptors could not be considered as maturity indexes.

Therefore, the qualitative volatile composition of the Perla grapes studied seemed to support the fact that bagging grapes will delay their ripening process.

CONCLUSIONS

Our experiment provided scientific data confirming that the practice of preharvest bagging of Perla grapes (red cultivar) caused: a) a uniform color of the whole bunch of grapes (lower values of standard deviation in all color parameters studied in bagged grapes compared to non-bagged grapes), b) a delay in ripening (lower values of maturity index and sugars, higher contents of organic acids, higher redish intensity but lower lightness and higher content of volatile compounds with leafy aroma descriptors (2-hexen-1-ol) in bagged grapes compared to non-bagged grapes.

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