# Effects of passive and active modified atmosphere packaging conditions on quality parameters of minimally processed table grapes during cold storage

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## Abstract

**BACKGROUND:** Table grape is a non-climacteric berry, sensitive to water loss and gray mold during postharvest storage.

**OBJECTIVE:** To assess the effect of passive and active modified atmosphere packaging on quality parameters of minimally processed table grapes.

**METHODS:** 'Red Globe' minimally processed table grapes were dipped in a sodium hypochlorite solution and irradiated with ultraviolet-C, before being packaged in a rigid polypropylene boxes, sealed with a micro perforated polypropylene film (PP) or a continuous polyethylene film, with: a) 5% $O_2$ + 15% $CO_2$ + 80% N (PET1); b) 20% $CO_2$ + air (PET2). Samples were stored at 5 °C for 21 days plus and additional 6-day period at 20 °C to simulate shelf-life.

**RESULTS:** No chemical and sensory changes during storage occurred in fruit sealed in PP packages. The high in-package $CO_2$ partial pressure which increased in PET1 and PET2 combined with the reduced concentration of $O_2$, increased decay incidence, stimulated anaerobic respiration, hastened soluble sugars degradation, produced higher weight loss and altered sensory quality.

**CONCLUSIONS:** Quality of minimally processed 'Red Globe' was better maintained in PP packaging where air-composition was not changed than in PET1 and PET2 where toxic levels of $CO_2$ and reduced tension of $O_2$ hastened quality loss and increased decay incidence.

**Keywords - Decay, MAP, ready-to-eat, Red Globe**

## 1. Introduction

Table grape is a non-climacteric berry with a low rate of physiological activity, very sensitive to water loss and gray mold (*Botrytis cinerea*) during postharvest handling and cold storage. Gray mold is the most aggressive postharvest disease, because of its ability to develop at low temperatures. Despite health hazard concerns and the great effort to find alternative means of control, the application of sulfur dioxide ($SO_2$) immediately after packing [1, 2, 3, 4, 5, 6] is still the standard practice to prevent gray mold.

Marketability of table grape may also be reduced by shatter, which is a physiological disorder leading to the loss of berries from the cap stem waterberry, which causes excessive softening and a watery and flabby texture in ripe grape, and rachis browning [7].

In the last decade consumers demand for fresh and high quality ready-to-eat products has increased exponentially, both for their uniform piece size and convenience as a nutritious snack alternative. Whole bunches of table grape are normally prepared for market in wooden, plastic or carton packages with paper strips separating bunches or also by individual wrapping of bunches in sponge or macro-perforated plastic bags [8]. Furthermore, table grape is particularly suitable to be minimally processed, as tissue injuries occurring during processing are quite limited. The product is, in fact, prepared either by pulling off the berries from the cap stem or by dividing a bunch in small clusters of few berries. Processed berries are then packaged in rigid plastic units or in plastic units wrapped within a polyolephinic film. The modified atmosphere created by the package prevents water loss, browning, and may significantly prolong the shelf-life [9] [10].

The aim of this work was to study the effects of a passive or active modified atmosphere packaging on quality parameters and decay incidence of minimally processed 'Red Globe' table grape during 21 days of cold storage plus 6 days at 20°C (shelf-life).

## 2. Materials and Methods

### *2.1 Plant material*

Red table grape (*Red Globe*) clusters were harvested from a 6-years-old commercial vineyard, located in Mazzarrone (37° 05' N, 14° 34' E, 128 m a.s.l., CT). Vines were grafted onto Ru 140 (*V. berlandieri* x *V. rupestris*) spaced 2.8 m × 2.8 m apart (1275 plants/ha) and trained to a "tendone" training system, covered with white net. The orchard received ordinary horticultural care in terms of fertilization, irrigation and soil management.

*Red Globe* table grape were hand-picked during the third week of September at commercially ripe stage (≥15.0 °Brix), suitable for the fresh fruit market.

### *2.2 Sample preparation and packaging*

After harvest, Red Globe bunches were immediately transported to the laboratory and selected on the basis of uniform size, berry color, firmness, lack of diseases and greenish rachises. Each bunch was then cut with sanitized scissors to obtain samples of clusters each of about 200 g. After selection, clusters samples were dipped in a sodium hypochlorite solution (150 ppm active chlorine) for 5 min to control microbial spoilage. After dipping clusters were irradiated with ultraviolet-C (UV-C) following previous studies and commercial practices [11]. Grapes were placed in a single layer on the processing line and the selected UV-C dose ($6.0 \pm 0.1$ kJ/m$^2$) was applied by establishing a specific exposure time (illumination steps of 2 min) at a fixed distance (60 cm) [12]. The fluence rate of the lamps at the level of the samples was $2.82 \pm 0.44$ mW cm$^{-2}$, measured with a Blak-Ray photometer (Ultra-Violet Products, Inc., San Gabriel, CA). Red table grapes were treated after harvest with ultraviolet-C light (UV-C) in combination with sodium hypochlorite solution, to check the effect of both treatments on quality and fungal decay of minimally processed table grapes. At end of the UV-C treatment, each cluster was packaged in a 15 × 8 cm rigid polypropylene retail boxes and sealed with two different films: a) 30 μm thick polypropylene film with laser micro

79 perforations of 60 μ diameter ($O_2$ permeability: 2.500 ml $m^{-2}$ $d^{-1}$ $atm^{-1}$ at 5 °C) and b) 30 μm thick
80 polyethylene continuous film ($O_2$ permeability: 1.300 ml $m^{-2}$ $d^{-1}$ $atm^{-1}$ at 5 °C).
81 The modified atmosphere in packages sealed with the micro-perforated film was achieved passively
82 (PP), while initial atmosphere in boxes sealed with continuous films was actively modified by two
83 different initial headspace gas compositions: 1) 5% $O_2$ + 15% $CO_2$ + 80% N (PET1) and 2) 20%
84 $CO_2$ + air (PET2). The sealed boxes were then stored at 5°C and 90% RH for 21 days followed by a
85 6-day retail at 20 °C.
86 A total number of 135 sealed boxes (3 blocks x 5 repl. x 3 treatments x 3 storage periods) were
87 evaluated for quality after storage on days 7, 14, 21 at 5 °C. 30 additional boxes were stored at 5°C
88 for weight loss evaluation during cold storage (10 boxes for treatments). At the end of the 21 days
89 storage period another set of fruit (3 blocks x 5 repl. x 3 treatments x 2 storage periods) were left at
90 20 °C for 6 days to simulate shelf-life conditions and evaluate for quality after 3 and 6 days at 20
91 °C; 30 additional boxes were stored at 20°C for weight loss evaluation during the shelf-life (10
92 boxes for treatments) and 90 additional sealed boxes (3 boxes x 3 treatments x 10 judges) were
93 evaluated by sensory analysis judges at the end of the shelf-life.
94
95 *2.3 Headspace gas composition*
96 In-package $O_2$ and $CO_2$ partial pressures were measured, immediately before quality
97 evaluation, using an $O_2$ and $CO_2$ portable analyzer (Checkpoint, Dansensor Italia, Segrate, Milano,
98 Italy) after 7, 14, 21 days at 5°C and at 3 and 6 days during the shelf-life at 20 °C using 15 packages
99 for each treatment.
100
101 *2.4 Quality parameters: firmness, soluble solids, titratable acidity, weight loss, decay and browning*
102 For each treatment, 15 randomly chosen boxes were used. Berry analysis included: weight
103 loss, total soluble solids content (TSS), juice pH and titratable acidity (TA), berry decay incidence
104 and browning severity.

105 Total soluble solids content (TSS) was determined by a digital refractometer (Palette PR-32, Atago
106 Co., Ltd); titratable acidity (TA) was measured by titration of 10 ml homogenized berry flesh juice
107 with 0.1 N NaOH to an endpoint of pH 8.1 and expressed as the percentage of tartaric acid (mod. S
108 compact titrator, Crison Instruments, Barcelona, Spain). Berry weight loss was calculated on 10
109 sealed boxes for each treatment (10 boxes x 3 treatments), and expressed as the percentage
110 reduction with respect to initial time.

111 Percentages of physical damage, decay caused by fungi and browning were calculated
112 separately, during the shelf- life period after 21 days of storage at 0 °C and 3 and 6 days at 20 °C,
113 by counting the number of rot berries; decay incidence was calculated as the percentage of rotten
114 berries.

115 Flesh browning was assessed by measuring the extent of browned area on each fruit on the
116 following scale: 0 = no browning; 1 = less than 1/4 browning; 2 = 1/4–1/2 browning; 3 = 1/2–3/4
117 browning; 4 = more than 3/4 browning. The browning index (BI) was calculated, using the
118 following formula: $[(1 \cdot N1 + 2 \cdot N2 + 3 \cdot N3 + 4 \cdot N4) \cdot 100/(4 \cdot N)]$, where N = total number of fruit
119 measured and N1, N2, N3 and N4 were the number of fruit showing the different degrees of
120 browning [13].

121

122 *2.5 Sensory evaluation*

123 After 21 days of cold storage plus 6 days at 20 °C (shelf-life), 3 sealed boxes for treatment
124 (2 berries in each box) were evaluated by each of a ten judges trained panel that generated a list of
125 descriptors in a few preliminary meetings. Sensory analysis was focused on firmness, crunchiness,
126 juiciness, sweetness, sourness, off-flavor, off-odor and visual appearance of the berries [14]. The
127 different descriptors were quantified using a ten point intensity scale where the digit 1 indicates the
128 descriptor absence while the digit 10 the full intensity [15]. The order of presentation was
129 randomized between judges. Water was provided for rinsing between samples.

130

*2.6 Total phenolics, total anthocyanins and antioxidant capacity*

Grape samples were chopped into small sections and analyzed in the same day for each considered chemical parameter. Analytical grade products (Fluka and Sigma-Aldrich Chemie, Italy) were used without any purification. The relative standard deviation (RSD) for each triplicate analytical determination was also considered.

In order to evaluate the total phenolic amount 2 g of homogenized sample were added with 10 mL of pure ethanol. The extraction was made using a vortex mixer mod. RX3 for 60 seconds. The mixture was filtered and the filtrate was taken into a test tube. The Folin-Ciocalteau micro method of Waterhouse [16] was used to determinate the total phenolic content (TPC). 300 µL of the filtrate were diluted in 4.8 ml of Milli-Q grade water and 300 µL of Folin-Ciocalteau reagent was added. After 8 min, 900 µL of 20 % $Na_2CO_3$ solution was added and mixed. The absorbance values were measured at 765 nm using SHIMADZU UV mini- 1240 spectrophotometer after a chemical reaction time of 30 min at 40 °C. A calibration curve of gallic acid (3, 4, 5- trihydroxybenzoic acid) was prepared in the concentration range from 0 - 50 µg $mL^{-1}$ and used as a standard. The results were given as mg gallic acid equivalent per g of fresh weight.

Antioxidant capacity was determined based on the ability of grape sample to scavenge free radical 2,2-diphenylpicrylhydrazyl (DPPH). DPPH free radical scavenging activity was determined using a modified method of Ohnishi [17] and Matthaus [18] 50 µl of filtered grape juice were added to the same amount of DPPH solution (3 mM). Then 95 % ethanol was added to final volume of 1 ml. The mixture was mixed thoroughly and allowed to stand in the dark for 10 min at room temperature. Absorbance values were then determined at 515 nm using the above mentioned spectrophotometer. The radical scavenging activity was expressed as % of inhibition considering A0 the absorbance of the control and A1 absorbance of the sample one [19].

Total anthocyanins amount in the extracts was determined according to the procedure described by, with some modifications. In order to achieve anthocyanins extraction, 25 ml of Ethanol:HCl 1.5 N (85:15) solution were added to 2 g of grape sample and allowed to stand at 4 °C for one night. After

157 extraction, the solution was filtered. The absorbance was measured using the above mentioned
158 spectrophotometer at 530 and 657 nm. The formula (1) was used to compensate for the contribution
159 of chlorophyll and its degraded products to the absorption at 530 nm. The anthocyanins content was
160 expressed as milligrams of Cya-3-glucoside equivalent per 100 g fresh sample weight.
161
162 *2.7 Microbiological analysis*
163 Microbiological analyses were carried out after 21 days of cold storage plus 3 days of shelf-
164 life. Samples of 25 g (5 berries) from each sealed box and for each treatment (3 boxes x 3
165 treatments) were obtained under sterilized conditions, homogenized in 225 mL of sterile distilled
166 water and shaken for 30 min at 200 rpm on a rotary shaker. Serial dilutions were carried out, and 1
167 mL was added to plate count agar for mesophilic aerobic and for mold and yeast counts (Petrifilm
168 Aerobic Count Plate, Laboratories 3M Sante´, France). Samples were prepared in triplicate, and
169 only counts of 30-300 colony forming units (CFU) were considered. All plates were incubated for 3
170 days at 30 °C.
171
172 *2.8 Statistical analysis*
173 Data were submitted to one-way analysis of variance (ANOVA) and means were separated
174 with Tukey's test at $P \leq 0.05$. The statistical analysis was carried out using Systat 10 (Systat, USA).
175
176 **3. Results**
177
178 *3.1 In-package gas composition*
179 In-package $CO_2$ increased during cold storage and even during the shelf-life period in all
180 treatments (Fig. 1A). However, while in PP packages changes were very slight in cold storage and
181 peaked to 2.7 kPa after 6 days at 20 °C, in PET1 and PET2 samples the levels of $CO_2$ increased
182 dramatically since the first sampling date, with values in both treatments significantly higher than

183 initially. At the end of cold storage the $CO_2$ partial pressure was 33 and 41 kPa in PET1 and PET2,
184 respectively, and further increased in shelf-life conditions with final values of 44 kPa in PET1 and
185 60 kPa in PET2. The in-package levels of $O_2$ were complementary to $CO_2$: in PP packages slightly
186 declined with storage and shelf-life and ranged between 20.5 kPa and 19 kPa; while in PET1 and
187 PET2 packages its partial pressure was always below 1 kPa (Fig. 1B).
188
189 3.1 *Decay and browning and Weight loss*
190 At the end of cold storage decay incidence was quite low in all treatments, with losses of
191 about 1 % in PP packages and around 3 % in PET1 and PET2 ones. During the six days of shelf-life
192 the percentage of rotted berries only slightly but not significantly increased (Fig. 2A). Rachis
193 browning occurrence was rare, did not increase during shelf-life and was significantly higher in
194 PET2 packages than in PP one, while its incidence did not significantly differ in PET1 from the
195 other two treatments (Fig. 2B). Weight loss increased gradually in cold storage and suddenly when
196 fruit were moved to -conditions, especially in PET1 and PET2 (Table 1). In particular, after 21 days
197 at 5 °C weight loss was 5.36 % in PP packages against 10.37 and 12.17 of PET1 and PET2, but
198 during the 6 days at 20 °C losses peaked to 24.6 % in PET2 and to 23.09 % in PET1, while in PP
199 packages the percentage of weight loss was slightly higher than 9 %. Despite in both packages with
200 continuous film, PET1 and PET2, losses were always much higher than in PP packages, differences
201 between the two were always significant, even at the last sampling date when differed of only 1.53
202 % (Table 1).
203
204 *3.2 Titratable acidity, total soluble solids, total phenols, total anthocyanins and antioxidant*
205 *capacity*
206 TSS did not change in PP packages while in cold storage and showed a slight, although
207 significant, reduction only after 6 days at 20 °C, when from the initial value of 16.7 ° Brix TSS
208 decreased to 15.8 ° Brix (Table 1). In contrast, in PET1 and PET2 the reductions in TSS were

209 marked and significant even from the first sampling time and continued at a faster rate during the
210 shelf-life period, with final values of 13.6 and 12.4, respectively in PET1 and PET2 packages
211 (Table 1). As a general trend, TSS seems to be slightly higher in PET1 packages than in PET 2
212 ones, but difference was significant only at the last sampling date.
213 Differently than TSS, TA was very stable over the whole course of the experiment and differences
214 among treatments were never significant, apart after 14 days at 5 °C, when values detected in PP
215 packages were slightly but significantly higher than in PET2 ones (Table 1).
216 No significant changes occurred, between treatments, in terms of total phenols (Fig. 3A) and total
217 anthocyanins content (Fig. 3B). The overall trend of total phenols seemed more stable or even
218 stimulated in PP packages, with the exception of results of day 14 at 5 °C. On the other hand,
219 phenolics average content was generally lower in PET1 and PET2 packages, even if data at the end
220 of cold storage, when the highest level of polyphenols was detected in PET1 packages, and at the
221 end of shelf-life, when polyphenols were not different among all treatments, are not consistent with
222 the overall trend.
223 The antioxidant capacity partially paralleled the changes in polyphenols; it was generally higher in
224 PP packages, albeit in some cases it was lower or similar to the other treatments (Fig. 3C).
225 Accordingly, differences between PET1 and PET2 were un-predictable and inconsistent.
226
227 *3.3 Sensory evaluation*
228 Sensory evaluation was conducted only after 3 days of shelf-life. All descriptors had the
229 highest scores in PP packages, with the exception of off-flavor and off-odor, which resulted higher
230 in PET1 and PET2 packages than in PP ones (Fig. 4).
231
232
233
234

235 *3.4 Microbiological analysis*

236 PP was the most effective packaging treatment in reducing mesophilic aerobic bacteria and
237 yeast counts (Fig. 5). Microbiological contamination did not change in PET1 and PET2 packages
238 for both kinds of microorganisms.

239

240 **4. Discussion**

241 Water loss, stem browning and, above all, gray mold (*Botrytis cinerea*), are the main cause
242 that reduce table grape marketability. Sulfur dioxide continue to be the most popular mean to
243 control gray mold [7] [8], but its application is not conceivable on ready-to-eat table grapes that will
244 be consumed within a few days from packaging and in most cases as soon as the package is opened.
245 The world-wide effort to replace sulfur dioxide has brought about to different alternative means
246 safer for consumers [21]. Controlled atmosphere storage is gaining more popularity than others
247 means for the absence of residue. High levels of $CO_2$ markedly reduce gray mold growth with a
248 complete inhibition with levels above 20-25 % [22]. Passive or active modified atmosphere
249 packaging showed some potential for ready-to-eat table grape. In cv Italia, no remarkable
250 differences were found in terms of appearance and marketable quality due to barrier films [23],
251 while a significant reduction of decay after 7 days at 0 °C followed by 4 days at 8° C plus 2 days at
252 20 °C was found in 'Superior seedless' table grape sealed in a micro perforated polypropylene with
253 in-package $CO_2$ levels never exceeding 10 kPa, [4]. Decay was completely suppressed in packages
254 made with continuous polypropylene films where $CO_2$ tension peaked to about 20 kPa [4].
255 However, in our study decay incidence was lowest in berries packed and sealed with micro
256 perforated film; in this treatment, in-package gas composition was similar to air. A higher decay
257 occured in active modified packages made with the continuous film, where in-package $CO_2$ ranged
258 between 25 and 40 kPa in cold storage and 35 and 60 kPa in shelf-life conditions.

High levels of $CO_2$ can be phytotoxic, especially in combination with low levels of $O_2$, particularly in sensitive species. Indeed, beneficial effects of a better control of pathogens by high levels of $CO_2$ could be offset by $CO_2$-induced injuries [9] [22].

In our experiment, the sanitizing effect of postharvest sodium hypochlorite combined with UV-C treatment, was very effective in preventing any gray mold infection. The high levels of in-package $CO_2$ added no advantage in reducing decay, but its toxicity might injure the tissue, making the berries sealed in PET1 and PET2 more susceptible to decay than those sealed in PP.

Despite water loss is considered the main cause of stem and rachis browning, [24] [25] other factors can significantly affect this disorder. Stem browning severity increased when 'Red Globe' grape was stored for 3 months at 0 °C with levels of $CO_2$ higher than 10 kPa [26]. In our study, despite all treatments were very effective in reducing browning severity, in accordance with the above cited ones, stem browning appeared rarely and slighter in PP samples than in PET1 and PET2 packages.

The most striking effect of continuous films was the shift from aerobic to anaerobic metabolism of berries, whose objectively measurable physiological consequences were the higher weight loss and TSS degradable rates. Water loss usually accounts for about 95-97 % of total weight loss [27]. In this study, based on water transmission rate of the used films, expected weight loss had to be much higher in PP packages than in PET1 and PET2 ones, but the results were exactly the opposite. This can only be explained by a marked anaerobic activity, which occurred in samples sealed with the continuous film (PET1 and PET2). This hypothesis is supported by the composition the head-space of PET1 and PET2 packages, which, since the first sampling time done after 7 days at 5 °C, showed levels of $CO_2$ of 23-34 kPa that continuously increased with final values of 44-60 kPa. In contrast, $O_2$ levels were always below 1 kPa. A further evidence that shows as the anaerobic metabolism might be the factor that mostly contributed to the higher weight loss of PET1 and PET2 packaged berries is given by the gradual but continuous decline in TSS which occurred in PET1 and PET2 berries, differently than in those packaged in PP film, which only at the last sampling showed a slight reduction in TSS with respect to harvest time. However, TA content was not affected either

285 by the storage time or the type of package, most likely because the main available substrates were
286 sugars and not organic acids.
287 Phenolic compounds and anthocyanins determine the characteristics of color and taste in fruit, such
288 as bitterness and astringency and have positively been correlated with fruit antioxidant capacity [28]
289 [29]. Polyphenols levels and changes over storage can be affected by various factors, as storage
290 temperature, interaction with other chemical compounds as well in-package gas composition [30].
291 A typical response to mechanical damages common to various fruits and vegetables is an increase
292 in phenylalanine ammonio-lyase (PAL) activity which stimulates new synthesis of phenolic
293 compounds that in turn may prevent pathogens' attacks [31] [32]. However, the response and its
294 magnitude depend on species, severity of mechanical damage and in-package gas composition. For
295 example, significant increases of soluble phenolic acids such as chlorogenic or caffeic acid,
296 associated with a PAL increase, were observed in carrots and in 'Lollo Rosso' lettuce after cutting
297 and during the early days of storage in air at 4 to 5 °C; on the other hand, phenolic synthesis was
298 halted when samples were packaged in modified atmosphere enriched in $CO_2$ (12% to 30%) with or
299 without $O_2$ (2%), or in an atmosphere containing 99% $N_2$ [33] [34]. Accordingly, total phenols of
300 ready-to-eat table grape cv Shahaneh berries were higher when single berries were prepared with
301 the stem removed, than in a 4-berry cluster, because processing operations induced more damage in
302 the former case, [35]**.** Our results showed a large variability in total phenols from one sampling date
303 to the others. This might be a consequence of the intrinsic variability in chemical composition of
304 table grape among bunches and even among berries of the same bunch, likely due to difference in
305 ripening stage of individual berries [32], which might have overwhelmed the effect of the
306 treatments. Nevertheless, the overall results showed a substantial stability of polyphenols in PP
307 packages alongside with overall lower levels detected in PET1 and PET2 treatments, whose
308 synthesis might be inhibited by the excessively high levels headspace $CO_2$ [33] [34].
309 Anthocyanins content, as polyphenols, was very variable and did not show a clear effect of the
310 treatments, although their overall level slightly increased with storage.

311 While low temperature is known to induce accumulation of anthocyanins in different fruit species
312 [36] [37] included table grape [31], modified atmosphere technology does not seem to be a suitable
313 tool for maintaining anthocyanins in fruit and vegetables [31] [34]. Yet, recently it was reported that
314 sanitation treatments with ultraviolet light (UV-C, λ 254 nm, 30 to 510 W) effectively increased
315 anthocyanin concentrations in products with high anthocyanin contents, such as red grapes [38]. It
316 is, then, possible that the overall stability of anthocyanins, especially in PET1 and PET2 packages,
317 might be the results of contrasting factors; the inhibitory effect of high levels of $CO_2$, from one side,
318 and the stimulating consequence of ultraviolet treatment and low storage temperature on the other.
319 Changes in antioxidant capacity matched well with those of polyphenols, even if, as a general trend,
320 it seemed to be slightly higher than polyphenols were in PP packages. In a previous study [35] the
321 antioxidant capacity of ready-to-eat individual berries without stem increased sharply during the
322 first week of storage, but declined at a higher rate than in a 4-berry cluster. The authors, in
323 accordance with previous results with ready-to-eat avocados [39], attributed this behavior to an
324 increased initial stress-induced antioxidant capacity in individual berries that subsequently declined
325 faster for the more oxidative process involving wounded tissues. A general decreasing trend
326 occurred over storage of ready-to-eat table grapes [31], and a positive correlation occurred between
327 flavonoids and antioxidant capacity [35]. In contrast, in this study the antioxidant capacity matched
328 better with total phenols than with total anthocyanins.
329 Berries sealed with PP films had the highest overall quality, since they were judged firmer, sweeter
330 than PT1 and PET2, and always got a higher score for the other descriptor with the exception of off-
331 flavor and off-odors. Nevertheless the overall sensory quality of PET1 and PET2 berries, despite
332 markedly worse than PP one, would have been still acceptable, but for the high perception of
333 fermentative volatiles which irremediably altered the eating quality.
334 Mesophilic microorganisms represent one of the most important indicators of food quality and their
335 level is extensively used to assess microbiological quality and safety of ready-to-eat produce. It
336 expresses the adequacy of temperature and sanitation control during processing, transport, and

storage, and reveals potential sources of contamination during manufacture [40]. Our results showed an overall lower population both of mesophilic aerobic microorganisms and of yeast and mold in PP packages. However, even in PET1 and PET2 packages despite the anaerobic conditions, the overall microorganism population was similar to the load detected in other studies with table grape [41], never exceeding $10^5$ cfu $g^{-1}$, and below the safe quality limits established in Europe, which suggested in the range of $10^5$-$10^8$ cfu $g^{-1}$ [40].

## 4. Conclusions

Objective of this study was to assess quality changes and decay incidence of ready-to-eat 'Red Globe' table grape using three different modified atmosphere packaging compositions. Indeed, PP packages, realized using a micro perforated film, rather than a "modified atmosphere packaging" was a "modified humidity packaging", as in-package $CO_2$ and $O_2$ partial pressures were slightly altered with respect to air. In contrast, in-package gas compositions in PET1 and PET2 packages were markedly different due to the relatively high barrier to gas exchange of the used continuous film but also for the initial composition of gases used to achieve the PET1 and PET2 active modified atmosphere packaging.

PET1 and PET2 were tested to confirm previous results reported in other studies [4] [22] [32] on the effectiveness of high levels of $CO_2$ to control decay incidence incited by *Botrytis cinerea* as feasible alternative to $SO_2$ fumigations. Results showed that in-package gas composition of PET1 and PET2, with high levels of $CO_2$ and very low concentrations of $O_2$ did not reduce decay incidence as PP did more effectively. Moreover, despite the excessive length of the storage period and the relatively high temperatures both during cold storage and shelf-life conditions for ready-to-eat produce, samples sealed in PP packages accused lower qualitative changes related to chemical composition, visual appearance and eating quality. In contrast in PET1 and PET2 berries overall quality declined very quickly and fermentative metabolism replaced aerobic respiration even in cold storage as shown by the high rate of TSS degradation and excessive weight loss.

363 Therefore the overall results show that when a proper pre-harvest fungicide spray program is
364 combined with a postharvest treatment with sodium hypochlorite associated with UV irradiation,
365 grey mold growth can effectively be controlled in ready-to-eat 'Red Globe' berries and that a
366 modified atmosphere packaging with high levels of $CO_2$ and reduced concentration of $O_2$, rather
367 than reducing, may sustain pathogens' growth and hastening quality loss. Similar results, in terms
368 of grey mold control, were obtained in several studies with ozone treatments in storage room [42],
369 but ozone penetration through the plastic cavity trays used in some experiments was partially
370 inhibited because of the mycelial growth on the surface of the fruit in contact with the plastic [43].
371 Therefore additional research is needed to evaluate the impact of current commercial packages on
372 the efficacy of ozone gas during cold storage.
373 Considering that, differently than other fruit, table grape does not gain any beneficial physiological
374 effects from an altered air gas composition and that transpiration rate is the major cause of
375 marketable quality, provided an effective sanitizing treatment is given, we conclude that a
376 "modified humidity packaging" contribute to maintain overall quality of ready-to-eat 'Red Globe'
377 table grape better than modified atmosphere packaging systems where in-package gas composition
378 is markedly altered with respect to air.
379
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Table 1 – Changes in titratable acidity, total soluble solids and weight loss in ready-to-eat grape sealed in a microperforated polypropylene (PP) film or a continuous polyethylene film under active modified atmosphere with 20 % $CO_2$ + 80% N (PET1) or 5 % $O_2$ + 15 % $CO_2$ + air (PET2).

| Treatments | Weight loss (%) | Total soluble solids (° Brix) | Titratable acidity (% tartaric acid) |
|---|---|---|---|
| | | *Harvest* | |
| | | 16.7±0.59 | 3.5±0.23 |
| | | 7 days at 5 °C | |
| PP | 0.83±0.039 c | 16.5±0.62 a | 3.5±0.21 a[1] |
| PET1 | 5.60±0.078 b | 15.7±0.61 b | 3.5±0.21 a |
| PET2 | 5.90±0.195 a | 15.8±0.82 b | 3.7±0.27 a |
| | | *14 days at 5°C* | |
| PP | 1.97±0.117 c | 16.2±0.47 a | 3.7±0.16 a |
| PET1 | 5.82±0.112 b | 15.1±0.66 b | 3.6±0.22 ab |
| PET2 | 6.70±0.035 a | 14.7±0.74 b | 3.4±0.25 b |
| | | *21 days at 5°C* | |
| PP | 5.36±0.039 c | 16.2±0.55 a | 3.8±0.18 a |
| PET1 | 10.37±0.312 b | 14.4±0.58 b | 3.8±0.20 a |
| PET2 | 12.17±0.273 a | 14.1±0.70 b | 3.8±0.23 a |
| | | *21 days at 5°C plus 3 days at 20 °C* | |
| PP | 6.90±0.310 c | 16.1±0.39 a | 3.4±0.13 a |
| PET1 | 15.44±0.660 b | 14.2±0.59 b | 3.6±0.19 a |
| PET2 | 18.11±0.390 a | 13.4±0.66 b | 3.3±0.22 a |
| | | *21 days at 5°C plus 6 days at 20 °C* | |
| PP | 9.36±0.552 c | 15.8±0.43 a | 3.4±0.14 a |
| PET1 | 23.09±0.980 b | 13.6±0.51 b | 3.4±0.17 a |
| PET2 | 24.62±0.431 a | 12.4±0.62 c | 3.5±0.21 a |

[1] *For each storage time means in columns followed by unlike letters are significantly different according to the Tukey's test at P<0.05. Each mean is followed by the standard deviation (n=15).*

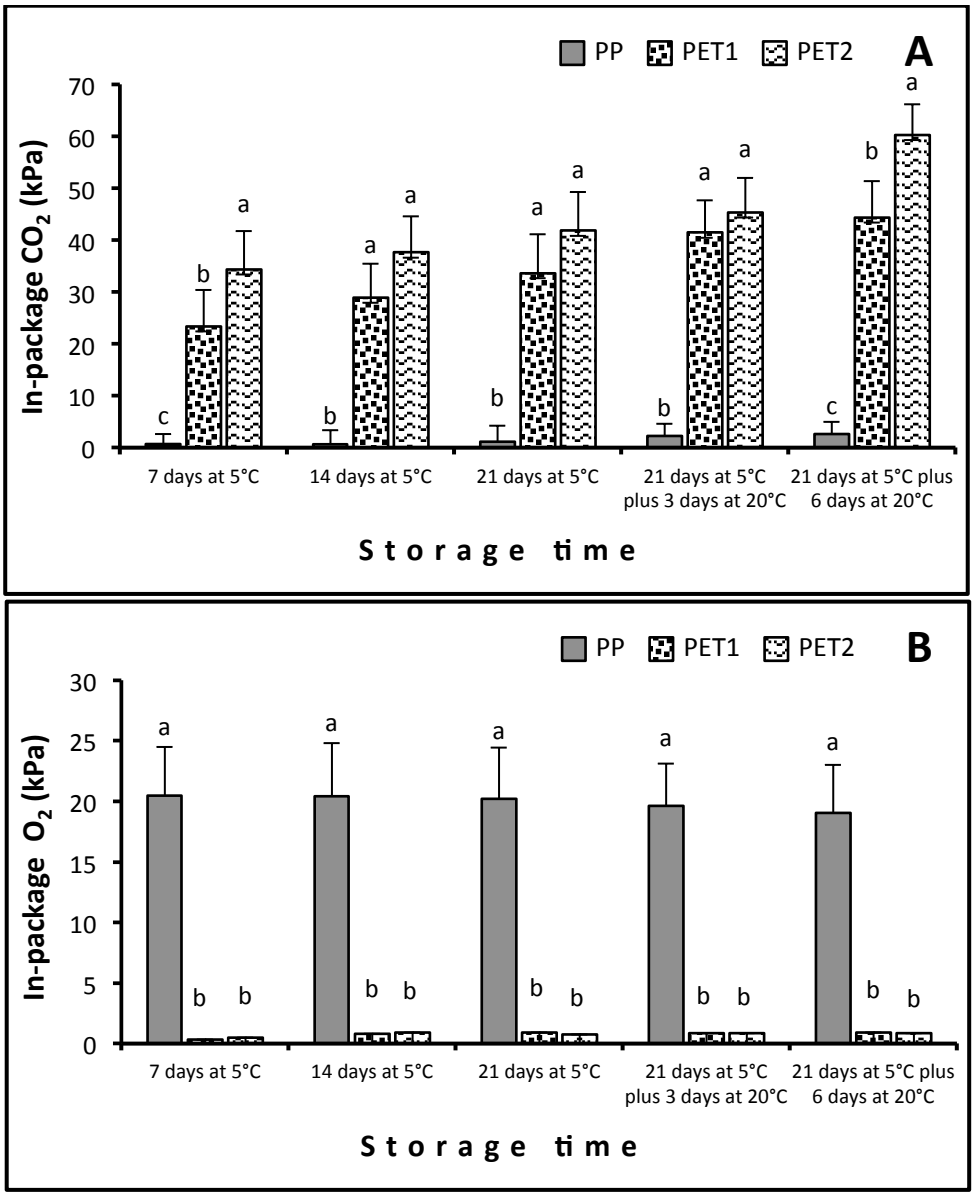


Figure 1 - Changes in $CO_2$ (A) and $O_2$ (B) in packages of ready-to-eat grape sealed in a microperforated polypropylene film (PP) or a continuous polyethylene film with an initial modified atmosphere of 20 % $CO_2$ + 80% N (PET1) or 5 % $O_2$ + 15 % $CO_2$ + air (PET2). For each storage time histograms with unlike letters are significantly different at $P<0.05$ according to the Tukey's test. Vertical bars are the standard deviations (n=15).

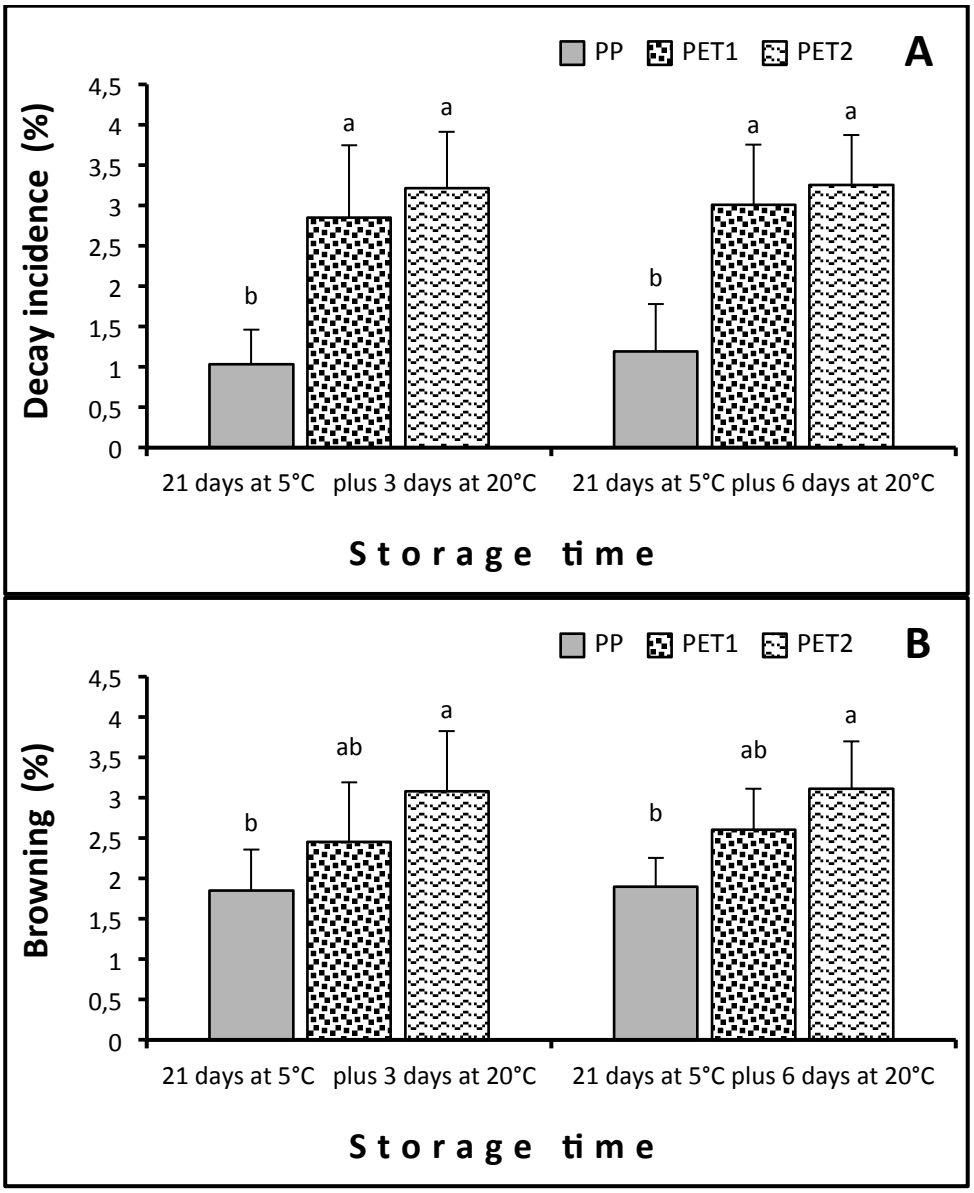


Figure 2 - Decay (A) and browning (B) rates of minimally processed grape berries sealed with microperforated polypropylene film (passive MAP) (PP), with a continuous polyethylene film (active MAP: 20% $CO_2$ + 80% N (PET1)) and with a continuous polyethylene film (active MAP: 5% $O_2$ + 15% $CO_2$ + air (PET2)) after 21 day storage at 5 °C plus 3 days at 20 °C. Data are Mean ± S.E (n=15). Column values marked with different letters are significantly different ($P \leq 0.05$).

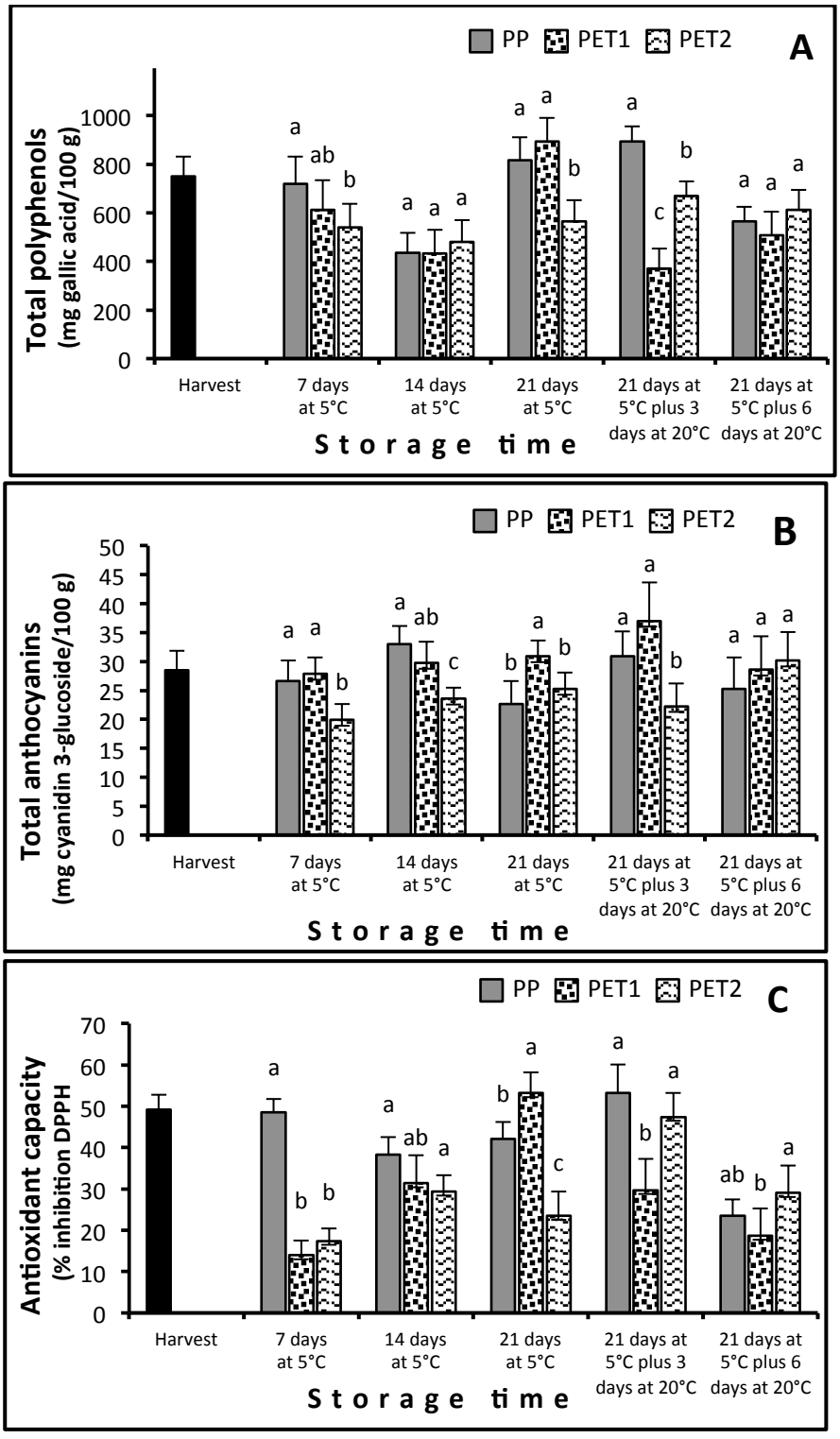


Figure 3 - Changes in total phenols (A), total anthocyanins (B) and antioxidant capacity (C) in ready-to-eat grape sealed in a microperforated polypropylene film (PP) or a continuous polyethylene film with an initial modified atmosphere of 20 % $CO_2$ + 80% N (PET1) or 5 % $O_2$ + 15 % $CO_2$ + air (PET2). For each storage time histograms with unlike letters are significantly different at $P<0.05$ according to the Tukey's test. Vertical bars are the standard deviations (n=15).

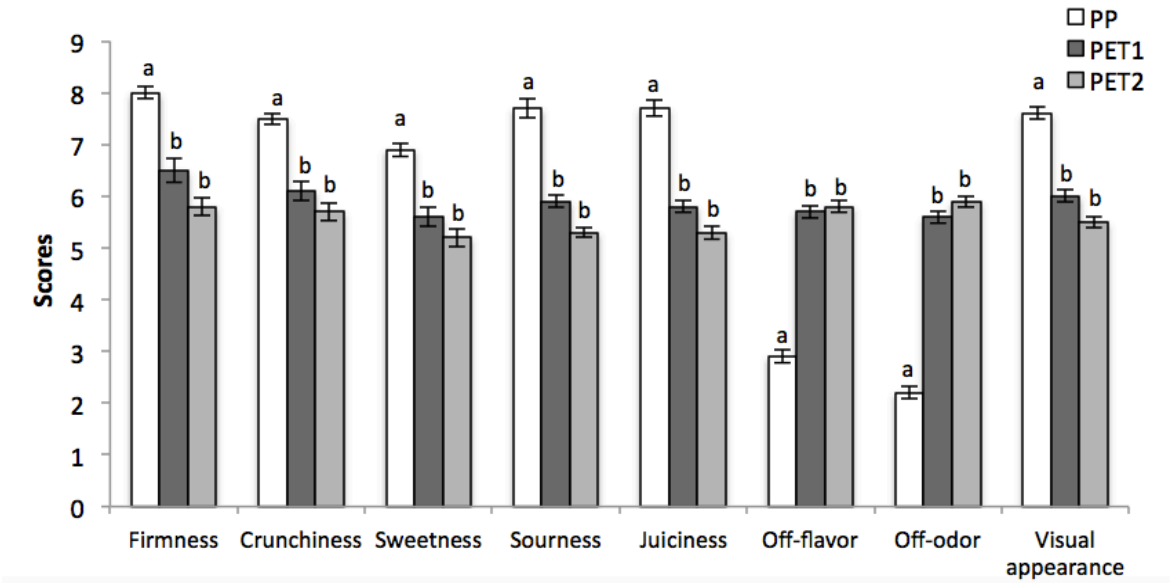


Figure 4 - Scores for the sensory analysis of minimally processed grape berries sealed with microperforated polypropylene film (passive MAP) (PP), with a continuous polyethylene film (active MAP: 20% $CO_2$ + 80% N (PET1)) and with a continuous polyethylene film (active MAP: 5% $O_2$ + 15% $CO_2$ + air (PET2)) after 21 days of cold storage at 5 °C plus 3 days at 20 °C (shelf-life). Data are means ± S.E. (n = 50). Column values marked with different letters are significantly different ($P \leq 0.05$).

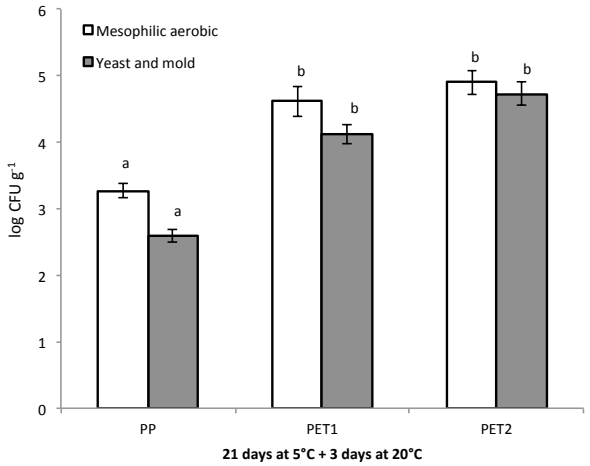


Figure 5 - Mesophilic aerobic and yeast and mold counts in minimally processed grape berries sealed with microperforated polypropylene film (passive MAP) (PP), with a continuous polyethylene film (active MAP: 20% $CO_2$ + 80% N (PET1) and a continuous polyethylene film (active MAP: 5% $O_2$ + 15% $CO_2$ + air (PET2)) after 21 day storage at 5 °C plus 3 days at 20 °C. Data are Mean ± S.E (n=5). Column values marked with different letters are significantly different within the same treatment ($P \leq 0.05$).