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Quality and safety of table grapes coated with HPMC edible coatings containing propolis extract

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Abstract

Edible coatings based on hydroxypropylmethylcellulose containing an ethanolic extract of propolis, are developed and applied to table grapes, cv. Muscatel, in order to improve quality and shelf life during storage, while taking advantage of the beneficial health properties of propolis. The weight loss, changes in soluble solids, phenol content, antioxidant capacity, respiration rates and the microbial counts of uncoated and coated samples were determined throughout cold storage. The sensory quality of samples was also analysed. Throughout storage, soluble solid contents sharply increased from 7 storage days onwards and phenols contents decreased, especially during the first 5 days. No effect of coatings was observed in the development of these variables. A decrease of clarity and hue values was observed during storage; the samples coated with the greatest amount of propolis being the lightest. The hue decrease was related with the a* colour coordinate increase, which was significantly more accused for uncoated samples. Regardless of their composition, coatings slowed down the weight losses and controlled the oxygen consumption of the samples. At 10 days of storage, coated samples maintained a better microbial safety than uncoated samples. Although no significant

effect of the propolis incorporation was observed on the preservation of grape quality during storage, its incorporation in the HPMC coatings contributes to enrich the health characteristics of the coated product.

Keywords: Propolis; Hydroxypropylmethylcellulose; Films; Grape

1. Introduction

Propolis has been known since ancient times for its interesting composition but currently its use is being re-evaluated for its application in biology, pharmacy and medicine (Burdock, 1998; Krell, 1996), with special emphasis on its use as a dietary supplement (Farré et al. 2004).

Propolis is a natural resinous substance collected from the leaf buds of different tree species by honeybees, which can be considered as a complex mixture of chemical constituents, whose composition depends on the constituents of the plant material and collection time. It usually contains resins (50%), composed of flavonoids and phenolic acids and their esters, waxes (30%), essential oils (10%), pollen (5%) and various organic compounds (5%) (Juliano et al. 2007). Thanks to its content in essential oils, propolis is usually aromatic and, depending on its botanical origin and the season in which it is harvested, varies in colour, taste or consistency (Krell, 1996). However, the main components of propolis are the flavonoids and the phenolic acids which are mainly responsible for most of its pharmacological properties (Bankova, 2000; Menghinello et al. 1999). It is qualified as an antiflammatory, immunostimulant, hepatoprotector and carcinostatic agent, and furthermore, its properties as an antimicrobial, antiviral, antifungal, analgesic and tissue regenerator have been demonstrated (Farré et al. 2004; Bankova, 2000; Choi et al. 1999; Scheller et al. 1999;

Durk, 1997; Krell, 1996). Moreover, propolis is a natural source of antioxidants, which protect oils and serum lipoproteins from oxidation (Isla et al. 2001; Krell, 1996).

As regards its application, propolis has long been used in the treatment of wounds and infections of the mouth, tooth cavities and throat. Currently, there are few studies about the incorporation of propolis into edible films. Juliano et al. (2007) prepared and characterized polymeric films containing propolis and showed that these films also had potential applications not only in pharmaceuticals but also in the agriculture and food industry. Budija et al. (2008) tested the use of ethanolic extracts as an additional component in natural wood finishes based on natural resins, waxes and oils. Drapak et al. (2006) argued in favour of the use of propolis films in various optoelectronic device applications.

In food technology, the antioxidant, antimicrobial and antifungal properties of propolis offer a great variety of applications in food preservation while being beneficial for human health (Farré et al. 2004). Since it has an unpleasant flavour and odour, its encapsulation in edible films to be applied in food preservation could represent an alternative way of consuming propolis. Additionally, propolis hydrophobic compounds may contribute to improve some properties of polymer films, such as the water vapour barrier.

Table grapes experience severe problems during postharvest such as weight and firmness losses, colour changes and berry decay. The main postharvest pathology is the gray mold caused by *Botrytis Cinerea*. To solve this problem, chemical fungicides, like SO_2, have been commonly used, but the increasing demand for more natural foods have stimulated new alternatives such as the use of modified atmospheres (Artés-Hernández et al. 2006) and thermal treatments combined or not with the application of natural or low toxicity compounds in edible coatings (Serrano et al. 2006). The Muscatel cultivar,

in particular, presents little colour homogeneity, a great tendency to browning and low gloss, all of which could be improved by applying edible coatings. In the revised literature, no data were found either about the application of edible coatings to the muscatel var. or the incorporation of propolis extract in coatings

The aim of this work was to analyse the effect of hydroxypropylmethylcellulose incorporated with propolis extract on the development of the physicochemical properties, respiration rate and microbial counts of table grapes, cv. Muscatel, throughout 22 storage days at 1-2ºC. Controls were carried out in all samples after 2 days at room temperature conditions to simulate the shop display period. Sensory quality was also evaluated.

2. Materials and methods

2.1. Raw materials

Organically grown table grapes (Vitis vinifera cv. Muscatel) were harvested in Pinos (Alicante, Spain) and immediately transported to the laboratory, washed in 10 mL/L sodium hypochlorite solution to remove residuals prior to coating, drained and dried at room temperature. The grapes, selected without signs of mechanical damage or fungal decay, were standardized in small clusters with grapes homogeneous in size, shape and colour.

Hydroxypropylmethylcellulose (Methocel® E15 Food Grade) was supplied by The Dow Chemical Company (Midland, USA) and crude propolis, from Bonamel Organic S.L. (Alquería de Aznar, Spain).

2.2. Film-forming dispersions methodology

The ethanolic extract of propolis (EEP) was prepared as follows: 10 g of propolis was extracted with 100 mL of 96º ethanol at 20ºC, for 7 days, using periodical manual shaking in dark conditions. The mixture was filtered through a Whatman Nº 1 filter paper and the filtered solution was used as EEP.

Hydroxypropylmethylcellulose (5% wt) was dispersed in distilled water at 80ºC for 2 h and stirred overnight at room temperature. After that, EEP was added to the hydroxypropylmethylcellulose solutions to reach final concentrations of EEP solids in the solution of 0, 0.5, 1 and 1.5% wt. Thus, the mass fraction of EEP in the dried films was 0, 0.091, 0.167 and 0.231, respectively. These film-forming dispersions were named M, M-0.5P, M-1P and M-1.5P, respectively. The mixtures were emulsified at room temperature using a rotor stator homogenizer ultraturrax (DI25 Yellow Line, IKA®, Germany) at 13,500 rpm for 4 min and then degasified at room temperature by means of a vacuum pump.

2.3. Application of the coatings

Selected clusters with 12-15 grapes were dipped in the film-forming dispersions for 1 min. Afterwards, they were hung up and dried at room temperature and natural convection for 2-3 h and then cold stored in perforated PET trays in an incubator (EC-1400-HR, Radiber S.A., Spain) at 1-2ºC and 85-90% R.H.

2.4. Grape characterization

Three different clusters for each time/treatment were characterized as to the different properties described below, at different cold storage times (3, 5, 8, 12 and 22 days). After each cold period, samples were placed under room temperature conditions for 2 days before the analyses, to simulate market operations.

2.4.1. Total soluble solids and pH

Seedless grapes were ground using an ultraturrax at 13.500 rpm for 1 min. Total soluble solids were measured by a refractometer (3T, Atago Co., Ltd., Japan) and measurements of pH were carried out by means of a pH-meter (GLP21+, Crison Instruments, Spain). Both analyses were carried out at 20ºC.

2.4.2. Total phenols

Total phenols were extracted according to the method described by Tomás-Barberán et al. (2001). 35 g of seedless grapes, with 40 mL of methanol plus 10 mL of HCl 6N and 4.2 mg of NaF, to inactivate polyphenol oxidases and prevent phenolic degradation, were ground using an ultraturrax at 9,500 rpm for 5 min. Then, the homogenate was centrifuged at 10,000 rpm, 4ºC and 10 min to obtain the supernatant. Total phenols were quantified by using the method reported by Selvendran and Ryden, (1990) and Benzie and Strain, (1999), based on the Folin-Ciocalteu method. 250 μL of supernatant was mixed in a volumetric flask of 25 mL with 15 mL of Milli-Q water, plus 1.25 mL of Folin-Ciocalteu reagent for 8 min. Then, 3.75 mL of 7.5% Na_2CO_3, plus the required amount of Milli-Q water, were added. After thorough mixing, the volumetric flasks were incubated in darkness for 2 h at room temperature. Absorbance was measured at

765 nm by using a spectrophotometer (Helios Zeta UV-VIS, Thermo Fisher Scientific, UK). The total phenolic content was expressed as mg of gallic acid equivalent per gram of sample, using a standard curve range of 0-800 mg/mL of gallic acid (Sigma-Aldrich, Germany).

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2.4.3. Antioxidant activity

Antioxidant activity was assessed using the free radical scavenging activity of the samples evaluated with the stable radical 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 160 according to the method described by Sánchez-Moreno et al. (2003). 10 g of seedless grapes with 10 mL of methanol were ground using an ultraturrax at 9,500 rpm for 5 min. Then, the homogenate was centrifuged for10 min at 10,000 rpm and 4ºC to obtain the supernatant. 0.1 mL of supernatant was added to 3.9 mL of DPPH solution (0.03 g DPPH/L methanol; DPPH, Sigma-Aldrich, Germany; Methanol, Panreac, Spain). 165 Absorbance (A) at 515 nm was measured by using a spectrophotometer (Helios Zeta UV-VIS, Thermo Fisher Scientific, UK) at 10 s intervals until the reaction reached a plateau (time at the steady state). The percentage of DPPH was calculated as the ratio of the A_t minus A_0 with respect to A_0 where, A_0 is absorbance at time t=0 and A_t is absorbance at time t.

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2.4.4. Weight loss

Weights of coated and uncoated grape clusters were controlled at different storage times. Cumulative weight losses were expressed as a percentage loss of the initial 175 weight (cold storage time=0)

2.4.5. Measurement of mechanical properties

The mechanical properties were measured by using a texture analyser (TA-XTplus, Stable Micro Systems, UK) with a 50 kg load cell, using a 75 mm diameter cylindrical probe. Grapes from each cluster (fifteen per treatment and time of storage) were placed longitudinally with the peduncle on the left of the texture analyser and 50% compressed at a speed of 2 mm/s. Force and distance at the failure point were used as mechanical parameters. The area (N.mm) under the force-distance curve till failure was also determined in each case.

2.4.6. Colour measurement

Colour was measured using a spectrocolorimeter (CM-3600d, Minolta Co., Japan) with a 10 mm diameter window. Measurements were taken in the different grapes (15: 5 of 3 different clusters) for each throughout the complete storage period. To avoid the effects of heterogeneity in the fruit, measurements were always carried out in the same previously marked sample zone in the grape. CIE-L*a*b* coordinates, hue (h^*_{ab}) and chrome (C^*_{ab}) (CIE, 1986) were obtained from the reflection spectra of the samples using D65 illuminant/10º observer.

2.4.7. Respiration rate

In order to measure the respiration rate, a closed system was used (Castelló et al. 2006). At each sample time during storage, grape clusters (each one about 150-200 g) were

placed in 0.655 L hermetic glass jars with a septum in the lid for sampling gas in the headspace at different times. Gas sampling was carried out every 30 min for 10 h by means of a needle connected to the gas analyser. O_2 y CO_2 contents were measured using an O_2 and CO_2 meter (Checkmate 9900, PBI Dansensor, Denmark).

This headspace gas analyser is based on an electrochemical sensor to record the 0_2 content and a mini-IR spectrophotometer to record CO_2 content (Rocculi et al. 2005). Experimental points were considered in the time range where a linear relationship was observed between gas concentration and time. This means that no changes in the respiration pathway of the samples occurred in this period and so changes in the headspace composition did not produce notable alterations in their metabolism. Respiration rate (RRi, mg kg-1 h-1) of the samples in terms of CO_2 generation and O_2 consumption was determined from the slope of the fitted linear equation, as described by Fonseca et al. (2002).

Respiration rates at time 0 were only determined in uncoated samples while, for all treatments, they were measured at 5, 8, 12 and 22 days of cold storage.

2.4.8. Microbiological analysis

Total aerobic mesophilic microorganisms, yeast and mould populations were evaluated periodically throughout storage. In sterile conditions, 10g of sample was homogenized for 2 min with 90 mL of tryptone phosphate water (Scharlab, Spain) with a stomacher blender (Bag Mixer 400, InterScience, USA). Serial dilutions of fruit homogenates were poured in plate count agar (PCA, Scharlab, Spain) and chloramphenicol glucose agar (CGA, Scharlab, Spain) for enumerated mesophilic aerobic bacteria (ISO 4833, 2003) and yeasts and moulds (ISO 7954, 1987). PCA and CGA plates were incubated

respectively at 30ºC for 48 hours and at 25ºC for 5 days. All tests were run in duplicate.

2.4.9. Sensorial analysis

The difference-from-control test was used to determine whether a difference exists between five samples (including a blind control) and a control and to estimate the size of any such differences in gloss, odour, firmness and flavour. Uncoated grapes were defined as control (C) and all the other samples were evaluated with respect to how different each one is from that control (Meilgaard et al. 1991). This test is appropriate to evaluate heterogeneous samples (Aust et al. 1985). Sensory properties of fruit can be affected not only by postharvest coatings, but also by the position of a segment within the fruit, the position of a fruit within the tree, or even the application of preharvest coatings (Denver et al. 1995).

The verbal category scale with its corresponding numerical scale used was: no difference (0), very slight difference (1), slight/moderate difference (2), moderate difference (3), moderate/large difference (4), large difference (5) and very large difference (6).

One hundred grapes per treatment were sampled in the sensory analysis session. Two grapes were placed on white pots identified by a random three-digit code or five grapes labeled C in the case of control. The 40 judges were 23-50 year-old volunteers, selected among the Universidad Politécnica staff. The order of presentation of the pots was randomized for each judge. All evaluations were conducted in individual booths under white illumination at room temperature in an EU homologated sensory room. Mineral water was used as palate cleanser between samples (AENOR, 1997).

2.4.10. Statistical analysis

Results were analysed by a multifactor analysis of variance with 95% significance level using Statgraphics® Plus 5.1. Multiple comparisons were performed through 95% LSD intervals.

To analyze the sensory data the ANOVA procedure appropriate for randomized (complete) block design was used. The 40 judges were the "blocks" and the 5 samples were the treatments in the design. A Dunnett's test for multiple comparisons with a control was used to reveal whether the tested samples were significantly different from the blind control or not (O'Mahony, 1986).

3. Results and discussions

3.1. Development of weight loss and physicochemical properties

Figure 1 shows the development of weight loss, ºBrix, total phenols and antioxidant activity for uncoated and coated samples with the different films throughout the cold storage time. The pH values did not change significantly during storage and, although there were differences among samples, these are attributable to the natural variability of the product and not to the treatment, since no clear tendencies associated with the coating type were observed. These values ranged between 3.63-3.69 for the different treatment/time; the mean value being 3.67 ± 0.10. Due to the nature of fruit organic acids, the usual decrease in fruit acidity (Vargas et al. 2006) did not provoke notable changes in pH.

Weight loss of the grapes (Figure 1a) was significantly higher in uncoated samples,

although no significant effect of the type of coating was observed for the rest of the samples. It is remarkable that this occurred mainly during the first 3-7 days of cold storage and only slightly increased in the rest of the period, probably due to the reduction of the process driving force, since the chamber relative humidity and the water activity of the product tend to be nearer. The protector role of the coating could be observed. Nevertheless, no clear differences among samples whose coatings contained or not propolis were detected, despite the fact that previous studies on isolated films revealed a significant reduction in the water vapour permeability of the films when propolis extract was incorporated in similar ratios to those used in this study (Pastor et al. 2010). This could be due to the differences in the film extensibility on the fruit skin and the subsequent degree of coating uniformity (Villalobos-Carvajal et al. 2009; Vargas et al. 2006). The lack of coating uniformity causes inefficiency of the film in a stochastic way, which made the observations of the different effects difficult. An acceleration of weight loss was detected on the last day of storage which can be attributed to an increase of the fruit metabolic activity, associated to the tissue senescence at long storage times, which is slowed down by coatings. Previous studies have remarked on the effectiveness of polysaccharide coatings as a water barrier in citric fruits and its enhancement by the incorporation of lipids (Rojas-Argudo et al. 2009; Valencia-Chamorro et al. 2009).

Total solids of the samples (Figure 1b) increased during storage, from about 18.5ºB till the 3th cold storage day to about 20ºB from the 7th day onwards. The effect of sample coating was not detected; the natural variability probably inhibits the observations of possible, small differences induced by treatments. On the other hand, the phenol content (Figure 1c) sharply decreased during the first 3 cold storage days for all treatments, regardless of the coating treatment, and a progressive, slow decay occurred afterwards.

In this sense, it is remarkable that the initial variability (at t=0) in the phenol content in the samples was greater than at longer storage periods, which is coherent with the natural phenol content decay that occurs in grape maturation and postharvest stages. The activity of phenylalanine ammonia-lyase (PAL) is key in the phenol compound accumulation in grapes and this activity decreases in the maturation and postharvest stages (Meng et al. 2008).

After 3 cold storage days, the phenol contents of all samples reached very similar values, which progressively reduce during cold storage, as was previously observed in grapes (Meng et al. 2008) and in other non-climateric fruits, such as strawberry (Ferreyra et al. 2007). Nevertheless, since non-red colour compounds are present in this white cultivar, this did not lead to a decrease in grape colour, as commented on below.

Antioxidant capacity of the samples (Figure 1d) slightly increased from the 7^{th} cold storage day on, regardless of the treatment, which could be attributed to the development of Maillard compounds in line with the development of the brown colour described below. Enzymatic browning could also contribute to the formation of antioxidant compounds, since an increase in PPO and POD activities was observed in grapes during postharvest storage (Meng et al. 2008). Of all the grape phenol compounds, it is the phenolic acids (cynnamic and benzoic, esterified or not with tartaric acid) which are mainly present in white grapes. These compounds are highly oxidable, producing brown compounds which also show antioxidant activity.

3.2. Mechanical and colour properties

Compression test on both uncoated and coated samples that had been cold stored for different times did not reveal any significant differences in the mechanical response

provoked by coating or storage time. The mean values and deviation of force and distance at failure and the area under the curve were 22 ± 8 N, 10.5 ± 0.9 mm and 98 ± 35 N.mm, respectively. Although a loss of firmness during postharvest cold storage was observed for other grape varieties (Valverde et al. 2005), strawberry (Vargas et al. 2006; del Valle et al. 2005; Mali and Grossmann, 2003), apple (Moldao-Martins et al. 2003) and sweet cherry (Yaman and Bayindirh, 2002) that was uncoated or coated with different edible coatings, this was not observed in muscatel grapes, in all likelihood masked by their hard skin, the break of which during the mechanical test would be principally responsible for the mechanical response.

The detected changes in total phenols and the development of brown compounds associated with enzymatic and non-enzymatic browning, typical of the fruit's development to senescence, will be associated with visible or measured changes in external colour. Figure 2 shows the development of different colour coordinates for the different coated and uncoated samples. Clarity (L*) and hue (h^*_{ab}) decreased with storage time, which reflects the development of sample browning. This decrease was significantly greater in uncoated samples, whereas using different coatings did not lead to any detectable differences. This implies that coatings slow down the non-enzymatic and enzymatic browning probably due to the oxygen barrier effect, since oxygen actively participates in these processes. The increase in the a* coordinate (greater in uncoated samples) was coherent with the hue changes and with the sample browning rates. When the highest amount of propolis was added to the film, this significantly increased L^* values which can be explained by the notable increase in the film´s opacity (Pastor et al. 2010) that inhibits light absorption of the grape surface, thus smoothing its colour.

Studies into the enzymatic activity of grapes during cold storage reflect a great increase

in polyphenoloxidase (PPO) and peroxidase (POD) activities (Meng et al. 2008) which explain both the decrease of polyphenol content as well as the development of brown colour in this white grape cultivar.

3.3. Respiration rates

The effect film application has on fruit respiration was evaluated through oxygen consumption and CO_2 generation, and the respiration quotient (RQ) was calculated. In raw materials, these values expressed as mL/kg.h were 18±2 and 21±3 for oxygen consumption and CO_2 generation, respectively, while the respiration quotient was 1.16±0.06, near 1, which reflects the normal respiration pathway of the fruits.

Figure 3 reflects the development of respiration rates as a function of the cold storage time. In the case of oxygen consumption, no significant differences among samples were observed at short storage times while the respiration rate decreased due to cold storage. Nevertheless, at longer times, where an acceleration of the respiration rates occurred in the process of fruit senescence, significant differences were detected between uncoated and coated samples, but there was no observed significant effect of the amount of propolis in the coating, as occurred in the weight loss. This indicates that coatings represent an oxygen barrier that limit the acceleration of aerobic respiration rates. In general, respiration rates are linked to sample weight loss, as has been reported in previous studies (Fallik et al. 2005; Porat et al. 2005; Valverde et al. 2005).

All the samples showed an increase in the CO_2 generation rate from 5 days of cold storage on, which agrees with the increase in the metabolic activity of samples at long storage times related with tissue senescence and cell breakdown. A reduction of CO_2 production in coated fruits has been described in grapes (Valverde et al. 2005) and other

fruits such as avocado (Maftoonazad and Ramaswamy, 2005) and sweet cherry (Alonso and Alique, 2004). Nevertheless, this was not observed in muscatel grapes coated with HPMC which could be related with a greater permeability of this polymer to CO_2, as described for pure HPMC coatings (Miller and Krochta, 1997).

Nevertheless, as compared with other grape cultivars and fruits, very low respiration rates were observed throughout storage, with few changes in these rates, these being only remarkable at the end of the storage period (24 days). This suggests that, under these conditions, it is likely that no significant modification of the internal atmosphere was caused in coated samples. The respiration quotients varied between 1.3 and 1.5 and, as expected, slightly increased throughout the storage time, especially in the coated samples where the oxygen consumption was mildly inhibited.

3.4. Microbiological analysis

Figure 4 shows the results of the microbiological analysis for coated and un-coated grapes. It is remarkable that the microbial quality of grapes was maintained after 24 days of storage for both uncoated and coated fruit. Nevertheless, a slightly lower microbial growth could be observed in coated (especially in treatment M-1.5P) than in uncoated samples, for both mesophilic aerobic counts and yeast and mould counts.

Likewise, although stochastic decreases in total counts were observed for determined times, in general, the microorganisms were observed to grow as expected , without once reaching the allowed levels, since they remain below 0.4 logs UFC/g.

3.5. Sensorial analysis

The results of the sensory analysis were summarized in Table 1 and Figure 5. As expected, coated grapes were significantly glossier than the uncoated grapes. This result is coherent with what was observed with dried films. In contrast, the judges found no significant differences in firmness between uncoated and the different coated grapes, which is in line with the instrumental determinations described above. Significant differences in odour and flavour appreciation were found between the uncoated samples and those coated with HPMC containing propolis, due to the impact of the taste of propolis on the overall flavour and odour. Nevertheless, differences were much less appreciable than when pure propolis was used to cover the grapes directly (unpublished results), which indicates that HPMC encapsulates propolis compounds, thus diminishing their sensory appreciation.

4. Conclusions

HPMC coatings prevent weight losses and browning of muscatel table grapes during cold storage, while improving their gloss and microbial safety and controlling the increase in oxygen consumption during cold storage. Propolis incorporation increased the colour clarity of the grapes due to the greater film opacity, which contributes to homogenize the grapes' appearance, this being considered positive for their quality. Nevertheless, no significant effect of the incorporation of propolis was observed on the preservation of grape quality during storage. However, if the health properties of propolis are taken into account, its incorporation in the HPMC coatings contributes to enrich the nutritional characteristics of the coated product and, in this sense, coating can be considered as a way to obtain healthier fruits.

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Figure Captions

Figure 1. Development of (a) weight loss, (b) the total soluble solids, (c) the total phenol contents and (d) the antioxidant activity throughout cold storage of samples. Mean values and LSD intervals.

Figure 2. Development of Luminosity (L*), a* coordinate, chrome (C^*_{ab}) and hue (h^*_{ab}) throughout cold storage of samples. Mean values and LSD intervals.

Figure 3. a) Development of O_2 (RRO_2) throughout storage for uncoated (C) and coated samples. b) Development of CO_2 ($RRCO_2$) throughout storage for all samples. Mean values and LSD intervals.

Figure 4. a) Development of growth of yeast and moulds and aerobic mesophilic microorganisms throughout storage for all samples (Mean values for all samples which did not show significant differences among them). b) Development of growth of yeast and moulds and aerobic mesophilic microorganisms for uncoated (C) and coated samples. Mean values and LSD intervals.

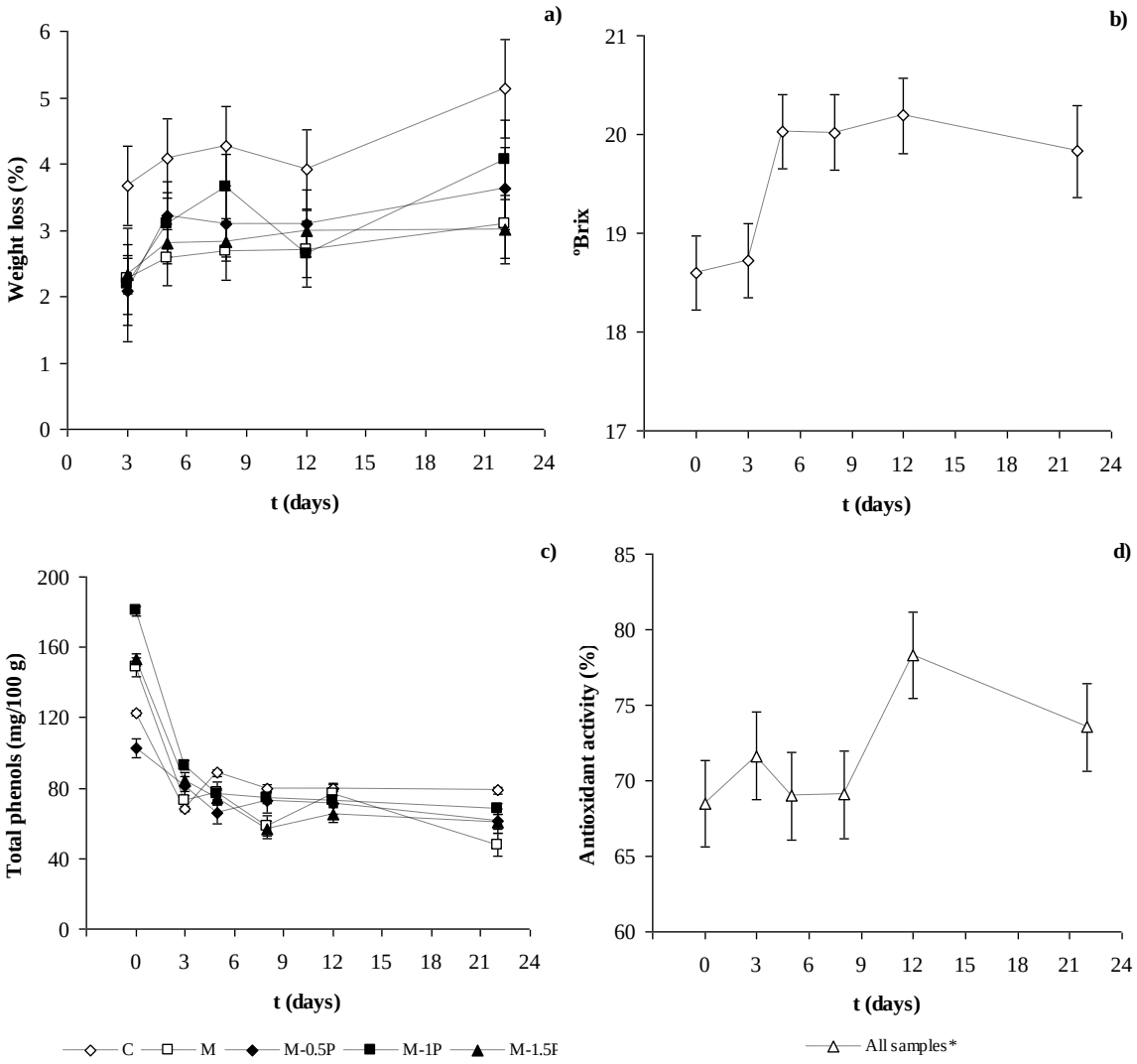
Figure 5. Average sensory scores of samples

Table 1.- Dunnett´s test values for uncoated and coated grapes.

Atributes	d[z]	C	M	M-0.5P	M-1P	M-1.5P
Gloss	0.56	0.62a[y]	3.52b	2.05b	1.20b	1.37b
Odour	0.71	0.36a	0.54a	2.49b	2.77b	2.85b
Firmness	0.73	1.33a	2.03a	1.87a	1.58a	1.48a
Flavour	0.75	1.00a	1.15a	1.90b	2.25b	2.38b

[z] Dunnett´s test parameter
[y] Means within rows followed by unlike letters are different according to Fisher's Protected LSD test (P = 0.05).

Figures



*Mean values for all samples which did not show significant differences among them.

Figure 1

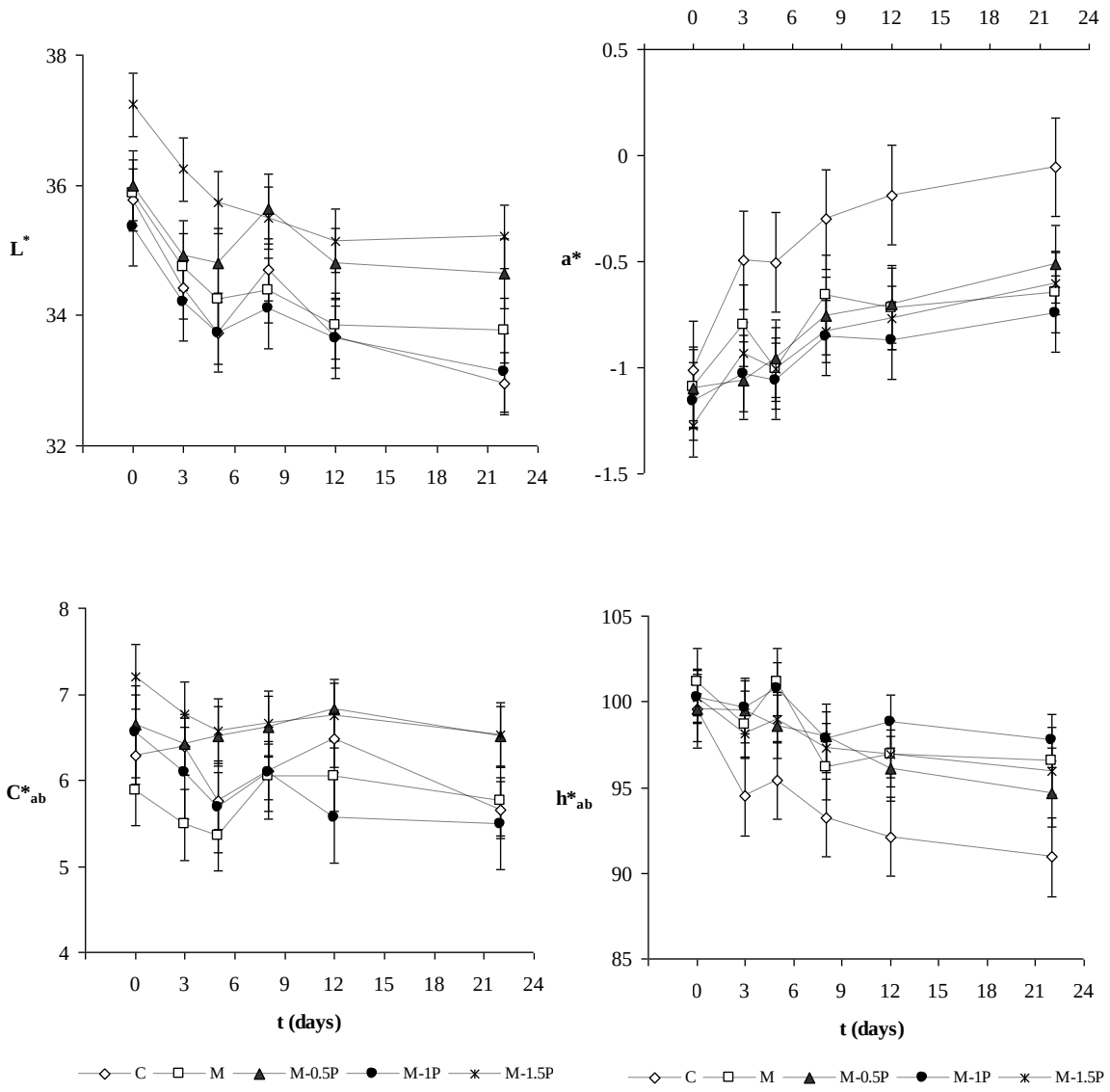
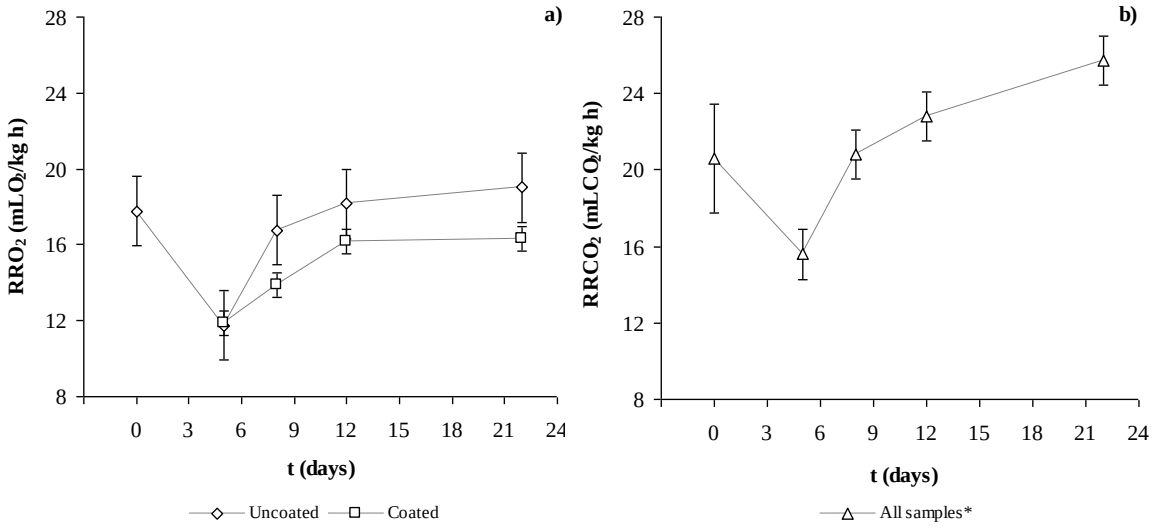


Figure 2



*Mean values for all samples which did not show significant differences among them.

Figure 3

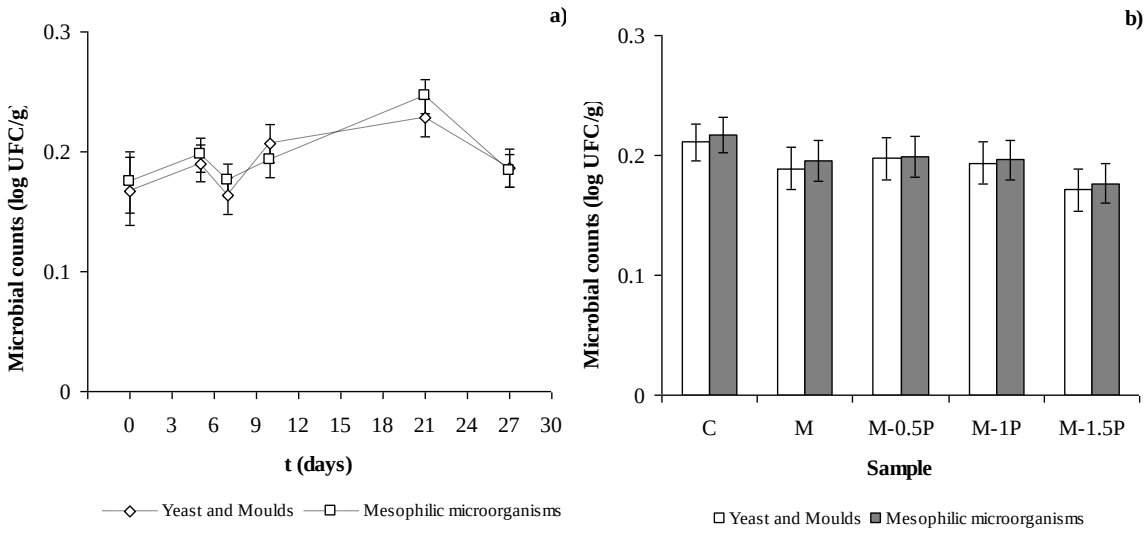

a)
b)
Microbial counts (log UFC/g)
0.3
0.2
0.1
0
0 3 6 9 12 15 18 21 24 27 30
t (days)
Yeast and Moulds
Mesophilic microorganisms
C
M
M-0.5P
M-1P
M-1.5P
Sample

Figure 4

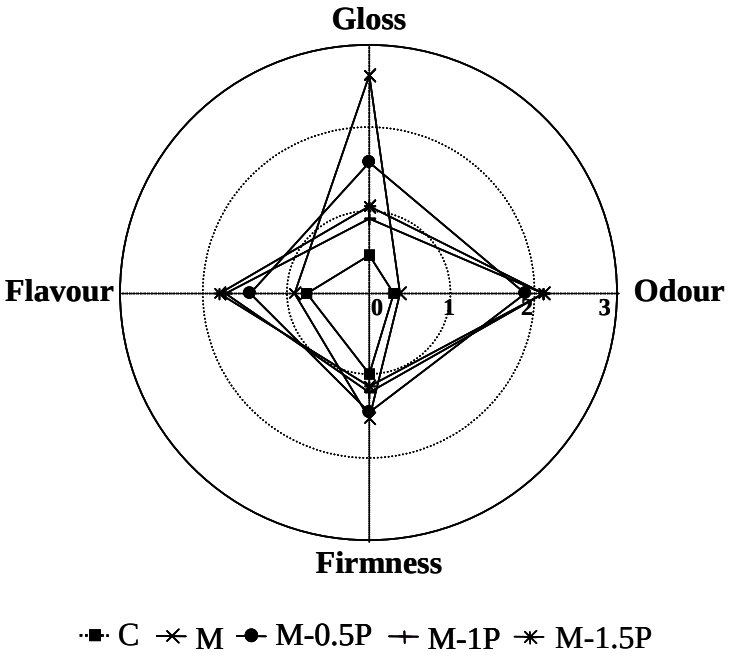


Figure 5