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Influence of minimally processed grapes washing with lemon essential oil

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Abstract

The increased interest in ready to eat products along with the great production of grapes make necessary to find a new way of presentation of this product in order to ease its consumption. The main goal is to keep quality and extend the shelf-life of grains of table grapes by applying different concentrations of lemon essential oil (LEO) which were applied in a preliminary stage of immersion. Samples were stored in PET trays at 5 ºC for 21 days. Soluble solids content, pH, acidity, antioxidant capacity, optical and mechanical properties and microbiology counts were periodically analysed. Noteworthy was that the application of LEO in the washing stage did not keep the preservation of the grapes' colour and texture. All samples would be considered safe according to microbiology requirements and based on the period of study, regardless of the concentration of LEO applied.

Introduction

According to FAOSAT data, Spain is the fifth largest worldwide producer of grapes in 2011 (FAOSTAT, 2013). Production of table grapes in Spain in 2011 was 243435 tonnes (MAGRAMA, 2013). Table grapes are a non-climacteric fruits with low physiological activity. In addition, these fruits are sensitive to water loss and fungal infection (mainly by Botrytis cinerea) during postharvest handling (Artés-Hernández *et al*., 2004). From the nutritional point of view, grapes are considered important sources of phenolic compounds, which are mainly responsible for their antioxidant properties (Baiano and Terracone, 2011; Melgarejo-Flores *et al*., 2013). Moreover, the increasing growth of minimally processed products, easy to consume and the globalization of food trade and distribution imply major challenges to food safety and quality (Appendini and Hotchkiss, 2002). This makes necessary to research new methods to achieve these objectives.

The modified atmosphere packaging (MAP) is one of the most used methods of preservation. The MAP allows the modification of the gas atmosphere inside the package due to the respiration of the product and transfer of gases through the packaging (Fonseca *et al.*, 2002). Products will have a longer shelf-life if the permeability of the packaging material is suitable for the respiration of the product, and also, an equilibrium modified atmosphere can be reached inside the package (Sandhya, 2010). The use of MAP to maintain the quality of table grapes has been studied by several researchers (Martínez-Romero *et al.*, 2003; Artés-Hernández *et al.*, 2004; Valverde *et al*., 2005; Valero *et al.*, 2006; Artés-Hernández *et al*., 2006; Guillén *et al*., 2007). Furthermore, there are many research that have studied the combination of MAP with the application of certain additives on minimally processed products in order to maintain its quality and shelf-life (Rocculi *et al*., 2004; Sapers and Miller, 1998).

Essential oils (EOs) are very complex natural mixture of different components and come from aromatic plants (Bakkali *et al*., 2008). In fact, Fisher and Phillips (2006) analyzed the main components of lemon, orange and bergamot essential oils by gas chromatography. This gas chromatographic determination indicated that the most important component of lemon essential oil (LEO) was limonene (95%), followed by linalool and citral. Many researchers have studied the combined use of MAP and natural antimicrobial compounds on different fruits, as table grapes (Valverde *et al.*, 2005; Valero *et al.*, 2006; Guillén *et al.*, 2007;) and sweet cherry (Serrano *et al*., 2005). All of them used these antimicrobial substances inside the packages, avoiding the contact with the fruits. Valverde *et al.* (2005) observed that from the microbiological point of view, the use of these natural antimicrobial components with MAP implied a microbiological reduction. In addition, this antimicrobiological effect was more effective on molds and yeasts counts than for mesophilics aerobics. Other authors, Melgarejo-Flores *et al.* (2013) used cinnamon leaf oil (CLO) applied in different ways to table grapes (water emulsions, vapors or incorporated into pectin coatings). They concluded that CLO as vapors or coatings could be used to control decay and increase the antioxidant health benefits of grapes due to CLO's antifungal and antioxidant properties. Based on the

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previous information, the aim of this study was to evaluate the antioxidant and antimicrobial effects of lemon essential oil applied in a dispersion solution at different concentrations on table grapes. Additionally, its influence on the compositional, physiological, optical and mechanical characteristics of this fruit was studied.

Materials and Methods

Raw materials

Fruits of grapes (*Vitis vinifera* L.) of the variety Moscatel Italiano (Monforte del Cid, Alicante) were used to carry out these experiments. They were acquired 24 hours prior to use and stored at 4ºC before being processed. Grapes from different clusters were mixed and also the stalks of the grapes were removed. Fruits were selected based on their size, colour, absence of defects and general appearance in order to increase the uniformity of these samples. The essential oil used in this study was lemon (*Citrus limonum* L.) (Soria Natural, Soria, Spain), which was acquired from herbalism (Valencia, Spain).

Application of essential oils

After selecting the grape, they were washed with solutions of LEO at different concentrations (150 and 300 ppm) for 10 minutes. While the samples were immersed in these solutions they were gently stirred, following which the samples were drained for 1 minute. Samples washed only with tap water were used as a control. In all cases, the temperature of the solution was 5ºC.

Storage conditions and sample size

130 g of grapes were packaged in polypropylene terephthalate (PET) trays. There were approximately 12 units of grapes per tray. Grapes packaged were stored at 4ºC over the full storage period. This experiment was carried out for 21 days. All containers were analyzed periodically (each 7 days): ºBrix, pH, acidity, weight loss, antioxidant capacity, colour, texture and microbiological. A total amount of 108 trays of grapes were used to this study. Three trays were used for each treatment and time. Concretely, 36 trays were intended for analyzing changes in ºBrix, pH, acidity and antioxidant capacity; 36 trays were aimed at analyzing optical and mechanical properties and 27 trays were used to follow the microbiology growth. Finally, the measurement of changes in gas composition of the headspace was carried out in 3 trays per treatment.

ºBrix, pH, acidity and weight loss

Soluble solids (expressed as ºBrix) were measured in previously homogenized samples using a refractometer (Zeiss, ATAGO model NAR-3T, Japan) at 20ºC and pH was obtained directly from the homogenized sample using a pH-meter ("Seven Easy") with contact electrode. Titratable acidity was determined by potentiometric titration with 0.1 N NaOH (Panreac, Barcelona, Spain) of up to pH 8.1-8.2. Results were expressed as g of tartaric acid per 100 g of sample.

Antioxidant capacity

Antioxidant capacity was determined by means of the DPPH method (Brand-Williams *et al*., 1995), which is based on the antioxidant capacity to match free radicals. DPPH (2,2-diphenyl-1-picryhyldrazyl) is a free radical which can react directly with antioxidants and be blocked by them (Smith *et al.*, 1987; Jiménez *et al*., 1998; Koleva *et al*., 2002). The reduction of DPPH-H is controlled by the decrease in absorbance of a characteristic wavelength at a given time during the reaction. In the radical form (DPPH•), it absorbs at 515 nm, but when it is reduced by an antioxidant (AH) or radical species (R•), absorption disappears.

For analysis of the samples, the grapes were peeled, separating on the one hand the skin, and on the other hand the pulp. 5 grams of sample (skin or flesh) diluted in methanol in a 1:2 (w/v) ratio were shaken for 5 min. Then, samples were centrifuged for 20 min at 4ºC, keeping the supernatant. A solution of 0.024 g/L DPPH was prepared. The absorbance of 3.9 mL of the DPPH solution was read at 515 nm in a spectrocolorimeter (Helios Zeta UV-VIS). Then 1 mL of supernatant of the spreadable sample diluted in methanol was added to the DPPH solution and absorbance was read again after 30 min. The analysis of antioxidant capacity was determined in triplicate. Antioxidant capacity results were expressed as inhibition of DPPH (%) (equation 1):

$$\text{Inhibition DPPH}\,(\%) = \left[\frac{(A_{control} - A_{sample})}{A_{control}} \text{x}100\right] \quad (1)$$

Where:

$A_{control}$ = DPPH solution absorbance at 515 nm before adding sample

A_{sample} = DPPH solution absorbance at 515 nm 30 minutes after adding the sample

Analysis of optical parameters

The colour of persimmon samples was measured using a spectrocolorimeter Minolta (CM-3600 d) with a window of 7 mm in diameter. For each treatment, half of the units of grapes in each tray were analyzed of each package were analysed (18 replicates) due to

the high variability in colour of samples. CIE-L*a*b* coordinates were obtained using D65 illuminant and 10º observer as reference system. These values were then used to calculate hue (h = artg [b*/a*]) and chrome ($C^* = [a^{*2}+b^{*2}]^{1/2}$).

Measurement of mechanical properties

Mechanical properties were analysed using a texture analyzer (TA/XT/PLUS Aname) by means of a puncture test (2 mm diameter punch) at a speed of 1 mm/s and considering a penetration distance of 10 mm. As in the colour analysis half of the samples of each tray were used (18 replicates) for each treatment. The parameters analyzed were: maximum force (F, N) and distance at which the maximum force took place (d, mm).

Microbiology

Serial dilutions were prepared by homogenising 10 g of grapes with 90 mL of 1% sterile peptone water in a stomacher bag, using sterile techniques. Mesophilic aerobic populations were analysed in Plate Count Agar (Scharlau Chemie, 1-329, Barcelona, Spain) incubating samples for 72 h at 31ºC. Yeast and moulds were determined in Sabouraud Chloramphenicol Agar (Scharlau Chemie, 1–166, Barcelona, Spain) plates for 5 days at 31ºC. Samples for analysis were taken on the processing day (0 day) and at 14 and 21 days. Sample dilutions were prepared, and after the incubation time, Petri dishes with a number of colonies between 30 and 300 for total mesophilic aerobic count and between 0 and 30 for moulds and yeast, were considered. Experiments were carried out in triplicate.

Statistical analysis

An ANOVA analysis using Statgraphics Centurion Software was performed to evaluate the effect of process variables (dose of essential oil and time of storage) on the results obtained.

Results and Discussion

The evolution of compositional changes (solid content expressed as ºBrix, pH and titratable acidity) and also the weight loss of grape samples throughout the storage of control and EO treated samples are shown in table 1. The soluble solids content remained constant during time storage and besides, the treatment used did not have influence in this compositional analysis. The values registered after the washing stage, were slightly lower in this study than those reported by Valverde *et al.* (2005), who obtained 20.59 ± 0.10ºBrix in table grapes of the variety *Vitis vinifera* L. cv "Crimson Seedless"

Table 1. Values soluble solid expressed as ºBrix, pH, titratable acidity and weight loss of grapes washed with tap water and lemon essential oil (LEO) throughout storage

Sample	Time (d)	º Brix	pH	Acidity (g tartaric acid/100 g)	Weight loss (%)
Tap water	0	17.6 (0.6)^{a}	4.07 (0.01)^{ab}	0.300 (0.106)^{b}	
	7	18 (0.4)^{a}	4.16 (0.03)^{b}	0.390 (0.000)^{c}	0.31 (0.12)^{b}
	14	18.1 (0.7)^{a}	4.06 (0.02)^{ab}	0.329 (0.008)^{bc}	0.3 (0.3)^{c}
	21	17.87 (0.15)^{a}	3.98 (0.03)^{a}	0.36 (0.02)^{bc}	0.4 (0.3)^{d}
150 ppm LEO	0	18.3 (0.2)^{a}	4.10 (0.02)^{b}	0.23 (0.00)^{a}	
	7	18.45 (0.07)^{a}	4.20 (0.01)^{c}	0.32 (0.04)^{b}	0.175 (0.002)^{a}
	14	17.6 (0.4)^{a}	4.09 (0.06)^{b}	0.33 (0.02)^{bc}	0.2 (0.2)^{bc}
	21	17.57 (0.15)^{a}	4.01 (0.11)^{a}	0.3425 (0.0115)^{bc}	0.3 (0.3)^{cd}
300 ppm LEO	0	18.45 (0.1)^{a}	3.97 (0.01)^{a}	0.23 (0.00)^{a}	
	7	18.6 (0.4)^{a}	4.18 (0.01)^{b}	0.36 (0.04)^{bc}	0.21 (0.07)^{ab}
	14	18.5 (0.8)^{a}	4.01 (0.03)^{ab}	0.36 (0.02)^{bc}	0.3 (0.2)^{c}
	21	18.0 (0.2)^{a}	3.88 (0.01)^{a}	0.35 (0.02)^{bc}	0.4 (0.3)^{d}

Parentheses indicate standard deviation
Same letters indicate homogeneous groups

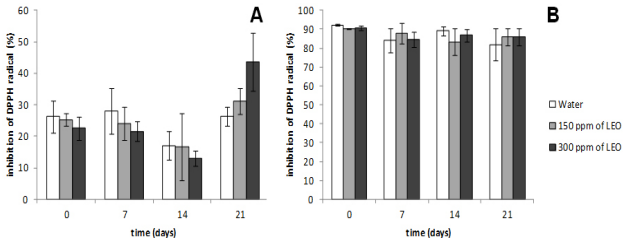

Figure 1. Evolution of antioxidant capacity in grapes samples (pulp = A and skin = B) washed with tap water and lemon essential oil (LEO) throughout storage.

at harvest and also, Valero *et al.* (2006) who detected levels of TSS of 18.34 ± 0.16ºBrix in the variety *Vitis vinifera* L. cv "Autumn royal" at harvest. Regarding pH value, it hardly changed during the studied. Artés-Hernández *et al.* (2004) obtained a similar pH value (4.06) in table grapes of the variety *Vitis vinifera* L. cv "Autumn Seedless" at harvest. On the other hand, acidity showed only a slight increase after the first week of storage remaining constant throughout the following weeks. The treatment used did not have influence in this quality parameter. In addition, the acidity values in this study were also slightly lower than those measured by Valverde *et al*. (2005) and Valero *et al*. (2006) at harvest. Along with the fact that they were other cultivars, in the present study the stalk was removed and that could mean the entrance of water into the matrix of the fruit with a consequent minor acidity and lower content of soluble solids.

There was a weight loss during the storage time for all treated samples. However, this loss was relatively small (0.55% at 21 days) with no significant difference among them. Guillén *et al*. (2007) noted that the weight losses on grapes bunches stored at MAP condition were lower than those stored at atmospheric air. There also was no influence of the type of film or treatment applied. Moreover, Valverde *et al*. (2005) who studied the effects of the combination of MAP with different components of essential oils on table grapes, observed that the application of these components inside the packages reduced more the weight loss than in the control samples. However, in the present study, the treatment with lemon essential oil (under the used conditions) did not influence in the

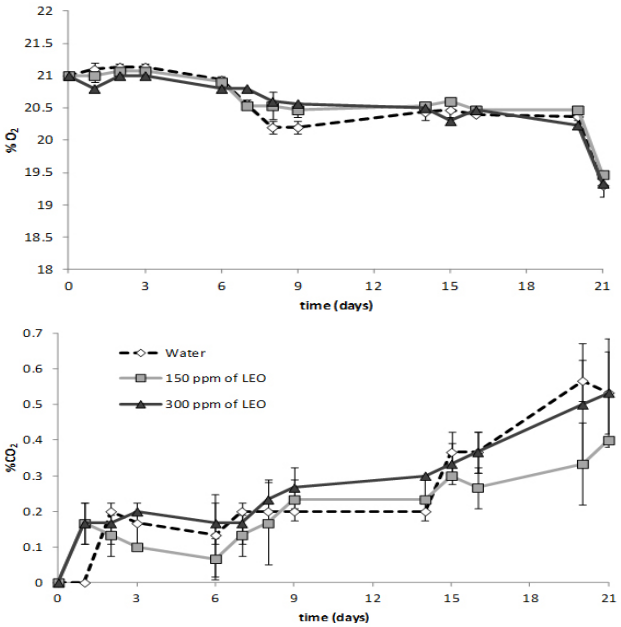

Figure 2. Evolution of the gases in the headspace atmosphere in grapes samples washed with tap water and lemon essential oil (LEO) throughout storage

mass loss during storage possibly due to the different way of application of the LEO.

Antioxidant capacity

Figure 1 shows the antioxidant capacity both pulp (A) and skin (B) of the grape throughout storage. As can be seen in Figure 1.B, in most of the cases, the antioxidant capacity of the grape skin was approximately three times higher than the in pulp. These results are in agreement with those obtained by Valero *et al.* (2006) who measured the total antioxidant activity (TAA) and the content of total phenolics in both skin and pulp of grapes at harvest, observing that both parameters were quite higher in the skin than in the pulp. In the present study, there was also a slight decrease in the antioxidant capacity of the grape skin samples at the end of storage. However, more changes were observed in pulp samples than in skin grape (Figure 1.A). A diminish was noted up to two weeks of storage. Moreover, no effect of the LEO on this parameter was observed during this period of time. Nevertheless, there was a significant increase in antioxidant capacity at the highest concentration of LEO at the end of storage in the pulp. This could be likely to the differences in the properties to LEO transport through these different phases. Thus, the more compacted distribution of cells in the skin implied a barrier to the transfer of the LEO to the pulp. If there had been more contact between LEO and the skin of the grape in the washing stage, it would have been easier to penetrate through the skin. In addition, the longer the storage time the more chances to the LEO to arrive to the pulp

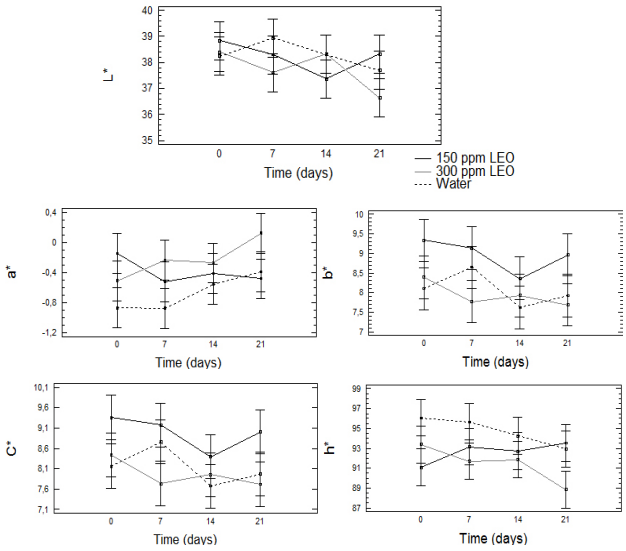

Figure 3. Changes in luminosity (L^*), a^* and b^* coordinates, hue and chrome in grape samples washed with tap water and lemon essential oil (LEO) throughout storage

which explains the increase in antioxidant capacity at 21 days of storage for grapes treated with 300 ppm of LEO. To sum up, there were no evidences of the antioxidant effect of the LEO in the conditions applied in grapes of this study.

Gas atmosphere evolution

Figure 2 shows the evolution of the gases (O_2 and CO_2) inside the containers of the treated grapes. No differences were shown in the evolution of the gas atmosphere between the different washing treatments. Thus, the LEO at the concentrations used did not change significantly the respiratory activity of the grapes. However, there was a slight decreased in O_2 concentration (from 21% to 19.5%) and a minor increased in CO2 (approximately to 0.6%) inside the packages. These changes in the headspace atmosphere were not great enough to affect the respiratory behaviour of the grapes. This could be due to the packaging material used. The package used in this experiment was made of PET which may not prevent the entry or outside air through the container. On the other hand, an atmosphere of equilibrium was not reached inside the container probably due to the high permeability of the package. In contrast, Martínez-Romero *et al*. (2003) who used two types of films of oriented polypropylene (nonperforated and perforated) for table grapes cv. Flame Seedless packaging, observed that the steady state was reached after 7 days of cold storage at 1ºC.

Guillén *et al.* (2007) studied the combination of MAP with natural antimicrobial compounds (eugenol, thymol and carvacol) on table grapes. They observed an increase in CO_2 and decrease in O_2 levels during the storage time at 1ºC. The atmospheric composition

Table 2. F-ratio values obtained from factorial ANOVA analysis for optimal and mechanical parameters. The factors for the analysis were: storage time and treatment (concentration of essential oil) and their interactions.

Analysed parameters	Factors		Factors Interaction (AB)
	Concentration LEO (A)	Storage time (B)	
Luminosity (L*)	1.25^{NS}	1.76^{NS}	1.43^{NS}
a* coordenate	5.71^{**}	1.46^{NS}	1.43^{NS}
b* coordenate	7.85^{***}	1.71^{NS}	0.66^{NS}
Chrome (C*)	7.86^{***}	1.74^{NS}	0.77^{NS}
Hue (h*)	6.29^{**}	1.15^{NS}	1.36^{NS}
Maximum force (F_{max}) (N)	5.18^{**}	2.55^{NS}	1.73^{NS}
Distance (mm)	2.27^{NS}	9.64^{***}	1.87^{NS}

NS: non statistical differences ($P \geq 0.05$).
* Confidential level: $P < 0.05$.
** Confidential level: $P < 0.01$.
*** Confidential level: $P < 0.001$.

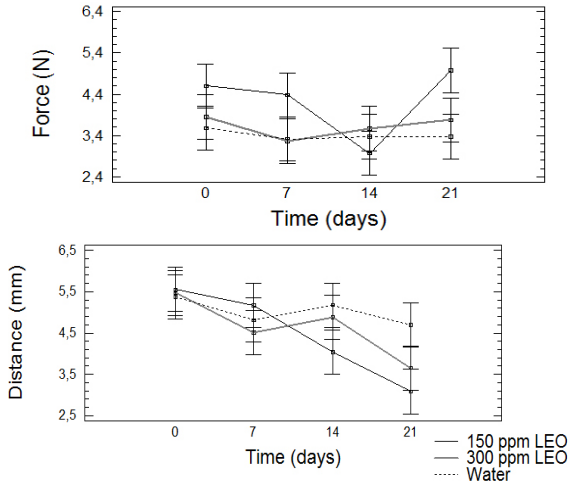

Figure 4. Changes in maximum force expressed in Newton (N) and the distance (d) at which it occurs in millimetres in grape samples washed with tap water and lemon essential oil (LEO) throughout storage.

inside the packages depended more on type of the film used than the addition of these natural compounds. Valverde *et al.* (2007) also observed an increase of CO_2 and a decrease in O_2 inside both the control packaging and in those which contained different natural antimicrobial components. However, those that contained menthol inside the package reached the highest values of CO_2 and the lowest values of O_2.

Optical properties

Figure 3 shows changes in luminosity (L^*), a^* and b^* coordinates, hue and chrome coordinates L^* a^* b^*, chrome (C^*) and hue (h^*) of the washed grapes along the storage. Moreover, table 2 shows the values of F-ratio of all the colour and mechanical parameters analysed in this study obtained according to a multifactorial ANOVA considering as factors: dose of essential oil and time of storage. No significant differences were observed in luminosity of the studied samples. However, the treatment had a significant effect on a^* and b^*, causing a decrease in hue of the samples washed with LEO at the studied concentrations, but no influence of time was observed in any optical properties studied. Moreover, samples washed with LEO at 150 ppm, had the higher values of b^* coordinate and chrome than grapes washed only with tap water and treated at 300 ppm of LEO. The results observed in other studies are different from these. In this regard, Guillén *et al*. (2007) observed that the use of MAP in grapes of the variety *Vitis vinifera* L. Cv. Aledo reduced the colour changes and the application of eugenol, thymol and carvacrol inside the package also implied a minor luminosity losses and an increase in chroma index. Besides, Valverde *et al.* (2005) observed that the appliance of eugenol, thymol or menthol implied a lower increase in hue angle and also, a lower decrease in L^* than the control on table grapes packaged. However, in the present study, the addition of LEO did not have so beneficial effect in terms of colour conservation.

Mechanical properties

Figure 4 shows values of maximum force and distance of grape samples throughout storage time. Grapes washed with 150 ppm of LEO (excluding values which were obtained after 2 weeks storage) had a more significant increase in maximum force than grapes washed with tap water or 300 ppm of LEO. In fact, as can be seen in table 2, the dose of the LEO had a greater influence than storage time in this mechanical property. Regarding storage time, no changes were observed in the breaking force of the grape berries, which could imply that the samples remained rather stable firmness in the period considered. Other authors, such as Guillén *et al.* (2007) noticed that grapes storage at atmospheric air softened more and faster than those which were stored in MAP with or without natural antimicrobial components inside the package. In fact, the use of these natural antimicrobial components reduced more the softening. Furthermore, Valverde *et al.* (2005) obtained a delay in the loss of firmness in those containers in which antimicrobial components (eugenol, menthol, thymol) were incorporated inside them, being eugenol the most effective.

Respect to the distance at which the maximum force took place, multifactorial ANOVA indicated the more influence of storage time than dose of LEO (Table 2), on samples washed with LEO especially with 150 ppm. Nevertheless, this parameter in grapes washed only with tap water was steady in time. Therefore, LEO would not keep the quality of mechanical properties. However, Guillén *et al.*, (2007) and Valverde *et al*. (2005), observed that grapes packaged with MAP and natural compounds of EO maintained better the mechanical properties than those without these compounds.

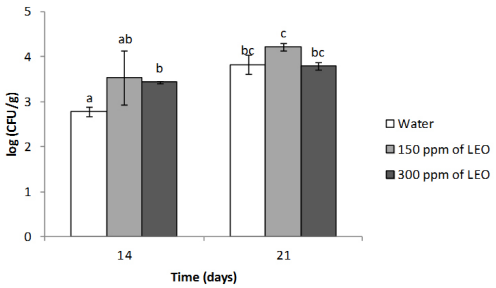

Figure 5. Microbial counts (decimal logarithm of colony forming units (log CFU/g)) in table grape samples washed with tap water and lemon essential oil (LEO).
Same letters indicate homogeneous groups.

Microbiology

Disinfection of fruits is usually carried out with liquid chlorine and hypochlorite (Rico *et al.*, 2007). In spite of the broad activity spectrum of chlorine against bacteria, moulds and yeasts (Krasaekoopt and Bhandari, 2001), it is remarkable that that chlorine and hypochlorite solutions can be corrosive especially at low pH, shortening the shelf life of tanks and others stainless steel equipment used in the processing of fresh cut products (Sapers, 2009). On the other hand, chlorine can react with different organic compounds in foods, producing trihalomethanes (Sánchez-Zafra, 2008; Huang and Batterman, 2010). Therefore, it is necessary to search for alternatives to chlorine treatment in minimally processed products.

As a reference for determining shelf-life from a microbiological point of view, the Spanish regulation for hygienic processing, distribution and commerce of prepared packaged meals with raw vegetables (BOE 12-1-2001, RD 3484/2000) was used. This law sets forth the microbiological limits at the manufacture date for aerobic mesophilic ($1•10^5$-$1•10^6$ CFU/g) as well as the expiration date ($1•10^6$-$1•10^7$ CFU/g). In the present study, the count of mesophilic aerobics, molds and yeasts did not exceed the microbiological limit in all cases. There was no microbial growth at the manufacture date. Microbial counts of mesophilic aerobics throughout the storage of control and LEO treated samples are shown in Figure 5. As can be seen, there was microbial growth during storage period. In addition, no antimicrobial effect of LEO was observed. However, the difference in mesophilic aerobics growth was very small between 14 and 21 days at the concentration of 300 ppm. This could indicate that this EO would have some antimicrobial activity at this concentration, which might be related to the aforementioned higher penetration of this concentration of LEO in the pulp in the antioxidant capacity part. Nevertheless, it would be necessary to extend the storage period to test the antimicrobial effect at this concentration. The application of the concentration of 150 ppm of LEO would not have been sufficient to exert antimicrobial activity. Furthermore, there was no growth of moulds and yeast in any of the treatments applied during the storage. On the other hand, application of natural antimicrobial compounds inside the package reduced considerable the microbiological growth. In relation to this, Guillén *et al.* (2007) obtained that viable counts were lower in grapes stored in MAP condition and with eugenol, carvacrol and thymol inside the container during storage time. The reduction in microbial populations of treated grapes with eugenol or thymol inside the package, were also observed by Valero *et al.* (2006). To sum up, higher concentrations of this EO would be necessary to observe its potential benefits as both microbiological and antioxidant agent.

Conclusions

LEO at the studied conditions did not affect the evolution of soluble solids, acidity and pH of grapes or their weight loss. As was expected, the skin of grapes showed a higher antioxidant capacidity than pulp, regardless the use of LEO. Considering the gas composition inside the package, no steady state was reached. LEO did not keep optical and mechanical properties of table grapes. Finally, despite the fact that LEO did not imply a lower count of aerobic mesophilic, it meant a lower rate in the growth of these microorganisms between 14 and 21 days when 300 ppm were used.

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