

## Effect of the application of thyme and lemon essential oils in packaging of minimally processed persimmon

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### Article history

Received: 27 January 2014

Received in revised form:

4 May 2014

Accepted: 8 May 2014

### Keywords

Persimmon

Essential oils (EOs)

Antioxidant capacity

Colour

Texture

Microbiology

### Abstract

The aim of this research was to develop a minimally processed persimmon product by applying different volumes (50, 250 and 500  $\mu\text{L}$ ) of thyme essential oil (TEO) or lemon essential oil (LEO) inside the package in order to increase shelf life. Samples were stored at 4°C for 13 days. Analyses were periodically carried out on moisture content, soluble solids content, antioxidant capacity, total phenols, pH, optical and mechanical properties and microbial counts. The results showed no improvements in antioxidant capacity, total phenol content, colour or texture resulting from the use of these essential oils (EOs) inside the package. Hence, the application of 500  $\mu\text{L}$  of LEO actually browned the samples. However, the highest doses of LEO (250 and 500  $\mu\text{L}$ ) did lead to a reduction of mesophilic aerobic which could give rise to a new strategy for extending the shelf life of cut fresh-persimmon products but considering the use of some antibrownings.

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### **Introduction**

The new way of commercialising persimmon with a firm texture after applying a treatment to remove its astringency (Arnal and Del Río, 2003) has increased the amount of overstocks of this product (GVA, 2011). Therefore, studying different ways in which this product can be offered to consumers is useful. Besides, it is known that most of edible plant essential oils have antimicrobial and antioxidant properties, which could greatly aid in the extension of the shelf life of fresh-cut products (Valero *et al.*, 2006; Serrano *et al.*, 2008).

Owing to the requirements of society nowadays, the increase in the demand of minimally processed fruits is widely known. In fact, there has been an expansion of these kinds of products worldwide (Fruit logística, 2011). In Spain, they are especially demanded in catering and restaurant enterprises, not only for fast food but also for canteens in companies, schools, hospitals, residential halls, etc. Moreover, they are also welcome in homes. This sector is continuously growing (6% more sales in 2010 than in the same period in 2009); especially considering the amount of fresh-cut fruit sold (10% more than 2009) (AFHORLA, 2012).

One of the main stages in the preparation of fresh-cut products is disinfection. This is usually performed using liquid chlorine and hypochlorite (Rico *et al.*, 2007). Chlorine has a broad activity spectrum, acting against bacteria, moulds and yeasts and virus (Krasaekoopt and Bhandari, 2001). Despite

its extended use, it is important to remark 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). In addition, chlorine can react with different organic compounds, increasing the risk of producing trihalomethanes, which are carcinogenic for human beings (Sánchez-Zafra, 2008). Therefore, it is necessary to search for alternatives to chlorine treatment in minimally processed products.

One way in which to reduce the microorganism burden in fresh-cut products could be through the use of plant essential oils since they have antimicrobial, antifungal, and antiviral properties (Dorman and Deans; 2000; Fisher and Philips, 2006; Bakkali *et al.*, 2008). Recently several research groups have investigated the antimicrobial effect of different essential oils applied directly to foods, such as on fruit salads (Belletti *et al.*, 2008), on peppers (Uyttendaele *et al.*, 2004), on lettuce, carrots, cabbage and parslane (Singh *et al.*, 2002; Karagözlü *et al.*, 2011; Scollard *et al.*, 2013), on cherries (Serrano *et al.*, 2005), on table grapes (Valero *et al.*, 2006), and on peach fruit (Montero-Prado *et al.*, 2011).

Also significant is the potential high antioxidant capacity of essential oils, depending on their nature. In this sense, Lee *et al.* (2005) identified the aromatic compounds of different essential oils, reporting that thymol, carvacrol, 4-aliphenol and eugenol showed a high level of antioxidant activity, inhibiting hexanal oxidation by 95-99% at 5  $\mu\text{mL}$  over 30 days.

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Furthermore, the activity of this antioxidant was very similar to that of other known antioxidants such as  $\alpha$ -tocopherol and butylated hydroxytoluene (BHT). It is also remarkable that essential oils have the power to reduce the activity of determined enzymes such as peroxidase (Ponce *et al.*, 2004; Mousavizadeh *et al.*, 2011).

Recently, thyme essential oil and lemon essential oil were included on the EAFUS list (Everything Added to Food in United States) (FDA, 2012), which highly increases the possibilities of using these additives for the development of new ways to commercialize products. However, their special flavour could affect their acceptability. Therefore it is necessary to determine the minimum amount these essential oils required to effectively reduce the microbiological burden, increasing their antioxidant activity and consequently their shelf life and value. Moreover, recently some compounds from essential oils have been included in the list of flavourings which are allowed by European Commission (Regulation (EC) N° 2232/96). Nevertheless this list is continuously updated removing certain substances which have been tested as toxic or including new ones.

Therefore, the aim of this paper was to evaluate the effect of thyme essential oil (TEO) and lemon essential oil (LEO) applied inside the package of minimally processed persimmon with regard to antioxidant capacity, changes in optical and mechanical properties and the growth of microorganisms.

## Materials and Methods

### Raw materials

Fruits of persimmon (*Diospyros kaki*) of the variety "Rojo Brillante" were used to carry out these experiments. They were acquired 24 hours prior to use and stored at 4°C before being processed from a local market. Fruits were selected based on their maturity, colour and general appearance in order to increase the uniformity of these samples. The essential oils used in this study were thyme (*Thymus vulgaris* L.) and lemon (*Citrus limonum* L.) (Soria Natural, Soria, Spain), which were acquired from herbalism (Valencia, Spain).

### Treatment of persimmon samples

After selecting the persimmon, they were washed in tap water with commercial sodium hypochlorite (Amukina, Laboratories Angelini, Farma-Lepori, Barcelona, Spain) using the recommended dose: 0.02 (v/v) for 1 minute. After this, the samples were dried with absorbent paper and the fruits were cut

into slices which were approximately 1.5 cm thick, after which the slices were divided into quarters. All samples were mixed to minimize the variability of this raw material.

### Application of essential oils

Different volumes of TEO or LEO (50, 250 and 500  $\mu$ L) were poured onto sterile gauze that had been previously glued to the package. This was to be a kind of "active package" the purpose of which was to evaluate how an atmosphere with high levels of EO could affect the product. Control samples without EO were also taken into consideration.

### Storage conditions and sample size

150 g of persimmon chunks were packaged in polypropylene trays hermetically sealed by means of a tray sealer (VAC-STAR S220MP, Sugiez, Switzerland) and stored at 4°C over the full storage period. There were 10-12 chunks of persimmon per tray. This experiment was carried out for 13 days.

A total amount of 112 trays of fresh-cut persimmon from the same batch (approximately 220 fruits) were used to this study. Two trays were used for each treatment and time. Concretely, 70 trays were intended for analyzing changes in composition, pH, antioxidant capacity, total phenols, optical and mechanical properties (initially for the raw material and at 1, 3, 6, 9 and 13 days) and 42 trays were used to follow the microbiology growth (initially for the raw material and at 1, 7 and 13 days).

### Analytical determinations

#### Moisture content, soluble solids content and pH

Moisture content was determined by drying to constant weight at 60°C in a vacuum oven at 10 kPa for 72 h (adaptation of method 934.06 AOAC, 2000). Soluble solids were measured in previously homogenized samples using a refractometer (Zeiss, ATAGO model NAR-3T, Japan), and pH was obtained directly from the homogenized sample using a pH-meter ("Seven Easy" METTLER TOLEDO - United States) with contact electrode. These analyses were carried out in triplicate.

#### 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-picrylhydrazyl) (Sigma Aldrich, Germany) 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.

5 g of samples diluted in methanol in a 1:2 (w/v) ratio were shaken for 5 min. Then, samples were centrifuged for 20 min at 4°C, 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 spectrophotometer (V-630 Jasco Easton, MD 21601 USA). 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):

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

Where:

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

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

#### Content of total phenols

Total phenols were measured using spectrophotometry by means of the modified colorimetric Folin-Ciocalteu method (Chang *et al.*, 2006). Phenols of samples were extracted with methanol (Panreac, HPLC-gradient grade, PAI-ACS, Barcelona, Spain), in a 1:2 (w/v) ratio and then continually stirred at 200 rpm for one hour. Test tubes were centrifuged for 5 minutes at 4000 rpm (Medifriger BL-S, P-Selecta, Barcelona, Spain) and 125  $\mu\text{L}$  were taken from the supernatant. 500  $\mu\text{L}$  of distilled water and 125  $\mu\text{L}$  of Folin-Ciocalteu reactive (Panreac, Barcelona, Spain) were added and the mixture was left resting for 6 minutes. Then, both 1.25 mL of a sodium carbonate solution at 7% and distilled water were added until reaching 3 mL of the full amount.

Samples were left resting for one hour and a half at room temperature and absorbance at 750 nm was measured using a spectrophotometer (V-630 Jasco, Easton, MD 21601 USA). Phenol concentration was estimated on a standard curve, using gallic acid (Sigma Aldrich, USA) as standard from 0 to 500  $\mu\text{g}/\text{mL}$ . Results were expressed as mg of equivalents of gallic acid per 100 g of sample. The analysis of phenol was carried out in triplicate using ground and frozen samples (-20°C).

#### Measurement of mechanical properties

Mechanical properties were analysed using a texture analyzer TA.XT2 (Texture Analyzer Aname, Stable Micro Systems, Haslemere, England) by means of a puncture test (2 mm diameter punch) at a speed of 1 mm/s until crossing completely the sample. Ten replicates were performed for each treatment. The parameters analyzed were: maximum force (F, N) and distance at which the maximum force took place (d, mm).

#### Analysis of optical parameters

The colour of persimmon samples was measured using a spectrophotometer Minolta (Minolta CM-3600 d, Tokyo, Japan) with a window of 7 mm in diameter. For each treatment, all the samples (approx. 10-12) of each treatment were analysed due to the high variability in the colour of samples. Colour was initially determined and then re-examined each three days. CIE-L\*a\*b\* coordinates were obtained using D65 illuminant and 10° observer as reference system. These values were then used to calculate hue ( $h = \text{argt} [b^*/a^*]$ ) and chrome ( $C^* = [a^{*2} + b^{*2}]^{1/2}$ ).

#### Microbiological analysis

Serial dilutions were prepared by homogenising 10 g of persimmon tissue 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 31°C. Yeast and moulds were determined in Sabouraud Chloramphenicol Agar (Scharlau Chemie, 1-166, Barcelona, Spain) plates for 5 days at 31°C. Samples for analysis were taken on days 0 (processing day), 1, 7, and 13. Sample dilutions were prepared, and after the incubation time, Petri dishes with a number of colonies between 30 and 300 for total count and between 0 and 30 for moulds and yeast, were considered. Microbial counts were expressed as  $10 \log \text{CFU g}^{-1}$ . Experiments were carried out in duplicate.

#### Statistical analysis

A multifactorial ANOVA analysis using Statgraphics Centurion Software was performed to evaluate the effect of two factors: treatment (volume and type of essential oil) and storage time to have an overview of the global results. Then other two multifactorial ANOVA analysis were performed to study the influence of the volume of each essential oil and time. A significance level of 95% was considered to determine statistically influence of the factors studied.

## Results and Discussion

### Compositional, pH, phenol content and antioxidant capacity changes

The results shown by the compositional analysis of the raw material were a moisture content of  $0.815 \pm 0.003$  (g water/g) and soluble solid content of  $0.169 \pm 0.002$ . Besides, the average pH of the persimmon fruits studied was  $5.92 \pm 0.05$ . Regarding the antioxidant properties, the total phenols in the raw material were  $13 \pm 2$  mg of equivalents of galic acid (GAE) and the fruit had an antioxidant capacity of  $42 \pm 2\%$  of inhibited DPPH. These values were similar to others reported in previous studies (Castelló *et al.*, 2006, 2011). Phenol content was slightly lower than those presented by Gorinstein *et al.* (2001), who obtained  $19.3 (\pm 1.4)$  mg of galic acid in persimmon flesh of the “Triumph” variety.

Figure 1 shows the evolution of water and soluble solid mass fraction over 13 days of storage. Furthermore, table 1 and table 2 show the values of F-ratio obtained considering all the results and only specific results for each essential oil respectively. Multifactorial ANOVA indicated the influence of both variables studied (treatment and storage time), the treatment applied having a greater effect. Moisture content decreased slightly in control samples while samples treated with EO, especially those with thyme showed slightly greater values with regard to control from the sixth day of storage. This behaviour could be associated with the influence of EO on the vapour pressure of headspace.

The soluble solid content of the control samples was constant along the period considered, while samples treated with EO showed a lower content in almost all the period considered, especially in TEO samples with 250  $\mu$ L. This is in accordance with the F-values showed in tables 1 and 2 which the highest values are considering in both, the treatment and the volume of each essential oil used. Figure 2 shows the evolution of pH of samples in the storage period. pH of control samples remained constant, while samples treated with EO showed lower values which decreased during storage, especially in LEO treated samples. This is coherent with the F-values show in tables 1 and 2 for pH.

Antioxidant capacity and phenol content changes can be observed in Figure 3. It is remarkable that there was a decrease in control and TEO treated samples after 1 day of storage. However, LEO treated samples showed a higher antioxidant capacity, above all those with 50 and 250  $\mu$ L. Throughout storage, values of control samples were constant except for the final day of the period studied, when it increased.

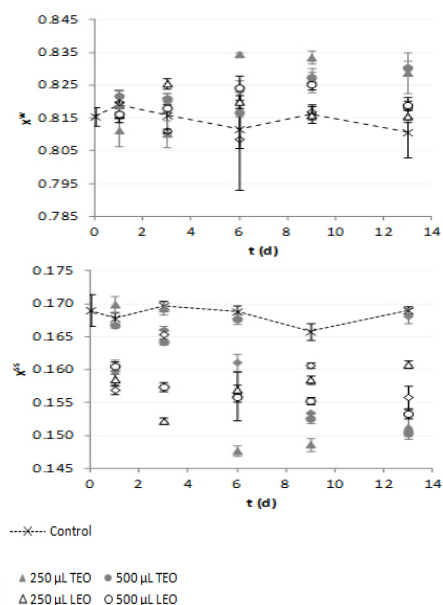


Figure 1. Evolution of water and soluble solid mass fractions ( $x^w$  and  $x^{ss}$  respectively) in cut persimmon samples with thyme (TEO) and lemon essential oil (LEO) put directly inside the package throughout storage. The discontinuous line represents the evolution of control samples.

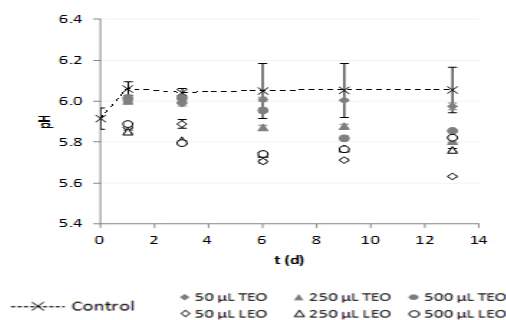


Figure 2. Evolution of pH in cut persimmon samples with thyme (TEO) and lemon essential oil (LEO) put directly inside the package throughout storage. The discontinuous line represents the evolution of control samples.

At the beginning the values of all the TEO treated samples were very similar. Despite the fact that their antioxidant capacity abruptly decreased on the third day, it increased again on the sixth day, remaining at this constant level from then on. No influence of concentration of TEO was found at the end of storage. From the beginning, the samples treated with LEO had slightly higher values than the control and TEO treated samples, especially for 50  $\mu$ L of LEO. However, samples with 250 and 500  $\mu$ L of LEO showed a lower % of inhibited DPPH at the end of storage. These results differ from those obtained by Valero *et al.* (2006) who used eugenol and thymol (one of the major components in TEO) and observed a higher antioxidant activity not only in the flesh of grapes but also in their skin as opposed to the control sample which showed a decrease.

As for total phenol content, on the first day of

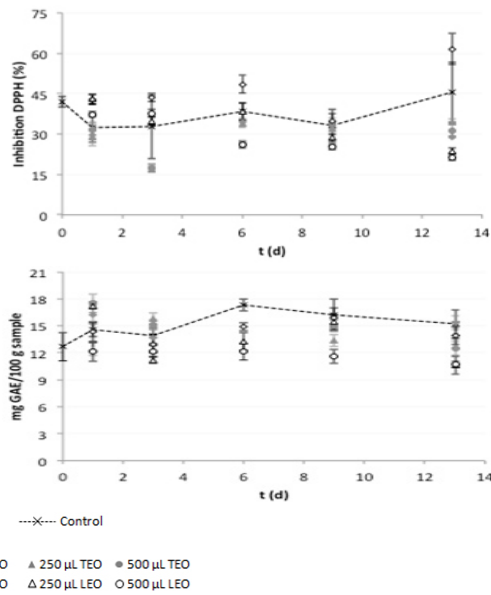


Figure 3. Evolution of antioxidant capacity and total phenol content in cut persimmon samples with thyme (TEO) and lemon essential oil (LEO) put directly inside the package throughout storage. The discontinuous line represents the evolution of control samples.

storage there was a slight increase in all samples except for those treated with 500 µL of LEO and 250 µL of TEO, whose values were similar to those of the raw material. Phenolic content in control samples tended to increase until reaching a maximum the sixth day of storage. Afterwards, it started to decrease. TEO samples, concretely 50 and 500 µL, showed the highest values (along with samples with 250 µL of LEO) the first day of storage. As time progressed, there was a decrease for samples with 250 and 500 µL of TEO. They had values below control at 13 days. For the phenol values for the LEO samples were lower than for the control samples after 3 days and especially after 6 days of storage. Moreover, it is noteworthy that the highest dose of this oil always led to values which were lower than in the control with a decreasing trend throughout storage. This fact is also reflected in the antioxidant capacity of these samples. However, Valero *et al.* (2006) were able to reduce the loss rate of phenols in the skin of grapes by using eugenol and thymol, although not in the flesh, where it was increased.

The increase of phenol content in samples could be due to the synthesis of phenylalanine ammonia lyase (PAL) as a consequence of the cut stress. Activity of this enzyme has been widely studied (Mateos *et al.*, 1993; López-Gálvez *et al.*, 1996; Pereyra *et al.*, 2005; Roura *et al.*, 2008). Wounds are one of the typical abiotic stress producing signals which are propagated from injured tissues to adjacent non injured tissues, causing the synthesis of new specific proteins resulting from damage. Some of these are

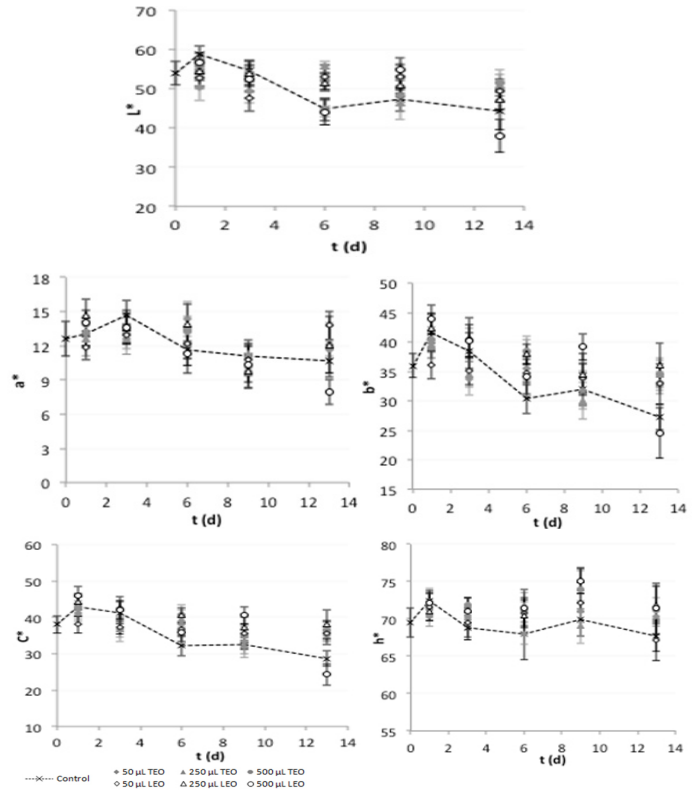


Figure 4. Evolution of luminosity ( $L^*$ ),  $a^*$  and  $b^*$  coordinates, Hue and Chrome in cut persimmon samples with thyme (TEO) and lemon essential oil (LEO) incorporated directly inside the package throughout storage. The discontinuous line represents the evolution of control samples.

phenol metabolism enzymes such as PAL, whose increase in activity gives rise to the accumulation of phenol compounds (Salveit, 2000). Most of them come from phenylalanine, which by removing one molecule of ammonium from the cynamic acid. PAL is the enzyme which catalyses this reaction (Taiz and Zeiger, 2006).

On the other hand, there was an increase in phenol content during the first days in the case of EO treated samples, especially with TEO, possibly because their own components are of a phenolic nature. In this regard, Lee *et al.* (2005) identified volatile compounds of basil and thyme leaves, also measuring antioxidant activity of their aromatic compounds. Among the chemical products identified in their extracts, thymol, carvacrol, 4-aliphenol and eugenol showed higher antioxidant activity, inhibiting the oxidation of hexanal by 95-99% at 5 µg/mL (amount of volatile compound) over 30 days. The level of antioxidant activity was similar to  $\alpha$ -tocopherol and butilated hiroxitoluene (BHT). Among the main constituents of thyme aroma, thymol (8.55 mg/g) and carvacrol (0.681 mg/g) were found.

Besides, the fact that along with the TEO treated samples, samples with greater doses of LEO showed a decrease in the content of total phenols could be

due to the decrease in PAL production. In this regard, Fujita *et al.* (2006), inhibited the activity of PAL from lettuce using transcinamaldehyde, this inhibition being dose-dependent. This compound could be found in cinnamon essential oil. Therefore, it is possible that some volatile compounds of the EO studied might interfere in its synthesis. Nevertheless, this decrease could also be attributable to the oxidation of phenolic compounds due to polyphenoloxidase (PPO) since samples with 500  $\mu\text{L}$  of LEO presented remarkable changes in colour, as will be explained in the section on optical properties.

#### Evolution of optical properties

Figure 4 shows changes in luminosity,  $a^*$  and  $b^*$  coordinates, hue and chrome. Luminosity of control samples decreased until the sixth day of storage and then remained constant. Samples treated with TEO and LEO showed slightly lower values than the control sample for the first three days of storage, especially in samples with lower dose of EO (50  $\mu\text{L}$ ). At six days of storage, samples treated with TEO (50  $\mu\text{L}$ ) showed the same values as the control sample. In contrast, samples with higher dose of TEO (500  $\mu\text{L}$ ) and LEO (50 and 250  $\mu\text{L}$ ) showed higher values of luminosity than the control sample after six days of storage. This is consistent with the results obtained by Valero *et al.* (2006), who reduced changes in  $L^*$  and  $a^*$  coordinate in grapes by applying eugenol and especially thymol at 150  $\mu\text{L}$  inside the package.

Regarding  $a^*$  coordinate, the first day of storage all samples showed the same values. The values for samples treated with TEO were constant for all doses used. Samples with 500  $\mu\text{L}$  of LEO experienced a progressive decrease showing the lowest values at the end of storage. In fact, these samples showed also the lowest values of luminosity, which would evidence the appearance of browning. All samples initially had the same values of  $b^*$  coordinate, although it decreased in control samples over time. On the whole, at the end of storage there were no differences between the TEO doses in  $b^*$  coordinate, whereas the highest concentration of LEO implied the lowest value of  $b^*$  coordinate. According to these results, it is likely that high LEO concentrations may negatively affect the colour of persimmon in the long run modifying the pigments. This behaviour could be related to the cytotoxic effects of EO or their components. In this regard, Bellitti *et al.* (2008) observed changes in the colour of fruit salads, especially those with cut apples, when they were treated with citral at high concentrations. Nevertheless, these cytotoxic effects were not observed when citron EO was used. On the other hand, as was previously explained, samples

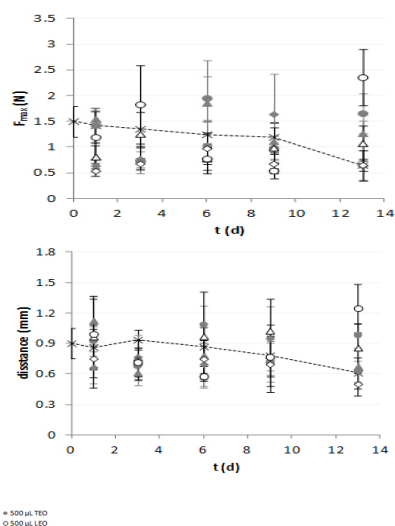


Figure 5. Evolution of maximum force expressed in Newton (N) and the distance (d) at which it occurs in millimetres in cut persimmon samples with thyme (TEO) and lemon essential oil (LEO) put directly inside the package throughout storage. The discontinuous line represents the evolution of control samples.

treated with 500  $\mu\text{L}$  of LEO showed a progressive decrease in total phenol content, most likely because of the polyphenoloxidase action. This would explain the colour's tendency to turn browner.

With respect to hue, the control samples showed a slight decrease during storage. However, hue values were kept higher in samples packaged with EO. No significant differences were found among EOs, or in the dose used. On the other hand, there was a gradual decline of chrome values in the control samples. The EO inside the container kept the value of this parameter higher in samples. Nevertheless, samples with the highest dose of LEO showed lower values at the end of the storage. This might be due to the reduction in both coordinates  $a^*$  and  $b^*$ , at the same period of time.

#### Evolution of mechanical properties

Figure 5 shows values of maximum force and distance of persimmon samples throughout storage time. The maximum force in control samples remained stable until the ninth day of storage, after which there was a decrease. This behaviour was also observed in the distance at which maximum force took place. No clear tendency was shown when the EO was applied directly to the container, although samples treated with 50  $\mu\text{L}$  of LEO showed a slight softening with respect to the control samples. However, Serrano *et al.* (2005) obtained a lower decrease in the firmness of sweet cherry samples in packages with thymol and menthol in comparison with control samples, although the best result was obtained when eugenol was used.

Table 1. F-ratio values obtained from factorial ANOVA analysis for composition, pH, phenol content, antioxidant capacity, optimal and mechanical parameters and microbiological count. The factors for the analysis were: storage time, treatment (volume and type of essential oil) and their interactions.

Analysed parameters	Factors		Interaction (AB)
	Storage time (days) (A)	Treatment (B)	
Moisture content (x <sup>m</sup> )	6.08**	12.71***	6.29***
Soluble solids content (x <sup>m</sup> )	175.29***	320.68***	85.89***
pH	19.28***	82.20***	3.20***
Phenol content	26.69***	34.78***	14.79***
Antioxidant capacity	11.28***	43.10***	11.45***
Luminosity (L*)	45.49***	12.92**	11.41***
a*	47.33***	3.53**	5.96***
b*	87.96***	14.76***	11.13***
h*	11.72***	11.59***	3.49***
C*	93.76***	14.83***	12.55***
Maximum force (F <sub>max</sub> )	1.71 NS	8.88***	6.39***
Distance	2.09 NS	1.94 NS	1.73*
Microbiological counts	845.59***	128.13***	37.41***

NS: non statistical differences (P ≥ 0.05).

\* Confidential level: P < 0.05.

\*\* Confidential level: P < 0.01.

\*\*\* Confidential level: P < 0.001.

Table 2. F-ratio values obtained from factorial ANOVA analysis for composition, pH, phenol content, antioxidant capacity, optimal and mechanical parameters and microbiological count. The factors for the analysis were: storage time, volume of each essential oil and their interactions.

Type essential oil	Analytical determinations	Factors		Interaction (AB)
		Storage time (days) (A)	Volume (B)	
LEMON ESSENTIAL OIL (LEO)	Moisture content (x <sup>m</sup> )	0.86 <sup>NS</sup>	5.89**	2.87**
	Soluble solids content (x <sup>m</sup> )	6.45**	543.45***	28.83***
	pH	6.88**	96.85***	2.35*
	Phenol content	24.47***	72.54***	10.26***
	Antioxidant capacity	9.93***	46.21***	13.18***
	Luminosity (L*)	48.29***	4.62**	13.37***
	a*	32.29***	6.00**	8.08**
	b*	68.91***	18.67***	11.50***
	h*	12.79***	15.46***	2.68**
	C*	77.73***	21.48***	15.46***
	Maximum force (F <sub>max</sub> )	4.46**	12.78***	7.68***
	Distance	0.78 <sup>NS</sup>	2.95*	1.97*
	Microbiological analysis	672.35***	177.40***	108.47***
THYME ESSENTIAL OIL (TEO)	Moisture content (x <sup>m</sup> )	9.13***	20.82***	9.38**
	Soluble solids content (x <sup>m</sup> )	333.81***	464.44***	141.02***
	pH	5.69**	18.81***	2.32*
	Phenol content	3.17*	6.76**	13.67***
	Antioxidant capacity	10.98**	10.83**	2.00*
	Luminosity (L*)	24.56***	22.21***	8.87***
	a*	23.19***	0.60 <sup>NS</sup>	4.16***
	b*	57.68***	15.37***	9.81***
	h*	4.48**	11.89***	3.86**
	C*	57.11***	14.92***	8.42***
	Maximum force (F <sub>max</sub> )	7.50***	5.09**	5.81***
	Distance	2.00 <sup>NS</sup>	0.65 <sup>NS</sup>	2.00*
	Microbiological analysis	561.21***	62.35***	7.39**

NS: non statistical differences (P ≥ 0.05).

\* Confidential level: P < 0.05.

\*\* Confidential level: P < 0.01.

\*\*\* Confidential level: P < 0.001.

Microbiological analysis

Microbial counts of mesophilic aerobics throughout the storage of control and EO treated samples are shown in figure 6. 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. According to this law, these products must not exceed 10<sup>5</sup>-10<sup>6</sup> CFU/g on the day of manufacture and 10<sup>6</sup>-10<sup>7</sup> CFU/g on the expiry date. In this case, the microbial count of mesophilic aerobic the day of manufacture was 300(±141) CFU/g, which was lower than the limit established. The samples treated with EO showed a higher microbial count than the control samples after one day of storage,

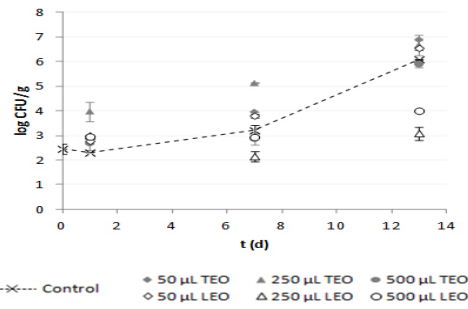


Figure 6. Microbial counts (decimal logarithm of colony forming units (log CFU/g)) in cut persimmon samples with thyme (TEO) and lemon essential oil (LEO) put directly inside the package throughout storage. The discontinuous line represents the evolution of control samples.

especially when 250 µL of TEO in packages was applied. After one week samples treated with 500 µL of TEO and with 250 and 500 µL of LEO showed lower values of mesophilic aerobic than control samples. Nevertheless, at 13 days of storage nearly all the treated samples had counts similar to the control sample, exceeding the limit established by RD 3484/2000 on the expiry date. Table 1 and 2 indicate that the storage time was the factor with the most significance effect on the microbial counts. On the other hand, it is remarkable that samples treated with LEO in packages at 250 and 500 µL showed lower counts of these microorganisms, which would mean that they could be still commercialized. Therefore, the volatile components of LEO would be more effective than those of TEO. Furthermore, the lowest value of pH in these samples could also have helped to extend their shelf life. These results are supported by the higher values of F-ratio in table 2 for the volume of LEO than for TEO. However, Fisher and Phillips (2006), who studied the antimicrobial effect of lemon vapours, did not report growth inhibition of any bacteria tested *in vitro*, although they observed an inhibition effect when citral and linalool vapours were used both *in vitro* and *in vivo*. On the other hand, Ramos *et al.* (2012) used antimicrobial active films based on polypropylene, which were prepared by adding thymol, carvacrol and a combination of an equimolar mixture of both additives at different concentrations in all cases. They saw that films with thymol at the highest concentration were the most effective against *Staphylococcus aureus* using the agar disk diffusion method. There was no growth of mould or yeast on the day of manufacture, not even during storage. Serrano *et al.* (2005) obtained a reduction in the microbial population in sweet cherry packages with eugenol, thymol, menthol and eucalyptol against control samples. Furthermore, this reduction was more effective for mould and yeast counts.

## Conclusions

LEO and TEO added to the package did not improve antioxidant capacity or total phenol content in samples. From a microbiological point of view high LEO volumes had a greater antimicrobial effect at the end of storage than when TEO was applied. This could be associated with the lower pH values of LEO samples. However, in the case of LEO at 500  $\mu$ L, the fruit showed a tendency to turn brown which could be due to the oxidation of phenols and other antioxidants.

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