MICROBIAL CONTROL IN FRESH HORTICULTURAL PRODUCTS USING ACTIVE PAPER SHEETS WITH ESSENTIAL OILS. A CASE STUDY IN LEMON CV. VERNA

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Abstract: *Active packaging with essential oils (EOs) encapsulated in β-cyclodextrins is a strategy to achieve a controlled EOs release, whose antimicrobial and antioxidant properties are widely known. The microbial loads of lemons (cv. Verna) packaged in a box including an active paper sheet (EOs encapsulated in β-cyclodextrins) was studied during refrigerated storage (8 ºC) with supplementary commercialization periods (5 days at room temperature). Lemons preserved with active sheets showed 0.5 and ≈3 log CFU cm $^{-2}$ lower mesophilic loads than control samples after 5 weeks of refrigeration and their respective commercialization periods. In addition, the active sheets reduced Enterobacteriaceae and yeasts/molds growth by ≈1, 1.23, and 0.1 log CFU cm $^{-2}$, respectively. On the other side, the physicochemical quality (soluble solid content, pH and titratable acidity) of lemons was not affected by the active packaging. In conclusion, the active packaging preserved the microbial quality in lemon while physicochemical quality was not affected.*

Keywords: carvacrol, eugenol, cyclodextrins, inclusion complex, active packaging.

1. INTRODUCTION

Lemon is produced in Spain in the Mediterranean area, concentrating most of the production in the areas of Murcia and Orihuela, being the "Verna" variety native from Region of Murcia [1]. Lemon is a non-climacteric fruit with low respiration and ethylene production rates. However, during the lemon postharvest life, physiological changes occur (e.g., consumption of organic acids and sugars) leading to quality losses, and as a consequence, the reduction of its shelf life. *Penicillium* genus is considered as the most important pathological disorder in citrus fruits, although other microbial groups can also proliferate in lemons. For this reason, chemical products, such as Imazalil, are usually applied to reduce these microbial infections. However, today's consumer is increasingly concerned about purchasing foods free of chemical products [2].

The use of waxes can help maintain the quality of lemons, thus reducing water loss through transpiration and respiration. When the washing process takes place before entering the processing line, the lemons lose their layers of natural wax. Therefore, the application of waxes is a usual method to avoid dehydration and control respiration during postharvest conservation. In this way, weight loss and cold damage are reduced with waxes [3]. In addition, natural antimicrobial agents can be also applied to further extend the shelf life of lemons.

The use of essential oils (EOs) is an alternative to antimicrobials of chemical origin to control microbial growth. Thus, the antimicrobial properties of EOs have been widely reported [4]. Specifically, EOs have compounds such as terpenes, terpenoids and low molecular weight aromatic and aliphatic compounds responsible of the high antimicrobial and antioxidant

properties of these natural plant extracts. In addition, the combination of the pure EO together with its major component (e.g., oregano oil and carvacrol) can increase the antimicrobial activity up to 10-30 % [5]. However, EOs are easily evaporated and are easily degraded under atmospheric conditions (oxidations, etc.). Therefore, the formation of inclusion complexes of EOs with β-cyclodextrins allows a controlled release of EOs. Thus, the incorporation of these EO inclusion complexes in active packaging will potentially extend the shelf life of horticultural products as previously observed [6].

The aim of this study was to study the microbial and physicochemical quality of lemons packaged with active paper sheets during refrigerated storage (up to 5 weeks at 8 ºC and relative humidity (RH) of 88%), and supplementary commercialization periods (5 days at room temperature).

2. MATERIAL AND METHODS

2.1. Material

Lemons (*Citrus×limon*, var. Verna) were supplied by the company Fruca Marketing S.L. (Beniaján, Murcia) in April 2021. Lemons were waxed with carnauba wax at the company's industrial facilities. Afterwards, they were transported to the cold rooms of the food technology pilot plant of the Universidad Politécnica de Cartagena where they were distributed in different boxes for the packaging treatments: active packaging consisting of boxes containing a paper (paper Kraft) sheet in the box bottom; and control packaging (box without the active paper sheet). The active sheets were prepared by coating at 1 g m^{-2} of encapsulated EOs (a mixture of carvacrol:eugenol 80:20, *weight* (*w*)/*w*) with β-cyclodextrins in a ratio of 1:7.6 *w:w* as previously described [7]. Each box contained 16 lemons. Boxes were stored at 8 ºC and 88 % RH for 5 weeks, with supplementary commercialization periods of 5 days at room temperature, after each week of refrigerated storage. Three replicates (3 boxes) were made for each sampling time and packaging treatment (control or active).

2.2. Physicochemical analyses

Soluble solids content (SSC) was determined on the extracted (using a domestic juice squeezer) lemon juice using a digital refractometer (Master Refractometer Automatic, Atago; Tokyo, Japan) and expressed in °Brix. The pH of the juice was measured with a pH meter (Crison Basic20; Alella, Spain). Titratable acidity (TA) was determined using an automatic titrator (model T50 Metter Toledo; Milan, Italy) with NaOH (0.1 N) to pH 8.1 of the diluted lemon juice (1 mL juice + 49 mL distilled water) and was calculated by equation 1:

$$\text{TA (\% citric acid)} = \frac{0{,}064 * Vol * N}{v} * 100 \quad (1)$$

where 0.064 is the equivalence factor of citric acid (major organic acid in lemons), *Vol* is the mL of NaOH used in the titration, *N* is the normality of NaOH (0.1 N) and *v* is the volume (mL) of juice used.

2.3. Microbiological analyses

Surface microbial loads of lemons were analyzed as previously described [8]. Briefly, three lemons were introduced in buffered peptone water (1:1 *w:volume*) and allowed to stand for 1 h at 4 °C. The pertinent dilutions were then made in buffered peptone water. Subsequently, aliquots of the dilutions were pour-plated on PCA (plate count agar) agar for mesophiles and psychrophiles, and violet red bile dextrose agar for enterobacteria. Yeasts and molds were surface-plated on rose bengal agar. The incubation conditions for mesophiles, psychrophiles, enterobacteria, yeasts and molds were: 31 ºC (48 h), 4 ºC (7 days), 37 ºC (24 h), 25 ºC (5 days) and 25 ºC (7 days), respectively. Results were expressed as log of colony-forming units (CFU) per cm^{-2}. Each of the three replicates was analyzed in duplicate.

2.4. Statistical analysis

Statistical analysis was performed using SPSS software (v.19 IBM, New York, USA) using multifactorial analysis of variance (ANOVA) (packaging treatment × storage time) with Tukey's test (p=0.05).

3. RESULTS

3.1. Physicochemical analyses

The initial values of SSC, pH and TA were similar to those obtained by other authors [9]. Regarding SSC, significant differences were observed between treatments in the 2nd and 3rd week of refrigerated storage (Table 1). For pH, there were observed significant differences in the 1st and 3rd week between active and control treatments (Table 1). For TA, no significant differences between treatments (control and active) were observed at the different sampling times of the refrigerated storage (Table 1).

During the commercialization periods, SSC significant differences were observed among active and control treatments after the commercialization periods corresponding to samples previously stored for 3 and 4 weeks. However, with regard to pH and TA, no significant differences were observed among treatments. As observed, no high differences were obtained for the analyzed physicochemical parameters among control and active treatments. Thus, the released EOs seemed not remarkably affect the physicochemical quality of lemon during refrigerated storage and supplementary commercialization periods at room temperature.

3.2. Microbiological analyses

Lemons showed initial levels of mesophiles, psychrophiles, enterobacteria, moulds and yeasts of 2.24, 0.87, 0.15, 0.61 and 1.23 log CFU cm^{-2}, respectively (Table 3). The initial values of total mesophilic aerobes, enterobacteria, yeast and moulds were similar to those obtained by other authors in lemons [10] [11]. However, psychrophilic loads of our study were higher than those reported in previous studies [8]. Lemons with active sheets showed 0.5 log units lower mesophilic counts than control samples after 5 weeks of refrigerated storage (Table 3). After commercialization, samples with active sheets showed 3 log CFU cm^{-2} lower mesophilic loads than control samples after the commercialization period corresponding to previous 5 weeks of refrigerated storage (Table 4).

Table 1. Soluble solid content (SSC, º Brix), pH and titratable acidity (TA, %) of lemons preserved in active (A) or control (C) packaging for 5 weeks at 8 ºC and 88 % RH.

SSC	**Initial**	**1st week**	**2nd week**	**3rd week**	**4th week**	**5th week**
C	7.1± 0.3 ^{Aa}	6,5 ± 0.0 ^{Aab}	6.2 ± 0.3 ^{Bb}	6.2 ± 0.3 ^{Bb}	7.0 ± 0.0 ^{Aa}	6.3 ± 0.3 ^{Ab}
A	7.1± 0.3 ^{Aab}	6.5 ± 0.5 ^{Abc}	7.0 ± 0.3 ^{Aab}	7.2 ± 0.3 ^{Aa}	6.8 ± 0.6 ^{Aabc}	6.2 ± 0.3 ^{Ac}
pH	**Initial**	**1st week**	**2 nd week**	**3 rd week**	**4 th week**	**5 th week**
C	2,2 ± 0.0 ^{Ac}	2,5 ± 0.0 ^{Ab}	2,5 ± 0.0 ^{Ab}	2,5 ± 0.0 ^{Ab}	2,5 ± 0.1 ^{Ab}	2,5 ± 0.1 ^{Aa}
A	2,2 ± 0.0 ^{Ac}	2,3 ± 0.0 ^{Bb}	2,5 ± 0.0 ^{Aa}	2,3 ± 0.0 ^{Bb}	2,5 ± 0.0 ^{Aa}	2,5 ± 0.0 ^{Aa}
TA	**Initial**	**1st week**	**2 nd week**	**3 rd week**	**4 th week**	**5 th week**
C	5,2 ± 0.3 ^{Aa}	5,3 ± 0.1 ^{Aa}	4,8 ± 0.1 ^{Ab}	4,7 ± 0.1 ^{Ab}	5,1± 0.2 ^{Aa}	4,5 ± 0.3 ^{Ab}
A	5,2 ± 0.3 ^{Aa}	5,1 ± 0.2 ^{Aa}	5,3 ± 0.3 ^{Aa}	5,1± 0.2 ^{Aa}	4,7 ± 0.1 ^{Ab}	4,6 ± 0.2 ^{Aa}

Different capital letters indicate significant differences ($p < 0.05$) between packaging treatments for the same storage time. Different lowercase letters denote significant differences ($p < 0.05$) between sampling times for the same packaging treatment.

Table 2. Soluble solid content (SSC, ºBrix), pH and titratable acidity (TA, %) of lemons stored in active (A) or control (C) packaging for 5 weeks at 8 ºC and 88 % RH, followed by supplementary commercialization periods (COM) of 5 days at room temperature.

SSC	1st week + COM	2 nd week + COM	3 rd week + COM	4 th week + COM	5 th week + COM
C	7.0 ± 0.5 ^{Aa}	6.5 ± 0.0 ^{Ab}	5.8 ± 0.3 ^{Bc}	5.7 ± 0.3 ^{Bc}	6.3 ± 0.3 ^{Ab}
A	6.8 ± 0.3 ^{Aa}	6.8 ± 0.3 ^{Aa}	6.3 ± 0.3 ^{Ab}	6.3 ± 0.3 ^{Ab}	6.3 ± 0.3 ^{Ab}
pH	**1st week + COM**	**2 nd week + COM**	**3 rd week + COM**	**4 th week + COM**	**5 th week + COM**
C	2.4 ± 0.1^{Ac}	2.5 ± 0.0 ^{Ab}	2.5 ± 0.1 ^{Ac}	2.6 ± 0.0 ^{Aa}	2.6 ± 0.0 ^{Aa}
A	2.5 ± 0.1 ^{Ab}	2.4 ± 0.3 ^{Ac}	2.5 ± 0.1 ^{Ab}	2.6 ± 0.0 ^{Aa}	2.6 ± 0.0 ^{Aa}
TA	**1st week + COM**	**2 nd week + COM**	**3 rd week + COM**	**4 th week + COM**	**5 th week + COM**
C	5.1 ± 0.3 ^{Aa}	5.3 ± 0.0^{Aa}	4.9 ± 0.1 ^{Aa}	4.9 ± 0.4 ^{Aa}	4.5 ± 0.2 ^{Ab}
A	5.3 ± 0.1 ^{Aa}	5.2 ± 0.3 ^{Aa}	4.5 ± 0.1 ^{Ac}	4.8 ± 0.2 ^{Ab}	5.1 ± 0.1 ^{Aa}

Different capital letters indicate significant differences ($p < 0.05$) between packaging treatments for the same commercialization time. Different lowercase letters denote significant differences ($p < 0.05$) between sampling times for the same packaging treatment.

Attending to psychrophiles, lemons with active packaging showed 1.3 log units lower counts than control samples (Table 3) after 5 weeks of refrigeration, although differences were not significant. However, this effect was not observed during commercialization, with similar ($p>0.05$) psychrophilic loads for both control and active samples (Table 4).

The enterobacteria loads of lemons packaged with active sheets was lower compared to control samples, with values of 2.7 and 3.1 log CFU cm^{-2}, respectively at week 5 (Table 3). At the end of the commercialization period, enterobacteria loads in lemons from active packages were also lower than the control samples with values of 2.9 and 3.7 log CFU cm^{-2}, respectively (Table 4).

For moulds, the active lemons showed 1.2 log units lower loads than control samples at the 5 th week of refrigerated storage (Table 3), although such trend was not significant ($p>0.05$). At the end of commercialization, no clear trends were observed for moulds (Table 4). A mild yeast reduction was observed after 5 weeks of refrigerated storage, while during commercialization there was no decrease in the microbial loads (Tables 3 and 4). No significant ($p>0.05$) differences were observed related to yeast loads (Tables 3 and 4).

As has been observed, the EOs released from the active sheets aimed to control the microbial growth in samples. The high antimicrobial properties of EOs have been widely reported, which are attributed to their components like terpenes, terpenoids and low molecular weight aromatic and aliphatic compounds [11].

Table 3. Count of total mesophilic aerobes, total mesophilic psychrophiles, enterobacteria, fungi and yeasts (log CFU cm^{-2}) of lemons preserved in active (A) or control (C) boxes for 5 weeks at 8 ºC and 88% RH.

Microorganism	Packaging	Initial	1st week	2 nd week	3 rd week	4 th week	5 th week
Total mesophilic aerobes	C	2,2 ± 0.3 ^{Ab}	3,3 ± 0.5 ^{Ab}	4,5 ± 0.8 ^{Aa}	4,7 ± 0.5 ^{Aa}	5,3 ± 0.4 ^{Aa}	5,1± 0.1 ^{Aa}
	A	2,2 ± 0.3 ^{Ac}	4,3 ± 0.2 ^{Ab}	4,6 ± 0.4 ^{Aa}	5,1 ± 0.0 ^{Aa}	5,3 ± 0.1 ^{Aa}	4,6 ± 0.6 ^{Aa}
Psychrophiles	C	0,9 ± 0.1 ^{Ac}	2,7 ± 0.3 ^{Ab}	3,6 ± 0.3 ^{Aab}	4,2 ± 0.5 ^{Aab}	4,4 ± 0.6 ^{Aab}	4,9 ± 0.3 ^{Aab}
	A	0,9 ± 0.1 ^{Ab}	3,0 ± 0.5 ^{Aa}	3,1 ± 0.7 ^{Aa}	4,4 ± 0.4 ^{Aa}	4,3 ± 0.3 ^{Aa}	3,6 ± 0.6 ^{Aa}

Microorganism	Packaging	Initial	1st week	2 nd week	3 rd week	4 th week	5 th week
Enterobacteria	C	0,2 ± 0.0 ^{Ab}	3,0 ± 0.1 ^{Aa}	2,0 ± 0.7 ^{Aa}	2,6 ± 0.0 ^{Aa}	3,3 ± 0.4 ^{Aa}	3,1 ± 0.4 ^{Aa}
	A	0,2 ± 0.0 ^{Ab}	2,3 ± 0.3 ^{Aa}	2,1 ± 0.0 ^{Aa}	2,1 ± 0.5 ^{Aa}	3.0 ± 0.3 ^{Aa}	2,7± 0.7 ^{Aa}
Moulds	C	0,6 ± 0.1 ^{Ac}	2,1 ± 0.3 ^{Ab}	2,4 ± 0.7 ^{Aab}	3,0 ± 0.2 ^{Aa}	3,2 ± 0.0 ^{Aa}	3,8 ± 0.4 ^{Aa}
	A	0,6 ± 0.1 ^{Ac}	2,9 ± 0.6 ^{Ab}	2,7 ± 0.4 ^{Ab}	3,6 ± 0.2 ^{Aa}	2,8 ± 0.4 ^{Aab}	2,6 ± 0.8 ^{Ab}
Yeasts	C	1,2 ± 0.2 ^{Ab}	3,1 ± 0.6 ^{Aa}	3,2 ± 0.4 ^{Aa}	4,0 ± 0.4 ^{Aa}	3,4 ± 0.4 ^{Aa}	3,6 ± 0.4 ^{Aa}
	A	1,2 ± 0.2 ^{Ab}	3,0 ± 0.2 ^{Aa}	3,2 ± 0.3 ^{Aa}	3,6 ± 0.4 ^{Aa}	3,7 ± 0.3 ^{Aa}	3,5 ± 0.1 ^{Aa}

Different capital letters indicate significant differences ($p < 0.05$) between packaging treatments for the same storage time. Different lowercase letters denote significant differences ($p < 0.05$) between sampling times for the same packaging treatment.

Table 4. Count of total mesophilic aerobes, total mesophilic psychrophiles, enterobacteria, fungi and yeasts (log CFU cm^{-2}) of lemons preserved in active (A) or control (C) boxes for 5 weeks at 8 ºC and 88% RH, followed by supplementary commercialization periods (COM) of 5 days at room temperature.

Microorganism	Packaging	1st week + COM	2 nd week + COM	3 rd week + COM	4 th week + COM
Total mesophilic aerobes	C	4,3 ± 0.3 ^{Ac}	5,3 ± 0.3 ^{Ac}	4,2 ± 0.2 ^{Ac}	7,4 ± 0.7 ^{Aa}
	A	4,8 ± 1.4 ^{Aa}	4,5 ± 0.2 ^{Aa}	4,6 ± 0.5 ^{Aa}	4,2 ± 0.6 ^{Ba}
Psychrophiles	C	4,1 ± 0.8 ^{Aa}	4,5 ± 0.2 ^{Aa}	4,2 ± 0.1 ^{Aa}	4,1 ± 0.2^{Aa}
	A	3,9 ± 0.4 ^{Aa}	4,1 ± 0.4 ^{Aa}	3,6 ± 0.5 ^{Aa}	4,5 ± 0.1 ^{Aa}
Enterobacteria	C	4,1 ± 0.3 ^{Aa}	3,4 ± 0.4 ^{Aa}	3,1 ± 0.6 ^{Aa}	3,7 ± 1.1 ^{Aa}
	A	3,5 ± 0.2 ^{Aa}	0,8 ± 0.1 ^{Ab}	2,3 ± 0.8 ^{Aa}	2,9 ± 0.6 ^{Aa}
Moulds	C	2,7 ± 0.3 ^{Ab}	3,3 ± 0.5 ^{Aa}	3,8 ± 0.2 ^{Aa}	3,1 ± 0.3 ^{Aa}
	A	2,6 ± 0.1 ^{Ab}	2,5± 0.4 ^{Ab}	2,8 ± 0.6 ^{Ab}	4,8 ± 0.2 ^{Aa}
Yeasts	C	2,8 ± 0.5 ^{Ab}	3,6 ± 0.1 ^{Aa}	3,2 ± 0.4 ^{Aa}	3,6 ± 0.6 ^{Aa}
	A	2,8 ± 0.3 ^{Ab}	2,9 ± 0.1 ^{Ab}	3,3 ± 0.4 ^{Aa}	3,8 ± 0.0 ^{Aa}

Different capital letters indicate significant differences ($p < 0.05$) between packaging treatments for the same commercialization time. Different lowercase letters denote significant differences ($p < 0.05$) between commercialization times for the same packaging treatment.

4. CONCLUSIONS

In conclusion, the active paper sheets controlled the microbial growth of lemons with lower mesophilic counts after a commercialization period (5 days at room temperature) following 5 weeks of refrigerated storage. Similar trends were observed for other microbial groups, although they were not significant. The released EOs did not affect the physicochemical quality of lemons, remaining the soluble solid content, pH and titratable acidity not highly affected. As observed, active packaging is a complimentary technique to conventional ones (e.g. waxes) to extend the shelf life of lemons during refrigerated storage and subsequent commercialization periods at room temperature.

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