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EVALUATION OF TWO ANTIMICROBIAL PACKAGING FILMS AGAINST *Escherichia coli* O157:H7 STRAINS *IN VITRO* AND DURING STORAGE OF A SPANISH RIPENED SHEEP CHEESE (ZAMORANO)

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Abstract

The antimicrobial activity of two packaging films (polypropylene –PP- and polyethylene terephthalate –PET-) coated with different concentrations (0, 4, 6 and 8%) of essential oil from *Origanum vulgare* (OR) and Ethyl Lauroyl Arginate HCl (LAE) was tested against two *E. coli* O157:H7 strains using *in vitro* systems and a raw milk sheep cheese model (Zamorano). The influence of the antimicrobials on the sensory attributes of cheese was also evaluated. For both strains, the MBC (minimum bactericidal concentration) values were identical to their respective MIC (minimum inhibitory concentration) values and lower for LAE (25 mg/l) than for OR (200-400 mg/l). PP and PET films coated with OR were tested by a vapour phase assay and the Japanish Standard method (JIS Z 2801:2000). Both films coated with LAE were tested by an agar diffusion method. Overall, *in vitro* tests were effective against both strains. The inhibitory activity depended on the active compound concentration, the target strain and the packaging material, PET being more effective than PP. For inoculated cheese slices, OR and LAE PP films did not effectively decrease *E. coli* O157:H7 counts after 7-days cold storage. PET films incorporating 6 and 8 % of OR and LAE significantly ($p < 0.05$) decreased the numbers of both strains and also did 4% for the reference and wild strain depending on the antimicrobial. LAE PP, OR PET and LAE PET did not significantly ($p > 0.05$) affect sensorial characteristics of Zamorano cheese. Packaging with PET films coated with ≥ 6% LAE concentrations might be useful in reducing *E. coli* O157:H7 numbers in sheep cheese.

Keywords: Antimicrobial active food packaging; *E. coli* O157:H7; sheep cheese; oregano essential oil; Ethyl Lauroyl Arginate; LAE

1. Introduction

Pathogenic *E. coli* strains are categorized into six specific groups or pathotypes based on their virulence determinants. One of them is enterohaemorragic *E. coli* (EHEC), which is considered a subset of Shiga-toxin producing *E. coli* (STEC) also named verocytotoxic-producing *E. coli* (VTEC) (Nataro & Kaper, 1998). Although several STEC serotypes can cause serious human illness and are recognized as EHEC, serotype O157:H7 is an important food borne pathogen and the predominant cause of EHEC-associated disease worldwide. In humans, low numbers (10-100 cells) of serotype O157:H7 strains can cause severe illnesses such as diarrhoea and haemorrhagic colitis (HC) that may progress into a life-threatening sequel called haemolytic uremic syndrome (HUS), especially in young children and the elderly. HUS is the most common cause of acute renal failure in young children and an important cause of morbidity and mortality in the elderly (Meng, Lejeune, Zhao, & Doyle, 2013).

The main reservoir of this pathogen appears to be wild and domestic ruminants such as deer, cattle, goats and sheep (Ferens & Hovde, 2011). In addition to undercooked beef hamburgers and other meat products, as well as water, fruits and vegetables, cheeses made from raw milk have been implicated in infections of *E. coli* O157:H7 (Espié et al., 2006; Farrokh et al., 2013). The occurrence of EHEC strains in sheep cheeses and raw sheep milk dedicated to cheesemaking has been reported by a number of authors in Spain and also in other countries (Caro, Mateo, Rúa, & del Rosario García-Armesto, 2011; Caro & García-Armesto, 2007; Farrokh et al., 2013; Rey et al., 2006). Ewes' breeding is an important activity in Spain (*ca.* 22 % of the EU), which is concentrated particularly in the inner regions. According to the Spanish Ministry of Agriculture, Food and Environment (http://www.magrama.gob.es; last accessed 30 July 2013), the Castilla y León region is the first producer of ewes' milk,

with more than 50 % of the Spanish production, most of this production being dedicated to manufacturing of raw sheep milk cheeses such as Zamorano cheese.

Active packaging has been defined as packaging which performs some desired functions other than merely providing a barrier to the external environment (Rooney, 2005). According to Commission Regulation (EC) No 450/2009 (European Commission, 2009), "active materials and articles means materials and articles that are intended to extend the shelf-life or to maintain or improve the condition of packaged food; they are designed to deliberately incorporate components that would release or absorb substances into or from the packaged food or the environment surrounding the food."

Antimicrobial packaging is a form of active food packaging which is beneficial to the consumers as well as to the food industry since it can extend product shelf life and/or maintain food safety by killing or reducing the growth rate of target microorganisms. Several antimicrobial agents could be incorporated into conventional food packaging systems (Cha & Chinnan, 2004; López, Sánchez, Batlle, & Nerín, 2007a; Nerin, 2012; Suppakul, Miltz, Sonneveld, & Bigger, 2003) including chemical agents, natural agents and probiotics (Han, 2005). A number of these compounds have been proposed and tested for antimicrobial activity in food packaging including oregano essential oil (OR) and Ethyl Lauroyl Arginate HCl (LAE). Antimicrobial films with OR and LAE have been tested against some *Salmonella* serovars, *Listeria monocytogenes* and sometimes against a non pathogenic *E. coli* strain in an infant milk formula (Muriel-Galet, López-Carballo, Gavara, & Hernández-Muñoz, 2012a), packaged salads (Muriel-Galet et al., 2012b) and cooked sliced ham (Theinsathid, Visessanguan, Kruenate, Kingcha, & Keeratipibul, 2012). However, information on the effect of packaging films

coated with OR or LAE on the behaviour of strains belonging to the pathogenic serotype *E. coli* O157:H7 is not available.

This study was undertaken to evaluate the anti-*E. coli* O157:H7 activity of two packaging films coated with different concentrations of OR and LAE. The activity was studied by *in vitro* tests and also by direct contact with artificially contaminated samples of a Spanish ripened sheep cheese (Zamorano cheese). The effects of the antimicrobials on the sensory attributes of cheese were also studied.

2. Materials and methods

2.1. Bacterial strains

Two strains of *E. coli* O157:H7 were used: non pathogenic strain CECT (Spanish Type Culture Collection) 5947 and strain M364VO isolated by us from a tank sheep milk farm in Castilla y León (Spain). The latter strain carried the *stx*2 and *eae* genes.

Both strains were stored at -18ºC in Nutrient Broth (NB, Scharlab, Barcelona, Spain) with 40% Glycerol. Subcultures were grown overnight in Tryptone Soya Broth (TSB, Scharlab) at 37ºC.

2.2. Antimicrobial compounds

Oregano essential oil obtained from *Origanum vulgare* L (OR; Chemical Abstracts Service (CAS) Registry Number 8007-11-2), supplied by Argolide Química SL. (Barcelona, Spain), and Ethyl Lauroyl Arginate HCl (LAE; CAS Registry Number 60372-77-2), supplied by Lamirsa (Barcelona, Spain), were used.

2.3. Active films

The coating technology used to produce the active materials was a process protected by the European Patent EP1657181 (Nerin & Garcés, 2006). The active films were manufactured and supplied by the Spanish company ARTIBAL S.A. (Sabiñánigo,

Spain). They consisted of coating layers with known concentrations of OR (4, 6 and 8%) and LAE (4, 6 and 8%). The plastic film PET (polyethylene terephthalate) was 23 μm thick with a density of 18.73±0.02 g/m^2. The PP (polypropylene) film was 40 μm thick. The active films contained the antimicrobial compounds expressed as a percentage of weight active agent/weight wet active layer. ATOX (active coating) and ACRIL (acrylic antimicrobial coating) are the industrial denominations of varnishes. The gramage of the coating was in all cases 4 g/m^2

2.4. Antimicrobial susceptibility assays

2.4.1. Broth dilution assays

A broth dilution assay was used to determine the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) for both OR and LAE (Becerril, Gomez-Lus, Goni, Lopez, & Nerin, 2007).

OR: Serial twofold dilutions, between 2.5 and 160 mg/ml, were made in dimethyl sulfoxide (DMSO) (Sigma-Aldrich Química, S.A., Madrid, Spain) (Sacchetti et al., 2005). Then, 10 μl aliquots were added to 890 μl of TSB (Scharlab) plus 0.6% Yeast Extract (Scharlab) (TSBYE), and inoculated with 100 μl of the bacterial suspensions (*ca*. 10^{6}cfu/ml) in TSBYE. The OR final concentrations ranged from 0.025 to 1.6 mg/ml. To determine whether DMSO would be inhibitory to the bacteria, controls with 10 μl of the solvent instead of OR dilutions were performed for both strains. The cultures were incubated at 37°C for 24 h while shaking. The bacterial growth was determined by measuring the optical density at 625 nm. Samples (100 μl) were also taken and serial dilutions were plated on Tryptone Soya Agar (TSA, Scharlab) and incubated at 37ºC for 24 hours.

LAE: Serial twofold dilutions, between 1.25 and 160 mg/ml were made in sterile distilled water and tested as above (LAE final concentrations ranged from 0.0125 to 1.6 mg/ml), cultures being incubated without shaking.

MIC was defined as the lowest concentration of antimicrobials at which bacterial growth was not detected. The MBC was the lowest concentration of antimicrobials at which bacteria failed to grow in TSBYE and were not cultured after plating onto TSA. Both were expressed as mg of antimicrobials per litre. Tests were performed in triplicate.

2.4.2. Vapour phase assay

The vapour phase activity between OR incorporated in the packaging materials and both *E. coli* strains was tested. Tests were carried out in triplicate by inoculating TSA plates with 100 µl of TSBYE containing 10^6 cfu/ml of each organism. The Petri dish covers were replaced by the active films as described by López et al. (2007b) and the growth examined after incubation at 37 ºC for 24 h. Controls with PP and PET without active compounds were also tested. When the growth of the target strains covered by the active films was compared with that of the controls, the antimicrobial effect of the atmosphere derived from the different concentrations of OR was categorised as: -, no inhibition; +, partial inhibition and ++, total inhibition.

2.4.3. Direct contact assays

OR: The Japanese Industrial Standard method JIS Z 2801:2000 (Anonymous, 2000) was used to evaluate the antibacterial activity of the active films by direct contact. Briefly, three pieces (50 x 50 mm) of each test film coated with 4, 6 and 8% of OR were placed, with the active surface up, on Petri dishes, inoculated with 400 µl of bacterial suspensions (*ca*.10^5cfu/ml) and covered with 40x40 mm pieces of uncoated sterile PP and PET-films. Six untreated subsamples of each film (coated only with the varnish)

were processed as above. Half of them were used for the determination of the initial cell count directly after inoculation. After incubation for 24 h at 37 ºC, the bacteria were harvested from the films surfaces using 10 ml of Buffered Peptone (Scharlab), which was serially diluted and plated on TSA. After 24 h at 37 ºC, the antimicrobial activity was expressed as the R value. The R value was calculated using the formula R=log (B/C) where, B: average of colony forming units (cfu) on the untreated films and C: average of the cfu found on the treated ones.

LAE: Since the JIS method could not be used for LAE, the evaluation of the antibacterial activity of this compound by direct contact was carried out using samples of coated PP and PET films (4, 6 and 8% of LAE) and an agar diffusion method as described by Iseppi et al. (2008). The varnish (ACRIL) coat weight was 4g/m^2. The coated films (40×40 mm) were placed onto TSA (Oxoid) plates seeded with 10^8 cfu/ml from overnight TSBYE (Scharlab) cultures. The plates were incubated at 37 °C for 24 h and the antagonistic activity was quantified by a clear zone of inhibition in the indicator lawn around and in contact with the coated plastic film. Films coated with varnish not containing LAE were also tested as negative controls

2.5. Antimicrobial activity in Zamorano sheep cheese

Because of the source of strain ECVT M364VO, wedges of commercial Spanish Zamorano cheese were used. Zamorano cheese is a hard cheese, made with the milk of the Churra and Castilian sheep breeds.

Commercial cheese wedges, prepared for an estimated domestic consumption of a week, were purchased and sliced. The slices were contaminated on the surface by dipping in a 10^6 cfu/ml TSBYE broth to achieve final levels of *ca*. 10^4 cfu/g. Control and contaminated cheese slices were placed between two pieces of PP or PET films coated with the varnish alone and three different concentrations of each antimicrobial

(4, 6, and 8%). Slices were then placed in uncoated PP bags and stored at 3 ºC to reflect domestic storage conditions. Numbers of the *E. coli* O157:H7 strains were counted, just after inoculation, and after one and seven storage days on MacConkey Agar with Sorbitol (SMAC, Scharlab) after 24 h incubation at 37 ºC. Each experiment was performed six times

2.6. Statistical analysis of data on Zamorano sheep cheese

E. coli counts were transformed and expressed as log cfu/g. Basic descriptive statistics of each parameter (mean and standard deviation) were calculated and linear regression analysis was used to determine the relationship between parameters. The potential influence of strain, film, antimicrobial and storage day was analyzed by using multi-factor analysis of variance (ANOVA). Subsequently, *post-hoc* pairwise comparisons were performed through the Fisher Least Significant Difference (Fisher-LSD) test. Data analysis was carried out with the "Statistica for Windows release 7.0" software (Statsoft Inc.,Tulsa, OK, USA).

2.7. Sensory analysis

A panel of ten members was recruited for evaluating the effect of the coated films on the smell and taste of non contaminated Zamorano cheese. All were selected on the basis of commitment and motivation. Panellists were asked to rank the samples in descending order based on overall acceptability. Control and wrapped cheese samples (PP with 4, 6 and 8% OR; PP with 4, 6 and 8% LAE; PET with 4, 6 and 8%OR; and PET with 4, 6 and 8% LAE) were analysed after 1 and 7 days of chilled storage in four different sampling days. Data were analysed and interpreted using the Friedman test according to ISO standard 8587:2006 (Anonymous, 2006; Anonymous, 2013).

3. Results and discussion

3.1. Determination of MIC and MBC values against E. coli *O157:H7*

The MIC and MBC values of OR and LAE for both *E. coli* O157:H7 strains are given in Table 1. MBC values of each compound were identical to their respective MICs. Similar data were reported by Becerril et al. (2007) for OR against the non-STEC strain *E. coli* ATCC 25922 (MIC and MBC values of 190 mg/l). For one *E. coli* O157:H7 strain, the OR MIC and MBC values were 625 µl/l (Burt &. Reinders, 2003). In this study, LAE showed stronger antibacterial activity than OR, the latter being two-fold more efficient against the reference culture than against the sheep milk strain. For LAE, the MIC and MCB values found by Muriel-Galet et al. (2012a) against the non-STEC strain *E. coli* ATCC 25922 were 20 ppm and 32 ppm, respectively.

The high antimicrobial activity of LAE on Gram-negative bacteria has been attributed to alterations which involve both the cytoplasm membrane and the external membrane without causing cellular lysis (Rodriguez, Seguer, Rocabayera, & Manresa, 2004). Among a number of plant essential oils, essential oil from *O. vulgare* has been found to exhibit the strongest *in vitro* bacteriostatic and bactericidal activities against non-verotoxigenic and verotoxigenic *E. coli* O157:H7 strains (Burt & Reinders, 2003; Marino, Bersani, & Comi, 2001).

3.2. Vapour phase assay

Table 2 shows the inhibitory effect of the atmosphere derived from OR incorporated in the packaging materials (PP and PET). Oregano essential oil did not inhibit the growth of both *E. coli* O157:H7 strains under 4%, but did under 6% (partial inhibition) and 8% (partial or total inhibition). A number of studies (Becerril et al., 2007; Gutiérrez, Batlle, Sánchez, & Nerín, 2010; López et al., 2007b; Rodriguez, Nerin, & Batlle, 2008) have reported that, in the vapour phase assays, the antimicrobial activity of active packaging containing essential oils (EOs) is produced by the volatile compounds present in the headspace of the Petri dish and the amount of active

compounds present in the agar. For oregano essential oil, these compounds have been mainly identified as the phenolic compounds carvacrol and thymol although minor components appear to play a significant role (Burt, 2004; Gutiérrez et al., 2010).

The effectiveness of EOs incorporated into packaging materials tested by vapour phase assay has been related to the concentration of the active compound, the packaging material and the target microorganism (Gutiérrez et al., 2010; López et al., 2007b; Rodriguez, Nerin, & Batlle, 2008). In this study, higher concentrations of OR in the active coatings resulted in higher antimicrobial activity, 6% OR being the minimum concentration showing inhibition against both *E. coli* O157:H7 strains. At 8% OR, differences were observed between PP and PET. Thus, total inhibition by 8% OR PP was observed against both *E. coli* O157:H7 strains while 8% OR PET completely inhibited only *E. coli* O157:H7 reference strain. Testing the behaviour of different films, Gutiérrez et al. (2010) concluded that a critical point in the design of an active packaging is the material used because not only the concentration of the active compound, but also the kinetic of release and the polymer play an important role since it is necessary to reach a minimum concentration of the active compounds in the solid medium during the lag phase of the microorganisms to inhibit their growth, and this occurs faster with certain active polymers. Overall, EOs are more effective against moulds and yeasts than against bacteria and among the latter, they appear to be more efficient against Gram-positive species (Burt, 2004). In addition, it has been reported that target microorganisms have a clear influence on the composition of the atmospheres generated by EOs. This finding has been attributed to diverse interactions and/or biotransformation routes (Lópezet al., 2007b).

3.3. Direct contact assays

Table 3 gives the quantitative antimicrobial activity of PP and PET films coated with ATOX varnish containing different concentrations of OR and tested under the JIS Z 2801:2000 method (Anonymous, 2000). This standard was developed to measure the antibacterial activity of antibacterial-treated plastic products and other non-porous materials. In this study, the OR PET film coated with 4%, 6% and 8% of the antimicrobial compound did not allow recovering of cultivable cells from both *E. coli* O157:H7 strains after 24h incubation at 37 ºC. These results demonstrate that the OR-containing PET films had a strong antibacterial *in vitro* direct contact activity against both *E. coli* O157:H7 strains. Evaluation of PP films with ATOX varnish coating containing OR showed that 6% and 8% OR also completely inhibited the *E. coli* O157:H7 type strain, which under 4% OR, had a R value of 0.20 (a reduction of 38%). However, for the milk strain *E. coli* O157:H7 M364VO, the PP films containing 4% and 6% OR allowed *E. coli* O157:H7 growth (R values of -0.41 and -0.23, respectively) although under OR concentrations of 8% produced a reduction of 91.7% (R value =1.08).

Our results show that by using the JIS Z 2801:2000 method, the OR PET films were more efficient than the OR PP films, the behaviour of the latter depending on the origin of the tested strain and the concentration of the active compound.

Table 4 shows the antimicrobial activity of LAE incorporated in the ACRIL varnish coating PP and PET films. No activity was observed against the tested strains at 0 and 4% LAE. Results obtained with 0% LAE PP and 0% LAE PET films suggest that, under the conditions of this test (incubation at 37 ºC), manipulations required to prepare coated films without LAE did not affect the antibacterial activity of the coated films. The activity, which was revealed by a clear zone of inhibition under and around the active films (between 1 and 2.5 mm) demonstrated that 6% LAE PET films were more

289 efficient than 6% LAE PP films although 8% LAE PET and 8% LAE PP films gave
290 identical results (2.5mm of inhibition zone).

291 As observed with the vapour phase assay, results obtained with the direct contact
292 assays appear to depend on the packaging materials, the concentration of the active
293 compound and the target microorganism.

294 *3.4. Antimicrobial activity in Zamorano sheep cheese*

295 The change in numbers of the *E. coli* O157:H7 strains during cold storage of
296 inoculated Zamorano cheese slices placed between two pieces of PP or PET films
297 coated with the varnishes alone and three different concentrations of each antimicrobial
298 (4, 6, and 8%) is given in Table 5. Statistical analysis demonstrated that counts were
299 significantly ($p < 0.05$) affected by the film, the antimicrobial compound, the storage day
300 and the tested strain.

301 After seven days of cold storage, 4% OR PP films significantly ($p < 0.05$)
302 decreased numbers of both *E. coli* O157:H7 strains. PP films coated with 6% and 8%
303 OR did not significantly ($p > 0.05$) reduce the counts. Overall, these results are not in
304 agreement with data obtained with the *in vitro* tests (vapour phase assay and JIS Z
305 2801:2000 method) where the inhibition of the PP films increased with increasing OR
306 concentrations (Tables 2 and 3).

307 In the cheese model, LAE PP films were only statistically significantly efficient
308 ($p < 0.05$) in reducing *E. coli* O157:H7 numbers when coated with 6% although the
309 highest inhibition zones in the *in vitro* direct contact assay was obtained with 8% LAE
310 PP films. The slight statistically significant decreases in cheese models (Table 5) when
311 stored under 4% OR PP and 6% LAE PP films at 3º C may be regarded as so small that
312 they may not reflect meaningful inhibition. These data suggest that using PP
313 antimicrobials food packaging films based on the release of OR and LAE, do not appear

314 to be efficient against the target *E. coli* O157:H7 strains inoculated in Zamorano cheese.
315 When the application of packaging films based on the release of LAE and OR from a
316 number of films were studied against other Gram-negative pathogenic mesophilic
317 strains (*Salmonella*) inoculated in food models, the reduction reported was much higher
318 (Muriel-Galet et al., 2012a; Muriel-Galet et al., 2012b).

319 Data obtained with PET films coated with OR show that they significantly ($p < 0.05$)
320 reduced levels of the tested strains when coated with 4% (type strain), and 6% and
321 8% (both strains). Similar results were observed for PET films coated with LAE
322 although for 4% LAE, the significant ($p < 0.05$) reduction was for the milk strain. It
323 should be noted that 6% OR and 6 % LAE PET were more efficient than 8% OR and
324 8% LAE PET. Overall, in the Zamorano cheese model, the antimicrobial capacity of
325 PET films was greater than that of PP films.

326 When studying the effectiveness of antimicrobial food packaging films, many
327 antimicrobial systems that have shown strong activity when tested on model systems, do
328 not demonstrate similar activity when tested in real food products. This, which is very
329 common, is due not only to the target microorganisms but also to the interactive effects
330 of a number of factors (Han, 2005). Amongst them are characteristics of the food such
331 as pH, a_w, fat and protein content, antioxidants, preservatives, salt and other additives
332 and also extrinsic determinants such as the storage temperature and the atmosphere
333 composition. Moreover, each food has its own characteristic microflora (Burt, 2004;
334 Han, 2005; Quintavalla & Vicini, 2002). The average physicochemical composition of
335 Zamorano cheese wedges was: fat over dry matter, 45%; protein, 25%; pH 5.3; a_w, 0.95
336 and NaCl content, 1.94%. The dominant microflora, which is lactic acid bacteria, was
337 over 9 log units cfu/g. It should be noted that although *E. coli* O157:H7 strains are
338 mesophilic bacteria unable to grow under 7 ºC some strains appear to possess unique

genetic mechanisms enabling survival and proliferation under low temperature conditions (Vidovic, Mangalappalli-Illathu, & Korber, 2011).

3.5. Sensory analysis

The value of the correlated Friedman statistic was F´=12.59. For OR PP films, at 5% level of significance, the Friedman's test showed significant differences among samples (F´= 48.58). The pairwise comparison for control samples and those packaged with OR-PP films showed significant differences ($p<0.05$) after 24h storage and also after seven days storage under 6% OR. No significant differences ($p>0.05$) were established between OR PP packages of cold stored cheese slices kept for 24 h and seven days. The results indicate that for OR PP, panellists detected the OR flavour after 24h and to a lesser extent after seven days storage. Panellists did not find significant differences ($p>0.05$) for cheese slices packaged with LAE PP (F'= 7.21) neither for OR PET (F'= 9.70) and LAE PET (F'= 12.3). Our data suggest that LAE and/or PET did not significantly affect sensorial characteristics of Zamorano cheese.

In conclusion, overall, the *in vitro* tests demonstrated the antimicrobial activity of PP and PET films coated with OR and LAE when tested against a wild and a reference *E. coli* O157:H7 strains although the effect on the food model (Zamorano cheese) was moderately effective and most depend on the film and also on the antimicrobial agent. Our data suggest that PET films coated with ≥ 6% LAE concentrations might be useful in reducing *E. coli* O157:H7 numbers in sheep cheese.

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Table captions

Table 1. MIC[a] and MBC[b] values for two *E. coli* O157:H7 strains in broth dilution assays

Table 2. Antimicrobial effect of the atmosphere derived from different concentrations of OR incorporated in two packaging films against two *E. coli* O157:H7 strains

Table 3. Antimicrobial activity tested by a direct contact assay[a] of different concentrations of OR[b] (4%, 6% and 8%) incorporated in two packaging films against two *E. coli* O157:H7 strains

Table 4. Antimicrobial activity tested by a direct contact assay[a] of different concentrations of LAE incorporated in two packaging films against two *E. coli* O157:H7 strains

Table 5. Effects of two antimicrobial packaging films against two *E.coli* O157:H7 strains inoculated on slices of Zamorano cheese

Table 1.

Antimicrobial activity	OR[c]		LAE[d]	
	Strain M364VO[e]	Strain CECT[f] 5947	Strain M364VO	Strain CECT 5947
MIC (mg/l)	400	200	25	25
MBC (mg/l)	400	200	25	25

[a]Minimum inhibitory concentration.
[b]Minimum bactericidal concentration.
[c]Oregano essential oil obtained from *Origanum vulgare.*
[d]Ethyl Lauroyl Arginate HCl.
[e]Strain from sheep milk.
[f]CECT, Spanish Type Culture Collection.

Table 2.

Films	Strain M364VO[a]	Strain CECT 5947[b]
0% OR[c] PP[d]	-[e]	-
0% OR PET[f]	-	-
4% OR PP	-	-
4% OR PET	-	-
6% OR PP	+	+
6% OR PET	+	+
8% OR PP	++	++
8% OR PET	+	++

[a]Strain from sheep milk.
[b]CECT, Spanish Type Culture Collection.
[c]Oregano essential oil obtained from *Origanum vulgare.*
[d]PP, Polypropylene.
[e]-, no inhibition; +, partial inhibition; ++, total inhibition.
[f]PET, Polyethylene terephthalate.

Table 3.

Strain	Film	OR concentration	Film control after inoculation (cfu/ml)	Film control after 24 h (cfu/ml)	Coated films after 24 h (cfu/ml)	R-value[c]
M364VO[g]	PP[f]	Varnish ATOX[d]	1.02 x 10^{5}[e]	5.40 x 10^{6}		
		4%			1.40 x 10^{7}	-0.41
		6%			9.20 x 10^{6}	-0.23
		8%			4.47 x 10^{5}	1.08
	PET[i]	Varnish ATOX	1.90 x 10^{5}	7.00 x 10^{4}		
		4%			NG[h]	
		6%			NG	
		8%			NG	
CECT[j] 5947	PP	Varnish ATOX	1.33 x 10^{5}	1.50 x 10^{7}		
		4%			9.43 x 10^{6}	0.20
		6%			NG	
		8%			NG	
	PET	Varnish ATOX	1.57 x 10^{5}	1.20 x 10^{5}		
		4%			NG	
		6%			NG	
		8%			NG	

[a]Activity tested by using Japanese standard JIS Z 2801:2000.
[b]Oregano essential oil obtained from *Origanum vulgare.*
[c]R-value was calculated using the formula R=log (B/C) where, B: average of colony forming units (cfu) on the untreated films and C: average of the cfu found on the treated ones after 24h incubation at 37 ºC.
[d]Films coated only with the varnish (0% OR).
[e]The values are means for counts obtained for three independent sample pieces.
[f]PP, Polypropylene.
[g]Strain from sheep milk.
[h]NG, no growth. Viable cells were not recovered from the inoculated coated films after 24h incubation at 37 ºC.
[i]PET, Polyethylene terephthalate.
[j]CECT, Spanish Type Culture Collection.

Table 4.

Strain	Film	LAE[b] concentration	Inhibition zone (mm)[c]
M364VO[f]	PP[e]	Varnish ACRIL[d]	0
		4%	0
		6%	1
		8%	2.5
	PET[g]	Varnish ACRIL	0
		4%	0
		6%	1.5
		8%	2.5
CECT 5947[h]	PP	Varnish ACRIL	0
		4%	0
		6%	1
		8%	2.5
	PET	Varnish ACRIL	0
		4%	0
		6%	1.5
		8%	2.5

[a]Activity tested by using an agar diffusion method as described by Iseppi et al. (2008).
[b]Ethyl Lauroyl Arginate HCl.
[c]Clear zone of inhibition around the plastic film.
[d]Films coated only with the varnish (0% LAE).
[e]PP, Polypropylene.
[f]Strain from sheep milk.
[g]PET, Polyethylene terephthalate.
[h]CECT, Spanish Type Culture Collection.

Table 5.

Fil	Antimicr	Concentr	*E.coli* O157:H7 strain			*E.coli* O157:H7 strain		
PP	OR[d]	Varnish	Day 0	Day 1	Day 7	Day 0	Day 1	Day 7
		0%	[ef]4.37±0	4.42±0.	4.26±0	4.33±0.	4.37±0.	4.44±0
		4%	4.31±0.1	4.26±0.	4.07±0	4.40±0.	4.19±0.	3.95±0
		6%	4.32±0.2	4.35±0.	4.18±0	4.38±0.	4.48±0.	4.32±0
		8%	4.16±0.1	4.23±0.	4.16±0	4.36±0.	4.13±0.	4.30±0
	LAE[g]	Varnish						
		0%	4.41±0.0	4.30±0.	4.22±0	4.30±0.	4.29±0.	4.28±0
		4%	4.23±0.0	4.12±0.	4.24±0	4.33±0.	4.37±0.	4.19±0
		6%	4.47±0.1	4.25±0.	4.22±0	4.44±0.	4.30±0.	4.24±0
		8%	4.38±0.1	4.29±0.	4.31±0	4.47±0.	4.44±0.	4.53±0
PE	OR	Varnish						
		0%	4.44±0.0	4.44±0.	4.38±0	4.50±0.	4.32±0.	4.31±0
		4%	4.21±0.0	4.12±0.	4.29±0	4.45±0.	4.28±0.	3.99±0
		6%	4.46±0.2	4.39±0.	4.07±0	4.53±0.	4.42±0.	4.23±0
		8%	4.29±0.1	4.26±0.	4.01±0	4.36±0.	4.26±0.	4.11±0
	LAE	Varnish						
		0%	4.35±0.0	4.39±0.	4.21±0	4.32±0.	4.30±0.	4.21±0
		4%	4.30±0.0	4.27±0.	4.12±0	4.35±0.	4.43±0.	4.21±0
		6%	4.47±0.0	4.22±0.	4.13±0	4.44±0.	4.32±0.	4.17±0
		8%	4.50±0.0	4.23±0.	4.30±0	4.44±0.	4.40±0.	4.28±0

[a]Strain from sheep milk.
[b]CECT, Spanish Type Culture Collection.
[c]PP, Polypropylene.
[d] Oregano essential oil obtained from *Origanum vulgare.*
[e]Each mean±standard deviation represents an average of six determinations.
[f]means on the same line with different superscript numbers are significantly different ($p < 0.05$).
[g]Ethyl Lauroyl Arginate HCl.

[h] PET, Polyethylene terephthalate.

- Two antimicrobial packaging films were tested against two *E. coli* O157:H7 strains
- The films were Polypropylene (PP) and polyethylene terephthalate (PET)
- Antimicrobials were essential oil from oregano (OR) and ethyl lauroyl arginate (LAE)
- Overall, i*n vitro* tests showed that coated films were effective against both strains
- In a ripened sheep cheese model, only PET films coated with ≥ 6% LAE might be useful