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EVALUATION OF EVOH-COATED PP FILMS WITH OREGANO ESSENTIAL OIL AND CITRAL TO IMPROVE THE SHELF-LIFE OF PACKAGED SALAD

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ABSTRACT

The aim of this study was to improve the present packaging of salad by combining modified atmosphere packaging with a new antimicrobial active bag consisting of PP/EVOH film with oregano essential oil or citral, with the purpose of extending shelf-life and reducing possible microbiological risks. The (O_2) and CO_2 barrier properties of PP/EVOH, mechanical properties (Young's modulus, tensile strength and elongation at break) were determined and compared with those of standard PP films. Antimicrobial tests were carried out for enterobacteria, total aerobic counts, yeasts and moulds, and lactic acid bacteria and psychrotrophic bacteria, and the effect of the release of the antimicrobial agent on the sensory characteristics of the salads was also studied. The application of the EVOH coating results in an increase in the tensile resistance of the PP films and a reduction in the elongation at break. The results showed that microorganism counts bacteria decreased especially at the beginning of the storage period. OEO and CITRAL samples had reductions of 1.38 log and 2.13 log respectively against enterobacterias, about 2 log against yeasts and moulds. The total aerobic counts reduced 1.08 log with OEO and 1.23 log with CITRAL and the reduction of lactic acid bacteria and psychrotrophic was about 2 log.. Citral-based films appeared to be more effective than materials containing oregano essential oil in reducing spoilage flora during storage time. Sensory studies also showed that the package with citral was the most accepted by customers at the end of the shelf life

Keywords: *minimally processed salads, citral, oregano essential oil, antimicrobial packaging, mechanical properties, EVOH.*

1. INTRODUCTION

Consumer demand for healthy, fresh-like, easy-to-prepare products coupled with consumer lifestyles changes have led to the development of a variety of novel products, of which minimally processed salads are an example. Cut vegetables are more susceptible to chemical and microbiological deterioration because during the cutting, cells are destroyed and exudates rich in minerals, sugar, vitamins, and other compounds are released. These nutrients and storage conditions may allow growth of microorganisms (Froeder, et al., 2007). During the last decades food borne outbreaks associated with consumption of raw vegetables have been increasing and green leafy vegetables seem to be the most frequently implicated products (Santos, et al., 2012).

In order to extend the shelf life of minimally processed products, various technologies are available including well implemented equilibrium modified atmosphere packaging (MAP) (Carrasco, Perez-Rodriguez, Valero, Garcia-Gimeno, & Zurera, 2008; Oms-Oliu, Martinez, Soliva-Fortuny, & Martin-Belloso, 2008) and more recently antimicrobial active packaging. The combination of active antimicrobial packaging and MAP is a possible alternative to enhance the quality, safety and shelf-life of products of this kind, in which antimicrobial agents added directly to the packaging material are released exerting their activity on the packaged product (Sanchez-Gonzalez, Chafer, Hernandez, Chiralt, & Gonzalez-Martinez, 2011).

In a previous work (Muriel-Galet, et al., 2012), oregano essential oil and citral were incorporated in an ethylene-vinyl alcohol copolymer (EVOH) matrix and a preliminary study on the antimicrobial activity of the films and on the sensory attributes of salad was reported. Results showed that the antimicrobial activity increased with concentration of the antimicrobial agent in the film but also increased the sensory perception of off-flavors. EVOH materials have been used recently as matrices for the development of active packaging systems, where the polymer triggers their activity on exposure to a humid environment (the food product) (Lopez-de-Dicastillo, Alonso, Catala, Gavara, & Hernandez-Munoz, 2010a; Lopez-de-Dicastillo, Catala, Gavara, & Hernandez-Munoz, 2011; Lopez-de-Dicastillo, Gallur, Catala, Gavara, & Hernandez-Munoz, 2010b)

In this work, active EVOH coated polypropylene (PP) films were manufactured using gravure printing technologies and used for modified atmosphere packaging of fresh produce in a processing plant. The aim of this study was to improve the stability of a mixed vegetable salad with the new antimicrobial active packaging that released oregano essential oil or citral combined with MAP and to characterize the films and the antimicrobial and sensory effects on salad.

2. MATERIALS AND METHODS

2.1 Chemicals and reagents

Ethylene vinyl alcohol copolymer with a 29% ethylene molar content (EVOH) was kindly provided by The Nippon Synthetic Chemical Company (Osaka, Japan). Oregano essential oil and citral were purchased from Sigma (Madrid, Spain). Polypropylene films were provided by Envaflex (Utebo, Zaragoza, Spain). "*Four season salad*" (a mixture of cut iceberg lettuce, red cabbage and shredded carrot) was purchased from Verdifresh (Ribarroja, Valencia, Spain). Water was obtained from a Milli-Q Plus purification system (Millipore, Molsheim, France).

2.2 Film preparation

A coating technology based on gravure printing was used to produce the antimicrobial material at Envaflex at a production speed of 60 m/min. Based on the results of previous work (Virginia Muriel-Galet & Hernández-Muñoz, 2012), the antimicrobial films were produced by incorporating a known concentration (7.5 % w/w) of oregano essential oil (OEO) or citral (CITRAL) in the EVOH coating solution. Polypropylene films were treated with corona and a primer based on polyethylene imine (Mica Corp., Kansas City, USA) was applied to improve adherence.. EVOH coating was applied on the PP films, keeping an area uncoated to maintain sealability for the manufacture of the salad bags. Specifically, of the 1140 cm^2 of film that was used to manufacture a bag, only 750 cm^2 of surface was coated. Control films were obtained without active agents (CONTROL). Thicknesses of the films were measured at 10 points of samples. The thickness of the coating was calculated from the difference in thickness between coated and uncoated areas.

2.3 Packaging and storage

"Four season salad" was packed in individual bags manufactured with the various films with (OEO and CITRAL) and without agents (CONTROL) as shown in Figure 1. The packaging was done at Verdifresh (Ribarroja, Valencia, Spain) with a MULTIVAC A-NG 85021 packaging unit, and with an atmospheric composition of 12% CO_2 and 4% O_2. Bags of "*four season salad"* were stored for 5 days at 4ºC plus 3 days at 8ºC, simulating commercial conditions of production, transport and commercialization (Lopez-Galvez, Gil, Truchado, Selma, & Allende, 2010).

2.4 Identification and quantification of volatile compounds

The equilibrium concentration of carvacrol (main component of oregano essential oil) and citral in the polymer samples was determined by thermal desorption and GC analysis using a thermal-desorber (890/891 model, Dynatherm Analytical Inst., Supelco, Bellafonte, PA, USA) connected in series to the gas chromatograph (Lopez-Carballo, Cava, Lagaron, Catala, & Gavara, 2005). In brief, a portion of the tested film (ca. 1cm^2) was placed in the desorption cell and heated at 210 ºC for 7 min. A He gas stream carried the desorbed compounds to the GC through a transfer line heated at 230 ºC. The GC was equipped with a 30 m, 0.53 mm, 2.65 µm Agilent HP-1 semicapillary column. The chromatographic conditions were: He as the carrier gas, 210 ºC and 260 ºC injector and detector temperatures, 7 min at 45 ºC, heating ramp to 220 ºC at 18 ºC/min, and 12 min more at 220 ºC. At the end of the desorption process the sample was weighed with a 0.1 mg precision balance (Voyager V11140 model, Ohaus, Switzerland). Calibration was done by injecting known amounts of carvacrol or citral in the desorption cell.

2.5 Oxygen and carbon dioxide permeance

The measurements of O_2 and CO_2 permeance through the film samples were carried out using isostatic methods (Lopez-de-Dicastillo, et al., 2010b). The O_2 permeation rates of the materials were determined at 0 and 90% RH and 23ºC using an OXTRAN Model 2/21 ML Mocon (Lippke, Neuwied, Germany). The CO_2 permeation rates of the materials were determined at 0 and 90% RH and 23ºC using an assembly that makes use of a gas chromatograph as detector.

The evolution of headspace atmosphere composition during storage was carried out by withdrawal of 20 mL gas samples and injection on a Checkmaster gas analyzer (Lippke, Germany).

2.6 Mechanical properties

A Mecmesin model MultiTest 1-I universal machine (Landes Poli Ibércia, S.L., Barcelona, Spain) equipped with a 100 N static load cell was used to evaluate the maximum tensile strength (σ_m), percentage of elongation at break (ε_b) and Young’s modulus (E) of the films according to ASTM standard method D882 (ASTM, 2009). The films were conditioned at 50% RH and room temperature for at least 1 week prior to testing. Film samples were cut into strips 2.54 cm wide and 10 cm long. The grip separation was set at 5.08 cm and the cross-head speed at 25 mm·min^{-1}. At least 6 replicates from each sample were tested. The tensile properties were calculated from the plot of stress (tensile force/initial cross-section area) versus strain (elongation as a fraction of the original length).

2.7 Antimicrobial activity of films on microflora of minimally processed salad

The antimicrobial activity of the films was tested *in vivo*. Analyses were performed on the 1st, 4th and 8th days after packaging.. 25-g samples of the packaged salad were weighed in sterile Stomacher bags, diluted with 25 mL of peptone water (Scharlab, Barcelona, Spain) and homogenized in Stomacher for 3 min (IUL S.L., Barcelona). Ten-fold dilution series were made in the same saline solution for plating.

The enumeration of particular microbial groups was performed by using the following media (Scharlab, Barcelona, Spain) and culture conditions: a) Nutrient Agar for total aerobic plate count, pour-plated and incubated at 30 ºC for 48 h, and also for total aerobic psychrotropic count, pour-plated and incubated at 10 ºC for 10 days; b) MEA for yeasts and moulds, pour-plated and incubated at 25 ºC for 5 days; c) VRBD agar for total enterobacteria, pour-plated, overlaid with the same medium and incubated aerobically at 37 ºC for 48 h; d) MRS agar for lactic acid bacteria, pour-plated and incubated at 25 ºC for 5 days. The counts were performed in triplicate.

2.8 Sensory analyses

A group of 51 panellists, consumers of ready-to-eat fresh products with no previous experience, evaluated the smell, visual appearance, texture and general acceptability of the samples by paired comparison tests (ISO 5495-2005). A total of three pairs of samples were evaluated. Each pair consisted of two samples. The first pair were samples packaged with CONTROL and OEO films, the second pair were CONTROL and CITRAL, and the third pair were OEO and CITRAL.

Three pairs of samples were evaluated in each session and a total of two sessions was performed: on the 1st day, to determine the initial impact of the aromatic agent, and on the 8th day after packaging, to verify the quality and acceptability at the end of storage.. The tests were done in a standardized test room (ISO 8589-2007). Samples of *“four season salad”* were placed in PP/EVOH bags identified by three-digit codes. The Williams method for constructing such designs was applied to the sensory studies (MacFie, Bratchell, Greenhoff, & Vallis, 1989).

2.9 Statistical analysis

Statistical analysis of the data was performed by one-way ANOVA study. The SPSS® computer program (SPSS Inc., Chicago, IL, USA) was used. Differences in pairs of mean values were evaluated by the Tukey-b test for a confidence interval of 95%. Data are represented as mean ± standard deviation. The design of the sensory evaluation and the data analysis were carried out with Compusense Five software (release 5.5, Ontario, Canada).

3. RESULTS AND DISCUSSION

PP/EVOH films containing oregano essential oil (OEO) or citral (CITRAL) were prepared as described in the experimental section. PP/EVOH film without antimicrobial agent was also obtained as control (CONTROL). The EVOH coatings had a thickness of 1.15 ± 0.05 μm.

One of the major components of oregano essential oil is carvacrol (72.46%), which is responsible for its antimicrobial properties (Burt, 2004; Charai, Mosaddak, & Faid, 1996). Because of this, carvacrol was the only component of the OEO monitored in this study.. Films with 7.5% oregano essential oil had a final carvacrol concentration of 3.5 ± 0.2%. These results show a carvacrol loss of about 35%, which can be attributed to evaporation during the drying process. The films with citral had higher losses owing to its higher volatility. The final concentration was 2.6 ± 0.2%.

The film samples obtained were stored in desiccators with silica gel. In these dry storage conditions, the concentration of the agents was maintained constant for 3 months. EVOH is characterized by its excellent gas and organic vapour barrier properties when dry, and therefore the release of carvacrol or citral is highly impeded in these storage conditions (Aucejo, Catala, & Gavara, 2000; Lopez-de-Dicastillo, et al., 2010b).

3.1. Oxygen and carbon dioxide permeance

Permeance ($\wp$) to (O_2) and (CO_2) was evaluated under two relative humidity conditions: 0% and 90%. Table 1 shows the values for all the samples. As can be seen, the incorporation of the coating results in an increase in barrier which is very considerable in dry conditions. The permeance to both $O_2$and CO_2 is reduced by more than 100-fold. This effect is to be expected, owing to the well-known high barrier properties of EVOH. Nevertheless, the effect is severely reduced by humidity because of water plasticization of EVOH. The permeance decrease caused by the coating process on the PP film was 1.5-fold for O_2 and 4-fold for CO_2 in the coated area. Permeance values for the materials with the agents could not be measured because of the sensitivity of the Oxtran sensor to volatile organic compounds and their interference with the chromatographic detector used in the assays with CO_2. Effect of the agents in the barrier properties of the coatings should be negligible since, plasticization should not be expected from the addition of apolar substances to a polar matrix and agents with double functionality which could promote chain cross-linking are not present.

Considering that only part of the bag surface was coated, the effect of the coating on the transmission rate was even lower. An estimation of the O_2 (OER) and CO_2 exchange rates (CO_2ER) for the entire bag at the beginning of storage can be obtained by the use of the permeation equation for a bag constituted by two different materials, considering the permeance values for each film ($\wp_i$), the film surface (A_i) and the pressure gradient (Δp_i):

$$OER = \sum_{i=1}^{n} (\wp_i(O_2) \cdot A_i) \cdot \Delta p(O_2); \quad CO_2ER = \sum_{i=1}^{n} (\wp_i(CO_2) \cdot A_i) \cdot \Delta p(CO_2)$$

$$OER(coated) = 2.3 \cdot 10^{-10} \frac{m^3}{s}; \; OER(CONTROL) = 2.9 \cdot 10^{-10} \frac{m^3}{s}$$

$$CO_2ER(coated) = 6.5 \cdot 10^{-10} \frac{m^3}{s}; \; CO_2ER(CONTROL) = 1.3 \cdot 10^{-9} \frac{m^3}{s}$$

In technological units, the gas exchange rates for a finished bag are: 25 and 20 cc/day of O_2 and 112 and 56 cc/day of CO_2 for the CONTROL and coated sample, respectively.

Table 2 shows the evolution of the headspace composition for the three samples tested. In all cases, the O_2 was rapidly consumed as the product respired and there was a corresponding increase in CO_2 levels. The presence of the EVOH coating in the active packaging did not significantly influence the headspace composition during the storage period. EVOH generally acts as a high barrier material to gases when dry. However, when in contact with fresh vegetables, the EVOH layer becomes humid and the barrier properties are dramatically reduced. Thus the EVOH layer becomes permeable and the whole package behaves similarly to the reference one. Although the differences were not significant, it appears that the packages containing CITRAL as active agent had a lower CO_2 content than the other two samples, as if the CITRAL had an effect on the reduction in the respiration rate of the salad.

3.2. Mechanical properties

The mechanical parameters (σ_m, E and ε_b) of the CONTROL and treated films conditioned at 50% RH are shown in Table 3. As can be seen, the application of the EVOH coating results in an increase in the tensile resistance of the PP films and a reduction in the elongation at break. This effect can be explained as a consequence of the different morphology of the two materials. PP is a material with a glass transition temperature below room temperature, whilst EVOH is a semicrystalline glassy copolymer, therefore more rigid and more fragile than PP. No effect was observed as a result of incorporation of the agent in the EVOH layer and the corona-treated. The effect of the coating application would be severely diminished in humid conditions as a consequence of the plasticization of the EVOH coating. Unfortunately, no measurement could be carried out in these environmental conditions (90%RH and room temperature) with the equipment used.

3.3 Antimicrobial activity of active films on microflora of minimally processed salads

The samples were subjected to microbiological analysis on the 1st, 4th and 8th days of refrigerated storage. The results of microbiological counts of minimally processed salad samples are shown in Table 4.

On the 1st day of storage, the enterobacteria counts for *"four season salad"* packaged in the active samples showed a significant decrease in comparison with the control sample. OEO and CITRAL samples had reductions of 1.38 log and 2.13 log respectively. With storage time, there was no measurable effect. It should be noted that high levels of enterobacteria are common in raw vegetables and it is reported that they are not used to indicate the microbiological quality of fresh vegetables (Little, Roberts, Youngs, & Louvois, 1999).

The total aerobic counts were reduced only on the 1st day of storage; OEO films reduced count by 1.08 log and CITRAL films 1.23 log . The aerobic population increased to 5 log in all cases on the 4th day and to 6 log in all cases at the end of storage.

Table 4 also shows the antifungal effectiveness of the active films. The active compounds appear to be most effective on day 1. OEO and CITRAL samples had a reduction of about 2 log. At the end of the storage period, the coatings produced an inhibition of about 0.5 log. The role of yeasts and moulds in the spoilage of vegetables is not well studied, although they have been implicated in the spoilage of fermented vegetable products (Fleet, 1992)(Abadias, Usall, Anguera, Solson, & Vinas, 2008) and could cause off-odours and visual defects in minimally processed vegetables. Some authors (Tournas, 2005) have pointed out the possible health problems associated with the presence of moulds in fruits and vegetables. Their growth could be related with the quality of the salad and acceptability by the consumer.

The reduction of lactic acid bacteria was more significant for the films containing CITRAL, about 2 log on the 1st day of storage, and about 1 log reduction was observed on the 4th and 8th days. OEO films showed an inhibition of 0.5 log on day 1, which was just maintained during storage.

With respect to psychrotrophic bacteria that are able to grow at refrigerator temperature, the samples packaged with the OEO and CITRAL films showed an inhibition of approximately 2 log at the beginning of storage at 4 ºC. However, there was no measurable effect after 8 days of storage, because psychrotrophic bacteria counts increased at 8 ºC and they reached a stationary phase before the day of analysis (data not shown).

It can be concluded that these active films provided inhibition of microflora bacteria in the first stages of the storage period, when the concentration of the agent in the package headspace was higher and the storage temperature lower. The intrinsic properties of the food (fat/protein/water content, antioxidants, preservatives, pH, salt and other additives) and the extrinsic determinants (temperature, packaging in vacuum/gas/air, characteristics of microorganisms) can also influence bacterial sensitivity (Tassou, Drosinos, & Nychas, 1995). The antimicrobial activity of essential oil benefits from a decrease in storage temperature and the low fat content in salads. It is generally supposed that the high levels of fat in foodstuffs protect the bacteria from the action of the EO (Pandit & Shelef, 1994; Tassou, et al., 1995), therefore these factors could contribute to the successful results observed with essential oils (Burt, 2004).

CITRAL, which appears to be more effective than OEO, has already been successfully used for the inhibition of yeast growth and lactic acid bacteria in fruit-based salads (Belletti, Lanciotti, Patrignani, & Gardini, 2008). CITRAL can also be used to prolong the shelf-life of "*four season salads*". In this study with real foodstuffs, essential oil produced lower effects than in another test carried out "*in vitro*" in our laboratory (Muriel-Galet, et al., 2012), which can be attributed to partial sorption of the agent in the food, with a subsequent reduction in concentration in the vapour phase. A reduction in the antimicrobial activity of essential oils due to the nature of the food matrix has been documented (Belletti, et al., 2008; Burt, 2004; Lanciotti, et al., 2004; Muriel-Galet, et al., 2012). Therefore, higher concentrations of plant essential oils are generally required when added to food (Gutierrez, Barry-Ryan, & Bourke, 2008). However, the application of essential oils in food may be limited by the changes they produce in the organoleptic and textural quality of the food or interactions of these compounds with food components (Devlieghere, et al., 2004).

3.4. Sensory analyses

The organoleptic effect of oils is one of the most important factors that limit their application to real food products as antimicrobial agents, a property widely described in *in vitro* tests (Belletti, et al., 2008). Their use may have a significant sensory impact that could result in non-acceptance by the consumer. For this reason, sensory analyses were carried out on the *"four season salads"* packaged with the films developed.

The effects of the addition of different kinds of agents on the sensory properties of smell acceptability, visual appearance, texture and general acceptability were studied. On the 1st day of storage, the assessors perceived significant differences in odour acceptability, depending on the agents (OEO or CITRAL), in comparison with the CONTROL samples, as shown in Figures 2–4. In terms of visual appearance, no significant differences were observed between the samples. This can be explained by the fact that 1 storage day is not enough to cause deterioration of the sample.

On the 8th day of storage, no significant differences were recorded for smell, visual appearance, texture and general acceptability when the control was compared with OEO or CITRAL. Only differences between oregano essential oil and citral were found by the judges. The samples with CITRALwere clearly preferred on day 8 of storage.

OEO and CITRAL resulted in a pleasant flavour, compatible with final products. This is a prerequisite for the use of essential oils in foods for antimicrobial purposes.

4. CONCLUSIONS

In conclusion, the use of active packaging based on the release of volatile antimicrobials combined with modified atmosphere packaging is an alternative technology to improve the microbiological and sensory stability of salad. The addition of the active EVOH coating did not substantially affect the functional properties of the packaging film, since neither barrier nor mechanical properties had unacceptable values. Nevertheless, the packaged product did produce improved microbial stability when active films were used, especially with regard to growth inhibition of enterobacteria, lactic acid and psychrotrophic bacteria, and yeasts and moulds at the beginning of storage. The sensory studies showed that the active agent release resulted in a non-typical smell which led the judges to prefer the control samples, whereas after long-term storage the samples in active bags were preferred, especially the one containing citral. The results of the present work demonstrated that packaging of minimally processed salad in MAP bags manufactured with PP/EVOH with essential oils is an appropriate way to reach a balance between the demand for microbial safety and consumer acceptability.

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LEGENDS TO FIGURES

Figure 1. *"Four season salad"* bags in PP/EVOH films with oregano essential oil (A) and citral (B) in active modified atmosphere.

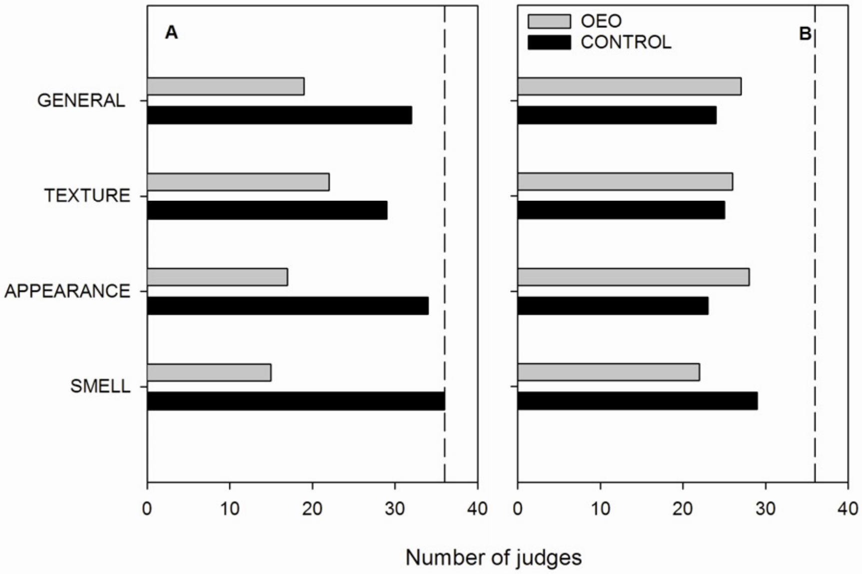
Figure 2. Sensory evaluation of the differences between the smell, appearance, texture and general preference of CONTROL and OEO on the 1st (A) and 8th (B) day of storage. The dashed line indicates the minimum number of responses for which the difference is significant.

Figure 3. Sensory evaluation of the differences between the smell, appearance, texture and general preference of CONTROL and CITRAL on the 1st (A) and 8th (B) day of storage. The dashed line indicates the minimum number of responses for which the difference is significant.

Figure 4. Sensory evaluation of the differences between the smell, appearance, texture and general preference of OEO and CITRAL on the 1st (A) and 8th (B) day of storage. The dashed line indicates the minimum number of responses for which the difference is significant

HIGHLIGHTS

- Development of antimicrobial film of PP/EVOH with oregano essential oil or citral.
- Active coating did not affect the functional properties of the packaging film.
- Antimicrobial films improved microbial stability at the beginning of storage.
- Consumers preferred salad packaged in active bags with citral at the end of storage.


A
B
OEO
CONTROL
GENERAL
TEXTURE
APPEARANCE
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0
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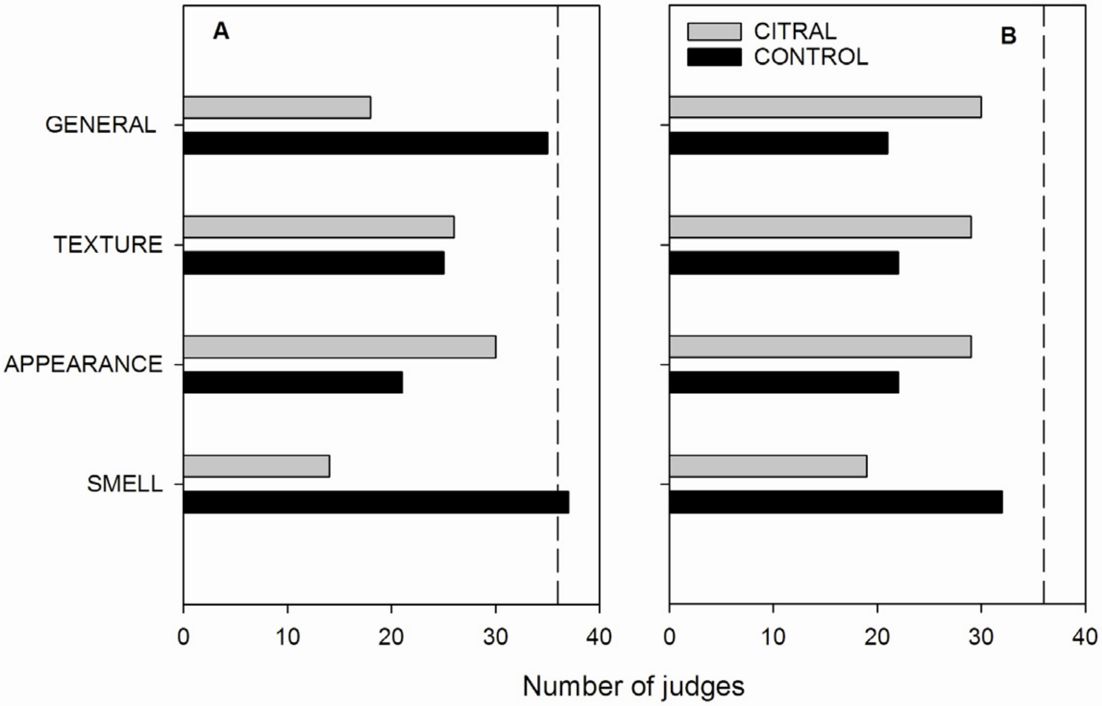
Number of judges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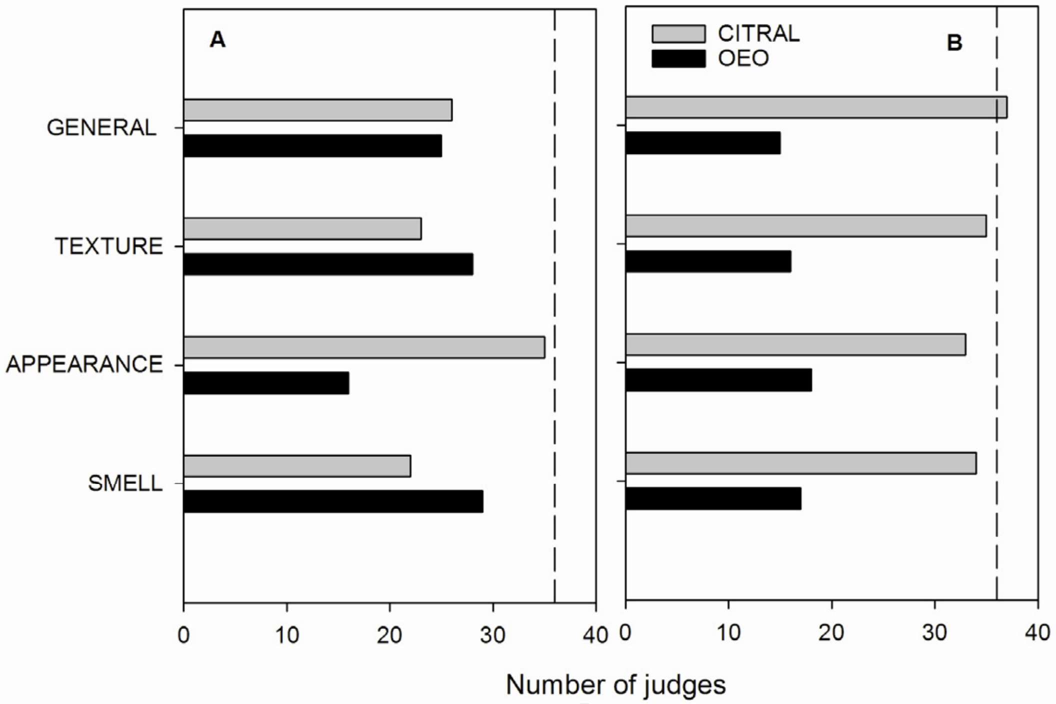
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40
Number of judges


A
B

Table 1. Values of O_2 and CO_2 permeance of PP films and PP/EVOH.

	Thickness (µm)	**$10^{14} \cdot 0_2$ permeance** ($m^3/m^2 \cdot s \cdot Pa$)		**$10^{14} \cdot C0_2$ permeance** ($m^3/m^2 \cdot s \cdot Pa$)	
		0% RH	**90% RH**	**0% RH**	**90% RH**
PP	30	13.54 ± 0.15	15.06 ± 0.20	117.80 ± 3.60	101.90± 1.50
PP+EVOH	30/1.15	0.085 ± 0.003	10.17 ± 0.08	0.78 ± 0.02	24.67 ± 0.22

Table 2. Atmospheric composition (% O_2 and CO_2) of headspace in mixed salad packages manufactured with the control film and the active films at different storage times.

	Day 1		Day 4		Day 8	
	O_2	**CO_2**	**O_2**	**CO_2**	**O_2**	**CO_2**
CONTROL	7.3 ± 1.3a	6.1 ± 1.4a	0.16 ± 0.08a	10.8 ± 1.4b	0.03 ± 0.01a	15.1 ± 1.1b
OEO	5.5 ± 1.5a	6.8 ± 1.3a	0.06 ± 0.03a	11.4 ± 1.7b	0.02 ± 0.01a	15.5 ± 1.4b
CITRAL	6.5 ± 1.5a	6.3 ± 1.0a	0.25 ± 0.12a	9.0 ± 1.0a	0.02 ± 0.01a	11.8 ± 1.2a

a, b: the letters are indicative of significance

Table 4. Enumeration of microbial population in mixed salad packages: enterobacteria, total aerobic, yeasts and moulds, lactic acid bacteria and psychrotrophic bacteria on 1st, 4th and 8th days of storage.

Coating	Day 1		Day 4		Day 8	
	Log	Reduction	Log	Reduction	Log	Reduction
ENTEROBACTERIA						
CONTROL	4.98 ± 0.65c		4.00 ± 0.00		4.87 ± 0.02	
OEO	3.60 ± 0.38b	–1.38	4.01 ± 0.14	-	4.73 ± 0.38	-
CITRAL	2.85 ± 0.09a	–2.13	3.96 ± 0.50	-	4.90 ± 0.03	-
TOTAL AEROBIC						
CONTROL	5.18 ± 0. 36 c		5.13 ± 0.02		6.17 ± 0.03	
OEO	4.10 ± 0.15 b	–1.08	5.03 ± 0.23	–0.10	5.89 ± 0.18	-
CITRAL	3.95 ± 0.11 a	–1.23	5.23 ± 0.07	-	6.05 ± 0.07	-
YEASTS AND MOULDS						
CONTROL	5.54 ± 0.12 c		4.28 ± 0.03 b		6.05 ± 0.16 b	
OEO	3.76 ± 0.25 b	–1.77	3.88 ± 0.11 a	–0.40	5.36 ± 0.32 a	–0.69
CITRAL	3.52 ± 0.53 a	–2.02	3.68 ± 0.00 a	–0.60	5.41 ± 0.04 a	–0.64
LACTIC ACID BACTERIA						
CONTROL	4.20 ± 0.14 c		4.05 ± 0.05 c		6.24±0.08 c	
OEO	3.53 ± 0.51 b	–0.67	3.71 ± 0.28 b	–0.34	5.80±0.20 b	–0.44
CITRAL	2.00 ± 0.00 a	–2.20	3.13 ± 0.16 a	–0.91	5.05±0.15 a	–1.19
PSYCHROTROPHIC						
CONTROL	5.74 ± 0.55 c		5.11 ± 0.00 b		6.11±0.06 b	
OEO	4.00 ± 0.01 b	–1.75	4.95 ± 0.28 a	–0.16	5.88±0.12 a	–0.23
CITRAL	3.74 ± 0.24 a	–2.01	4.93 ± 0.11 a	–0.18	5.82±0.60 a	–0.28

a,b,c: the letters are indicative of significance

Table 3. Mechanical properties of films fested at 50% RH.

Sample	**ó$_m$ (MPa)**	**å$_b$ (%)**	***E* (MPa)**
PP	125.65 ± 5.76 a	356.65 ± 29.74 b	658.48 ± 41.40 a
CONTROL	205.67 ± 42.56 b	303.76 ± 38.14 a	1729.02 ± 90.55 b
OEO	211.89 ± 10.32 b	274.44 ± 27.88 a	1746.98 ± 35.65 b
CITRAL	213.64 ± 9.29 b	281.51 ± 26.29 a	1754.90 ± 82.94 b

σ_m: Maximum tensile strength. ε_b: Elongation at break. *E*: Young’s modulus. Reported values are means of 6 replicates ± standard deviation.

a, b: the letters are indicative of significance