Characterization and antimicrobial activity of active polypropylene films containing Oregano essential oil **and *Allium* extract to be used in packaging for meat products.**

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Abstract

Cooked ham is more prone to spoilage than other meat products due to its properties, making the preservation techniques a key step in its commercialization. One of the most promising preservation strategies is the use of active packaging. Oregano essential oil (OEO) and Proallium® (an *Allium* extract) have previously shown to be useful in polylactic acid (PLA) active films for ready to eat salads. The present work aims to study the suitability of polypropylene (PP) films containing OEO and Proallium® in the preservation of cooked ham. Concerning the technological features of the studied material, no remarkable change in the mechanical or optical properties of PP films containing the active substances were recorded in comparison to the PP film without extracts. Although films containing both active substances were more flexible than the control film and less strong, highlighting the plasticization effect of the natural extracts. Moreover, physical properties changed when active substances were added to the film. The incorporation of 4% Proallium® affected in a higher extend the transparency of the film than in the case of 8% OEO, undergoing a decrease in the transparency of 40 and 45%, respectively. Moreover, only the film containing the highest amount of OEO (8%) decreased significantly the thickness. Both active substances showed antibacterial properties; however, Proallium® active films seemed to be more effective against *Brochotrix thermosphacta* than PP films containing OEO, with all percentages of Proallium® killing the bacterial population present in the ham after 60 days. In addition, materials containing the lowest Proallium® content exhibited the higher acceptability by the consumers in the sensory analyses with a willing purchase of 63-100%, even better than the control package (56-89%). In fact, 2% of Proallium® obtained the best results in the smell study performed by the panellists.

Keywords: polypropylene; oregano essential oil; *Allium* extract; active packaging

Abbreviations: Analysis of variance multifactor (ANOVA); *Brochothrix thermosphacta* (*B. thermosphacta*); colony forming units (CFU); crystallisation temperature (Tc); elastic modulus (E); elongation at break (EAB); enthalpy of crystallization (ΔHc); enthalpy of melting (ΔHm); essential oils (EOs); ethylene vinyl alcohol (EVOH); least significant difference (LSD); melting temperature (Tm); oregano essential oil (OEO); polylactic acid (PLA); polypropylene (PP); propyl thiosulphinate oxide (PTSO); Spanish Type Culture Collection (CECT); tensile strength (TS); tetrahydrofuran (THF); thermogravimetric analysis (TGA).

1. Introduction

Meat and meat products are good media for the growth of microorganism (Van Haute et al. 2016). Cooked ham is more prone to spoilage than other meat products due to its low salt content, pH near 6 and water activity higher than 0.945. In addition, cooked ham is usually sliced and packaged before being sold and consequently its shelf-life could decrease (Baños et al. 2012). Therefore, the preservation strategies for these foods are of great importance.

New trends in consumer demands, regarding minimally processed food or ready-to-eat fresh food, are driving a search for innovative ways to maintain food quality, freshness and safety (Appendini and Hotchkiss 2002). One of these new developments is active packaging, which was defined in Regulation (EC) No 450/2009. These materials include antimicrobial active films that can be classified in two categories: those made by using polymers inherently antimicrobial; and those that involve the direct incorporation of the antimicrobial additive into the packaging films (Suppakul, et al. 2003). Active food packaging systems containing antimicrobial additives are polymeric materials developed to release these active substances into the food in order to prevent microbial growth and to extend foodstuff shelf-life (Del Nobile et al. 2009). Polyolefins have been used as food packaging matrices in the development of active films due to their mechanical, barrier, optical and thermal properties (Ramos et al. 2012; Gigli et al. 2014). Polypropylene (PP) is a polyolefin with low water vapor transmission, medium gas permeability, good resistance to greases and chemicals, good abrasion resistance, high temperature stability and good gloss and high clarity. All of these features make PP suitable for food packaging (Robertson 2012; Ramos et al. 2012; Ramos et al. 2014; Muriel-Galet et al. 2013).

Regarding active substances, several studies have shown that consumers have recently become more informed about food additives and tend to choose those with additives of natural origin rather than their synthetic analogues (Carocho et al. 2015). In this regard, essential oils (EOs) and plant extracts have been the target of extensive research because these substances have demonstrated biological properties that exhibit benefits in food (Llana-Ruiz-Cabello et al. 2015; Maisanaba et al. 2017; Ribeiro-Santos et al. 2017). Therefore, new alternatives based on aromatic plants, such as EOs, have been studied (Ramos et al. 2014). The advantages observed for active packaging containing EOs compared with direct addition of these additives to the food, such as lower amount of compounds required or extended effects due to controlled migration from the film to the food are increasing the demand of these materials (Sanchez-Silva et al. 2014). Besides, when the additive is sprayed directly to food, the active substances could interact with food constituents causing their denaturalization. This phenomenon can be avoid also by using active packaging containing EOs (Sung et al. 2013). Among EOs, oregano essential oil (OEO) and its major compounds, carvacrol and thymol, have shown its usefulness as active substance in food packaging. A previous experimental study performed in our laboratory have proved the suitability of OEO when incorporated to a matrix of polylactic acid (PLA) as antioxidant agent in ready-to-eat salads (Llana-Ruiz-Cabello et al. 2016). Muriel-Galet et al. (2013) also showed the ability of ethylene vinyl alcohol (EVOH)-coated PP films containing OEO to improve the shelf life of packaged salad. Similarly, Ramos et al. (2012) reported the antimicrobial activity of PP films containing carvacrol and thymol against bacterial strains present in foods such as *Escherichia coli* or *Staphylococcus aureus*. Moreover, another plant extract, Proallium®, is a commercial product based on *Allium* extract that is composed of organosulphur compounds. Garlic EO and its major organosulphur compounds have shown antimicrobial activity when they are included in active packaging (Sung et al. 2014; Teixeira et al. 2014). Specifically, we have previously observed that films of PLA containing Proallium® showed antimicrobial activity against bacteria, yeast and molds

(Llana-Ruiz-Cabello et al. 2015b). However, this is the first work dealing the use of active packaging containing Proallium® in the preservation of ham.

The aim of the present work was to develop new active films based on PP containing OEO and Proallium® and further characterize them though optical, mechanical and thermal properties. Moreover, the effectiveness of these films in the preservation of cooked ham was also evaluated.

2. Materials and Methods

2.1 Supplies and Chemicals

Polypropylene ISPLEN® PP 050 G2M was purchased from Repsol YPF S.A. (Madrid, Spain). Oregano essential oil was acquired from El Jarpil (Almería, Spain). Proallium® with 14.5% of PTSO (Llana-Ruiz-Cabello et al. 2015b) as active agent was provided by Domca S.L. (Alhendin, Granada, Spain).

Chemicals for the different assays were purchase from Sigma-Aldrich (Spain) and VWR International Eurolab (Spain). Ham for the *in vivo* assays was purchase in a local market (Valencia, Spain).

2.2 Film preparation

Two types of active PP films were obtained, one of them containing nominal OEO contents of 2, 5 and 8 % (w/w) and the other containing nominal Proallium® contents of 2, 3 and 4 % (w/w). Films were prepared by melt blending in a twin-screw extruder (DSE 20-40D, Brabender, Germany). Extrusion temperatures were set at 200-205 ºC working at a screw sped of 70 min^{-1}. Active substances were introduced in the mixer once the polymer was melted and were fed into the barrel through the lateral liquid port at L/D 10 to reduce possible volatility and degradation losses. The average width and thickness of the films were around 10 cm and 80 µm, respectively.

2.3 Quantification of the overall content of OEO and Proallium® incorporated into PP films

The amount of OEO and Proallium® contained in PP films was evaluated using Thermogravimetric Analysis (TGA). Briefly, samples were heated to 900ºC and the loss of mass was measured in a thermal analyser Q5000IR (TA Instruments, New Castle, DE, USA) as described in Llana-Ruiz-Cabello et al. (2015b).

2.4 Quantification of carvacrol, thymol and propyl thiosulphinate oxide incorporated into PP films

The main constituents of OEO (i.e. carvacrol and thymol) and Proallium® (i.e. propyl thiosulphinate oxide, PTSO) incorporated into the PP films were extracted using tetrahydrofuran (THF) as solvent. Then, Thermo Finnigan gas chromatograph (GC) equipped with a DB-5MS column (Agilent Technologies, USA) (30 m, 0.25 mm, film thickness 0.25 µm) using helium as a carrier gas (flow rate of 1 ml min−1) was used for the analyses of active substance. The quantification of carvacrol and thymol was performed following the method described by Llana-Ruiz-Cabello et al. (2016) and the quantification of PTSO was carried out according to Llana-Ruiz-Cabello et al. (2015b).

2.5 Physical properties

The light transmission and transparency of films were measuredat selected wavelengths between 200 and 800 nm, using a Jasco V-630 UVeVisible recording spectrophotometer (Madrid, Spain) according to the method described in Llana-Ruiz-Cabello et al. (2015b). The transparency of the films was calculated according to Han and Floros (1997), as follows:

Transparency values = (-logT600)/x; where T600 is the fractional transmittance at 600 nm; and x: is the film thickness in mm.

Thickness were measured was measured in at least six different locations on each film using a digital micrometre Mitutoyo 547-400 Absolute (Kawasaki, Japan). The results are expressed as the median ± standard deviation.

2.6 Mechanical properties

Films were conditioned for 48 h at 25 ºC and 50±5 % RH before testing their mechanical properties. Tensile strength (TS), elongation at break (EAB) and elastic modulus (E) were determined at room temperature using an M350-20CT Universal Testing Machine (Testometric, Rochdale, England) according to UNE-EN-ISO 527-1, with slight modifications.

2.7 Thermal analysis

A Differential Scanning Calorimeter DSC Q2000 (TA Instruments) was used to develop the thermal analysis of films. Crystallisation temperature (Tc), and melting temperature (Tm) of the different PP films were measured using a Differential Scanning Calorimeter DSC Q2000 (TA Instruments). Specimens weighing 6 mg were heated at the rate of 10 ºC min^{-1} from 20 ºC to 250 ºC. Then, the samples were cooled at 10 ºC min^{-1} from 250 ºC to -50 ºC. Finally, the samples were heated again at 10 ºC min^{-1} up to 250 ºC.

The crystallinity was calculated as the ratio between the enthalpy of the melting point to the corresponding theoretical enthalpy of 100 % crystalline PP (estimated as 165 J/g for PP according to Zhu (2002) in percentage basis.

2.8 Antimicrobial activity in cooked ham

2.8.1 Microbial strain

Brochothrix thermosphacta, supplied by the Spanish Type Culture Collection (CECT), was grown overnight on brain heart infusion (BHI, Sharlau, Barcelona, Spain) at 28 ºC in aerobic atmosphere.

2.8.2 Experimental design

Three slides of ham were placed in polystyrene trays (135 x 180 mm, BANDESUR, Alcalá la Real, Spain) for each treatment and inoculated with a suspension of *B. thermophacta* to yield a final bacterial level of 10^5 colony forming units (CFU)/cm^2 using a Digralsky spreader. In order to separate the slides the developed films were used as interlayer. Different trays were prepared using PP and PP containing OEO (2, 5 and 8 %) or PP containing Proallium® (2, 3 and 4 %).

Samples packaged were stored in PP/polyamide plastic bags under vacuum (Technotrip EVT-10-2-CD-SC, Barcelona, Spain) and stored at 5 ºC.

The antimicrobial activity of PP films was evaluated after 1, 6, 12, 21, 27 and 60 days of packaging by studying the evolution of *B. thermophacta* population in the cooked ham along the storage time.

2.8.3 Microbial analysis

Samples of the surface of each slide of ham were taken at selected times to measure viable counts of bacteria. Each slide (aprox. 14 g) was aseptically removed and homogenized with 125 ml of peptone water (0.1 % peptone) using a Masticator blender (IUR, Barcelona, Spain) for 1 min. Then, a decimal dilution series was prepared for plating on STAA agar (Oxoid LTD, Basingstoke, Hampshire, England). Finally, plates were incubated aerobically at 28 ºC for 48-72 h before bacterial counts were determined. Results were expressed as CFU/cm^2.

2.9 Sensory analyses

The sensory tests were performed on days 1, 4, 11, 18, 24, 32 and 60 after packing according to the method described by Llana-Ruiz-Cabello et al. (2016).

Finally, the panellists were asked to answer about their willingness to purchase the product.

2.10 Packaging and storage

Several packages were produced containing three slices of "sliced ham" (approximately, 15 grams per slice) packed in contact with the different PP films containing OEO and Proallium®. For this purpose, 4 pieces of dimensions 8 x 17 cm of each PP film were used in total per package; 2 of them inserted between the three slices of ham as interlayers and another two pieces of PP films used to cover the slices of ham at the top and at the bottom. The "sandwich" of PP films and sliced ham was vacuum packed into commercial bags of PP/polyamide using a Multivac Vacuum Chamber Machine (Germany) at 150 mbar.

Bags of ham, both control and samples, were stored at 4 ºC for 60 days, simulating commercial conditions of production, transport and commercialization. All developed films were stored at -20 ºC before their characterization.

2.11 Statistical analysis

All measurements were performed at least in triplicate. Results were presented as means ± SD; significant differences were consider when $p<0.05$. The analysis of variance (ANOVA) was used to evaluate the significance in the difference between factors and levels. Comparison of the means was done by Dunnet's test. The statistical analysis of the data was performed using GraphPad InStat software (GraphPad Software Inc., La Jolla, CA, USA).

For the sensory analysis STATGRAPHICS Plus for Windows statistical software was used to calculate the analysis of variance multifactor (ANOVA) and Fisher's least significant difference

(LSD) to evaluate the impact of the day and packaging material on sensory attributes. Significant differences were determined at $p<0.05$ (significantly different from the control) and $p<0.01$ (highly significantly different from the control).

3. Results and Discussion

3.1 Content of OEO and Proallium® and their main constituents incorporated in the films

The TGA curves obtained for OEO, Proallium®, PP and PP films containing different concentrations of OEO and Proallium® are displayed in Figure 1. Both, OEO and Proallium® showed a lower thermal stability than the PP films. OEO, which is mainly composed by a mixture of different volatile substances, showed a gradual loss of mass from room temperature up to 180 ºC. Proallium® also showed a gradual loss of mass occurring at temperatures around 150 ºC as consequence of the volatiles which represent almost an 80% of the Proallium® composition. The other 20% of the chemical composition of Proallium® is lost in a second degradation step occurring between 350 and 450 ºC. On the other hand, the TGA curve for PP film showed a degradation step at temperatures from 400 to 500 ºC.

The TGA curves for the PP films containing OEO and Proallium® showed two weight loss steps. The first one occurring from 100º C up to 250 ºC corresponds mainly to the loss of mass related with the OEO and Proallium® incorporated into the PP films. Therefore, the measurement of the weight lost at 250 ºC provides an estimation of the overall content of OEO incorporated into the PP during the manufacturing of the films.

In relation to this, the contents of OEO and Proallium® incorporated in the materials are found in table 1. The yield of incorporation of OEO into the PP films by melting extrusion achieved an average value near 80%, which can be considered quite good attending to the volatile nature of the OEO and the temperatures of the process (Ramos et al. 2012). The yield of incorporation for Proallium® was 85% except for the incorporation of 4% w/w Proallium® which was 70%.

These results are in agreement with those previously obtained for OEO and Proallium® included in another plastic matrix, PLA, with weight losses around 70-80% (Llana-Ruiz-Cabello et al. 2015b, 2016). Similarly, other authors have evidence similar behaviour explaining that the losses observed are also due to the volatilization of the components during the extrusion process (Altiok et al. 2010; Ramos et al. 2012).

On the other hand, the analysis of the main constituents of OEO and Proallium® showed that the higher the amount of active agent incorporated, the higher the content of active components detected. The values obtained are shown in table 1. In relation to the overall OEO content into the PP films, carvacrol represents the 60%-75% of the total while thymol represents the 7%-9%. Considering the composition of the OEO declared by the supplier is 55% carvacrol and 5% thymol (Llana-Ruiz-Cabello et al. 2017), the extrusion process led to a concentration of these two molecules probably due to the higher selective losses of the more volatile substances of the OEO (e.g. α-pinene, α- and β-terpinene or ρ-cymene) during the process. In fact, these substances show vapour pressure values from 1 to almost 5 mmHg while carvacrol and thymol have a vapour pressure around 0.02 mmHg.

Finally, concerning the content of PTSO in the PP films containing Proallium®, the results evidenced that PTSO represents almost a 10% of the overall Proallium® incorporated to the films. This value is slightly lower than the 14.5% of PTSO content declared by the supplier for Proallium®-SO-DMC (Llana-Ruiz-Cabello et al. 2015c) thus pointing out towards a selective loss of this active agent in comparison with other components of Proallium® such as propylenglycol.

3.2 Physical properties

Consumers require transparent packages in order to evaluate the aspect of the food product to take the most adequate buying decision (Sehrawet and Kundu 2007). Table 2 shows the transparency values. PP films not containing OEO and Proallium® showed a transparency value of 1.56. This value shows a moderate transparency of the PP films. In particular, transparency parameter was highly affected by the addition of high contents of OEO. Hence, the incorporation of 8% of OEO decreased the transparency in a 40%. On the other hand, Proallium® affected in a higher extend the transparency of the films than the incorporation of OEO. In this case, films containing 2% Proallium® had transparency values similar to films containing 8% OEO. This finding were also observed when these active agents were incorporated into PLA, Proallium® exhibiting changes in the transparency when included in PLA but OEO had no effect (Llana-Ruiz-Cabello et al. 2015b, 2016).

The thickness of the PP films was ranged between 80 and 97 µm. Although, the addition of OEO and Proallium® to PLA (Llana-Ruiz.Cabello, et al. 2015b, 2016) was found to decrease the thickness of the films due to the plastification of the polymeric matrix, in the case of PP such effect was not significant from a statistical point of view. Only the film containing the highest amount of OEO had a significant decrease of the thickness.

3.3 Mechanical properties

Table 2 shows the mechanical properties of the different active films in terms of tensile strength, Young's modulus and elongation at break. Mechanically, PP is a semi-rigid and though material. In relation to this, Young's modulus gives a measurement of the stiffness (rigidity). While increasing the content of OEO from 2% to 8% did not produce a significant variation in Young's modulus, the incorporation of different amounts of Proallium® provoked a significant variation on this parameter. Anyway, PP films containing these both extracts were less resistant to deformation than PP films thus becoming materials more flexible.

On the other hand, the incorporation of OEO and Proallium® significantly decreased the tensile strength. Elongation at break was also affected by the incorporation of the OEO and especially with Proallium® although non-significant statistical differences with regards PP films were actually found. Therefore, considering tensile strength and elongation at break, the incorporation of OEO and Proallium® produced films that were less strong due to a plasticisation effect of both extracts.

Similar results were obtained by Ramos et al. (2012) who observed that the incorporation of carvacrol and thymol to PP materials provoked a clear decrease in Young's modulus and tensile strength, and a slightly increase in elongation at break. However, EVOH-coated PP films containing OEO exhibited an increase in the tensile resistance of PP films and a reduction in the elongation at break (Muriel-Galet et al. 2013). Nevertheless, clear of evidence of the plasticisation effect of EO when incorporated to the plastic matrix have been observed by many authors (Altioket al. 2010; Ahmad et al. 2012; Marcos et al. 2014; Llana-Ruiz-Cabello et al. 2015b,2016).

3.4 Thermal properties

Table 3 shows the crystallization (T_c) and melting temperatures (T_m) as well as their corresponding enthalpies (ΔH_c and ΔH_m) and the degree of crystallinity (in %) for all the PP films developed.

The PP films incorporating OEO decreased all the thermal parameters in comparison with neat PP films. The enthalpies related to the crystallization and melting point also decrease as the content of OEO incorporated into the PP films increases. Crystallinity degree significantly decreased for the PP films containing the highest OEO levels (i.e. 5% and 8%). Similar results were obtained by Ramos et al. (2012) who observed that the incorporation of carvacrol and thymol to PP materials provoked a clear decrease in crystallinity and melting enthalpy while the other thermal parameters were slightly affected.

In the case of Proallium®, the addition of higher contents of Proallium® further decreased T_c up to 114 ºC but not T_m. The enthalpy related to crystallization decreased with the addition of Proallium®. On the other hand, the enthalpy related to the melting point and the crystallinity degrees were not significantly affected by the incorporation of Proallium® as it happened with OEO.

3.5 Antimicrobial activity in cooked ham

Brochotrix thermosphacta is an ubiquitous microorganism throughout the meat production chain, from animals to food. This microorganism can dominate the spoilage microbiota at the expense of other genera (Remenant et al. 2015). *B. thermosphacta* is responsible to spoilage meat products, including cooked ham, developing a disgusting, sour-sweet odour associated with acetoin production (Pin et al. 2002).

In order to evaluate the antimicrobial activity of developed films against this microorganism, samples were subjected to microbial analysis on the 1st, 6th, 12th, 21st, 27th and 60th day of refrigerated storage. Results of microbial counts of *B*. *thermophacta* in cooked ham are shown in table 4.

In control samples, no changes in the bacterial counts were observed along the storage time. The development of *B*. *thermophacta* could be stopped due to the vacuum-packed process because the growth of this microorganism is influenced by available oxygen remaining in the package (Remenant et al. 2015). The shelf-life of sliced cooked hams packed in a modified atmosphere or vacuum is limited to between 3-6 weeks (Leroy et al. 2009). In the present study, the active packaging containing Proallium® was able to eliminate the content of *B*. *thermophacta* from cooked ham after 8 weeks of storage. Moreover, PP-OEO films also decreased the microbial counts of this microorganism. Therefore, the use of these interlayer films suppose a novel tool that in combination with other hurdles, such as refrigeration or vacuum packaging, could extend the shelf life of meat products.

The ability of OEO, and its major compounds, incorporated in different film matrices to reduce microbial counts in food has been reported by several authors. Ramos et al. (2012) reported that PP films containing 8 % of carvacrol and thymol and films containing an 8 % mixture of both substances showed antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*. Antimicrobial activity was observed in salad packaged in EVOH-coated PP films containing OEO (Muriel-Galet et al. 2013) and polylactic acid (PLA) films containing OEO (Llana-Ruiz-Cabello et al. 2016).

3.6 Sensory analyses

Figure 2 shows the median scores for each sensory descriptor at the different storage times and packaging material as well as the box and whisker plot for the scores obtained along storage time for each packaging material.

Smell was in general the lowest scored descriptor in comparison to the others and thus the most critical one (Figure 2A). The panellists detected in most of the cases the inherent smell of the oregano and *Allium* extracts and they evaluated the smell as something unexpected. In fact, general acceptability and purchase intention were highly correlated to the smell.

Multifactor ANOVA showed a significant impact of both, packaging materials and days of storage ($p < 0.05$) on the sensory perception of the smell. Ham in the control package was above the threshold value of 5 from day 1 to day 11. From that point, smell was slightly below the threshold up to day 60. The active materials containing OEO were significantly below the scores given to the control package. Materials containing 3% and 4% Proallium® were below the control package although non-significant differences were found. The best results for the smell were obtained for materials containing 2% of Proallium®. This material was scored above 5 for the entire storage time.

Figure 2B shows the median scores for visual appearance. In this case, this sensory attribute was above or very close to the threshold value for the different materials and storage time. ANOVA identified the storage time as non-significant ($p > 0.05$). In the case of the packaging materials, just the material containing 2% OEO was significantly different from the other materials, scoring the worst. Surprisingly, visual appearance was better evaluated than smell, but scarcely influenced in the purchase intention of consumers.

General acceptability showed a similar trend to the smell (Figure 2C). ANOVA showed a significant impact of the storage time and packaging material ($p < 0.05$). Acceptability of the ham packaged in the control material decreased from day 1 to day 11 and remained close or above the threshold up to day 60. The active materials containing OEO and Proallium® showed values in general below the control package although with very few significant differences. The exemption was PP containing 2% Proallium®, which scored the best and above the threshold for the entire storage.

Finally, Table 5 shows the percentage of panellists willing to purchase or consume the ham packaged into the different materials and storage time. The best results were obtained for the control material and the PP containing 2% Proallium®. Purchase intention was higher than 50% for both materials and along the entire storage time. Materials containing OEO were in general below 50% including 0% purchase intention at some specific days.

4. Conclusions

Polypropylene (PP) films containing Proallium® or OEO showed similar thermical and mechanical properties as PP. Moreover, Proallium® active films seemed to be more effective against *B. thermosphacta* than PP films containing OEO. Regarding the sensorial analyses, materials containing the lowest Proallium® percentage was highly accepted by the consumers, even better than the control package. However, oregano flavour was noticed as strange to consumers, and had a negative impact in purchase intention.

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Figure caption

Figure 1. TGA curves obtained for OEO, Proallium, PP film and PP containing OEO and Proallium®.

Figure 2. Sensory analysis measured as smell (A), visual appearance (B) and general acceptability (C) of cooked ham in control and active packaging containing OEO (2, 5, 8 %) or Proallium® (2, 3 4 %). Pro: Proallium®.

Film sample	Overall content (%)	Carvacrol content (%)	Thymol content (%)	PTSO content (%)
PP 2% OEO	1.6 ± 0.3	1.10 ± 0.10	0.132 ± 0.011	
PP 5% OEO	3.9 ± 0.2	2.9 ± 0.3	0.33 ± 0.02	
PP 8% OEO	5.84 ± 0.11	4.2 ± 0.3	0.420 ± 0.011	
PP 2% Proallium	1.680 ± 0.014			0.183 ± 0.011
PP 3% Proallium	2.56 ± 0.06			0.196 ± 0.015
PP 4% Proallium	2.82 ± 0.10			0.25 ± 0.06

Table 1. Content of OEO, Proallium® and their main compounds incorporated into the PP films.

Film sample	Thickness (μm)	Transparency	Tensile Strength (MPa)	Young's modulus (GPa)	Elongation at break (%)
PP	90 ± 3[bc]	1.56 ± 0.10[a]	42 ± 6[cd]	1.43 ± 0.24[d]	500 ± 140[ab]
PP 2% OEO	86 ± 2[ab]	2.14 ± 0.03[b]	38 ± 4[bc]	0.83 ± 0.10[b]	490 ± 80[ab]
PP 5% OEO	88.3 ± 1.2[b]	2.16 ± 0.07[b]	38 ± 4[b]	0.85 ± 0.04[b]	560 ± 100[b]
PP 8% OEO	80 ± 2[a]	3.03 ± 0.12[c]	30 ± 4[a]	0.80 ± 0.12[ab]	530 ± 140[ab]
PP 2% Proallium	97 ± 5[c]	2.91 ± 0.18[cd]	45 ± 6[d]	1.28 ± 0.21[c]	510 ± 180[ab]
PP 3% Proallium	86 ± 6[ab]	3.26 ± 0.19[de]	37 ± 3[b]	0.89 ± 0.14[b]	420 ± 70[a]
PP 4% Proallium	89 ± 7[b]	3.4 ± 0.3[e]	36 ± 3[b]	0.68 ± 0.13[a]	420 ± 70[a]

Table 2. Thickness, transparency values and mechanical properties of PP films incorporated with OEO or Proallium® at various concentrations. TS: Tensile strength. EAB: Elongation at break. E: Young´s module. Values are given as mean ± SD from triplicate determinations for thickness and transparency and at least 6 replicates for mechanical properties. Values within each column (i.e. parameter) with at least a common letter at the superscript (i.e. a,b,c,d…) mean that they are not significantly different at 95% of confidence level.

Film sample	Tc (ºC)	ΔHc (J/g)	Tm (ºC)	ΔHm (J/g)	Crystallinity (%)
PP	120.5 ± 0.7^{d}	107.7 ± 0.3^{d}	162.5 ± 0.7^{c}	113.9 ± 0.4^{b}	69.0 ± 0.2^{b}
PP 2% OEO	120.0 ± 0.0^{d}	101.2 ± 0.4^{bc}	161.0 ± 0.0^{b}	109.9 ± 4.5^{b}	66.6 ± 0.8^{b}
PP 5% OEO	117.0 ± 0.0^{c}	94.4 ± 0.1^{a}	161.0 ± 0.0^{b}	100.8 ± 1.3^{a}	61.1 ± 2.0^{a}
PP 8% OEO	115.5 ± 0.7^{b}	95.0 ± 2.5^{a}	159.5 ± 0.7^{a}	99.8 ± 3.7^{a}	60.5 ± 0.9^{a}
PP 2% Proallium	115.5 ± 0.7^{b}	103.0 ± 2.0^{c}	160.0 ± 0.0^{ab}	112.1 ± 1.3^{b}	67.9 ± 2.7^{b}
PP 3% Proallium	114.5 ± 0.7^{ab}	99.6 ± 1.7^{b}	160.5 ± 0.7^{ab}	114.2 ± 3.3^{b}	69.2 ± 0.8^{b}
PP 4% Proallium	114.0 ± 0.0^{a}	100.3 ± 0.9^{bc}	161.0 ± 0.0^{b}	111.3 ± 1.4^{b}	67.5 ± 2.2^{b}

Table 3. Thermal properties of PP films incorporating OEO and Proallium® (2nd heating cycle). Tc: Crystallization temperature. ΔHc: crystallizing enthalpy. Tm: Melting temperature. ΔHm: melting enthalpy. Values within each column (i.e. parameter) with at least a common letter at the superscript (i.e. a,b,c,d…) mean that they are not significantly different at 95% of confidence level.

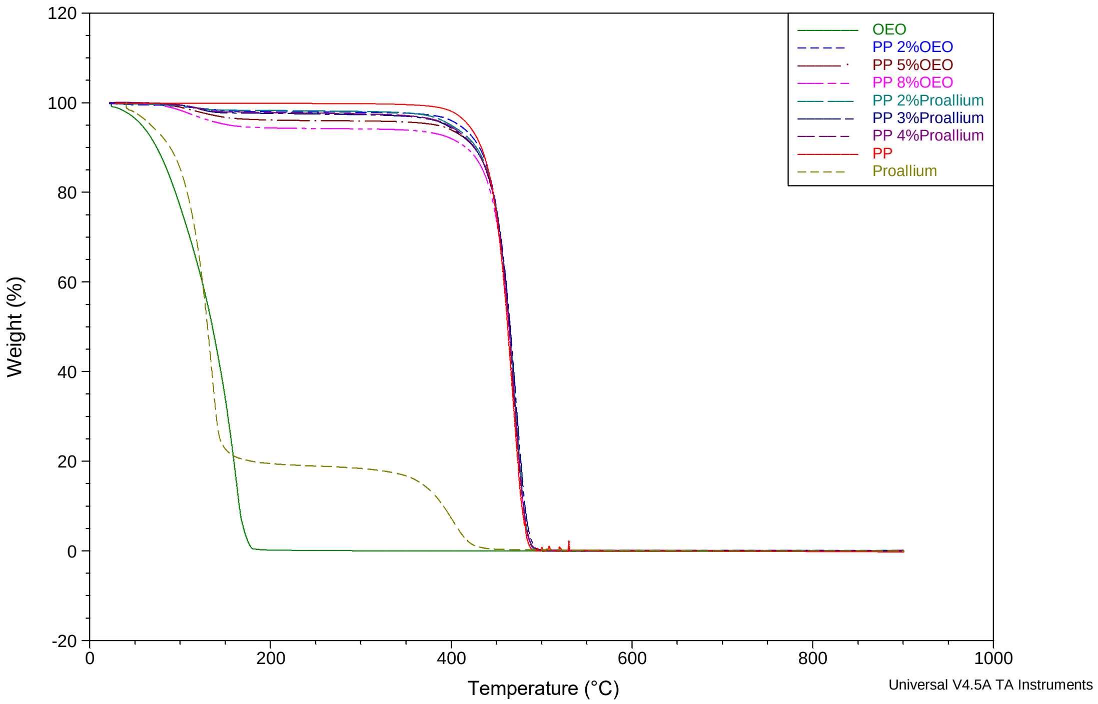
	Day 0	Day 1	Day 6	Day 12	Day 21	Day 27	Day 60
PP	4.7 ± 0.0	4.8 ± 0.2	4.7 ± 0.2	4.8 ± 0.07	4.6 ± 0.2	4.7 ± 0.2	4.8 ± 0.0
PP 2% OEO	4.7 ± 0.0	4.4 ± 0.1	3.5 ± 0.8*	2.9 ± 0.1**	2.7 ± 0.2**	2.3 ± 0.1**	2.3 ± 0.1**
PP 5% OEO	4.7 ± 0.0	3.8 ± 0.2*	2.9 ± 0.1**	1.6 ± 0.6**	2.0 ± 0.1**	1.6 ± 0.0**	1.8 ± 0.0**
PP 8% OEO	4.7 ± 0.0	2.6 ± 0.2**	1.7 ± 0.1**	1.2 ± 0.1**	1.1 ± 0.1**	1.0 ± 0.0**	1.2 ± 0.0**
PP 2% Proallium	4.7 ± 0.0	2.4 ± 0.4**	1.8 ± 0.1**	1.5 ± 0.3**	1.5 ± 0.1**	1.4 ± 0.1**	0.0 ± 0.0**
PP 3% Proallium	4.7 ± 0.0	1.7 ± 0.1**	1.5 ± 0.2**	1.2 ± 0.3**	1.2 ± 0.2**	1.0 ± 0.0**	0.0 ± 0.0**
PP 4% Proallium	4.7 ± 0.0	1.4 ± 0.1**	1.2 ± 0.2**	1.0 ± 0**	0.9 ± 0.2**	0.0 ± 0.0**	0.0 ± 0.0**

Table 4. Antimicrobial activity against *B. thermosphacta* of films containing OEO and Proallium® in cooked ham. Values are given as log CFU/cm^2. Results are the mean ± SD from three different experiments.

Day	PP	PP 2%OEO	PP 5%OEO	PP 8%OEO	PP 2%Prollium	PP 3%Proallium	PP 4%Prollium
1	83	50	50	67	83	83	83
4	89	67	0	50	63	67	80
11	61	33	50	17	67	50	67
18	61	17	0	0	83	67	83
24	56	17	33	33	100	33	17
32	67	50	0	33	100	50	33
60	67	33	33	50	50	50	17

Table 5. Percentage of panellists willing to purchase or consume the ham packaged into the different materials and storage time.

Figure 1



120
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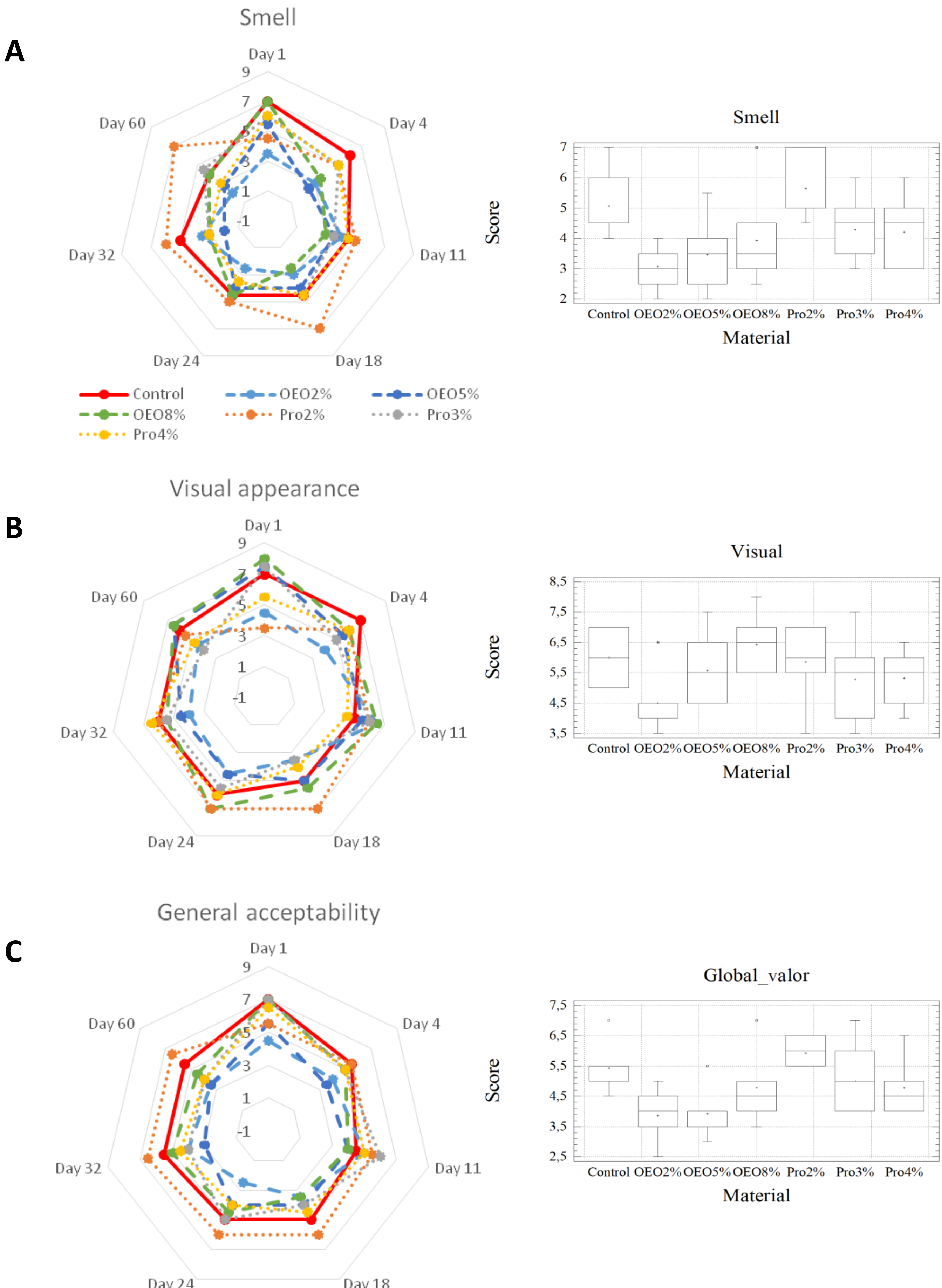
60
40
20
0
-20
Weight (%)
0
200
400
600
800
1000
Temperature (°C)
Universal V4.5A TA Instruments
OEO
PP 2%OEO
PP 5%OEO
PP 8%OEO
PP 2%Proallium
PP 3%Proallium
PP 4%Proallium
PP
Proallium

Figure 2


A
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Day 1
Day 4
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Control
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Control OEO2% OEO5% OEO8% Pro2% Pro3% Pro4%
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Control OEO2% OEO5% OEO8% Pro2% Pro3% Pro4%
Material