

Active packaging based on PLA and chitosan-caseinate enriched rosemary essential oil coating for fresh minced chicken breast application

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ARTICLE INFO

Keywords:

Antioxidant film
Rosemary essential oil
Chitosan
Chicken breast
Volatiles-organic-compounds
Malondialdehyde

ABSTRACT

Active packaging systems are considered an effective way to prolong the shelf life of fresh food products. This study compared five different biopolymer films for their ability to delay the lipid oxidation of raw chicken meat. New antioxidant poly lactic acid film was prepared by coating the film surface with chitosan or chitosan/caseinate blend enriched with rosemary essential oil at concentration of 1% and 2%. Films were characterized in terms of microstructure, water vapor permeability, mechanical properties, and antioxidant capacity. In vivo study was done using fresh minced chicken meats stored at 4 °C and analysed after 0, 4, 7, 11, 14, 21 days. Results indicated that water vapor transmission rate was reduced by the presence of the coating and assumed the lowest value ($1.23 \pm 0.03 \times 10^{-4} \text{ g m}^{-2} \text{ s}^{-1}$) with coating enriched with 2% of rosemary essential oils. The films showed an antioxidant capacity of maximum 6% equivalent to the antioxidant capacity of 6,25 µg of REO/mL. Results from *in vivo* test showed that the active films were able to reduce meat oxidation during storage in anaerobic modified atmosphere condition: samples packed with active film showed constant malondialdehyde (MDA) and colour up to 14 days and reduction of heptanal and ethanol concentration compared to samples packed with control film (72 % and 90 % respectively). Overall, this study has demonstrated that PLA films coated with an active coating are a promising delivery method for providing antioxidant effects in packaging for fresh meat products.

1. Introduction

Active packaging can be designed to deliberately incorporate components that would release or absorb substances into or from the packaged food or the environment surrounding the food to enhance the food preservation (Regulation (EC) 1935/2004). These substances can be active compounds to be released into the packaging atmosphere or packed food (i.e. antimicrobial or antioxidant) or component able to absorb undesirable substances from the packaging head space (scavengers). Among natural antioxidant compounds, essential oils have been extensively studied to develop active films (Llana-Ruiz-Cabello et al., 2018; Maisanaba et al., 2017; Ribeiro-Santos, Andrade, de Melo, & Sanches-Silva, 2017). Rosemary essential oil (REO) is known for its strong antioxidant properties and several studies have demonstrated the effectiveness of REO for controlling the oxidation of food products (Kahraman, Issa, Bingol, Kahraman, & Dumen, 2015; Pereira et al.,

2017; Qiu, Jacobsen, & Sørensen, 2018). Moreover, REO has a high antimicrobial efficacy against *Pseudomonas* spp., one of the predominant spoilage bacteria on poultry meat (Forsythe, 2020; Hao, Roy, Pan, Shah, & Mraz, 2021).

Essential oils have been incorporated into food packaging by including them in film forming solutions which are subsequently cast on plates (Pires, de Souza, & Fernando, 2018; Souza et al., 2019; Torrieri, Cavella, & Masi, 2015) or including them (rosemary extract, oregano essential oil, BHT) in polyolefin films (Llana-Ruiz-Cabello et al., 2018; Wrona et al., 2021).

To reduce its volatility and its thermal degradation when incorporating it into polymer matrix, usually shaped by extrusion, REO can be included in a biopolymer solution before being deposited as a coating on the food contact surface of the film. The coating acts as a carrier for the active compounds, helping to maintain high concentrations of preservatives on the surface of foods.

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Among biopolymers, chitosan has been extensively studied for coating applications because of its film-forming properties, biodegradability, selective permeability to gases, and good mechanical properties (Elsabee & Abdou, 2013). REO was successfully used as an active compound in food packaging biofilms based on chitosan (Abdollahi, Rezaei, & Farzi, 2012; Pires et al., 2018; Souza et al., 2019; Torrieri et al., 2015) or blend of chitosan and protein films (Volpe, Cavella, Masi, & Torrieri, 2017; Yeddes et al., 2020).

Poly (lactic acid) (PLA) is one of the most commercially available biopolymers (Nova-Institute, 2020) and is “Generally Recognized As Safe” (GRAS) by the Food and Drug Administration (FDA Regulation, at 21 C.F.R. § 184.1061) for use in food packaging (Conn et al., 1995). PLA has unique properties like good appearance, high mechanical strength, and low toxicity (Jamshidian, Tehrani, Imran, Jacquot, & Desobry, 2010). However, a weakness of PLA is its relatively high permeability to gases and vapours, which results in an increased risk of oxidation reactions occurring within PLA food packaging.

To improve functional properties of biopolymer film, active packaging is gaining increasing attention in the last ten years. Few studies have been conducted also on bilayer films incorporating antioxidant compounds (Scaffaro et al., 2020; Suryani, Rihayat, Nurhanifa, & Riskina, 2020).

There are many methods to measure the efficacy of antioxidant packaging materials (Shahidi & Zhong, 2015). Generally, in vitro antioxidant tests using free radical traps are relatively straightforward to perform. Among free radical scavenging methods, DPPH method is furthermore rapid, simple (i.e. not involved with many steps and reagents) and inexpensive in comparison to other test models (Alam, Bristi, & Rafiquzzaman, 2013). Due to the complexity of the oxidation process in food system, it is also important to evaluate their performance when in contact with a food matrix (Shahidi & Zhong, 2015). In case of meat samples, it is common to study the myoglobin oxidation by monitoring the colour of the meat (Robertson, 2009) and lipid oxidation process by evaluating changes in primary or secondary products (formation of carbonyls, aldehydes, volatiles, malondialdehyde) (Domínguez et al., 2019).

In a previous study, blend film based on chitosan and sodium caseinate has been optimized to improve the mechanical and water vapor barrier properties of chitosan film (Volpe et al., 2017). Moreover, the inclusion of rosemary essential oil in chitosan film was already optimized (Torrieri et al., 2015). Thus, the aim of this work was to develop an active film based on PLA and chitosan or chitosan and caseinate coating enriched with rosemary essential oil. Active films have been characterized in terms of microstructure, antioxidant properties, water vapor permeability and mechanical analysis. The antioxidant capacity of the film was also evaluated when in contact with real food, a fresh minced chicken breast, by monitoring the quality indices affected by oxidation reaction during storage at 4 °C.

2. Materials and methods

2.1. Materials

For preparation of the active films, medium molecular weight chitosan (CH) with a degree of deacetylation of 75–85%, sodium caseinate (SC), acetic acid puriss. p.a., rosemary essential oils (*Rosemary officinalis*- REO) and tween 80 were purchased from Sigma-Aldrich (Milan, Italy); Poly (lactic acid) (PLA) were kindly supplied by Icimendue srl, (Caserta, Italia). Chicken breast (Class A) meat was purchased from a local butcher (Dundee, UK). For meat packaging, PET tray sealed with lintop PE HB film were supplied by Linpac Packaging Pontivity (France). 2-Thiobarbituric acid, malondialdehyde bis (dimethyl acetal), trichloroacetic acid (TCA), 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and methyl butyrate internal standard were purchased from Sigma Aldrich (Dorset, UK).

2.2. Coating composition and active film preparation

CH solution with a concentration of 1% (w/v) and CH/SC blend solution at 2% and 4% (w/v), respectively, in ratio 1:1, were prepared following the method of Volpe et al. (2017). Rosemary oil and Tween 80 were emulsified at 4:1 ratio, by using a shaker (MS 3 Digital, IKA, Staufen, Germany) for 5 min at 2000 rpm, and added to the CH or CH/SC solutions to reach a final oil/tween 80 concentration in the solution of 1% and 2% (w/v). Solutions were finally emulsified for 4 min at 15500 rpm at room temperature using a rotor-stator homogenizer (Ultra Turrax®, T 18, IKA, Staufen, Germany) to obtain active coatings. PLA (21.0 cm × 29.7 cm) was layered with 20 mL of active coatings by using a Rod Coater (Gardco Company, Pompano Beach, USA). The coated PLA films were dried at 20 °C and 50 % relative humidity (RH) for 2 h in an environmental chamber (Climacell, MMM Group, Planegg Germany). Once the films were dried, they were coated again with a further 10 mL of coatings without REO to help protect the volatile essential oil from evaporation. Active PLA film were coded as CH + REO1%, CH + REO2%, CH/SC + REO1% and CH/SC + REO2%. The REO concentration per grams of solids and per surface unit were, respectively, 1.05 mL/g/m², 2.13 mL/g/m², 0.35 mL/g/m² and 0.71 mL/g/m². PLA sheets without coating were used as control sample. Dry coated film thickness was determined using a HO62 micrometre (Metacontrol Srl, Napoli, Italia) with a sensitivity of ±2 µm.

2.3. Scanning electron microscopy analysis

Cross-sectional images of film samples were taken with a scanning electron microscope LEO (EVO 40, Zeiss, Oberkochen, Germany). To do this, film samples were immersed in liquid nitrogen to obtain a fragile fracture. Thus, the films were first mounted on bronze stubs with the cross-section oriented upward and then coated with gold, by using DC sputter coater (AGAR B7340, Agar Scientific Ltd, Stansted, UK), under a low vacuum pressure (with an accelerating voltage of 10 kV). Digital images of film cross-sections were collected at a tilt angle of 0° to the electron beam using an acceleration voltage of 20 kV.

2.4. Physical properties of active film

2.4.1. Mechanical properties

Tensile tests were carried out according to D882-10 (ASTM (American Society for Testing and Materials) (2010)) using a dynamometer (Instron Universal Model No 4301, Instron Engineering Corp., Canton, MA) equipped with a 1000 N load cell. The films were cut into a strip with 100-mm in length and 25 mm in width using a sharp razor blade; the test was carried out at 50 mm/min. Before testing, the thickness of the strips was measured at five points. The Young's modulus (EM), tensile strength (TS) and elongation at break (ε%) were calculated. The test was carried out at only one REO concentration (CH + REO2% and CH/SC + REO2%), because it was shown that REO concentration did not have a significant effect on mechanical behaviour of a biopolymer-based film (Perone et al., 2014). The results are given by the average of five strips for each sample.

2.4.2. Water vapor permeability

The water vapor permeability (WVP) of the films was evaluated using a gravimetric test according to ASTM (2002) using a Payne permeability cup (Carlo Erba, Milan, Italy). Eight grams of silica gel was introduced into each cup. A film sample, with a diameter of approximately 6 cm, was placed on top of the cup and sealed using a top ring kept in place by three tight clamps. The film surface exposed to vapor transmission was 9.89 cm². The cups were weighed and then placed in desiccators containing a saturated KCl solution (aw = 0.85 ± 0.003). The desiccator was stored in a thermostatic incubator (MMM Medcenter Einrichtungen GmbH, Monaco di Baviera, Germany) at 20 °C. Cups were weighed at scheduled times, and the water vapor transmission rate

(WVTR) and water vapour permeability (WVP) was calculated as reported by [McHugh, Avena-Bustillos, and Krochta \(1993\)](#). The results are reported as the average of three replications of each sample.

2.5. Film antioxidant capacity

The DPPH method was used to determine the free radical scavenging capacity of rosemary essential oil and PLA active films. For rosemary essential oil samples, eight different dilutions of analysed samples in methanol were prepared at concentrations ranging between 0.025 – 0.3 %. The calibration curve of the REO in the studied range followed a linear relationship with a R^2 of 0.99. The IC_{50} of REO, calculated as the REO concentration to obtain a 50 % of scavenging activity reduction, was 75 $\mu\text{g/mL}$. DPPH assays were carried out to measure the effectiveness of REO as an active antioxidant component. DPPH and methanol stock solution were prepared at 100 mM concentration. For active films, the test was standardised using a scale of 12.5, 25, 50, 75 mg samples of each film. REO or film samples were placed into centrifuge tubes along with 4 mL of the DPPH stock solution and left to react in the dark at ambient temperature for 30 min. Once the reaction had occurred, the absorption of each sample was read at 517 nm on a dual wave spectrometer (Jasco V-550 UV/VIS, Lecco, Italy). Methanol was used as a blank and DPPH stock solution acted as the control sample. A decrease in absorption values should be observed if the test sample contains antioxidants. After absorption readings were obtained, the antioxidant capabilities of the active films were calculated using the following equation:

$$\text{DPPH Radical Scavenging Activity}(\%) = \left(1 - \frac{A_1}{A_C}\right) \times 100 \quad (1)$$

where A_C is the absorbance of the control and A_1 is the absorbance using the film samples ([Siripatrawan & Harte, 2010](#)).

2.6. Application on fresh minced chicken breast

2.6.1. Packaging and storage condition

Chicken breast (Class A) meat was purchased four days after slaughter and 3 days after processing. The meat was vacuum packed prior to purchase, so exposure to oxygen was kept as limited as possible. The samples were transported in cooled bag to Abertay University's laboratory in 20 min. The chicken was ground using a grinder (Chef KMC515 with a food mincer attachment, Kenwood Ltd., UK). Petri dishes (50 mm Diameter) were filled with 10 g of ground chicken. Film samples were then cut to cover the surface of the meat in each petri dish. The film was placed onto meat samples with the coated surface touching the meat. The resulting film to meat ratio was 4.8 mg/g.

The chicken samples were modified atmosphere packaged with a gas mixture of 80 % CO_2 and 20 % N_2 (T100 vacuum packer, Multivac, UK). The tray was sealed with a barrier film (13 × 16 cm) (LINTOP PE HB A 40, 270 mm, Linpac Packaging Pontivity, France). The headspace gas composition was monitored by using a gas analyser (Oxybaby, Witten, Germany). The packed chicken samples were stored at 4 °C for 21 days. After 0, 4, 7, 11, 14 and 21 days the samples were analysed by chemical and physical analysis. At each storage time, headspace gas composition (CO_2 %, O_2 %) was recorded before opening the packaging to control the effective gas composition.

2.6.2. Thiobarbituric acid reactive substances (TBARS)

Malondialdehyde is an organic compound which is highly reactive and can be used as a marker of lipid oxidation. To measure the concentration of malondialdehyde (MDA) present in each of the packed chicken samples, a TBARS assay was carried out according to the method of [Lau and King \(2003\)](#) with some modifications.

Trichloroacetic acid (TCA) and 2-Thiobarbituric acid (TBA) reagents were prepared at 25 % (w/v) and 0.6 % (w/v), respectively. One gram of

chicken sample was placed in a 50 mL centrifuge tube along with 10 mL deionized water. This mixture was homogenised using an (ULTRA-TURRAX® T25 Digital, Ika) 5000 rpm/ 30 s, then 2.5 mL of TCA (25 % w/v) solution was added and vortexed for 30 s to mix. Test samples were refrigerated for 5 min before being centrifuged at 4000 rpm for 10 min. The supernatant (11 mL) was then extracted from the sample and centrifuged for a further 5 min at 4000 rpm to remove any remaining meat fibres. Then, 1.5 mL of TBA (0.6 %) was added to the 3.5 mL of the extracted supernatant bringing the total volume to 5 mL. For the calibration curve, known concentrations of MDA (1 mM) stock solution were used in place of the supernatant. The samples were incubated for 15 min at 70 °C. When incubation was complete, the absorbance values for each sample were obtained using a Genesys 10S UV-vis dual wave spectrophotometer (Thermo Scientific, UK) at 532 nm against a blank containing 1 mL of TCA/1.5 mL TBA. The malondialdehyde concentration of the sample were calculated using the standard curve of MDA as a reference.

The linearity of the calibration curve was obtained in the range of 5–300 μM nominal concentrations with regression coefficient (R^2) 0.98.

2.6.3. Colour measurement

Image analysis was chosen to assess colour changes in the chicken over the 21 days of storage.

Images were obtained in a photography studio/dark room. Fluorescent lighting was set at a 45-degree angle to the sample. Camera positioning was also standardised, held directly above the samples to allow for consistent images. A Nikon D500 Digital SLR Camera was used to photograph the samples with a Nikon DX SWM Micro 1:1 ϕ 52 lens. Sample placement was kept consistent with the use of positioning indicators and standard matte black card was used as a background for the images.

Image J photo analysis program was used to analyse the changes in colour by converting the image into the LAB colour space system which allows for a more accurate analysis of colour. LAB scale extends from red to green on the **a** axis (a+/a-) and on the **b** axis blue to yellow (b+/b-). Brightness is measured on the **L** axis ([Kaewthong, Waiyagan, & Wattanachant, 2017](#)).

2.6.4. Release of volatile compounds

Solid phase micro extraction gas chromatography mass spectrometry (SPME GC/MS) was used to measure the release of volatile compounds from the packed chicken. An SPME method was developed based on previously developed protocols ([Brunton, Cronin, Monahan, & Durcan, 2000](#); [Watkins, Rose, Warner, Dunshea, & Pethick, 2012](#)). A GC/MS method was developed performed according the previously developed method of [Brunton et al. \(2000\)](#), [Rux, Luca, and Mahajan \(2019\)](#) and [Watkins et al. \(2012\)](#) and standardised to the packaging systems using a model system.

The model system was realized to replicate the conditions of the chicken samples packed in modified atmosphere packaging (MAP), as well as match the ratio of meat to film correctly. Briefly, samples of ground chicken breast (5 g) were wrapped in 24.4 mg of PLA (Control and active) film and inserted into 20 mL headspace vials with septum. Methyl butyrate internal standard was injected into vials at a volume of 200 μL with a concentration of 1 $\mu\text{g/g}$ of meat. The vials were then flushed with 80 % CO_2 gas for 10 s to simulate the controlled atmosphere used in the MAP system.

The samples were refrigerated and extracted over a 21-day period. Samples were frozen on their sampling date until the analysis. A total of 18 replicates were made for each film type meaning there would be triplicates of every sample.

SPME analysis was carried out using a Shimadzu GCMS-QP2010 SE with DB Wax column. Various SPME fibres were tested; 50/30 μm DVB/CAR/PDMS, 65 μm PDMS/DVB and 85 μm polyacrylate. The polyacrylate fibre was chosen for the final analysis. Sample runs were carried out in batches of 8 over a week. Transfer time between refrigeration and

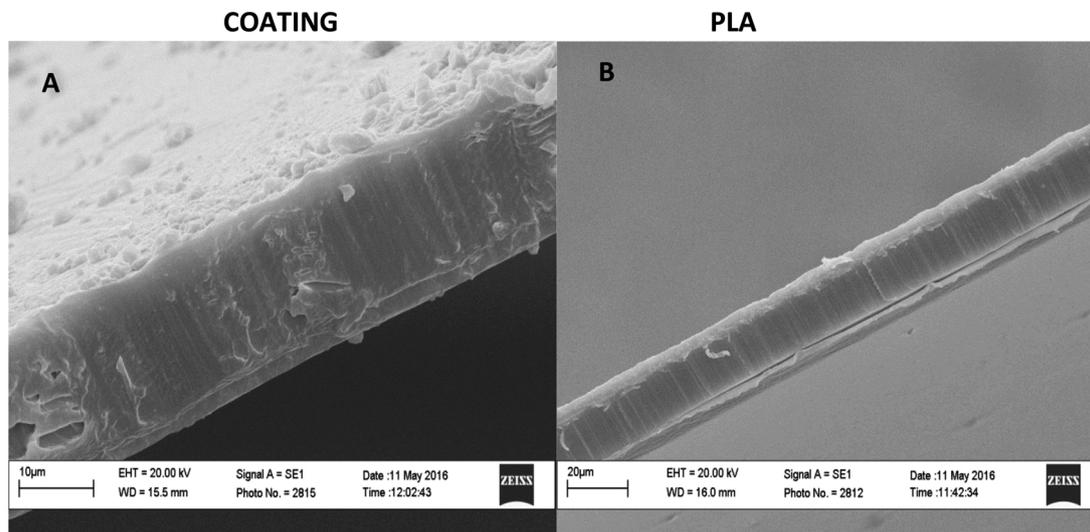


Fig. 1. SEM micrographs of (a) CH + REO1%, PLA film coated with chitosan incorporated with REO at 1% (5 kx) and (b) CH + REO2%, PLA film coated with chitosan incorporated with REO at 2% (2kx).

Table 1
Mechanical and water vapor barrier properties of films.

	E MPa	TS MPa	ϵ %	WVTR ($\times 10^4$) $\text{g m}^{-2} \text{s}^{-1}$	WVP ($\times 10^{14}$) $\text{g m}^{-1} \text{s}^{-1} \text{Pa}^{-1}$
Control	1133 \pm 136 ^a	93 \pm 9 ^a	0.6 \pm 0.1 ^b	1.70 \pm 0.20 ^b	8 \pm 1 ^b
CH + REO2%	2073 \pm 244 ^b	140 \pm 19 ^b	0.2 \pm 0.1 ^a	1.58 \pm 0.06 ^{a,b}	8 \pm 3 ^{a,b}
CH/SC + REO2%	1323 \pm 89 ^a	160 \pm 28 ^b	0.4 \pm 0.1 ^a	1.23 \pm 0.03 ^a	6 \pm 2 ^a

^a Control, PLA film without coating; CH + REO2%, PLA film coated with chitosan incorporated with REO at 2%; CH/SC + REO2%, PLA film coated with a blend of chitosan and sodium caseinate incorporated with REO at 2%.

^b The values are the means \pm SD obtained from five independent for the mechanical test and three independent replication for the WVTR and WVP. Means within a column with the same lower-case letter are not significantly different ($P > 0.05$).

analysis was kept consistent for all samples.

2.7. Statistical analysis

Three replications were performed for each test and data was presented as mean values with standard deviation. The mean values were calculated using the results of the treatment replicates and the technical replicates.

Statistical analysis was performed on SPSS Statistic 24 software (SPSS Inc. Westland's Road, Quarry Bay, Hong Kong) and Graphpad Prism 8 software (GraphPad Software, Northside Dr. San Diego, CA 92108). For the image analysis data was obtained using Image J software version 2017 (LOCI, Wisconsin).

ANOVA analysis of variance was used, at 95 % confidence level, to determine if the difference between factors and levels was significant. Mean values which displayed significant differences were analysed using Tukey's HSD multiple comparisons test. Normality of data was tested using the Shapiro Wilks test of normality. Differences between sample groups were regarded as significant at $p < 0.05$.

3. Results and discussions

3.1. Structure and physical properties of active films

SEM analysis was carried out to verify the homogeneity and adhesion of the biopolymer coating on the PLA surface. By way of example, samples CH + REO1% and CH + REO2% were reported (Fig. 1). The cross-sectional views of the coated PLA samples confirm the firm adherence of the chitosan-based coating over the PLA surface. Same results were observed for CH/CS based coating (data not showed). The

strong interactions between the two layers may be related to the high interfacial tension of the two layers. However, the addition of coating has resulted in a rough and uneven surface, with some impurities. The thickness of the coating for all the films was very thin and was not possible to calculate it from the image. In fact, no differences were observed among films in thickness which showed an average value of $20 \pm 1 \mu\text{m}$.

The mechanical properties are reported in Table 1. The elastic modulus (EM) of films ranged from 1133 ± 136 MPa for control sample to 2073 ± 89 MPa for CH + REO2%. No significant differences were observed between control and CH/SC + REO2% samples ($p > 0.05$), the PLA film coated with chitosan showed a significantly higher elastic modulus ($p < 0.05$).

Regarding TS, control film showed a TS value of 93 ± 9 MPa, whereas the CH + REO2% and CH/SC + REO2% showed a value of 140 ± 19 MPa and 160 ± 28 MPa, respectively. Significant differences ($p < 0.05$) were observed only between the control sample and the active films. Moreover, the control film showed the higher $\epsilon\%$ equal to 0.6 ± 0.1 % compared to active films which assumed an average value of 0.29 ± 0.1 %.

These results suggest that by adding a layer of CH or CH/SC blend to PLA, the films were notably reinforced, as compared to the uncoated PLA film. In agreement with previous findings (Li et al., 2020), the tensile strength of the PLA film increased with the number of layers. On the other hand, it seems that the coating limits the extension capacity of PLA. However, no differences in terms of biopolymer composition were observed.

The WVTR and WVP values of film samples are reported in Table 1. The control film showed the highest WVTR ($1.7 \pm 0.2 \times 10^{-4} \text{g m}^{-2} \text{s}^{-1}$) whereas PLA coated film showed lower values, but only for CH/

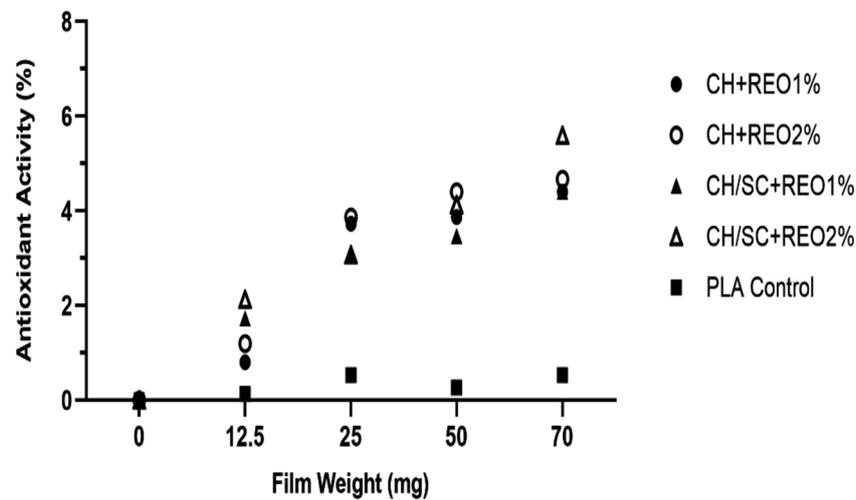


Fig. 2. Antioxidant activity (%) of film samples: PLA Control, PLA film without coating; CH + REO1%, PLA film coated with chitosan incorporated with REO at 1%; CH + REO2%, PLA film coated with chitosan incorporated with REO at 2%; CH/SC + REO1%, PLA film coated with a blend of chitosan and sodium caseinate incorporated with REO at 1%. CH/SC + REO2%, PLA film coated with a blend of chitosan and sodium caseinate incorporated with REO at 2%.

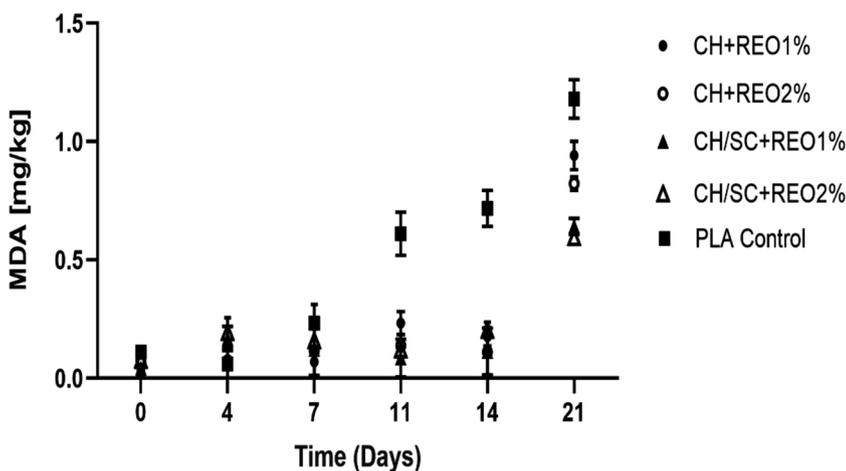


Fig. 3. MDA concentrations of meat in contact with different films during storage for 21 days at 4 °C. PLA Control, PLA film without coating; CH + REO1%, PLA film coated with chitosan incorporated with REO at 1%; CH + REO2%, PLA film coated with chitosan incorporated with REO at 2%; CH/SC + REO1%, PLA film coated with a blend of chitosan and sodium caseinate incorporated with REO at 1%. CH/SC + REO2%, PLA film coated with a blend of chitosan and sodium caseinate incorporated with REO at 2%.

CS + REO2% ($1.23 \pm 0.03 \times 10^{-4} \text{ g m}^{-2} \text{ s}^{-1}$) the differences observed in terms of WVTR were statistically significant. Same results were observed in terms of WVP. This result can be justified by the lower water vapour permeability of the CH/CS coating respect to CH ones (Volpe et al., 2017). The polyelectrolyte complexes between chitosan and sodium caseinate were able to neutralize the biopolymer charges, which reduced the hydrophilic nature of the film. These observations disagreed with previous results showing a strong increase of the WVTR of PLA coated with chitosan or pea starch, ascribed to the strong hydrophilicity of the biopolymers (Li et al., 2020; Zhou, Yang, Wang, & Chen, 2019). Our results showed no effect or a small improvement of the water vapour barrier properties.

3.2. Antioxidant activity

The antioxidant capacity of films ranged between 1%–6% as function of mass of film tested. A clear rise in antioxidant activity was observed as the weight of the active film was increased, as shown in Fig. 2. Blank samples containing no active film were used as a control, and therefore displayed 0% antioxidant activity after the 30 min reaction time. The antioxidant capacity of the film at 18,75 mg/mL (75 mg of film) was equivalent to the antioxidant capacity of 6,25 µg of REO/mL. ANOVA results showed that there was a significant difference in antioxidant activity between all samples ($p < 0.0001$). The Tukey HSD test

identified that the significant differences were found between all samples ($p < 0.0001$) except CH + REO1% and CH/SC + REO1% samples which had similar antioxidant activity scores ($p = 1.0$). The data for this test was found to be normally distributed ($p < 0.68$). When 75 mg of film were tested, the samples CH/SC + REO2% showed the highest antioxidant capacity among active film. Previous results showed that sodium caseinate can be a good substrate for dispersion active compounds, mainly for rosemary essential oil, for the developing of bioactive coating with good antioxidant properties (Valentino, Volpe, Di Giuseppe, Cavella, & Torrieri, 2020). Moreover, REO incorporated in chitosan film maintained its ability to scavenge the DPPH radical after the casting and migration assay, showing antioxidant properties in the range of 2–10 % as function of REO concentration and food simulant (Souza et al., 2019).

3.3. Application on fresh minced chicken breast

3.3.1. Malondialdehyde concentration

Fig. 3 shows the variation of MDA of samples packaged with the different active films and without the active film during storage at 4 °C for 21 days.

The initial MDA value of the chicken meat was 0.15 mg MDA/kg, which is within the normal range of MDA for fresh samples as reported in previous studies (Domínguez et al., 2019; Kahraman et al., 2015). All

Table 2

Volatile organic compounds (VOCs) extracted from raw chicken breast stored in different bio-based active packaging systems for 21 days at 4 °C.

ID	Volatile organic compounds (VOCs)	rt (min)	m/z
1	Methyl butyrate (Internal Standard)	3.4	102
2	Acetic acid	13.5	60
4	Heptanal	17.567	114
5	Hexanal	17.419	100
6	Ethanol	20.887	88
7	Butanal	17.192	86
8	Octanal	26.34	128
9	Pentanal	23.734	86
10	1- Hexanol 2- ethyl	13.6	130
11	Propylene glycol	11.406	76
12	Dimethyl sulfone	15.04	94
13	Hexanoic acid	18.921	116
14	Decanoic acid methyl ester	18.933	186
15	Butanoic acid	15.5	88
16	Hexadecanoic acid	19.896	256
17	Paraldehyde	17.427	132
18	Tridecanoic acid methyl ester	18.936	228
19	Propanoic acid	15.65	286
20	2,2,4-Trimethyl-1,3-pentanediol disobutyrate	15.088	213
21	1-Tetradecanamine	25.92	100
22	1-Octanol, 2-methyl	18.709	284
23	Pentanoic acid	18.93	128
24	Heptadecanol	19.948	256
25	Butyric acid 4-pentadecyl ester	17.106	298
27	Undecanal	18.377	170
28	Octadecanoic acid, methyl ester	22.371	298
29	Oelic acid	17.325	282

MAP systems displayed an increase of MDA over the 21 days of storage. Control samples had the highest concentrations of MDA and most steady increase throughout the analysis. When compared to the control, all packages containing the active film displayed a lower average concentration of MDA over the 21 days.

From day 0–7 no significant difference in MDA concentration was

observed between samples. At day 11 a significant difference in MDA concentrations has been observed when comparing the control to the active film samples ($p < 0.0832, 0.3810, 0.4478, 0.8233$). We continue to see this trend on day 14 ($p < 0.0047, 0.0104, 0.0051, 0.0137$) and 21 ($p < 0.0465, 0.0234, 0.0070, 0.0083$)

On day 21 we also see a significant difference between the chitosan and blend samples, with the CH1% and CH2% samples having higher MDA concentrations than either of the blend samples ($p < 0.0171, 0.0163, 0.0026, 0.0113$).

MDA concentration is a direct marker of lipid peroxidation (Varzaru, Untea, & Saracila, 2020) therefore lower concentrations of MDA suggests that the presence of REO is contributing some inhibitory effects to the oxidation of the chicken meat. Oxidation of lipids gives rise to rancid odours and flavour in meat (Campo et al., 2006). According to Sheard et al. (2000), the threshold of off-odour perception by consumers corresponds to a TBARS value of 0.5 mg MDA/kg sample for pork patties. Analysing the results, control samples reached the off-odour threshold at the 11th day of storage, while meat packed with the active film only on day 21. Among active film, CH/SC + REO1% and CH/SC + REO2% showed the lowest value (0,6 mg MDA/kg samples) after 21 days.

In the present study, results on day 21 showed a 50 % decrease in the amount of MDA in CH/SC + REO1% and CH/SC + REO2% treated meat sample compared to the control. Similar results were previously reported with rosemary essential oil used in combination with MAP (Kahraman et al., 2015) and with chitosan film incorporated with rosemary essential oil applied in a meat product (Souza et al., 2019), and with chitosan-based coating incorporated with essential oil, namely: chitosan solution with REO coating fresh silver carp (Abdollahi, Rezaei, & Farzi, 2014), chitosan film with Thyme EO in fish fillets (Yang et al., 2015).

Zeid, Karabagias, Nassif, and Kontominas (2019) reported a decrease of 20.2 % in the amount of MDA in rosemary treated fish sample packed with PLA films containing different EO's (thyme, rosemary, oregano). However, EO's were incorporated into the film by solvent casting method, thus was less available to act on the product surface. Wrona

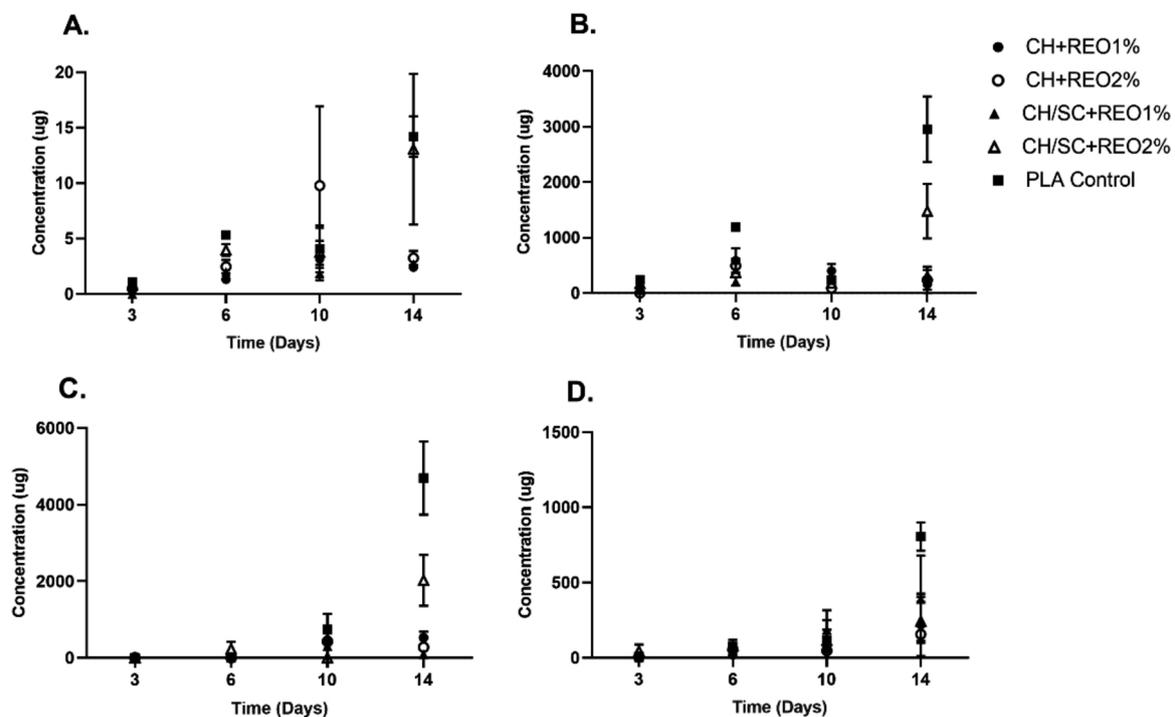
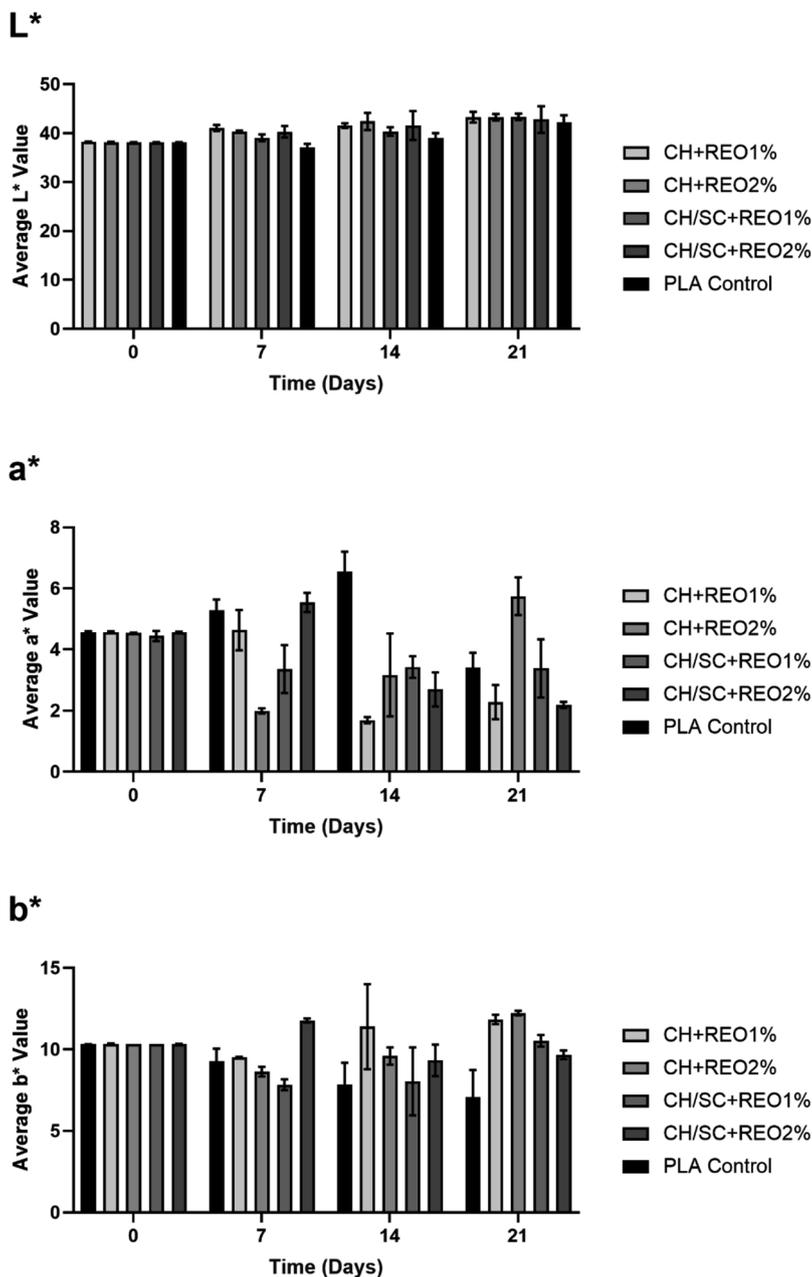


Fig. 4. Volatiles organic compounds of meat in contact with different films during storage for 14 days at 4 °C. PLA Control, PLA film without coating; CH + REO1%, PLA film coated with chitosan incorporated with REO at 1%; CH + REO2%, PLA film coated with chitosan incorporated with REO at 2%; CH/SC + REO1%, PLA film coated with a blend of chitosan and sodium caseinate incorporated with REO at 1%. CH/SC + REO2%, PLA film coated with a blend of chitosan and sodium caseinate incorporated with REO at 2%. (A. heptanal, B. butanoic acid, C. ethanol, D. acetic acid) for the different samples.



(L*) Lightness, (a*) green / magenta and (b*) blue/ yellow values throughout shelf life.

Fig. 5. Colour change of meat (L, a, b) of meat in contact with different films during storage for 21 days at 4 °C. PLA Control, PLA film without coating; CH + REO1%, PLA film coated with chitosan incorporated with REO at 1%; CH + REO2%, PLA film coated with chitosan incorporated with REO at 2%; CH/SC + REO1%, PLA film coated with a blend of chitosan and sodium caseinate incorporated with REO at 1%. CH/SC + REO2%, PLA film coated with a blend of chitosan and sodium caseinate incorporated with REO at 2%.

et al. (2021) reported an extension of 22 % of the shelf life of fresh meat (measured on the base of appearance and smell of meat) when packed with 50 µm LDPE film with 25 % of masterbatch with flaxseed oil.

The use of an active coating on PLA seems to be more effective thanks to the fast availability of the active substance at contact with the meat samples. In fact, a similar antioxidant activity was observed by Contini et al. (2012) for cooked turkey meat packaged in PET trays coated with citrus extract. Results showed that a 47.8 % decrease in amount of MDA in turkey meat were observed in the presence of citrus extract. The effectiveness of the citrus extract coating was attributed to its high surface roughness, and to the high level of release (solubility) of the antioxidant in water.

3.3.2. Release of volatile compounds

The SPME GCMS analysis identified many volatile compounds of interest, which were recorded for analysis (Table 2).

Acetic acid, ethanol, heptanal, hexanal, butanoic acid, hexanoic acid were chosen for further analysis as these were the most consistently occurring VOC's throughout the analysis. Ethanol and hexanal are connected to the spoilage of raw chicken breast and have been linked to the metabolic processes of *Pseudomonas* spp, *Moraxella* spp. and *B. thermosphacta* spp. (Bowman, Freeman, Later, & Lee, 1983; Freeman, Silverman, Angelini, Merritt, & Esselen, 1976; Lee, Smith, & Freeman, 1979; Thomazini & Miyagusku, 2006). Acetic acid is a common marker for primary stage oxidation in chicken meat (Mikš-Krajnik, Yoon, & Yuk, 2015). Butanoic and hexanoic acid have both been linked to the natural

spoilage of meat and are commonly occurring in products stored in MAP (Casaburi, Piombino, Nychas, Villani, & Ercolini, 2015; Ercolini et al., 2011; La Stora et al., 2012).

Two-way ANOVA with Tukey's multiple comparisons test were used to identify significant differences between samples. The factors considered for the multiple comparison test were sampling time point and film type. Since all samples were prepared from the same source of chicken, samples were set as linked in the multiple comparison test. The results of this analysis were as follows and summarised in Fig. 4.

Control samples displayed significantly higher concentrations of heptanal (A.) when compared samples stored in packaging systems CH + REO1%, CH + REO2% and CH/SC + REO1% after 3 ($p < 0.0001$, 0.0001, 0.0120), 6 ($p < 0.0002$, 0.0316, 0.0003) and 14 days of storage ($p < 0.0167$, 0.0115, 0.0173). No significant differences found at day 10. Control samples displayed significantly higher concentrations of butanoic acid (B.) when compared samples stored in packaging systems CH + REO1%, CH + REO2% and CH/SC + REO1% after 3 ($p < 0.0349$, 0.0008), 6 ($p < 0.0241$, 0.0005) and 14 ($p < 0.0287$, 0.0190, 0.0334) days of storage. Significantly higher concentrations of Ethanol (C.) were found in the control samples after 14 days of storage when compared to all other samples ($p < 0.0001$, 0.0001, 0.0001, 0.0001) where the ethanol concentration was reduced by the 90 % compared to the samples packed with control film. No significance was identified at earlier time points. No significant differences were identified for; acetic acid (D.) throughout shelf life.

3.3.3. Colour change

Clear visual changes were observed in the chicken samples over the duration of the 21-day period. Samples became lighter and began to gain a distinctive yellow colouring around their edges as well as a slimy surface texture. Fig. 5 displays clear changes in the LAB values over the 21 days, most noticeable an increase in (L^*) lightness. Mean values for a^* generally decreased after the first 7 days as yellowing began to occur.

Throughout shelf life, a slight increase in (L^*) lightness has been observed, however no significant differences were identified between samples. A significant difference in green / magenta (a^*) values were identified at day 14, however this was not consistent throughout shelf life so is likely due to sample variation. No significant differences were found between blue / yellow (b^*) values throughout shelf life. Control samples generally displayed lower b^* values throughout shelf life, indicating a greater loss of red pigment.

These results are in line with the findings of Kahraman et al. (2015), particularly for the (a^*) coordinate (an average of 6), where they used a Colorflex HunterLab spectrophotometer which differed from our system. Therefore, by using a semi-professional camera Nikon DX SWM Micro 1:1 ∞ 52 lens with a colour analysis software, we can generate comparable results with other tristimulus colorimeter.

However, during our study, we did not observe a significant difference by visual observation during the shelf life of the chicken samples, this observation confirms the data observed for the (b^*) coordinate. Same applies for the development of red pigment before 14 days.

4. Conclusions

To conclude, four bio-based active packaging systems using were successfully developed utilizing PLA films coated with CH and a blend of CH/SC containing different amount of REO. A model system was developed to measure the packaging's effectiveness at preserving the quality of fresh chicken.

DPPH assays indicated that the film samples loaded with REO were able to provide antioxidant effects, with the CH/SC 2% REO demonstrating the greatest radical scavenging activity. TBARS assay supported these results, with active film meat samples displaying significantly lower concentrations of MDA throughout shelf life when compared to the PLA control sample. CH/SC 2% REO also demonstrated the lowest concentrations of MDA confirming its greater scavenging capability.

SPME testing further supported these findings showing lower concentrations of heptanal, butanoic acid and ethanol throughout shelf life when compared to control samples. Colour analysis indicated no significant differences throughout shelf life, suggesting that the visual changes observed were uniform across all sample sets. Overall, this study has demonstrated that PLA films coated with chitosan based active coatings are a promising delivery method for providing antioxidant effects in packaging for fresh meat products.

Funding

This work was supported by the European Europe [PON03PE_00180_1] and Abertay University R-LINCS funds.

Authors statement

Steven Park: investigation, writing-original draft preparation. Stefania Volpe: validation, visualization, formal analysis, Alberto Fiore: conceptualization, writing, review & editing, funding acquisition; Elena Torrieri: conceptualization, writing, review & editing, supervision; Paolo Masi: founding acquisition.

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