Effect of shellac coating on the properties of egg white protein (EWP) films for cherry tomato packaging.

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Abstract

Egg white protein (EWP) films have limited applications for the modified atmosphere packaging (MAP) of fresh fruits and vegetables due to their poor water vapour barrier properties compared to commonly used petroleum-based polymers. There are some strategies to improve this drawback such as nanoparticles addition, layer intercalation, or coatings application. Shellac is a natural non-toxic polymer with good film-forming properties, biodegradability, and noted moisture resistance. For these reasons, in this study shellac was used as coating material for EWP films obtained by compression moulding and was applied in two thicknesses (24 and 40 µm) on one or both sides of the film. The objective was to improve the barrier properties of these films and bring them closer to those of bio-based commercial films such as polylactic acid (PLA), and to compare with petroleum-based materials such as oriented polypropylene (OPP). At 23 °C, the oxygen transmission rate (OTR) of EWP films was lower than that of PLA and OPP films, while the water vapor transmission rate (WVTR) was significantly higher. Shellac coating reduced the WVTR of EWP films up to values close to those of PLA and did not change OTR at 50% relative humidity. The colour of coated EWP films differs from that of commercial films, especially in lightness and in yellowish tone, which decreased and increased, respectively, with the thickness and number of coating layers. Cherry tomatoes were kept for 21 days at 4 °C in bags made with these films to verify with real-product situations these improvements. The product experienced a similar weight loss in shellac-coated EWP packages compared with that of PLA, so in this regard it could be considered an alternative to this commercial film. However, further improvement needs to be addressed in order to make them comparable to water barrier properties of OPP.

Keywords: edible film, egg white protein, shellac, compression moulding, food packaging, modified atmosphere, quality.

INTRODUCTION

Modified atmosphere packaging (MAP) is a technology used in food to prolong its shelf-life. This technology consists of changing the air surrounding the food in the package to another composition (Sandhya, 2010). MAP involves the use of packages, and most of these packages are made from petroleum-based polymers because of their low cost, easy processability, and excellent physiochemical properties for food packaging. However, petroleum-based materials are not renewable, and to satisfy the increasing expectations for sustainability and biodegradability, the research community is focusing on, for example, identifying biodegradable packaging materials and finding innovative methods to make plastics degradable (Siracusa *et al.*, 2008; Ierna *et al.*, 2017). Bio-based materials could be an option to replace petroleum-based materials. However, for certain applications biobased materials cannot be fully competitive with conventional petroleum-based plastics. Bio-based materials drawbacks such as poor processability, brittleness, hydrophilicity, or poor moisture and gas barrier properties limit their extensive application (Mekonnen *et al.*, 2013).

Among other biobased materials, proteins have proven to be appropriate raw materials for bioplastics development (Hernandez-Izquierdo and Krochta, 2008). Depending on the sequential order of the amino acids and the presence of bonds and interactions, the proteins can acquire different shapes, structures, and functionalities, which can be modulated by various physical and chemical agents (Krochta, 2002). However, protein-based films are not different to other bioplastics, being very sensitive to humidity and featuring low barrier properties to water, thus limiting the application to a wide range of food products.

Wheat, soy, corn zein, and other proteins have been widely studied as potential bioplastics (Siracusa *et al.*, 2008), while research conducted in EWP is limited. However, studies have shown that it is also possible to produce bioplastics from EWP by using different processing technologies (Jerez *et al.*, 2007; Lee *et al.*, 2013; Pranata *et al.*, 2019). EWP films have good mechanical properties (Gennadios *et al.,* 1996), good oxygen barrier performance (Gómez-Estaca *et al.,* 2016) and are more transparent than other protein films such as whey, soybean or zein (Gennadios *et al.*, 1996). However, EWP based materials are very sensitive to water, since its hydrophilic nature, and have a low water barrier property. Thus, further research is needed to overcome the main shortcomings of protein-based packages.

Coatings have been successfully applied to protein-based films for a long time (Gontard *et al.*, 1995). A wide range of compounds may be used to develop bio-based and/or edible coatings, including lipids and polysaccharides (Avramescu *et al.*, 2020). Natural waxes such as carnauba, shellac, or beeswax, have proven to be useful in the development of edible coatings (Gutiérrez-Pacheco *et al.*, 2020). Particularly, shellac is a product obtained from lac insect (*Laccifer lacca),* an arthropod that parasites certain trees (Wang *et al.*, 1999). Food and Drugs Administration (FDA) has declared shellac as 'generally recognized as safe' (GRAS) and European Food Safety Authority also has declared shellac as a food additive (E904) (Thombare *et al.*, 2022). Shellac has a complex chemical composition, consisting of a mixture of resin, wax, and pigments (Tamburini *et al.*, 2017), showing a great film forming ability and good barrier properties (Soradech *et al.*, 2012). For these reasons, shellac has been applied as a layer on bio-based films for improving the water vapour barrier performance of bio-based films (The *et al.*, 2008; Wei *et al.*, 2015). However, to the best of our knowledge, shellac has not been applied on EWP films.

MAP is widely used to maintain the quality of fresh or fresh-cut fruits and vegetables. Therefore, in this range of products it is interesting to incorporate bio-based, biodegradable and/or compostable films, as indicated by the increasing number of studies in this regard (Almenar, 2021). These types of packages and materials could be advantageous in the development and distribution of more sustainable products. However, more studies about the adaptation and improvement of bioplastics for a wider applicability should be undertaken (Khalil *et al.*, 2018).

Therefore, the objectives of this study were: 1) to develop a novel bio-based packaging based on EWP films, improving their water vapour barrier properties by adding shellac layers, 2) to test the performance of developed packaging films for the MAP storage of fresh cherry tomato, and 3) to compare the novel packaging with bio-based and petroleum based commercial films in terms of material barrier properties and its performance preserving fresh products.

MATERIALS AND METHODS

Materials

EWP powder was purchased online (Sports Supplements Limited, Colchester, UK). Food-grade glycerine (GLY) was supplied by Barcelonesa Global Chemical Solutions (Cornellà de Llobregat, Spain). Two different shellac products were acquired (Restaurar&Conservar, Lisboa, Portugal). Shellac A (SHA) is less pigmented and has a little bit lower quantity of wax than Shellac B (SHB). Oriented polypropylene (OPP) film was provided by Envaflex (Utebo, Spain), while polyactid acid (PLA) film was provided by Sidaplax (Ghent, Belgium). Cherry tomatoes (*Solanum lycopersicum* var. *cerasiforme*) were produced in Almeria (Spain) and purchased from a local distributor.

EWP films production

EWP powder and a mixture of glycerol and water in 1:0.5:0.5 (in weight) ratio were blended in an orbital mixer (Kitchen Aid, USA) under vacuum conditions during 30 min. The obtained film forming solution (FFS) was stored for 24 h at 5°C. After this conditioning time, 10g of the FFS was distributed in an aluminium foil and was compression moulded using a hot press (LP-S-50, Labtech Engineering Co., Phraeksa, Thailand) applying a heating cycle (130°C at 150 bar for 5 min) followed by a cooling cycle (20°C at 150 bar for 5 min).

After aluminium foil removal, EWP films were coated with shellac. First of all, shellac was dissolved in ethanol in 1:2 w/w proportion. Shellac coatings were applied in the EWP films with K Hand coater bars (RK Print Coat Instruments, Litlington, UK) with different wire diameter (24 and 40 μm). Finally, EWP films were conditioned at 23°C and 55% RH for at least 2 days before its use. A brief processing scheme is shown in Figure 1.

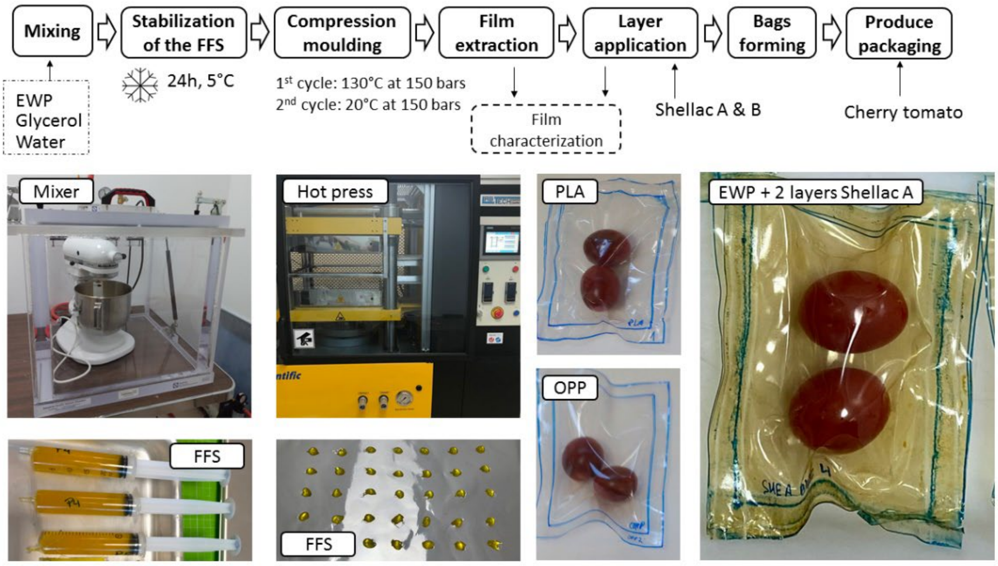

Figure 1: Manufacturing process for EWP films for packaging cherry tomatoes.

EWP films characterization

Oxygen and water vapour transmission rates (OTR and WVTR)

The oxygen transmission rates (OTR) of the different films were measured with a Mocon OX-TRAN 2/22, and the water vapour transmission rates (WVTR) with a Mocon PERMATRAN-W 3/34 (Minneapolis, USA). To avoid system sensor saturation, films were masked with adhesive aluminium foil, leaving an area of $7.9 \cdot 10^{-5}$ m^2 for the measurement. Developed films and commercial films were tested at 23°C and 50% relative humidity. Analysis were conducted in triplicate, and two samples per replicate were tested. Obtained results were expressed in $g\ m^{-2}\ d^{-1}$.

Films colour

Films colour was measured using a CR-200 colorimeter (Konica Minolta Sensing INC., Tokyo, Japan), placing the sample on a white plate. Readings taken were expressed as L*, a* and b* parameters. Three measurements of 3 different replicates were performed for each material. Obtained films were scanned to record the differences in the colour.

Cherry tomato packaging and weight loss

Obtained films were thermosealed using an HSR-01 heat sealer (Pubtester Instruments, Jinan, China) to obtain bags of 0.075 m x 0.1 m. Two cherry tomatoes (average weight of 24.96±3.09 g) per bag were packaged on bags formed with uncoated EWP films, EWP films coated with SHA or SHB (both made with 2 layers and using 40-µm applicator bar), PLA films, and OPP films. A macroperforated OPP bag was used as control. Three replicates were prepared for each condition. Bags were stored at 4 °C for 21 days, and weight loss was determined at the end by subtracting the final weight from the initial weight (expressed as %).

Statistical analysis

Experiments were conducted in triplicate, and data were expressed as the mean value ± standard deviation. The analysis of variance was performed using one-way analysis of variance (ANOVA) followed by Duncan's post-hoc test, using the Statistical Package for Social Sciences v. 26 (Chicago, USA). Differences were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Barrier properties of EWP films

For uncoated EWP films, a thickness of 167±10 µm was measured. Thicknesses of monolayer EWP films using the 24-µm bar were 172±11 µm and 156±10 µm for SHA and SHB, respectively, while the ones with the 40-µm bar were 158±14 µm and 149±13 µm for SHA and SHB, respectively. Thicknesses of bilayer EWP films using the 24-µm bar were 172±15 µm and 172±15 µm for SHA and SHB, respectively, while the ones with the 40-µm bar were 197±18 µm and 156±12 µm for SHA and SHB, respectively. Commercial OPP and PLA films were thinner than the developed films (38 and 43 µm, respectively).

The OTR of non-coated EWP film was 0.098±0.006 g m^{-2} d^{-1}. This value was lower than that obtained in commercial PLA (0.502±0.001 g m^{-2} d^{-1}.) and OPP (2.572±0.498 g m^{-2} d^{-1}). There were no significant differences ($p>0.05$) between the OTR of the uncoated EWP film and those of these films coated with shellac regardless of the number of layers applied or the wire diameter of the bar used to apply it (Figure 2). Oxygen permeability (OP) of EWP uncoated film, calculated from the OTR value, resulted in $1.90 \pm 0.498 \cdot 10^{-18}$ kg m m^{-2} s^{-1} Pa^{-1}. Pranata *et al.* (2019) obtained similar OP values ($2.39 \pm 0.08 \cdot 10^{-18}$ kg m m^{-2} s^{-1} Pa^{-1}) in egg protein films measured at 23°C and 55% RH.

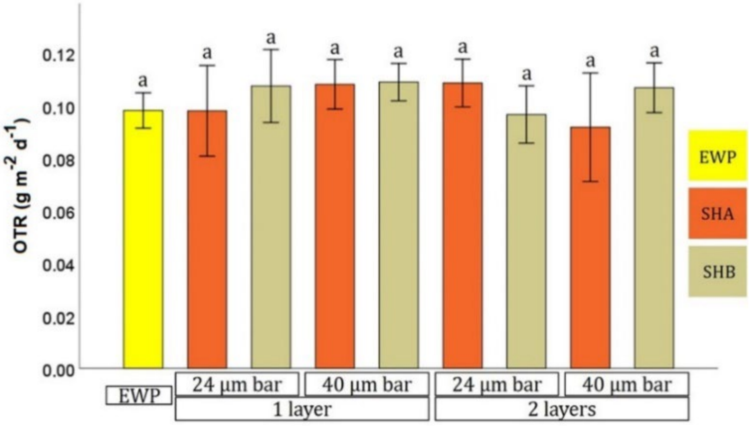

Figure 2: Oxygen transmission rate (OTR) of EWP films with and without shellac coating applied with different number and thickness of layers.

The WVTRs of coated and uncoated EWP films are shown in Figure 3. In the case of EWP films, the WVTR is 253.351±11.247 g m^{-2} d^{-1}. Due to the hydrophobic characteristic of shellac, all the coated samples with this lac showed a significant reduction in the WVTR compared to the uncoated EWP film. For the two shellacs used, the WVTR decreases as the number of layers and their thickness increase. On the other hand, SHA had more effectiveness in the WVTR reduction than SHB except for the case of two layers-40 µm bar. When EWP films are coated with two layers of shellac using the 40 µm bar, the WVTRs obtained (69.383± 10.826 g m^{-2} d^{-1} and 80.531±4.603 g m^{-2} d^{-1} for SHA and SHB, respectively) are very close to those of commercial PLA films (36.325±0.106 g m^{-2} d^{-1}), but the reduction in WVTR is not enough to be comparable to that of the OPP (0.547±0.001 g m^{-2} d^{-1}).

Similar results were obtained for other bio-based films. Shellac coating reduced the water vapour permeability (WVP) in agar and cassava starch films by 2-20 times compared to uncoated films, depending on the shellac layer thickness and the composition of the coating (The *et al.,* 2008). The WVP of konjac glucomannan films was also reduced applying shellac coating with different concentrations of stearic acid as solvent (Wei *et al.,* 2015). Shellac coating could be an option to reduce the WVP of biobased films.

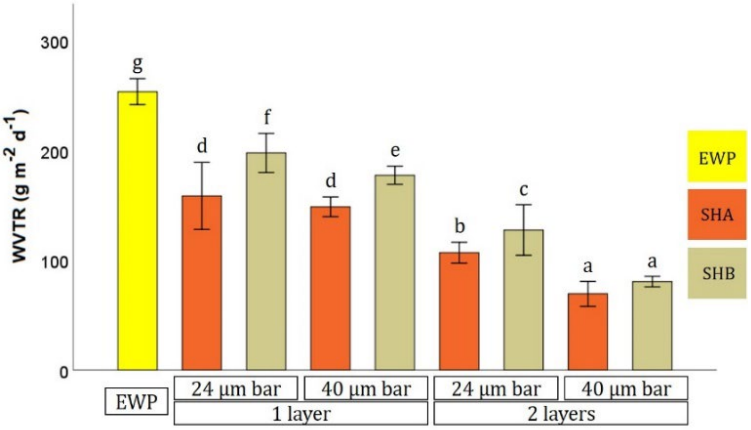

Figure 3: Water vapour transmission rate (WVTR) of EWP films with and without shellac coating applied with different number and thickness of layers.

Films Colour

Figure 4a shows the values of the coordinate L* for coated and uncoated EWP films. Uncoated films had higher luminosity than shellac coated films ($p<0.05$). In general, the luminosity decreases as the number of layers and their thickness increase. L* values are similar for films coated with 1 layer-40 µm bar and those coated with 2 layers-24 µm bar. Significant differences ($p<0.05$) were found between SHA and SHB. Films coated with SHA have a lower luminosity than those coated with SHB, and these differences increase with the number and thickness of the layers. Not only the coatings affect the lightness of EWP films, but also the thickness of the film influences the lightness, reducing it as the film thickness increases (Lee *et al.,* 2013).

The b* values for EWP films without and with different type of shellac coatings are shown in Figure 4b. In uncoated EWP films samples, the coordinate b* is slightly higher than 0. This result is similar to that obtained by Pranata *et al.* (2019) for EWP films conditioned at 23 °C at 55 % HR (b*=2.11). Due to shellac colour, in all coating cases was significant differences in b* value ($p<0.05$) comparing with uncoated EWP films. In all samples coated with SHA, b* was higher than those coated with SHB. These differences can be attributed to the colour of the lac which in the case of SHA is yellowish-orange and in the case of SHB is brownish. The coordinate b* was influenced by the type of bar and the number of layers. For both shellacs b* values increased with increasing the bar thickness and the number of layers.

The highest b* value was for films having 2 layers coating applied with 40-µm bar (44.64 for SHA and 28.03 for SHB) while the lowest b* was for films having 1-layer coating applied with 24-µm bar (17.34 for SHA and 13.95 for SHB). For the two shellacs, similar b* values are obtained in samples coated with 1 layer-40 µm bar than in those covered with 2 layers-24 µm bar. In other studies, add waxes as carnauba had similar effect in the coordinate b*, increasing the values in chitosan films from 10.73 to 22.63 (dos Santos *et al.,* 2017) and in gelatine films from 0.93 to 3.7 (Zhang *et al.,* 2018).

The results obtained for L* and b* values were reflected in the scanner images of the plastics (Figure 4c), as a greater yellow coloration was observed in the plastics that have a coating of SHA compared to SHB.

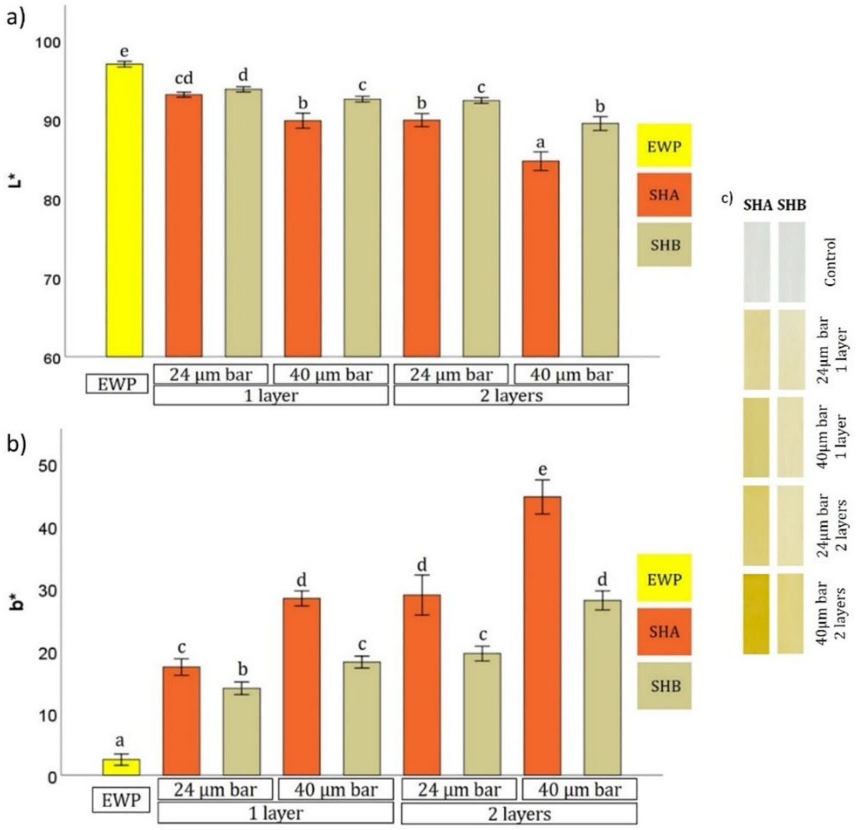

Figure 4: L* values (a), b* values (b) and scan images (c) of EWP films with and without shellac coating applied with different number and thickness of layers.

Cherry tomato packaging

The weight losses of cherry tomatoes packaged in the different materials, after 21 days of storage at 4°C, are represented in Figure 5. The control tomatoes showed the highest weight loss. There are no significant differences in weight loss between tomatoes packaged in the different EWP bags and those packaged in commercial PLA bags, with values around 6%. Although shellac-coated films had lower water vapour permeability than uncoated EWP films, these differences did not translate into weight loss. However, tomatoes packaged in OPP had a weight loss of less than 1%, indicating a significant difference from the other materials. In the case of OPP, the low permeability of this film to water vapour affects the weight loss of tomatoes, linking WVTR of the package with weight loss as shown by other studies (Batu and Thompson, 1998).

A higher weight loss of 8-10% was found in cherry tomatoes stored for 22 days in PLA packages, but in this case, the storage temperature was 20°C (Briassoulis *et al.,* 2013).

However, cherry tomatoes packaged using bio-based intact bitter cassava film lost 4.38% of their weight after 19 days at 10°C and only 0.38% using OPP films (Tumwesigye *et al.,* 2017).

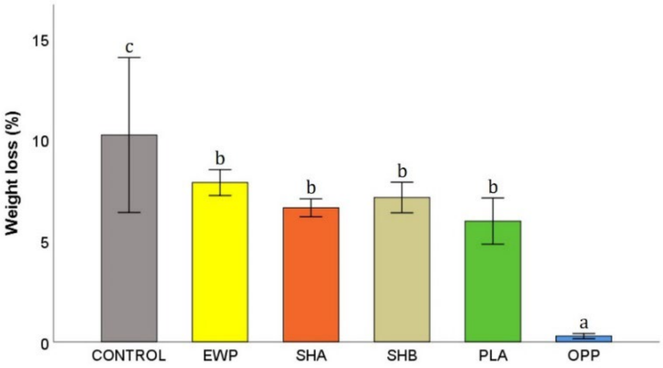

Figure 5: Weight loss of cherry tomatoes after 21 days stored at 4°C in different bags.

CONCLUSION

The formulation and the processing conditions allowed us to develop stable FFSs and then homogeneous EWP films to carry out this study.

The application of a shellac coating on EWP films significantly improved their water vapour barrier properties, possibly due to the hydrophobicity of the lac. The WVTR was reduced by increasing the number of layers, from one to two, and by increasing their thickness when applied with 24 or 40 µm bars, reaching a decrease up between 3.1 and 3.6 times, which approximates the WVTR of shellac-coated EWP films to that of commercial PLA, but still keeps it far from the water vapour barrier properties of OPP. SHA had a greater positive effect on water vapour barrier properties than SHB. However, no significant differences ($p>0.05$) were found in the OTR between the coated and uncoated films for either of the two types of shellac.

Shellac coatings modified the colour of EWP films, reducing lightness and increasing the b* coordinate (coated films have a more yellowish tone). These changes were more remarkable for SHA than for SHB and increased with the number and thickness of the layers.

The EWP bags limited cherry tomato weight loss during 21 days of storage at 4 °C in a similar way to shellac-coated EWP and PLA bags, reducing the losses with respect to a storage exposed to the air. As expected, the weight losses for tomatoes in OPP bags were much lower. Presence of natural waxes in the skin of tomatoes may be the reason why differences in weight loss are not observed between packages with different WVTR, and that losses are only reduced when the WVTR is two orders of magnitude lower, as in the case of OPP.

The results obtained allow us to advance in the knowledge of EWP films as packaging materials and show the progress and what remains to be improved to extend its use.

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