Sustainability & Circular Economy

Antioxidant and antimicrobial properties of PLA-based active packaging with pomegranate peels and extract

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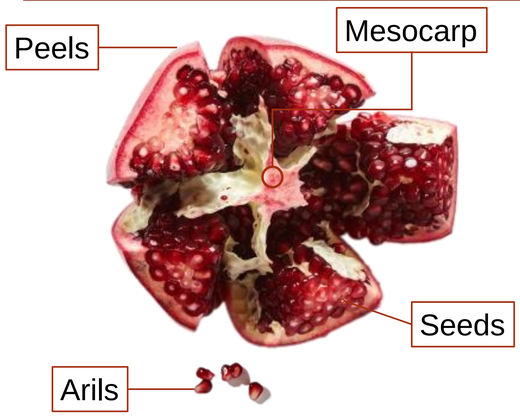
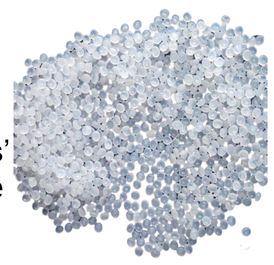
INTRODUCTION



- 50% of pomegranate are by-products, making pomegranate a major source of by-products.
- These by-products are a major source of phenolic compounds with antioxidant and antimicrobial activities
- Punicalagin (A+B) and ellagic acid are the major phenolic compounds

- Polylactic acid (PLA) is a biopolymer, derived from starch or sugarcane
- Active food packaging primary goal is to extend foods' shelf-life through the dynamic interaction between the package and the packaged food.



OBJECTIVE

- ✓ Evaluate the antioxidant properties of PLA-based active packaging with 3% of pomegranate peels extract (PLA/3PPE) and with 3% of pomegranate peels (PLA/3PP).
- ✓ Determine the content in punicalagin (A+B) and ellagic acid.
- ✓ Evaluate the antimicrobial properties of PLA-based active packaging PLA/3PPE and PLA/3PP against *Staphylococcus aureus, Enterococcus faecalis, Listeria monocytogenes,* and *Escherichia coli.*

MATERIALS & METHODS

- For the *in vitro* antioxidant activity of the films, 9.08 cm^2 of the films were submersed in ethanol 95% (v/v) and kept at 40 ºC for 10 days. At the end of this period, the DPPH radical scavenging assay [1], was performed as well as the total content in phenolic compounds [2] and the total content in flavonoids [3] were determined.
- For the determination of the total content of punicalagin (A+B) and ellagic acid by UHPLC-DAD [4], the films were submersed in methanol.
- The antimicrobial activity of the films was determined according to the ISO 22196:2011 [5], against *S. aureus* (ATCC 25923), *E. faecalis* (NCTC 775), *L. monocytogenes* (ATCC 13932), and *E. coli* (ATCC 25922).

RESULTS & DISCUSSION

Determination and quantification of punicalagin (A+B) and ellagic acid:

- ➢ Both PLA/3PPE and PLA/3PP presented similar content in ellagic acid (**Table 1**).
- ➢ Punicalagin was not found in either organic solvents; this might be due to the high molecular weight of punicalagin (1084.71 g/mol), which prevents the migration of the molecule to the food simulant/solvent or due to the hydrolyzation of punicalagin into ellagic acid.
- ➢ Significant differences in the extraction of ellagic acid can be observed between the ethanol (95%) and methanol extraction. Methanol, although it is not an approved solvent extraction for migration assays by the European Union, it is clear that this solvent can significantly extract more ellagic acid from PLA-based films than the appointed solvent for fatty foods [8].
- ➢ Temperature plays a significant role in the extraction of these compounds in PLA-based packaging that the pomegranate peels have higher compatibility with PLA than the PPE-FD.

Table 1. Antioxidant capacity and total content of phenolic compounds and flavonoids of the active PLA films.

Samples	Punicalagin (A+B) mg/dm^2	Ellagic Acid mg/dm^2
PLA/3PPE 40 °C, ethanol 95 %, 10 days	< LoD	0.30 ± 0.01 ^{b}
PLA/3PP 40 °C, ethanol 95 %, 10 days	< LoD	0.31 ± 0.01 ^{b}
PLA/3PPE 40 °C, methanol, 24 h	< LoD	0.40 ± 0.03 ^{c,d}
PLA/3PP 40 °C, methanol, 24 h	< LoD	0.42 ± 0.02 ^{d}
PLA/3PPE 25 °C, methanol, 24 h	< LoD	0.39 ± 0.00 ^{c}
PLA/3PP 25 °C, methanol, 24 h	< LoD	0.27 ± 0.01 ^{a}

Legend: LoD – Limit of Detection; PLA/3PPE – PLA with 3 % of pomegranate extract; PLA/3PP – PLA with 3 % of pomegranate peel

Antioxidant activity of the films:

- ➢ PLA/3PPE significantly presented a higher inhibition percentage and a higher content in total phenolic compounds than the PLA/3PP. PLA/3PP presented a significantly higher content in flavonoids.
- ➢ PLA is a hydrophobic polymer and the introduction of polar and hydrophilic groups, such as pomegranate peels, may lead to the decrease of the PLA contact angle and, consequently, promote its hydrophilicity [6,7]. Concerning active food packaging, PLA' hydrophobicity is considered a limitation due to its low ability to encapsulate and gradually release active compounds [6]. The higher release of phenolic compounds in the PLA/3PPE (**Figure 1**) suggests that the pomegranate peels have higher compatibility with PLA than the PPE-FD.

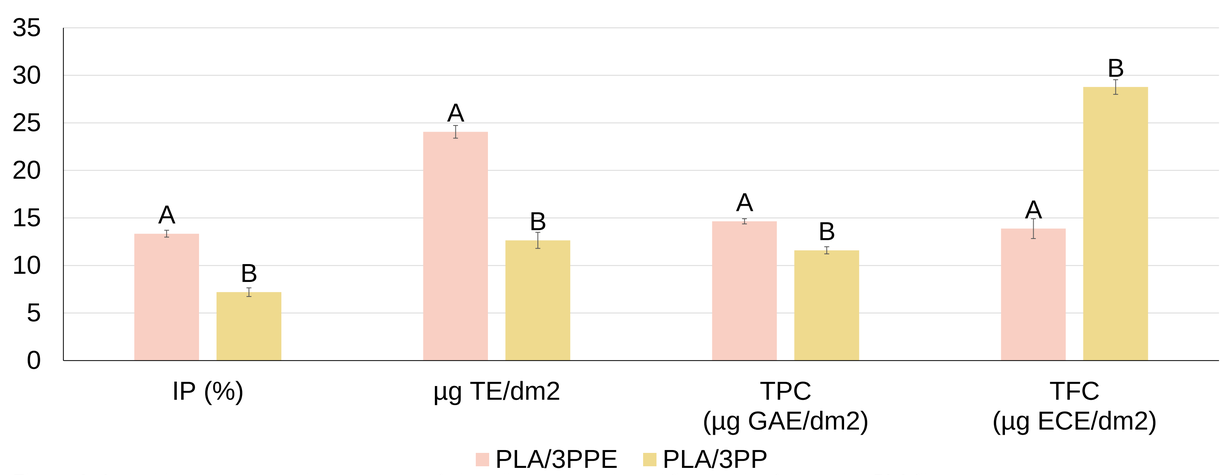


Figure 1. Antioxidant capacity and total content of phenolic compounds and flavonoids of the active PLA films.
Legend: PLA/3PPE – PLA incorporated with 3 % of pomegranate extract; PLA/3PP – PLA incorporated with 3 % of pomegranate peel; IP – inhibition percentage; TE – Trolox equivalents; TPC – Total content in Phenolic compounds; GAE – Gallic acid Equivalents; TFC – Total content in Flavonoids; ECE – Epicatechin Equivalents.
Different letters represent results with significant differences.

Antimicrobial activity of the films:

- ➢ The PLA/3PPE and PLA/3PP presented antimicrobial activity against *S. aureus* (**Table 2**).
- ➢ Also, a decrease in the count of bacteria in the *L. monocytogenes* with PLA/3PPE seems to indicate a potential antimicrobial activity against this microorganism. However, neither the PLA/3PPE nor the PLA/3PP presented antimicrobial activity against *E. coli* and *E. faecalis.*

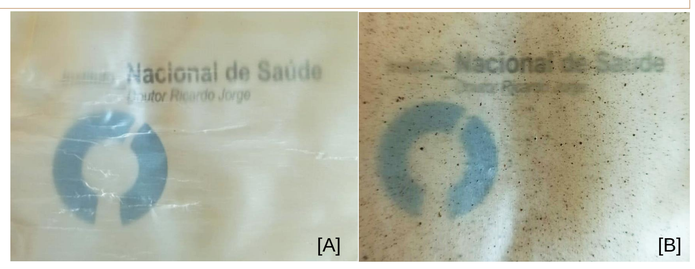


Figure 2. PLA-based active packaging with 3% of pomegranate peels extract (PLA/3PPE) [A] and with 3% of pomegranate peels (PLA/3PP) [B].

Table 2. Antimicrobial activity of the control (PLA) and active (PLA/3PPE and PLA/3PP) films.

Samples	Dilution	*S. Aureus* (Log/cm^2)	*L. Monocytogenes* (Log/cm^2)	*E. Coli* (Log/cm^2)	*E. faecalis* (Log/cm^2)
PLA	1 10^{-1} 10^{-2}	>4.27	>4.27	>4.27	>4.27
PLA/3PPE	1 10^{-1} 10^{-2}	0.97	3.95	>4.27	>4.27
PLA/3PP	1 10^{-1} 10^{-2}	0.49	>4.27	>4.27	>4.27

Legend: PLA/3PPE – PLA with 3 % of pomegranate extract; PLA/3PP – PLA with 3 % of pomegranate peel; CFU – colony forming units.

CONCLUSION

Pomegranate peel extract and pomegranate peel were successfully incorporated into active polylactic acid-based packages (PLA/3PPE and PLA/3PP). The two films presented antioxidant activity however, the PLA/3PPE presented higher values and a higher content in phenolic compounds. Ellagic acid was quantified in both active films, both in the food simulant (ethanol 95%, v/v) and extraction solvent (methanol). The active films only presented antimicrobial activity against *S. aureus*, suggesting that the PLA traps the antimicrobial compounds.

ACKNOWLEDGMENTS

Cássia H. Barbosa thanks the Fundação para a Ciência e Tecnologia (FCT), Portugal for the Ph.D. Grant 2021.08154.BD. **Funding**: This work was carried out under the MobFOOD Project (POCI-01-0247-FEDER-024524 and LISBOA-01-0247-FEDER-024524), funded by POCI (Operational Programme "Competitiveness and Internationalization") and POR Lisboa (Lisbon Regional Operational Programme), through ANI, and by the Programa de Cooperación Interreg-A España–Portugal (POCTEP) 2014–2020 (project 0377_IBERPHENOL_6_E). This research was also funded by PT national funds (FCT/MCTES, Fundação para a Ciência e Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) through the grant UIDB/00211/2020).

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