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INFORME DE LA ACTIVIDAD:

En este artículo se estudia la posibilidad de emplear recubrimientos biopoliméricos conteniendo levaduras con actividad antifúngica como alternativa sostenible para mitigar la pérdida de frutas y verduras causada por enfermedades fúngicas poscosecha, como tratamiento complementario al ActiBioMulch objeto de este proyecto. Estos recubrimientos poliméricos se desarrollaron mediante nanofibras electrohiladas derivadas de pululano, goma de anacardo, FucoPol y poli(óxido de etileno) (PEO) y se cargaron con *Meyerozyma caribbica* (GenBank ID: JQ398674). Se caracterizadon las propiedades morfológicas, térmicas y químicas de la goma de anacardo (CG:PEO), FucoPol (FP:PEO) y nanofibras de pululano. Se evaluó la viabilidad de *M. caribbica* dentro de las fibras y su actividad antifúngica in vitro contra seis fitopatógenos comunes en frutas y verduras. El análisis morfológico mostró la presencia de nanofibras que encapsulan *M. caribbica*. La espectroscopía ATR-FTIR identificó la ausencia de interacciones entre la levadura y los polímeros. Las fibras que contienen *M. caribbica* exhibieron efectos fungistáticos in vitro sobre la germinación de las esporas. Las nanofibras de pululano mostraron la mayor viabilidad de *M. caribbica* y el mayor porcentaje de inhibición del crecimiento frente a los hongos evaluados. Estas prometedoras nanofibras que encapsulan levaduras de control biológico podrían usarse como recubrimientos comestibles o ayudas agrícolas, que ofrecen una alternativa para el tratamiento poscosecha para controlar enfermedades fúngicas y reducir la pérdida global de alimentos.

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Development of antifungal electrospun nanofiber mats containing *Meyerozyma caribbica*

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ABSTRACT

To mitigate vegetable and fruit loss caused by post-harvest fungal diseases, polymeric antifungal coatings encapsulating biocontrol yeasts offer a sustainable alternative. Nanofibers derived from pullulan, cashew gum, FucoPol and poly (ethylene oxide) (PEO), were electrospun and loaded with *Meyerozyma caribbica* (GenBank ID: JQ398674). The morphological, thermal and chemical properties of cashew gum (CG:PEO), FucoPol (FP:PEO), and pullulan nanofibers were characterized. The viability of *M. caribbica* within the fibers and their *in vitro* antifungal activity against six phytopathogens were assessed. Morphological analysis exhibited the presence of nanofibers encapsulating *M. caribbica*. ATR-FTIR spectroscopy identified the absence of interactions between the yeast and polymers. Fibers containing *M. caribbica* exhibited *in vitro* fungistatic effects on spore germination. Pullulan nanofibers showed the highest *M. caribbica* viability and the highest percentage of growth inhibition against the evaluated fungi. These promising nanofibers encapsulating biocontrol yeasts could be used as edible coatings or agricultural aids, which offer an alternative for post-harvest treatment to control fungal diseases, reducing global food loss.

1. Introduction

Governmental and international organizations have raised public attention on food loss and waste, and according to the Sustainable Development Goals calls, the objective is to reduce these losses to the half by 2030 (*United Nations*, 2015).

Fruits and vegetables are one of the most consumed commodities globally, accounting for more than 42% of total food wastage (Ganesh et al., 2022). Among them, phytopathogens result in an annual estimated loss of 10–15% of the world's major crops (Peng et al., 2021). Particularly, the predominant postharvest pathogens responsible for spoilage in fruits and vegetables primarily include fungi categorized under the genera *Botrytis*, *Penicillium*, *Aspergillus*, *Alternaria*, *Colletotrichum* spp., and *Rhizopus* (Parafati et al., 2015; Yang et al., 2017), which are naturally present in fruits and vegetables. These pathogenic fungi use different mechanisms to bind to the surface of the host plant. In any case, the penetration of the fungal onto the plant requires the contact and adhesion of the spores to the plant surface. Fungi possess the capability to generate enzymes that modify the plant surface, thereby aiding in adhesion. Subsequently, these phytopathogens induce various forms of rot in the fruit during the post-harvest stage resulting in compromised quality and rendering them unsuitable for sale (Rodrigues et al., 2021). In addition, some fungi species are capable of producing mycotoxins in food and agricultural commodities (Zain, 2011), which can cause adverse health effects to humans and/or livestock.

Addressing these pathogens typically involves the application of chemical fungicides, both pre and post-harvest, directly onto the fruits or vegetables. Nevertheless, the utilization of chemical fungicides has revealed drawbacks such as their harmful impact on human health, the emergence of resistant strains, and their considerable adverse effect on

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the fungus developed after 24 h.

In this case, FP:PEO nanofibers showed the highest percentage spores germination inhibition (*P. italicum*, 84.2 ± 4.6%, *P. digitatum*, 82.5 ± 3.5% and *T. roseum*, 100.0 ± 1.8%) in comparison with CG:PEO and Pul nanofibers. This phenomenon was caused by the nutrient's depletion provided by yeast, which limits the germination of fungal spores (Bautista-Rosales et al., 2013; Zhang et al., 2020). Additionally, Iñiguez-Moreno et al. (2020) found that the main VOC's emitted by *M. caribbica* include ethanol, ethyl acetate, isobutyl alcohol, 1-butanol, 3-methyl, 2-methyl, isoamyl acetate, and phenethyl alcohol, suppressed the mycelial growth and sporulation, due to damage to the DNA and cell membranes (Zhang et al., 2020). However, despite the high percentage of spore inhibition, it was not enough to avoid the development of the fungus after the evaluated time.

4. Conclusions

Nanofibers of CG:PEO, FP:PEO and Pul encapsulating *M. caribbica* were obtained by the electrospinning process. SEM analysis showed smooth and homogeneous nanofibers, completely covering the yeast *M. caribbica*. CG:PEO, FP:PEO and Pul fibers showed an average size of 719 ± 140 nm, 192 ± 50 nm and 137 ± 20 nm, respectively. Pul nanofibers showed the highest viability of *M. caribbica*, persistent even after 25 days at 26 °C (8.38 × 10^6 CFU/ml). For this reason, Pul nanofibers showed the highest antifungal inhibition percentage against the 6 tested fungi. In addition, all the three different polymeric nanofibers showed a fungistatic effect against the spores. ATR-FTIR analysis did not show any strong interactions between *M. caribbica* and the used polymers since no peak displacement was observed. These promising results make biopolymeric electrospun nanofibers a viable alternative to produce biocontrol tools, such as a protective antifungal coating on the surface of fruits or for agricultural applications. Since electrospinning is already available at industrials scale, the technology transfer to the market could be possible in a short period of time. However, further studies are required to evaluate *in vivo* the potential of this novel material, paying special attention to the antifungal properties, the interaction between the polymers (CG:PEO, FP:PEO and Pul) and the surface of the fruits, and the effect of the coating on fruit quality parameters. Additionally, the evaluation of the kinetics of yeast release from nanofibers into the surface of the fruit and the effect of polymer matrix on the antifungal activity of the yeast will be of great interest.

Author statement

Conceptualization Y.V.G., C.P., and J.M.L.; data curation, formal analysis, investigation and methodology was carried out by Y.V.G., writing-original draft preparation and writing-review and editing was performed by Y.V.G., C.P., M.C.S., J.A.R.S and visualization, supervision, project administration, funding acquisition was carried out by J.M. L.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodhyd.2023.109343.

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