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Original Article

Development and characterization of pectin-based antifungal edible coatings with entrapped biocontrol agent *Meyerozyma guilliermondii* LMA-Cp01 for the management of *Colletotrichum gloeosporioides* from papaya fruit

Desarrollo y caracterización de recubrimientos comestibles de pectina con el agente de biocontrol atrapado *Meyerozyma guilliermondii* LMA-Cp01 para el manejo de *Colletotrichum gloeosporioides* de papaya

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ABSTRACT:

This study aimed to formulate gels using 1% (w/v) low-methoxyl pectin, 0.5% (w/v) glycerol, and 0.5 mM calcium chloride to develop films, alone or in combination with *M. guilliermondii*, through the casting method, to obtain an antifungal edible coating. The gels obtained were characterized rheologically by frequency sweep and gelation kinetics. The effect of yeast addition on thickness, morphology, differential ΔE*, tensile strength, percent elongation at break, elastic modulus of films, and its antifungal activity against *Colletotrichum gloeosporioides* from papaya fruit were evaluated. The film-forming dispersions proved to be true gels, showing viscoelastic behavior and narrow molecular structure typical of cross-linked polymers. These gels presented structural stability, and their texture could allow immersion or atomization applications. Films alone and those added with *M. guilliermondii* showed thickness values of 0.034 and 0.02 mm, respectively. ESEM revealed changes in the film's morphology with yeasts, demonstrating the entrapment of the biocontrol agent. The yeast's addition to the films improved all the mechanical parameter values and achieved a complete inhibition of *C. gloeosporioides*. This research provides new pectin-based systems which are viable candidates to produce antifungal edible-coating with entrapped *M. guilliermondii* that could protect fruits and vegetables against postharvest diseases.

Keywords: Edible films, biological control, rheology, gelation kinetics.

RESUMEN:

Este trabajo tuvo como objetivo formular geles de pectina de bajo metoxilo al 1% (p/v), glicerol al 0.5% (p/v) y cloruro de calcio 0.5 mM para producir películas solas y combinadas con *M. guilliermondii* como recubrimiento comestible antifúngico. Los geles se caracterizaron reológicamente por barrido de frecuencia y cinética de gelificación. Se evaluó el efecto de la adición de levaduras en el espesor de las películas, cambio de color ΔE*, morfología, propiedades mecánicas y actividad antifúngica contra *Colletotrichum gloeosporioides* aislado de papaya. Las dispersiones formadoras de película mostraron un comportamiento viscoelástico y una estructura molecular estrecha de geles verdaderos. Estos geles débiles presentaron estabilidad estructural y su textura permitiría aplicaciones de inmersión o atomización. Las películas solas y adicionadas con *M. guilliermondii* mostraron espesor de 0.034 y 0.02 mm, respectivamente. ESEM mostró cambios en la morfología de las películas con levadura, lo que demuestra la encapsulación del agente de biocontrol. La adición de levaduras a las películas mejoró todos los parámetros mecánicos y logró una inhibición completa de *C. gloeosporioides*. Esta investigación proporciona nuevos sistemas basados en pectina viables para la elaboración de recubrimientos comestibles antifúngicos con *M. guilliermondii* que podrían proteger a las frutas contra enfermedades poscosecha.

Palabras clave: Recubrimientos comestibles, control biológico, reología, cinética de gelificación.

INTRODUCTION

Most fresh vegetables and fruits are highly perishable with relatively short shelf lives; they are susceptible to large losses during pre- and postharvest due to biochemical reactions, weight loss, and physical and fungal damage (Porat *et al*., 2018). Various approaches have been used to raise solutions to these issues. Preservation with chemical fungicides incites environmental concerns and leaves residuality involving toxicological hazards to human health. Also, fungi with chemicals acquired resistance have increased due to repeated applications (González-Gutiérrez *et al*., 2024)

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. One approach to prevent fungal spoilage is to delay the ripening process after the harvest. Low storage temperature is used to slow down chemical and enzymatic reactions and phytopathogen growth. Nonetheless, prolonged storage of fruits from warm regions under unsuitable conditions can cause chilling physiological injury (Kaur *et al*., 2022). Hence, it is necessary to search for cost-effective alternative methods to improve the postharvest shelf-life of fruits and vegetables. Packaging is considered a better option for fruit preservation as it provides a thin layer barrier against oxygen, carbon dioxide, moisture, pathogens, and light, thus delaying ripening and senescence (Pillai *et al*., 2024). Conventional packaging materials are usually composed of petroleum-based polymers such as low-density polyethylene (LDPE), polyethylene terephthalate (PET), polypropylene (PP), polyamide (PA), and polycarbonate (PC), which can lead to a significant environmental problem (Chettri *et al*., 2023). An appropriate alternative to plastic packaging is edible coatings, which can be consumed along with the food. These coatings are made from naturally occurring biopolymers including lipids, proteins, carbohydrates, and composites with excellent mechanical and rheological characteristics (Calderón-Santoyo *et al*., 2022; Pandya *et al*., 2023; Peerzada *et al*., 2023). Among these biopolymers is gelatin, which is usually used in edible coatings applied to fresh products (Estrella-Ozuna *et al*., 2024), sodium alginate, a polysaccharide widely used in the food industry (Íñiguez-Moreno *et al*., 2021), and alginate-chitosan bilayer films (Acuña-Pacheco *et al*., 2024).

Additionally, acidic sugar chains derived from galactose, such as pectins, are structural constituents in plant cells that provide support and protection by being a part of the defense system against phytopathogens (Rohasmizah and Azizah, 2022; Patil *et al*., 2023; Jhanani *et al*., 2024). This biological polymer forms a stable hydrogel that can perform a film on fruit surfaces, acting as a support for many additives such as antioxidants, dyes, texture improvers, phytoalexins, or antagonists of phytopathogenic fungi, such as lactic acid bacteria and yeasts (Aguilar-Veloz *et al*., 2022; Bomzon, 2022; Pandya *et al*., 2023). In recent years, antifungal edible coatings have emerge as an effective approach to mitigate vegetable and fruit loss caused by postharvest fungal diseases by forming a semipermeable film on the surface, which reduces mechanical damage and protects against fungal infections (González-Estrada *et al*., 2017; Zhou *et al*., 2023).

Meyerozyma guilliermondii (anamorph *Candida guilliermondii*) is an ascomycetous yeast whose biocontrol activity has been demonstrated against several fungal diseases on fruits, such as *Colletotrichum gloeosporioides* from mango fruit (López-Cruz *et al*., 2020), *Penicillium digitatum* and *P. italicum* from orange, *P. expansum*, *Rhizopus stolonifer* and *Botrytis cinerea* from apples and pears (López-Cruz *et al*., 2023), *Lasiodiplodia theobromae*, *Rhizopus* sp. and *Neofusicoccum batangarum* from jackfruit (Ayón-Macias *et al*., 2023) and, *B. cinerea* and *Cladosporium cladosporioides* from blueberries (Covarrubias-Rivera *et al*., 2024). *M. guilliermondii* exhibits multi-mode mechanisms of action, including competing for space and nutrients, production of cell wall-degrading enzymes, volatile organic compounds, induction of several defense-related genes, and parasitism (Yan *et al*., 2021; Ayón-Macias *et al*., 2023; Cheng *et al*., 2023; Herrera-Balandrano *et al*., 2023; López-Cruz *et al*., 2023).

Functionalized edible coatings using biopolymers with entrapped antagonistic yeasts play an important role in the fungal disease control trends during postharvest management, improving their efficiency and viability, being a sustainable and eco-friendly technology (Iñiguez-Moreno *et al*., 2021; Vázquez-González *et al*., 2024). An ideal edible coating must meet certain basic characteristics such as permeability properties to water vapor and gases to avoid an anaerobic environment, breaking strength, melting above 40 °C, low viscosity and non-sticky faces, being translucent, without affecting the quality of fruits and vegetables (Chettri *et al*., 2023; Miranda *et al*., 2024). Some edible coating characteristics could potentially be modified by the addition of biocontrol agents. Hence, there is still research needed to ensure the filmogenic capacity and compatibility of biopolymeric films and antimicrobials (Nunes *et al*., 2023). Based on these foundations, this is the first time the yeast *M. guilliermondii* LMA-CP01 was added to pectin-based edible-coatings. The objective of this work was to formulate pectin-base gels using calcium chloride as a cross-linking agent and glycerol as a plasticizer to produce support matrix edible films. The film-forming dispersions were characterized by low deformation rheology. Furthermore, the physical and mechanical properties and antifungal activity of films were studied.

MATERIALS AND METHODS

Materials and antagonistic strain

Low methoxyl pectin LA 410 was purchased from Danisco (Guadalajara, México), and glycerol and calcium chloride tetrahydrate were purchased from Sigma-Aldrich (Darmstard, Germany).

M. guilliermondii LMA-Cp01 strain was isolated from the papaya surface (*Carica papaya* L.) (LIIA, Instituto Tecnológico de Tepic) and stored in glycerol at -80°C. The yeast was inoculated in Trypticase Soy Broth (TSB) (BD Bioxon, Mexico) and harvested after three days at 28° C at 150 rpm. The stock solution of *M. guilliermondii* was transferred to a test tube with 10 mL of sterile TSB and maintained at 28 °C, 200 rpm for 48 h. Subsequently, this solution was incubated in a flask with 400 mL of TBS for 24 h under the same conditions. The yeast solution was centrifuged at 3000 xg for 10 min, in a Z326 K universal centrifuge (HERMLE, Wehingen, Germany) and resuspended in sterilized NaCl 0.85% solution. Yeasts were adjusted at $1x10^8$ cells/mL using a Neubauer chamber.

Film formation

Dispersions of pectin were prepared at 1% (w/v), then glycerol was added at 50% (w/w) related to pectin dry matter basis, and finally, calcium chloride tetrahydrate as a cross-linking agent was dosed at different concentrations (3.3, 2.7, 2.0, 1.3 and 0.5 mM) (Santana and Kieckbusch, 2013). After obtaining

the ideal concentration of calcium chloride that allowed the form of a weak gel with a continuous phase (0.5 mM), the obtained film-forming solution was placed in a Petri dish and kept in a desiccator (casting method) to obtain the film of cross-linked pectin with calcium. Similarly, cross-linked gels of pectin were prepared, and once the dispersion presented a homogeneous phase, the yeast *M. guilliermondii* solution was added at $1x10^8$ cell mL^{-1} to obtain the films of pectin + *M. guilliermondii* (Fig. 1).

Rheological characterization

Film-forming dispersions of pectin and pectin + *M. guilliermondii* were characterized on a hybrid rheometer Discovery HR-2 (TA instruments, New Castle, USA) using a cone-plate geometry (angle 2° 1′ 1″, 60 mm diameter). Frequency sweep (ω) was performed from 0.1 to 100 rad/s at 25°C and sweep time was performed during 180 min at 1 rad/s at 25°C (Phan *et al*., 2023).

Fourier-Transform Infrared Spectroscopy (FTIR)

Infrared spectra of pectin, pectin films, and pectin + *M. guilliermondii* films were analyzed using a spectrophotometer Nicolet FT-IR iS50 (Thermo Fisher Scientific, Waltham, USA) by a reflection technique (ATR) and recorded between 4000 to 500 cm^{-1}.

Color

Films were evaluated with CIE colorimeter CIE $L^*a^*b^*$ scale, using total color differences (ΔE^*_{ab}) according to equation 1:

$$\Delta E^*_{ab} = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad (1)$$

Where ΔL^*, Δa^*, *and* Δb^* are the differences between each color value of standard color plate and film samples, respectively. Color differences produced by films were evaluated with colorimeter CR-400 of Konica-Minolta (Tokyo, Japan), comparing surfaces with three tonalities: green (L^*= 33.83, a^*= -22.04, b^*=11.52), yellow (L^*= 82.57 a^*= -4.90 b^*= 32.52) and orange (L^*= 51.11 a^*= 40.03 b^*=28.78); readings by overlaying films over the same surfaces, simulating color changes from physiological to maturity stage of papaya fruit surface.

Thickness

The film thickness was determined as the average of 30 measurements for each sample using a Mitutoyo micrometer (293-230) of MAC (Aurora, USA) (Iñiguez-Moreno *et al*., 2021).

Films mechanical properties

Tensile data was obtained using a Stable brand texturometer Micro Systems TA-XT2 (Godalming, UK). Film samples (wide 1 cm and 5 cm long) were cut. Initial grip separation and cross-head speed were set at 3 cm and 0.05 cm/s, respectively. Tensile strength (RT), percent elongation at break (%E) and elastic modulus (Y) were calculated according to Jantrawut *et al*. (2017).

Environmental scanning electron microscope (ESEM)

Morphological examination of films was conducted by environmental scanning electron microscope ESEM (EVO LS10) of Carl Zeiss (Jena, Germany). Cross-sectional areas of films were analyzed. The cross-sectional microstructure of both films were observed after cryo-fragmentation. The samples were placed on a support at 90° and observed at 2000x and 8000x, at 40 Pa and with a voltage of 15 kV for the samples without yeast, and at 5000x and 15000x, 25 Pa, 15 kV and work distance 7.0 mm for the one with *M. guilliermondii*.

***In vitro* antifungal activity of films**

The antifungal activity of *M. guilliermondii* and pectin-based films were evaluated *in vitro* against the pathogen *Colletotrichum gloeosporioides* from papaya fruit. *C. gloeosporioides* was cultured on potato dextrose agar (PDA) (BD Bioxon, Mexico) plates for 7 days at 28 °C. Conidial suspension was adjusted to 10^5 spores/mL by counting in a hemocytometer using an optic microscope (BA310 Motic, USA). 50 mL of fungal spores were spread onto PDA disks (8 mm) and let dry for 15 min. After that, the disks were covered with films of pectin

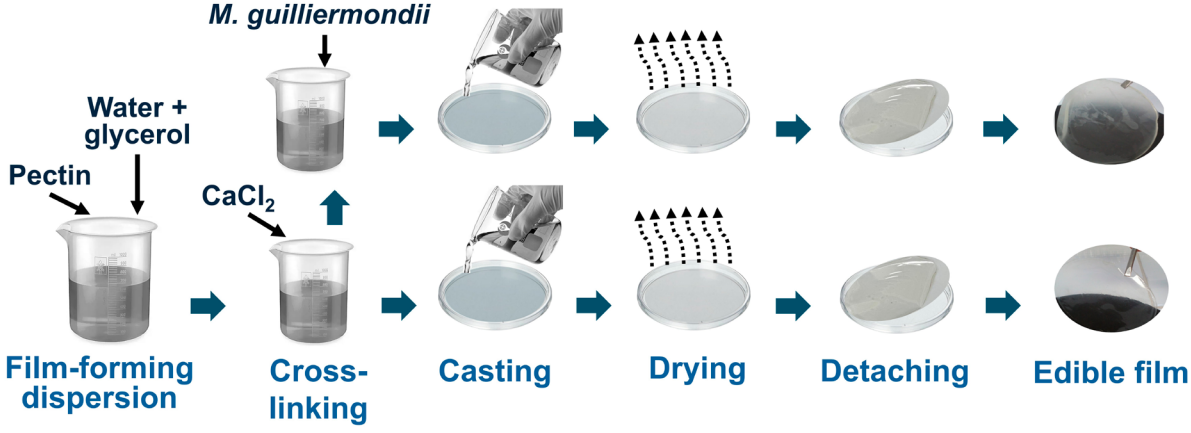

Figure 1. Schematic representation of edible films obtention with and without *M. guilliermondii*.
Figura 1. Representación esquemática de la obtención de películas comestibles con y sin *M. guilliermondii*.

or pectin + yeast and incubated at 28 °C. The germination rate of *C. gloeosporioides* was determined by microscopy by measuring the percentage of germinated spores for 12 h at 28°C in samples of approximately 200 spores. A spore was considered germinated when its germ tube was longer than half the length of the spore. The inhibition percentage was calculated according to Equation 2 (González-Estrada *et al*., 2017; Iñiguez-Moreno *et al*., 2020).

$$\%\ Inhibition = \frac{spores\ control - \ spores\ with\ treatment}{spores\ control} * 100 \ldots\ldots\ldots\ldots (2)$$

The mycelial growth inhibition of films against *C. gloeosporioides* was also evaluated. Plugs of 8 mm in diameter of fungal mycelia from 7 days old were placed at the center of PDA plates. Then, the plates were covered with films containing or not the yeasts and incubated at 28 °C. Petri plates were used as a positive control with only the fungi. The inhibition of mycelial growth was calculated according to Equation 3. The experiment was performed with three replicates and each experiment was repeated twice.

$$\%\ Inhibition = \frac{mycelial\ growth\ control - \ mycelial\ growth\ treatment}{mycelial\ growth\ control} * 100 \ldots\ldots\ldots\ldots (3)$$

Statistical analysis

Data analysis was carried out by Student's t-test using the statistical software STATISTICA v. 12.0 (StatSoft Inc., Tulsa, OK, USA). The analysis of variance (ANOVA) and Tukey's HSD test were used for comparing mean values of percentage inhibition of *C. gloeosporioides.* Statistical differences significance was judged at the level of $p < 0.05$.

RESULTS AND DISCUSSION

The main controlling parameter to achieve film-forming dispersions was the dose concentration of calcium chloride. The gel formulated with the lowest concentration of crosslinking agent (Ca^{2+} 0.5 mM) exhibited a more uniform phase in its maturity stage, facilitating pouring into the non-stick Petri dishes. This established a $CaCl_2$ concentration of 0.5 mM as the optimal condition for the film formation process using the casting method. At higher concentrations, the formation of agglomerates of macroscopic size was observed due to the high reactivity of the low-methoxyl pectin solution with divalent calcium cations. These agglomerates persist in the polymeric structure of the formed films, creating a disordered structure with a discontinuous texture.

Rheological properties

Gel-forming solution characterization is fundamental for the development of edible films to improve film uniformity (Shivangi *et al*., 2021). Gelation kinetics are shown in Fig. 2A. Graph depicts a constant increment on the elastic modulus enhancing a maximum point that resulted in a flexible gel or dispersion of cross-linked polymers as a result of the continuity in the formation of a structure with a low force. Damping material values, or tan δ, showed values lower than 1 (Fig. 2A). This fact confirms the gel state in both solutions used for

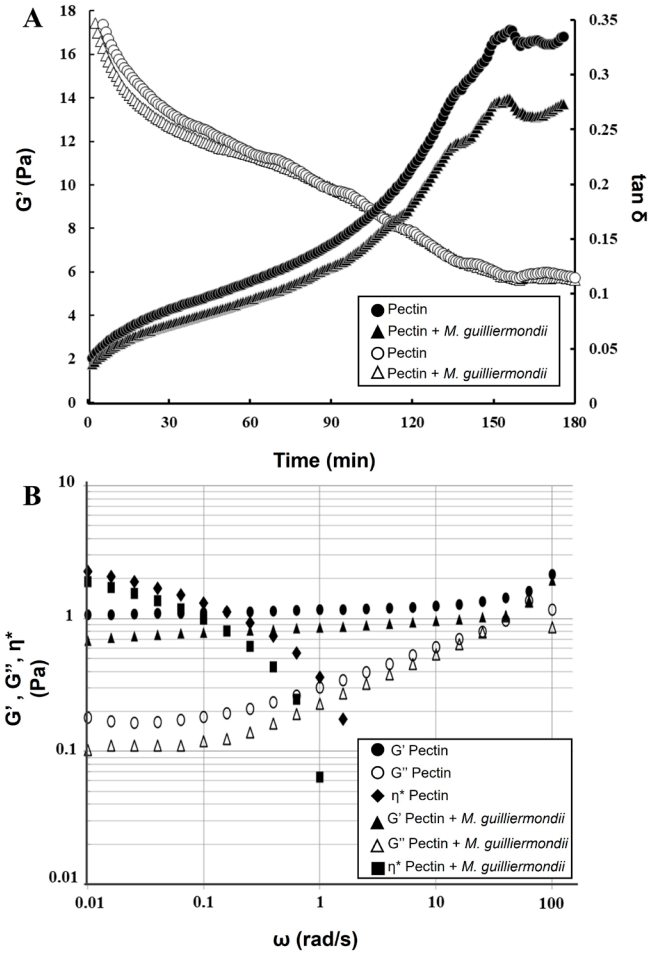

Figure 2. Gelation kinetics (A) on film-forming dispersions of pectin and pectin + *M. guilliermondii* and damping material values (tan δ) vs film-forming dispersions time at 25 °C and 1 rad/s, and frequency sweep (B) on film-forming dispersions of pectin and pectin + *M. guilliermondii* with 1% (w/v) of pectin and 0.5 mM of $CaCl_2$ at 25 °C and 1 rad/s.
Figura 2. Cinética de gelificación (A) en dispersiones formadoras de película de pectina y pectina + *M. guilliermondii*, y valores de material amortiguador (tan δ) vs tiempo de dispersiones formadoras de película a 25 °C y 1 rad/s, y barrido de frecuencia (B) en dispersiones formadoras de película de pectina y pectina + *M. guilliermondii* con 1% (p/v) de pectina y 0.5 mM de $CaCl_2$ a 25 °C y 1 rad/s.

film formation (Abbastabar *et al*., 2015), indicating that the particles are strongly associated with colliding forces. When the tan δ value is around 0.01 and 0.1, the structure of the gel is more flexible and deformable and not very susceptible to syneresis because in the frequency sweep (Figure 2B), G' and G" maintain a 10:1 ratio.

Fig. 2B depicts the behavior of film-forming dispersions of pectin and pectin + *M. guilliermondii* subjected to a frequency sweep. It is appreciated that elastic moduli G' predominates for both the pectin gel and the one containing the yeast. This condition implies that the analyzed solutions have both a viscoelastic behavior, typical of fluids that obey Maxwell's model (Hütter *et al*., 2020; Tseng and Chang, 2024), which indicates that the gels will flow like a liquid and will be propitious, from the rheological point of view, to form films

by the casting method or spraying to form a coating, but also have viscosity and adhesion to the surfaces by contact and thus, they are optimal for application by immersion.

Fig. 2B also illustrates how the storage module is independent of the shear rate during the test, while the loss module changes its behavior at frequencies close to 100 rad/s. At low frequencies and extended oscillation periods, the macromolecular chains can unravel, and the system behaves like a solution. Conversely, at high frequencies and short time interval, the macromolecular chain remains entangled, causing the system to display solid-like behavior (Okay, 2009). This phenomenon is evidenced by analyzing the behavior of the complex viscosity (η*). When at rest, the gel without yeast exhibits a significantly higher viscosity due to its more ordered structure ($p < 0.05$). As the shear frequency increases up to 1 rad/s, the complex viscosity of both gels fluctuates around values close to zero (Patova *et al*., 2017). The decrease in G′ and G″ values in the gel containing the yeasts is attributable to the microbial cells diminishing the strength of the molecular structure by interfering with the formation of binding sites. The behavior of the studied gels at high frequencies is characteristic of true gels, and at G′ values below 10 Pa, structural stability is assumed (Vityazev *et al*., 2020). This finding suggests that the gel is less prone to syneresis, avoiding the formation of an aqueous phase and preventing the proliferation of microorganisms. It is noticed that G′ > G″, a characteristic typical of cross-linked polymer gels, indicating their ability to absorb water without fully dissolving. In addition, it can be inferred that the structure is compact because there is a 10:1 ratio between the elastic and viscous modulus, respectively (Patova *et al*., 2017).

Molecular interactions by FTIR

Fig. 3 shows IR spectra of (A) pure pectin, (B) pectin film, and (C) pectin + *M. guilliermondii* film. All the IR spectra were normalized to unity, taking the most intense band as a reference band, which appears around 3200 cm^{-1} in all spectra. The three spectra showed similar signals: (i) an intense broad band located at 3200 cm^{-1} caused by stretching vibrations of the hydroxyl groups (absorption in the O-H region was due to inter- and intra-molecular hydrogen bonding of the galacturonic acid backbone) (Abid *et al*., 2017), (ii) a shoulder around 2905 cm^{-1} that is related to C-H absorption, these include CH, CH_2, and CH_3 stretching and bending vibrations, (iii) a fingerprint region located below 1500 cm^{-1} and (iv) a region between 1800 and 1500 cm^{-1}, depicted in the inset of the figure. To observe the cross-linking effect, spectra A and B are depicted since this allows the observation of infrared absorption by the carboxylic acid and the carboxylic ester groups of the pectin molecules. At 1726 cm^{-1} (esterified carboxyl groups) and 1600 cm^{-1} (free carboxyl groups), characteristic weak signals from bands of low methoxyl pectin are observed. Ca^{2+} dosage in pure pectin was able to displace the band centered on 1600 cm^{-1} (inset: spectrum A) to 1609 cm^{-1} (inset: spectrum B). This shift could be due to the modification of the functional group environment, suggesting an interaction

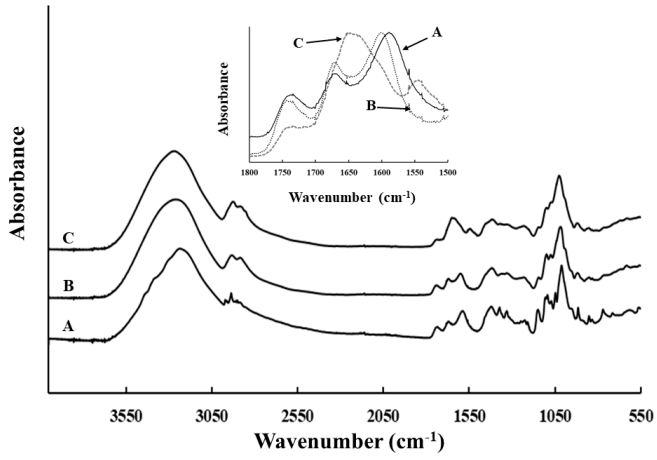

Figure 3. FTIR spectra of: pure pectin (A), film of pectin (B), and film of pectin + *M. guilliermondii* (C). Inset: partial IR spectrum on the region from 1500 to 1800 cm^{-1}.
Figura 3. Espectro FTIR de: pectina pura (A), película a base de pectina (B) y película a base de pectina + *M. guilliermondii* (C).

between free carboxyl groups from pure pectin and calcium ions that could generate sites that promote *M. guilliermondii* entrapment. From early studies, electrostatic interactions exist between Ca^{2+} and COO^- in the 'egg-box' model (Cao *et al*., 2020). As an electron-withdrawing ion, calcium induces a charge deviation, which results in a change in the band vibration. In addition, the ordering and arrangement of junction zones may be different under various gel conditions. Alternatively, on the spectrum C (inset), amide bands centered at 1643 cm^{-1} and 1542 cm^{-1} (amide I and amide II absorptions, respectively) were revealed. These bands are attributable to the vibrations of the amide groups from proteins and indicate their secondary structure. These signals are attributed by the amount of *M. guilliermondii* entrapped into the cross-linked pectin. These characteristic absorptions of yeasts on the spectrum A and B were not observed (Mihoubi *et al*., 2017). Returning to section (i), the band assigned to galacturonic acids, which are sources of substrate for *M. guilliermondii*, no important modifications are observed in this signal ca. 3200 cm^{-1} (spectrum B and C). These results suggest that a good coupling between the cross-linked pectin and the yeasts could be used as edible coatings.

Optical properties

Both films (pectin and pectin + *M. guilliermondii* films) exhibited a uniform superficial macroscopic texture. The film of pectin is translucent (Fig. 4A) due to the carbohydrate properties and occurs because they are capable of forming crystals that allow light waves to break through; and the one that contains the biocontrol agent is opaque with a yellowish tone, as reported by Iñiguez-Moreno *et al*. (2021). These characteristics in films with biocontrol agents (Fig. 4B) are attributed to their lipid and protein constituents, which are not translucent.

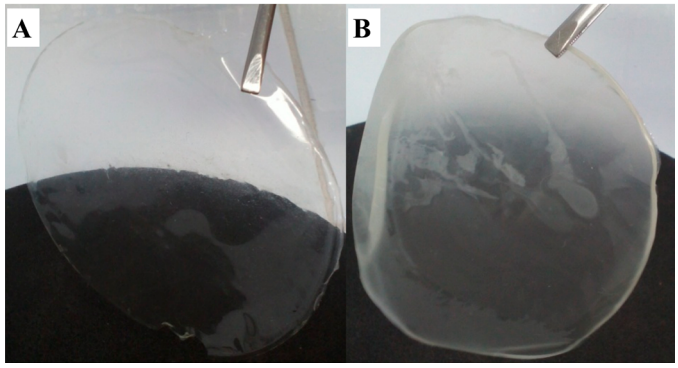

Figure 4. Films of pectin (A) and pectin + *M. guilliermondii* (B).
Figura 4. Películas a base de pectina (A) y pectina + *M. guilliermondii* (B).

The analysis of the films formed degree influencing the differential ΔE* are reported on Table 1. The color variation is less than 5 in the yellow spectrum, as a consequence of the transparency and coloration presented by the films formed, however, ΔE* exceeds the minimum perceptible in the orange and green tones. Therefore, the application of a pectin coating with *M. guilliermondii* will produce a change in the perceptible coloration in the zones of the papaya fruit that present these tonalities. Consequently, the application of a coating added with *M. guilliermondii* as a biocontrol agent will significantly modify the appearance of the fruit, except in cases where it presents a yellow tone, corresponding to an intermediate stage of maturity between physiological and consumer stages of papaya fruit.

Thickness

Films formed from the pectin gel without the biocontrol agent presented an average of 0.0343 mm in its cross-section and, for those containing *M. guilliermondii* have an average thickness of 0.0206 mm, significantly different ($p \leq 0.05$). The presence of the yeasts is presumably the cause in the decrease of the thickness, since these interrupt the continuity of the molecular structure in the films, decreasing the areas of cross-linking points with calcium ions between the chains of galacturonic acids, modifying the structure of the film as well as the quantity and location of the union points (Barbut and Harper, 2019).

Morphology by ESEM

Micrographs of film without *M. guilliermondii* showed smooth uniform morphology (Fig. 5A and B). The effect that yeast addition on the structure of polymer matrix on the cross-sectional area, shows irregular longitudinal zones, which evidenced the entrapment of *M. guilliermondii* (Fig. 5C and D). Probably, yeasts are negatively charged in their cell-wall, forming clusters that produce a different polymeric structure regarding to the original matrix with the cross-linked pectin (Casamorin *et al*., 2014). Nevertheless, yeast cells were homogeneously distributed in pectin film.

Tensile properties

Films added with *M. guilliermondii* proved to withstand almost twice as much force before yielding to rupture (F_{max}), increasing the tensile strength (RT) above 3x. The percent elongation at break (%E) is also increased by 30% because the yeasts decrease the crystallinity of the pectin film, and in turn, a diminish in the longitudinal elastic modulus was observed (Y). These values are shown in Table 2. The increase in tensile properties may be due to the interactions between pectin chains and yeast wall components, as was evidenced in FTIR analysis. The same pattern was reported by Iñiguez-Moreno *et al*. (2021).

Antifungal activity

The results of the inhibition of *C. gloeosporioides* are shown in Table 3. The pectin-based film without yeast did not inhibit the spore's germination or mycelial growth. Iñiguez-Moreno *et al*. (2020) reported that sodium alginate films did not inhibit the growth of *C. gloeosporioides* from avocado fruit, but, the films with the yeast *Meyerozyma caribbica* reached more than 99% of inhibition.

The yeast *M. guilliermondii* completely inhibited the germination and mycelial growth of *C. gloeosporioides*, as the same of the pectin film added with the yeast. The biocontrol efficacy of *M. guilliermondii* against several phytopathogenic fungi has been previously reported. Its biocontrol effectiveness is due to different mechanisms of action such as competing for space and nutrients, production of hydrolytic

Table 1. Mean values of L*a*b* and differential ΔE*.
Tabla 1. Valores promedio de L*a*b* y diferencial ΔE*.

	Film of pectin			**Film of pectin + *M. guilliermondii***		
	Green	**Yellow**	**Orange**	**Green**	**Yellow**	**Orange**
L*	42.52 ± 0.81[b]	81.37 ± 1.37[a]	54.81 ± 0.89[a]	37.47 ± 0.68[a]	80.31 ± 1.40[a]	53.95 ± 0.99[a]
a*	13.58 ± 0.61[b]	-3.64 ± 0.51[a]	32.68 ± 1.36[a]	-17.29 ± 0.85[a]	-3.90 ± 0.59[a]	33.47 ± 0.89[a]
b*	7.56 ± 0.59[b]	30.31 ± 0.95[a]	22.84 ± 0.93[a]	9.22 ± 0.89[a]	30.69 ± 1.03[a]	23.26 ± 0.78[a]
ΔE*	6.4 ± 0.5[b]	3.0 ± 0.3[a]	9.0 ± 1.1[a]	12.7 ± 0.5[a]	2.8 ± 0.3[a]	10 ± 1.2[a]

Values are expressed as means ± standard deviation (n = 15). Values in columns with the same name, followed by different lowercase letters, are significantly different according to Student's t-test at $p < 0.05$.

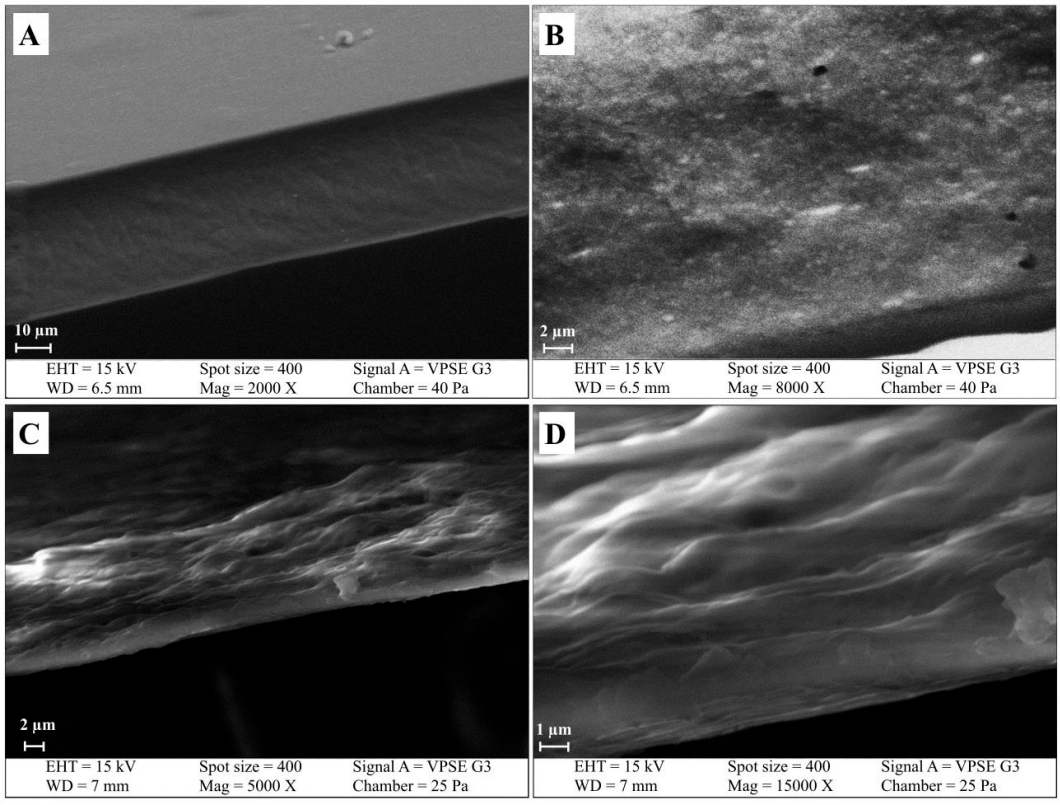

Figure 5. Cross-sectional area of films obtained by environmental scanning electron microscopy (ESEM) of pectin at 2000 x (A) and negative at 8000x (B) and, pectin + *M. guilliermondii* at 5000 x (C) and 15000 x (D).
Figura 5. Área de sección transversal de películas obtenidas mediante microscopio electrónico de barrido ambiental (ESEM) de pectina a 2000 x (A) y 8000x (B), y de pectina + *M. guilliermondii* a 5000x (C) y 15000x (D).

Table 2. Films tensile data of pectin and pectin + *M. guilliermondii*.
Tabla 2. Datos de tracción de películas de pectina y pectina + *M. guilliermondii.*

Film	F_{max} (N)	RT (MPa)	%E	Y (N/mm²)
Pectin	7.14 ± 0.55[b]	7.0 ± 0.6[b]	12 ± 0.9[b]	0.178 ± ±0.06a
Pectin + *M. guilliermondii*	13.41 ± 0.61[a]	22.35 ± 2.78[a]	43 ± ±2.1[a]	0.104 ± ±0.05a

Values are the mean ± standard deviation (n=5). Different letters in the same column indicate significant differences according to Student´s t-test ($p < 0.01$). Fmax= Maximum force. RT= tensile strength.

Table 3. Antifungal activity of films against *C. gloeosporioides*
Tabla 3. Actividad antifúngica de las películas contra *C. gloeosporioides*

Treatment	Percentage of inhibition	
	Spore's germination	Mycelial growth
M. guilliermondii	100 ± 0.0 a	100 ± 0.0 a
Film of pectin	0 ± 0.0 b	0 ± 0.0 b
Film of pectin + *M. guilliermondii*	100 ± 0.0 a	100 ± 0.0 a

Values are expressed as means ± standard deviation (n = 6). Values in columns with different lowercase letters are significantly different according to Tukey's HSD test at $p < 0.05$.

enzymes and volatile organic compounds (Ayón-Macías *et al*., 2025). The efficacy of the film added with *M. guilliermondii* demonstrates that pectin-based edible film is a support matrix for entrapped yeast to control pathogenic fungi.

CONCLUSIONS

A pectin-based gel combined with glycerol, using calcium chloride as a cross-linking agent was prepared, and exhibited rheological properties that facilitate its application through spraying or fruit immersion to create edible coatings. The

resulting films can serve as a support matrix for biocontrol agents such as *M. guilliermondii*. The incorporation of *M. guilliermondii* into the pectin-based films positively influenced their mechanical properties and thickness, while also demonstrating antifungal activity against *C. gloeosporioides* in papaya fruit. Pectin-based films enriched with *M. guilliermondii* present a potential alternative for controlling the phytopathogenic fungus *C. gloeosporioides*. Further studies on the application of pectin-based edible coatings containing *M. guilliermondii* on fruits are necessary.

CONFLICTS OF INTEREST

The authors declared they have no conflicts of interest.

REFERENCES

Abbastabar, B., Azizi, M.H., Adnani, A. and Abbasi, S. 2015. Determining and modeling rheological characteristics of quince seed gum. *Food Hydrocolloids*, 43, 259–264.

Abid, M., Cheikhrouhou, S., Renard, C.M.G.C., Bureau, S., Cuvelier, G., Attia, H. and Ayadi, M.A. 2017. Characterization of pectins extracted from pomegranate peel and their gelling properties. *Food Chemistry*, 215, 318–325.

Aguilar-Veloz, L.M., Calderón-Santoyo, M., Carvajal-Millan, E., Martínez-Robinson, K. and Ragazzo-Sánchez, J.A. 2022. *Artocarpus heterophyllus* Lam. leaf extracts added to pectin-based edible coating for *Alternaria* sp. control in tomato. *LWT*, 156, 113022.

Ayón-Macías, K.D., Ragazzo-Sánchez, J.A. and Calderón-Santoyo, M. 2025. Antagonistic effect of co-cultures involving *Meyerozyma caribbica* and *M. guilliermondii* against postharvest pathogens of jackfruit: Understanding the mechanisms behind their action. *Physiological and Molecular Plant Pathology*, 136, 102528.

Ayón-Macias, K.D., Ragazzo-Sánchez, J.A., Narváez-Zapata, J.A., Damasceno-Gomes, S. and Calderón-Santoyo, M. 2023. *Meyerozyma* strains as biocontrol agents against postharvest phytopathogens of jackfruit (*Artocarpus heterophyllus* Lam.). *Archives of Phytopathology and Plant Protection*, 56 (15), 1180–1204.

Barbut, S. and Harper, B.A. 2019. Dried Ca-alginate films: Effects of glycerol, relative humidity, soy fibers, and carrageenan. *LWT*, 103, 260–265.

Bomzon, P.S. 2022. Sources, origin and characterization of edible packaging. *In*: *Edible Food Packaging*. Singapore: Springer Nature Singapore, 27–37.

Calderón-Santoyo, M., Solis-Velazquez, O.A., Ragazzo-Sánchez, J.A. and Iñiguez-Moreno, M. 2022. Animal- and plant-based edible food packaging for perishable foodstuff. *In*: *Edible Food Packaging*. Singapore: Springer Nature Singapore, 39–85.

Cao, L., Lu, W., Mata, A., Nishinari, K. and Fang, Y. 2020. Egg-box model-based gelation of alginate and pectin: A review. *Carbohydrate Polymers*, 242, 116389.

Casamorin, J., Bennett, R. and Dedeles, G. 2014. Biosorption of Cd (II) by yeasts from ripe fruit peels in the Philippines. *Journal of Health Pollution*, 4 (7), 14–24.

Cheng, L., Zhou, L., Li, D., Gao, Z., Teng, J., Nie, X., Guo, F., Wang, C., Wang, X., Li, S. and Li, X. 2023. Combining the biocontrol agent *Meyerozyma guilliermondii* with UV-C treatment to manage postharvest gray mold on kiwifruit. *Biological Control*, 180, 105198.

Chettri, S., Sharma, N. and Mohite, A.M. 2023. Edible coatings and films for shelf-life extension of fruit and vegetables. *Biomaterials Advances*, 154, 213632.

Covarrubias-Rivera, L., Ragazzo-Sánchez, J.A., González-Gutiérrez, K.N., Narváez-Zapata, J.A. and Calderón-Santoyo, M. 2024. Isolation and identification of phytopathogenic fungi from blueberry (*Vaccinium corymbosum*) and their biocontrol by *Meyerozyma guilliermondii* LMA-Cp01. *Journal of Plant Pathology*, 107 (1), 525–536.

González-Estrada, R.R., Carvajal-Millán, E., Ragazzo-Sánchez, J.A., Bautista-Rosales, P.U. and Calderón-Santoyo, M. 2017. Control of blue mold decay on Persian lime: Application of covalently cross-linked arabinoxylans bioactive coatings with antagonistic yeast entrapped. *LWT - Food Science and Technology*, 85, 187–196.

González-Gutiérrez, K.N., Ragazzo-Sánchez, J.A. and Calderón-Santoyo, M. 2024. Field and postharvest application of microencapsulated *Yamadazyma mexicana* LPa14 : anthracnose control and effect on postharvest quality in avocado (*Persea americana* Mill. cv. Hass). *Pest Management Science*, 80 (7), 3459–3469.

Herrera-Balandrano, D.D., Wang, S.-Y., Wang, C.-X., Shi, X.-C., Liu, F.-Q. and Laborda, P. 2023. Antagonistic mechanisms of yeasts *Meyerozyma guilliermondii* and *M. caribbica* for the control of plant pathogens: A review. *Biological Control*, 186 (April), 105333.

Hütter, M., Carrozza, M.A., Hulsen, M.A. and Anderson, P.D. 2020. Behavior of viscoelastic models with thermal fluctuations. *The European Physical Journal E*, 43 (5), 24.

Iñiguez-Moreno, M., Ragazzo-Sánchez, J.A., Barros-Castillo, J.C., Solís-Pacheco, J.R. and Calderón-Santoyo, M. 2021. Characterization of sodium alginate coatings with *Meyerozyma caribbica* and impact on quality properties of avocado fruit. *Lwt*, 152 (April).

Jantrawut, P., Chaiwarit, T., Jantanasakulwong, K., Brachais, C. and Chambin, O. 2017. Effect of plasticizer type on tensile property and *in vitro* indomethacin release of thin films based on low-methoxyl pectin. *Polymers*, 9 (7), 289.

Jhanani, G.K., AlSalhi, M.S., T, N. and Shanmuganathan, R. 2024. As assessment of shelf life increasing competence of pectin (Zucchini) based edible coating on tomatoes. *Environmental Research*, 258, 119368.

Kaur, J., Gunjal, M., Rasane, P., Singh, J., Kaur, S., Poonia, A. and Gupta, P. 2022. Edible packaging: An overview. *In*: *Edible Food Packaging*. Singapore: Springer Nature Singapore, 3–25.

López-Cruz, R., Ragazzo-Sánchez, J.A. and Calderón-Santoyo, M. 2020. Microencapsulation of *Meyerozyma guilliermondii* by spray drying using sodium alginate and soy protein isolate as wall materials: a biocontrol formulation for anthracnose disease of mango. *Biocontrol Science and Technology*, 30 (10), 1116–1132.

López-Cruz, R., Segarra, G., Torres, R., Teixidó, N., Ragazzo-Sanchez, J.A. and Calderon-Santoyo, M. 2023. Biocontrol efficacy of *Meyerozyma guilliermondii* LMA-Cp01 against post-harvest pathogens of fruits. *Archives of Phytopathology and Plant Protection*, 56 (13), 1003–1020.

Mihoubi, W., Sahli, E., Gargouri, A. and Amiel, C. 2017. FTIR spectroscopy of whole cells for the monitoring of yeast

apoptosis mediated by p53 over-expression and its suppression by *Nigella sativa* extracts. *PLOS ONE*, 12 (7), e0180680.

Miranda, M., Bai, J., Pilon, L., Torres, R., Casals, C., Solsona, C. and Teixidó, N. 2024. Fundamentals of edible coatings and combination with biocontrol agents: A strategy to improve postharvest fruit preservation. *Foods*, 13 (18), 2980.

Nunes, C., Silva, M., Farinha, D., Sales, H., Pontes, R. and Nunes, J. 2023. Edible coatings and future trends in active food packaging–fruits' and traditional sausages' shelf life increasing. *Foods*, 12 (17), 3308.

Pandya, Y., Sharma, A. and Bakshi, M. 2023. Edible coatings in fruits: Effectiveness and applicability : A review. *FoodSci: Indian Journal of Research in Food Science and Nutrition*, 10 (1), 1–10.

Patil, V., Shams, R. and Dash, K.K. 2023. Techno-functional characteristics, and potential applications of edible coatings: A comprehensive review. *Journal of Agriculture and Food Research*, 14, 100886.

Patova, O.A., Golovchenko, V.V., Vityazev, F.V., Burkov, A.A., Belyi, V.A., Kuznetsov, S.N., Litvinets, S.G. and Martinson, E.A. 2017. Physicochemical and rheological properties of gelling pectin from Sosnowskyi's hogweed (*Heracleum sosnowskyi*) obtained using different pretreatment conditions. *Food Hydrocolloids*, *65*, 77–86.

Peerzada, J., Sinclair, B.J., Perinbarajan, G.K., Dutta, R., Shekhawat, R., Saikia, N., Chidambaram, R. and Mossa, A.-T. 2023. An overview on smart and active edible coatings: safety and regulations. *European Food Research and Technology*, 249 (8), 1935–1952.

Phan, C.-M., Ross, M., Fahmy, K., McEwen, B., Hofmann, I., Chan, V.W.Y., Clark-Baba, C. and Jones, L. 2023. Evaluating viscosity and tear breakup time of contemporary commercial ocular lubricants on an *in vitro* eye model. *Translational Vision Science & Technology*, 12 (6), 29.

Pillai, A.R.S., Eapen, A.S., Zhang, W. and Roy, S. 2024. Polysaccharide-based edible biopolymer-based coatings for fruit preservation: A Review. *Foods*, 13 (10), 1529.

Porat, R., Lichter, A., Terry, L.A., Harker, R. and Buzby, J. 2018. Postharvest losses of fruit and vegetables during retail and in consumers' homes: Quantifications, causes, and means of prevention. *Postharvest Biology and Technology*, 139 (September 2017), 135–149.

Rohasmizah, H. and Azizah, M. 2022. Pectin-based edible coatings and nanoemulsion for the preservation of fruits and vegetables: A review. *Applied Food Research*, 2 (2), 100221.

Santana, A.A. and Kieckbusch, T.G. 2013. Physical evaluation of biodegradable films of calcium alginate plasticized with polyols. *Brazilian Journal of Chemical Engineering*, 30 (4), 835–845.

Shivangi, S., Dorairaj, D., Negi, P.S. and Shetty, N.P. 2021. Development and characterisation of a pectin-based edible film that contains mulberry leaf extract and its bio-active components. *Food Hydrocolloids*, 121, 107046.

Tseng, H.-C. and Chang, R.-Y. 2024. The identification of the generalised Maxwell fluid for n -hexadecane liquids via non-equilibrium molecular dynamics simulations. *Molecular Simulation*, 50 (6), 463–469.

Vázquez-González, Y., Prieto, C., Calderón-Santoyo, M., Ragazzo-Sánchez, J.A. and Lagarón, J.M. 2024. Development of antifungal electrospun nanofiber mats containing *Meyerozyma caribbica*. *Food Hydrocolloids*, 147, 109343.

Vityazev, F.V., Khramova, D.S., Saveliev, N.Y., Ipatova, E.A., Burkov, A.A., Beloserov, V.S., Belyi, V.A., Kononov, L.O., Martinson, E.A., Litvinets, S.G., Markov, P.A. and Popov, S.V. 2020. Pectin–glycerol gel beads: Preparation, characterization and swelling behaviour. *Carbohydrate Polymers*, 238, 116166.

Yan, W., Gao, H., Qian, X., Jiang, Y., Zhou, J., Dong, W., Xin, F., Zhang, W. and Jiang, M. 2021. Biotechnological applications of the non-conventional yeast *Meyerozyma guilliermondii*. *Biotechnology Advances*, 46, 107674.

Zhou, C., Bai, J., Zhang, F., Zhang, R., Zhang, X., Zhong, K. and Yan, B. 2023. Development of mussel-inspired chitosan-derived edible coating for fruit preservation. *Carbohydrate Polymers*, 321, 121293.