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Electrosprayed hydroxypropyl methylcellulose microcapsules containing *Rhus microphylla* fruit extracts and their application in strawberry (*Fragaria × ananassa*) preservation

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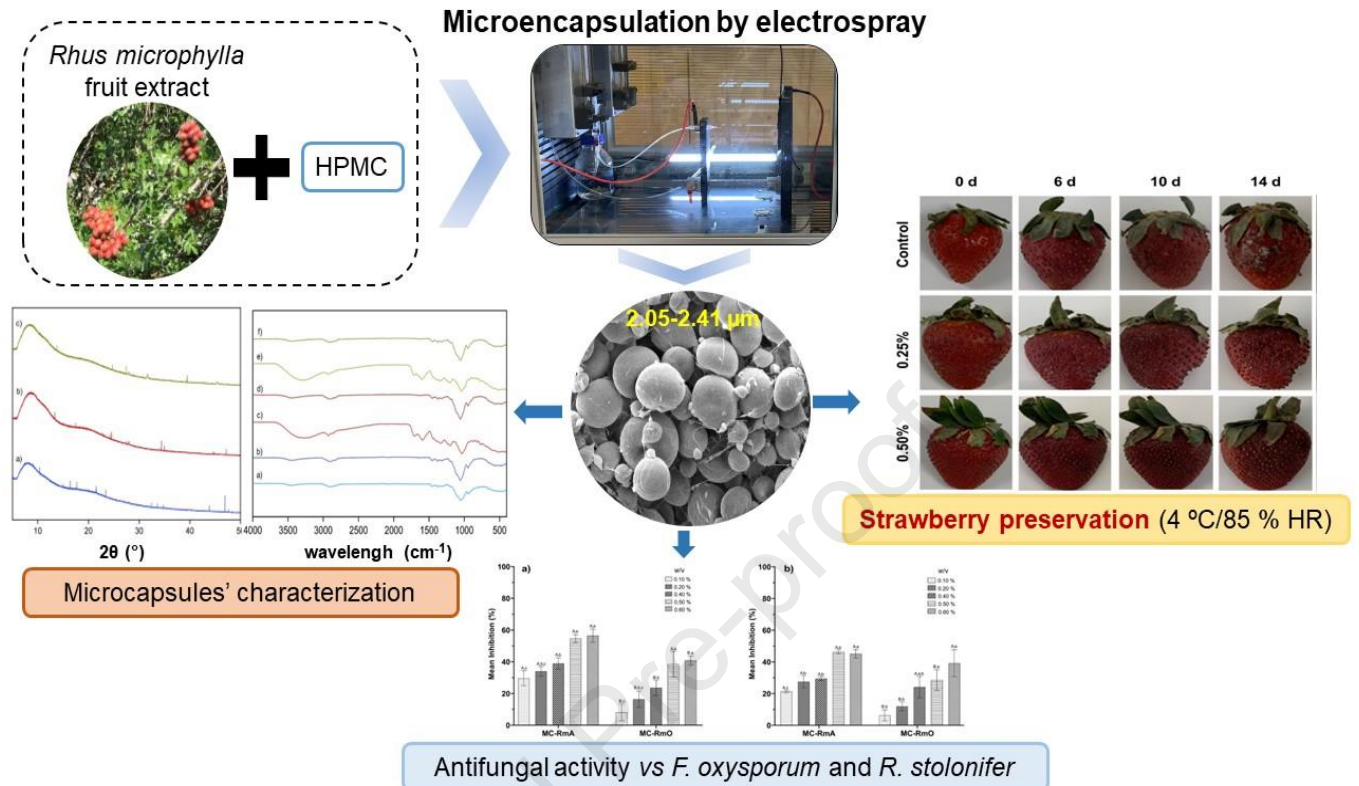
**Jorge L. Guía-García:** Methodology, Investigation, Writing – original draft, preparation. **Ana V.**

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## Graphical abstract



1 **Electrosprayed hydroxypropyl methylcellulose microcapsules containing *Rhus microphylla* fruit**  
2 **extracts and their **application** in strawberry (*Fragaria x ananassa*) preservation**

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23 **Abstract**

24 Encapsulation technology is used to incorporate a wide range of compounds, which is beneficial for  
25 protecting and improving the bioactivity of plant extracts. In this study, the objectives were to develop  
26 hydroxypropyl methylcellulose microcapsules containing two different extracts from *Rhus microphylla* fruit  
27 namely RmA (obtained by conventional agitation) and RmO (obtained by ohmic heating) using  
28 electrohydrodynamic processing. The microcapsules were then characterized through Scanning Electron  
29 Microscopy (SEM), ATR-Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), and  
30 thermogravimetric analysis (TGA). Additionally, the study aimed to evaluate their influence on strawberry  
31 quality. Spherical microcapsules with a particle size of 2.05-2.41  $\mu\text{m}$  were successfully obtained, and FTIR  
32 analysis confirmed the proper incorporation of the extracts. The microcapsules containing RmA extract  
33 (MC-RmA) exhibited superior antioxidant and antifungal activities *in vitro*. Consequently, their efficacy in  
34 preserving the quality of strawberry fruits during storage at  $4\pm 1$  °C and 85% relative humidity (RH) was  
35 evaluated at concentrations of 0.25% and 0.50% (w/v). After 14 days, the MC-RmA-treated fruits showed  
36 reduced weight loss, improved firmness, and unchanged color. Additionally, the gradual release of  
37 antifungal activity from MC-RmA suggests its potential as a novel solution to mitigate postharvest losses in  
38 strawberry fruits.

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40 **Keywords:** *Rhus microphylla*; electrospraying; microcapsules; strawberry; shelf life

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## 48 1. Introduction

49 Encapsulation is a process where a polymeric matrix surrounds another material, providing an  
50 enhancement and protection of its bioactivity, namely for compounds susceptible to degradation (Machado  
51 et al., 2019). It has been used in solids, liquids, and gaseous compounds (Papoutsis et al., 2018), mainly  
52 for the encapsulation of flavors (Dalmolin et al., 2016), aromas (Sanchez-Reinoso et al., 2017) and,  
53 recently, plant extracts (Pereira et al., 2018), demonstrating the versatility of this technology to be applied  
54 in various industries, such as: pharmaceutical, agri-food, among others. The size, shape, and functionality  
55 of the encapsulates strongly depend on the coating materials used, which can be mostly synthetic polymers  
56 (e.g., polycaprolactone and polyethylene glycol), gums (e.g., arabica gum and xanthan gum), proteins  
57 (e.g., zein and sodium caseinate) and polysaccharides (e.g., starch and maltodextrin) (Danafar, 2017;  
58 Sablania et al., 2018; Liu et al., 2019a). Also, the technique used has an important effect on the  
59 characteristics of the structures produced. Different techniques have been reported for the **obtention** of  
60 encapsulates, such as: spray-drying (Medina-Torres et al., 2019; Nunes et al., 2020), emulsification (Ishkeh  
61 et al., 2021), layer-by-layer (Pinheiro et al., 2015), coacervation (Ursache et al., 2018), and  
62 electrohydrodynamic processing (Bhushani et al., 2017), which differ in their mechanism and conditions of  
63 use, because there is no universal procedure that covers all core types and combinations of wall materials  
64 (Pellicer et al., 2019).

65 Electrohydrodynamic processing is a novel technique to produce capsules and fibers in micro and  
66 nanoscale and that can be used in two modes, electrospinning for the production of fibers and  
67 electrospraying to produce particles (Silva et al., 2022). Electrospraying presents some advantages  
68 comparing to others methods; for example, it is possible to encapsulate thermolabile compounds without  
69 affecting their integrity, it has a lower energy consumption, and it also provides greater homogeneity in the  
70 shape and particle size of the structures produced (Gómez-Mascaraque et al., 2017). During  
71 electrospraying process, the polymer solution containing the compounds of interest is atomized into a  
72 collector through a capillary employing a high electric field, where the electric energy promotes the  
73 atomization by a deformation of the droplet at the tip of the capillary nozzle, forming a structure known as  
74 Taylor cone (Silva et al., 2021). Proper atomization and formation of the Taylor cone is ensured by

75 employing electrical forces higher than the surface tension forces of the encapsulating solution (Nikoo et  
76 al., 2018). Electro spraying has been used for the microencapsulation of bioactive compounds such as  
77 anthocyanins (Atay et al., 2018),  $\beta$ -carotene (Gómez-Mascaraque et al., 2017), and curcumin (Gómez-  
78 Estaca et al., 2017), demonstrating its effectiveness to produce homogeneous structures using different  
79 wall materials and concentrations.

80 On the other hand, Mexico has a vast biodiversity of plants, being of great interest the plants that grow in  
81 arid and semi-arid zones, due to their phytochemical content (Vega-Ruiz et al., 2021), antioxidant  
82 (Santiago-Mora et al., 2017), antifungal (Charles-Rodríguez et al., 2020), and antiproliferative properties  
83 (López-Romero et al., 2018). Some extracts from *Larrea tridentata* and *Flourensia cernua* have shown  
84 noteworthy antifungal effects against *Rhizoctonia solani* (Castillo et al., 2010), while extracts from  
85 *Myrtillocactus geometrizans* showed interesting anti-hyperglycemic and anti-inflammatory activities *in vitro*  
86 (Montiel-Sánchez et al., 2021). The genus *Rhus*, belonging to the family Anacardiaceae is composed of  
87 about 35 species (Yi et al., 2007). Extracts of some of these species have shown remarkable antioxidant  
88 (Bursal & Köksal, 2011; Wu et al., 2013; Liu et al., 2019b), antifungal (Jasso de Rodríguez et al., 2015;  
89 Charles-Rodríguez et al., 2020), and anticancer properties (Kim et al., 2019). Nonetheless, the use of crude  
90 plant extracts is limited because they tend to be highly susceptible to degradation under certain  
91 environmental conditions, such as extreme temperatures, humidity, and light (Muhoza et al., 2019; Al-  
92 Maqtari et al., 2021). In this context, encapsulation has proven to be an excellent tool to protect the integrity  
93 and activity of bioactive compounds (e.g., phenolic compounds) and plant extracts by the formation of  
94 micro- or nanocapsules that have been effective in extending the shelf life of some fruits, such as avocado  
95 (Correa-Pacheco et al., 2017), bell pepper (González-Saucedo et al., 2019), tomato (Gutiérrez-Molina et  
96 al., 2021), and strawberry (Hesami et al., 2021), among others.

97 Strawberry (*Fragaria x ananassa*) is a widely consumed and appreciated worldwide fruit for its flavor and  
98 multiple nutritional benefits (e.g., antioxidant, anti-aging, and anti-tumor properties), representing a valuable  
99 economic market, with Mexico being the third largest exporter of fresh strawberries (Müller et al., 2010;  
100 Morales-Mora et al., 2019; Li et al., 2020). However, strawberries are highly perishable during postharvest  
101 due to their sensitivity to injuries and fungal infections, which affect their quality (e.g., firmness, color, flavor),

102 thus causing important product losses (Chu et al., 2020). The application of new technologies, such as the  
103 development of encapsulates containing bioactive plant extracts through electrospraying, emerges as an  
104 alternative to improve the postharvest quality of fruits and vegetables. Therefore, the aims of the present  
105 study were to develop and characterize microcapsules containing *R. microphylla* fruit extracts using food-  
106 grade hydroxypropyl-methylcellulose (HPMC), by means of electrospraying, and to evaluate their effect on  
107 the postharvest decay of strawberries, as model fruit. It is noteworthy that this is the first report about the  
108 development of HPMC microcapsules through electrospray containing *R. microphylla* fruit extract and the  
109 study of their effect on strawberry preservation.

## 110 2. Materials and methods

### 111 2.1. Materials and reagents

112 Hydroxypropyl-methylcellulose (methoxyl 28-30 %, hydroxypropyl 7-12 %, viscosity 2 % aqueous solution,  
113 viscosity range of 40-60 mPa/s, at 20 °C, 90kDa, CAS 9004-65-3) was purchased from Alfa Aesar GmbH  
114 & Co KG (Karlsruhe, Germany). Folin-Ciocalteu reagent (FC), 2,2-diphenyl-1-picryl hydrazyl (DPPH, CAS  
115 1898-66-4), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS, CAS 30931-67-  
116 0), 2,4,6-tri(2-pyridyl)-striaizine (TPTZ, CAS 3682-35-7), iron (III) chloride hexa-hydrate (CAS 10025-77-1),  
117 ascorbic acid (AA, CAS 50-81-7), potassium persulfate (K<sub>2</sub>S<sub>2</sub>O<sub>8</sub>, CAS 7727-21-1), sodium carbonate  
118 (Na<sub>2</sub>CO<sub>3</sub>, CAS 497-19-8) and gallic acid monohydrate (GA, CAS 5995-86-8) were purchased from Sigma-  
119 Aldrich (Steinheim, Germany). Absolute ethanol (>99.5%, CAS 64-17-5) was purchased from Honeywell  
120 (North Carolina, USA) and Sabouraud dextrose broth (SDB) was purchased from PanReac AppliChem  
121 (Darmstadt, Germany).

122 Strawberries (*Fragaria x ananassa*) var. Festival were obtained from local market (Saltillo, Coahuila,  
123 Mexico), twelve hours after harvesting and immediately transported to the laboratory of the Universidad  
124 Autónoma Agraria Antonio Narro (UAAAN). Fruit with uniform color and size, without physical damage or  
125 fungal infection were selected.



126 In this work, two hydroalcoholic *R. microphylla* fruit (Rm) extracts obtained by conventional agitation (RmA)  
127 and ohmic heating (RmO) were used for encapsulation tests, selected based on their outstanding  
128 antioxidant and antifungal capacities determined in previous work (Guía-García et al., 2021).

## 129 *2.2. Preparation of polymer solutions containing Rm extracts and electro spraying conditions*

130 For the selection of the most appropriate encapsulation conditions, different amounts of extracts and  
131 ethanol concentrations were tested (Table 1). The HPMC concentration (3.0 %, w/v) was selected based  
132 on a preliminary study (Silva et al., 2021). The work solutions were prepared by dissolving the specific  
133 amount of extract in the ethanol solution, then, HPMC was slowly added and mixed.

134 The equipment used for the electro spraying process was a Fluidnatek<sup>®</sup> LE-50 (Bioinicia S.L, Valencia,  
135 Spain) equipped with a variable high voltage power supply (0-30 kV). The solutions were placed in 10 mL  
136 plastic syringes (TERUMO<sup>®</sup>, Leuven, Belgium) coupled to a digitally controlled syringe pump and  
137 connected by a polytetrafluoroethylene tube to a blunt stainless-steel needle with a diameter of 0.60 mm  
138 (20 ga, FISNAR<sup>®</sup>, Glasgow, United Kingdom). The electro spraying process was performed in horizontal  
139 mode with a temperature and relative humidity (RH) maintained in a range between 20-25 °C and 45-65  
140 %, respectively. The flowrate and the distance between the needle and the collector were constant in all  
141 experiments based on preliminary tests (0.5 mL/h and 17 cm, data not shown). Voltage varied between 12-  
142 25 kV ensuring correct Taylor cone formation in all experiments.

## 143 *2.3. Microcapsules characterization and bioactivity*

### 144 *2.3.1. Morphology and particle size of microcapsules*

145 To select the best encapsulation conditions, the surface morphology of the particles obtained was examined  
146 by Scanning Electron Microscope (SEM) (Quanta FEG 650, FEI, USA). Briefly, 1.0-2.0 mg of sample were  
147 deposited on a double-sided conductive carbon tape, then analyzed at an acceleration voltage of 3.0 kV  
148 with a working distance of ~10 mm. After selecting the best treatment for each extract, 1.0-2.0 mg of specific  
149 samples were coated with gold under vacuum for 1 min (EM ACE200, Leica Microsystems Inc. Wetzlar,  
150 Germany) and analyzed in the SEM, with a voltage of 5 kV at the same working distance. The morphology  
151 of at least 150 microcapsules was analyzed using ImageJ software (version 1.53k, Maryland, USA), and

152 the particle size and the particle aspect ratio (PAR) were determined. PAR was calculated with the following  
153 equation:

$$154 \quad PAR = \frac{\text{Particle height}}{\text{Particle length}} \quad (1)$$

### 155 2.3.2. ATR-Fourier transform infrared (FTIR) spectroscopy analysis

156 FTIR assay was employed to analyze the bonding arrangements and functional groups of the constituents  
157 present in free and encapsulated extracts to determine the possible interactions. For the analyses, a Bruker  
158 FT-IR VERTEX 80/ 80v (Boston, USA) in Attenuated Total Reflectance mode (ATR) with a platinum crystal  
159 was used to obtain the FTIR spectra. The measurements were recorded from 4000 to 400  $\text{cm}^{-1}$   
160 wavenumber range, at a resolution of 4  $\text{cm}^{-1}$  and 32 scans.

### 161 2.3.3. X-Ray diffraction analysis (XRD)

162 XRD assay was performed to determine the presence of crystalline polymorphisms in the samples  
163 employing an X-Ray Diffractometer X Pert PRO MRD system (Malvern Panalytical Ltd., Royston, UK). The  
164 analyses were carried out at room temperature, and samples were observed at a voltage of 45 kV and  
165 40 mA using angular scans from 5.0° to 50° (2 $\theta$ ) with a Cu source, X-ray tube ( $\lambda$  of 1.54056 Å). The  
166 information was collected during 174 s. For 2 $\theta$  the fine calibration offset was -0.0372°.

### 167 2.3.4. Thermogravimetric analysis (TGA)

168 Measurements were performed using a simultaneous thermal analyzer and a differential scanning  
169 calorimeter (TGA/DSC 3+, Mettler Toledo, Columbus, USA). Each sample (2.5 mg) was placed in the  
170 equipment's scale on an alumina crucible, and heated at rate of 5 °C/min. The heating was from 30 to 500  
171 °C under a nitrogen atmosphere.

### 172 2.3.5. Extract release from microcapsules

173 To determine the extract release from the microcapsules (MC-RmA and MC-RmO), samples were treated  
174 using two treatments: ultrasound (U) or agitation (A). For the ultrasonic bath release, the methodology of  
175 Šturm et al. (2019) was followed with some modifications. Firstly, 10 mg of microcapsules were placed in  
176 0.5 mL of milli-Q water and sonicated for 5 min. Then, the solutions were centrifuged at 13,300 rpm for 15

177 min. In the second method, the same concentration was used, but the solutions were kept in agitation for 1  
178 h. The supernatant of the solutions was used for TPC, DPPH, ABTS, and FRAP assays.

### 179 2.3.6. Total phenolic content (TPC) by Folin-Ciocalteu

180 The TPC released from the microcapsules was determined using the Folin-Ciocalteu (FC) method, following  
181 the methodology of Müller et al. (2010) with minor modifications. Twenty microliters of the supernatant were  
182 mixed with 100  $\mu\text{L}$  of diluted FC solution (1:10 v/v, in water) for 5 min in a 96-well microplate, and 75  $\mu\text{L}$  of  
183  $\text{Na}_2\text{CO}_3$  (7.5 % w/v) were added. The reaction was incubated for 5 min at 40  $^\circ\text{C}$ , cooled and kept at room  
184 temperature for 30 min more under dark conditions. The absorbance was measured at 750 nm on a Sinergy  
185 H1 Hybrid Reader microplate equipment (Biotek, Vermont, USA), and the values were compared with a GA  
186 calibration curve (2.5-200 mg/L,  $R^2=0.9994$ ). The results were expressed as mg GA equivalents per gram  
187 of microcapsules (mg GA/g MC). All experiments were performed in quadruplicate.

### 188 2.3.7. Radical scavenging capacity

#### 189 2.3.7.1. DPPH radical scavenging activity

190 The scavenging capacity for DPPH was measured according to the method described by Guía-García et  
191 al. (2021), with minor modifications. Twenty-five microliters of the supernatant were placed in a 96-well  
192 microplate and mixed with 200  $\mu\text{L}$  of DPPH solution (150  $\mu\text{M}$ , dissolved in absolute ethanol). The reaction  
193 was incubated at room temperature for 30 min under dark conditions. The absorbance was measured at  
194 520 nm in a Sinergy H1 Hybrid Reader microplate equipment (Biotek, Vermont, USA), using absolute  
195 ethanol as control. The scavenging capacity was expressed as percentage of Radical Scavenging Activity  
196 (%RSA), using the following equation:

$$197 \quad RSA (\%) = \left( \frac{A_{control} - A_{sample}}{A_{control}} \right) \times 100 \quad (2)$$

198 where  $A_{control}$ = control absorbance and  $A_{sample}$ = sample absorbance. All assays were carried out in  
199 quadruplicate.

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### 202 2.3.7.2. ABTS radical scavenging activity

203 The ABTS assay was performed based on the method of Jesus et al. (2019), with minor modifications. The  
204 ABTS solution was prepared at concentration of 7 mM in milli-Q water and mixed with a potassium  
205 persulfate solution (2.45 mM) (1:1), the mixture was kept during 14-16 h at 4 °C under dark conditions to  
206 complete the reaction. Then, 10 µL of the supernatant were mixed with 200 µL of ABTS solution (adjusted  
207 with ethanol at 20 % to an absorbance of  $0.700 \pm 0.010$  at 734 nm) in a 96-well microplate and incubated  
208 for 10 min under dark conditions at room temperature. The absorbance was measured at 734 nm in a  
209 Sinergy H1 Hybrid Reader microplate equipment (Biotek, Vermont, USA), using water as control. The  
210 results were expressed as %RSA as described in section 2.2.7.1 according to Equation 2. All experiments  
211 were conducted by quadruplicate.

### 212 2.3.7.3. Ferric reducing capacity by FRAP assay

213 The ferric reducing capacity of the microcapsules content was evaluated following the method described  
214 by Guo & Jauregi (2018), with minor modifications. In a microcentrifuge tube was added 5 µL of the  
215 supernatant and mixed for 15 s with 150 µL of FRAP reagent (83.33 % of acetate buffer (300 mM), 8.33 %  
216 of TPTZ (10 mM) in HCl 40 mM, and 8.33 % of ferric chloride hexahydrate aqueous solution (20 mM)).  
217 Then, 100 µL were transferred to a 96-well microplate and the absorbance was measured at 595 nm in a  
218 Sinergy H1 Hybrid Reader microplate equipment (Biotek, Vermont, USA). The results were expressed as  
219 ascorbic acid equivalents (AA), using an ascorbic acid standard curve (1.5-400 mg/L,  $R^2=0.9992$ ). All  
220 assays were made by quadruplicate.

### 221 2.3.8. Antifungal properties

#### 222 2.3.8.1. Fungal strains

223 The *Fusarium oxysporum* strain (NCBI, accession no. MT001892) was acquired by CICY (Yucatan Center  
224 for Scientific Research, Yucatan, Mexico) and *Rhizopus stolonifer* strain (CDBC accession no. 1384) was  
225 purchased from CINVESTAV (Center for Research and Advanced Studies of the National Polytechnic  
226 Institute, CDMX, Mexico).

227

228 2.3.8.2. *Microdilution assay*

229 The antifungal activity was made following the method report by Flores-López et al. (2016) with minor  
230 modifications. First, spore's suspensions of each strain were prepared by pouring a sterile Tween-80  
231 solution (0.1%, w/w) onto a Petri dish containing 7-day-old fungi to release the spores. Then, the  
232 suspensions were mixed, and the spores were counted using a Neubauer chamber. Subsequently, sterile  
233 broth was added to the spore suspension to obtain the desired concentration of  $10^4$  spores/mL. After this,  
234 different amounts of microcapsules (0.10, 0.20, 0.30, 0.40, 0.50, and 0.60 %, w/v) were diluted with 100  $\mu$ L  
235 of SDB and placed in a sterile 96-well microplate, followed by the addition of 100  $\mu$ L of a spore's suspension  
236 of each strain. A positive control of 100  $\mu$ L of SDB and 100  $\mu$ L of spore's suspension was used. The samples  
237 were mixed and incubated at  $25 \pm 2$  °C for 36 h, the fungal growth was measured by changes in the optical  
238 density (OD) at 530 nm in a Sinergy H1 Hybrid Reader microplate equipment (Biotek, Vermont, USA). The  
239 percentage of growth inhibition (%) was calculated through Equation 3:

$$240 \quad \text{Inhibition (\%)} = \left( \frac{OD_{control} - OD_{sample}}{OD_{control}} \right) \times 100 \quad (3)$$

241 where  $OD_{control}$ , represents the optical density of the control and  $OD_{sample}$  represents the optical density of  
242 each treatment. All experiments were carried out in triplicate.

243 2.4. *Effect of microencapsulated extracts on strawberry fruit decay*

244 To evaluate the effect on strawberry fruit decay, only the microcapsules containing RmA were selected, as  
245 they presented the best *in vitro* results of antioxidant and antifungal activities.

246 A coating containing microcapsules (RmA) was prepared using a structured water vehicle, previously  
247 optimized for application in berries: 0.24 % (w/v) of lyophilized chia mucilage, 0.15 % (w/v)  $\text{CaCl}_2$  and  
248 0.05 % (w/v) glycerol (Charles-Rodríguez et al., 2021). Three treatments were evaluated: uncoated  
249 (control); coating with 0.25 % (w/v) and coating with 0.50 % (w/v) of microcapsules containing RmA,  
250 respectively. The treatments were applied on strawberry fruit by aspersion and left to dry in a convection  
251 oven at 25 °C for 25 min (Biobase Biodustry Shandong Co, Ltd., Jinan, SHG, China). For each treatment,  
252 three repetitions of 10 strawberries were evaluated (n=30, per treatment), the fruits were placed in

253 performed polypropylene plastic trays and stored at  $4 \pm 1$  °C and 85 % RH for 14 d. Physicochemical and  
 254 decay evaluations were analyzed at regular intervals (0, 2, 4, 6, 8, 10, 12, and 14 d).

## 255 2.5. Physicochemical analyses

### 256 2.5.1. Weight loss

257 Weight loss of strawberries (n=30, per treatment) during storage was evaluated by means of the mass  
 258 changing every two days in each fruit using an analytical balance (Ohaus, New Jersey, USA), and the  
 259 results were expressed as percentage using the following equation:

$$260 \quad \text{Weight loss (\%)} = \frac{W_0 - W_d}{W_0} \times 100 \quad (4)$$

261 where  $W_0$  is the initial weight, and  $W_d$  is the respective weight of every test day.

### 262 2.5.2. Texture analyses

263 The firmness of fruit was measured two times at different center region of seven fruit per replicate of each  
 264 treatment at day 0 and 14 of the experiment. A texture analyzer CT3 (Brookfield, USA), equipped with a 6  
 265 mm diameter size cylindrical probe was used. The conditions were the following: trigger force of 0.05 N,  
 266 penetration depth of 5.0 mm and test speed of 5.0 mm/s. The results were expressed in Newtons (N).

### 267 2.5.3. Color

268 The change in color parameters ( $L^*$ ,  $a^*$  and  $b^*$ ) of the strawberry surface was measured using a Minolta  
 269 colorimeter (CR-400, Minolta, Tokyo, Japan) every two days. The readings were made in two different  
 270 points on the fruit surface. The results were reported in function of chromaticity ( $C^*$ ), hue angle ( $H^*$ ) and  
 271 redness values ( $a^*/b^*$ ), calculated by the following equations (Quintana et al., 2021; Salas-Méndez et al.,  
 272 2019):

$$273 \quad C^* = \sqrt{(a^{*2}) + (b^{*2})} \quad (5)$$

$$274 \quad H^* = \tan^{-1} \left( \frac{b^*}{a^*} \right) \quad (6)$$

$$275 \quad \text{Redness} = \left( \frac{a^*}{b^*} \right) \quad (7)$$

#### 276 2.5.4. Fungal decay

277 For fungal decay evaluation, the stored strawberries (n=30) were visually inspected for the presence of  
278 mold growth every 2 d, and any fruit with visible spoilage was considered affected. The following equation  
279 was used to calculate the fungal decay percentage in each treatment (Quintana et al., 2021):

$$280 \quad \text{Fungal decay (\%)} = \frac{\text{Number of decay fruit}}{\text{Total number of fruit}} \times 100 \quad (8)$$

#### 281 2.6. Statistical analysis

282 The results were expressed as means  $\pm$  standard deviations. Minitab software version 17.0 (State College,  
283 PA, USA) and GraphPad Prism version 8.0.1 (La Jolla California, USA) were used for data analyses. One-  
284 way analyses of variance (ANOVA) were used to detect any significant differences followed by Tukey's  
285 mean comparison test ( $p < 0.05$ ).

### 286 3. Results and discussion

#### 287 3.1. Morphology and particle size of microcapsules

288 The ethanol and the extract amount were determining factors in the selection of the encapsulation  
289 conditions; the use of 50 % ethanol did not allow a correct evaporation of the solvent, leading to droplets,  
290 and the use of higher extract concentrations increased the presence of droplets in the samples (data not  
291 shown). On the other hand, with a concentration of 75 % ethanol and 1 mg/mL of extract and voltage of 14  
292 and 17 kV, the best structures were obtained for both the RmA and RmO extracts (T4 and T8, respectively).  
293 Fig. 1 shows that these processing conditions allowed to produce homogeneous and spherical structures  
294 with a smooth surface. **This was confirmed** by the PAR results (Table 2), where structures with values  
295 closer to 1 are more closely related to spherical shapes **for the encapsulates** (Silva et al., 2021). **On the**  
296 **other hand, the particle size ranged from 2.05 to 2.41  $\mu\text{m}$  as indicated in Table 2. This size range classifies**  
297 **the samples as microcapsules, given that they fall within the typical range of 1-1000  $\mu\text{m}$**  (Shishir et al.,  
298 2018). The significative differences in the particle size between MC-RmA and MC-RmO could be explained  
299 by their components which can influence in the conductivity of the solutions and affect the particle size  
300 (Bhushani et al., 2017). These results are in agreement with those obtained in the encapsulation of ferulic

301 acid, where spherical microcapsules were obtained through spray-drying using HPMC as wall material,  
302 having a suitable incorporation of phenolic acid within the structures (Yu et al., 2021).

### 303 3.2. ATR-FTIR analyses

304 In Fig. 2 is shown the FTIR spectra of HPMC powder, empty MC-HPMC, and the free (RmA and RmO) and  
305 encapsulated extracts (MC-RmA and MC-RmO). For the HPMC powder and MC-HPMC, the peak around  
306  $3460\text{ cm}^{-1}$  corresponds to the  $\text{—OH}$  stretching vibration, and the presence of  $\text{—CH}$  aliphatic stretching  
307 vibrations was confirmed by the absorption peak at  $2908\text{ cm}^{-1}$ , while the two absorption peaks of  $1454$  and  
308  $1371\text{ cm}^{-1}$  could be attributed to the  $\text{—CH}_3$  asymmetric vibrations (Sheng et al., 2021). Besides, it was  
309 observed a strong peak around  $1060\text{--}1020\text{ cm}^{-1}$  corresponding to the  $\text{—CO}$  stretching vibrations in all the  
310 samples (Wang et al., 2021). On the other hand, for unencapsulated extracts (RmA and RmO), the region  
311 around  $3270\text{--}3300\text{ cm}^{-1}$  indicated the  $\text{—OH}$  stretching vibrations from phenolic compounds and ethanol  
312 (extraction solvent), whereas the absorption peak of  $2926\text{ cm}^{-1}$  corresponds to the symmetric aliphatic  
313 stretching vibrations ( $\text{—CH}_2$ ) (Hu et al., 2019). The absorption peak at  $1709\text{ cm}^{-1}$  is related to the  $\text{—C=O}$   
314 stretching, and in the region of  $1590\text{ cm}^{-1}$  the absorption peak corresponds to the aromatic ring stretching  
315 (Zhao et al., 2022). However, in the microcapsules containing RmA and RmO some minor changes in the  
316 spectra occurred, as the absorption peaks ( $1590\text{--}1700\text{ cm}^{-1}$ ) of extracts were covered, indicating that  
317 extracts were correctly incorporated within the microcapsules (Sheng et al., 2021). Moreover, the intensity  
318 of the absorption peak around  $3270\text{--}3330\text{ cm}^{-1}$  (presented in the extracts) decreased, and it was displaced  
319 to the spectral area of  $3460\text{ cm}^{-1}$ . This could be explained by the hydrophobic interactions or hydrogen  
320 bonds formation between the polymer and the phenolic compounds from the extracts (Moreno et al., 2018).  
321 The FTIR results confirm the correct encapsulation of extracts inside the HPMC microcapsules produced  
322 by electrospray, providing protection, and reducing their susceptibility to environmental conditions.

### 323 3.3. XRD analyses

324 The XRD analysis is useful to identify the degree of crystallinity in samples, where a crystalline material  
325 exhibits specific and well-defined peaks in the diffractogram, while an amorphous material shows a rounded



326 and diffuse peak (Papoutsis et al., 2018). Amorphous materials have higher water-solubility and  
327 hygroscopicity compared with crystalline materials (Botrel et al., 2014).

328 Fig. 3A shows the X-ray diffractograms of the HPMC microcapsules and those containing RmA and RmO  
329 extracts. In general, all samples exhibited a diffuse peak around  $2\theta=8^\circ$  and, according to the shape of the  
330 peak in the diffractograms (i.e., long and flattened), all samples also showed an amorphous structure.  
331 These results are in agreement with Yu et al. (2021) that encapsulated ferulic acid using HPMC by spray-  
332 drying, observing a single diffuse and broad peak, representative of amorphous materials. These types of  
333 structures are desirable, as amorphous structures usually have higher fluidity and solubility (Dalmolin et al.,  
334 2016); meanwhile, crystalline structures dissolve more slowly because only the surface exposed to the  
335 solvent tends to dissolve first (Ban et al., 2020).

#### 336 3.4. TGA analyses

337 Commonly, the bioactive compounds present in plant extracts are thermally unstable; in specific,  
338 polyphenols are very sensitive to high temperatures, which can cause the breakdown of the glucosyl moiety  
339 of the aglycone present in these compounds, altering the bioactivity and bioavailability of the natural  
340 compound (Bedrníček et al., 2020).

341 Thermal analysis can provide information about the thermal stability of the samples and shows the amount  
342 of moisture and volatile compounds present in microparticles; and also, the thermal breakdown of the wall  
343 polymers (İnan & Özçimen, 2021). In Fig. 3B, the stage of major degradation occurred between 300-360 °C,  
344 and it is associated with the depolymerization and thermal breakdown of the polymer (Cho et al., 2019).

345 The results demonstrated that the HPMC polymer can effectively protect the *R. microphylla* extracts from  
346 high temperatures, thereby preventing their thermal degradation.

#### 347 3.5. TPC and antioxidant capacity of microcapsules

348 The different bioactive properties of microcapsules containing Rm extract depend on the effective release  
349 of the compounds from the polymeric matrix, since an unsuccessful release could cause a decrease in their  
350 bioactivity. Several works have reported the correlation between the TPC and the antioxidant capacity of  
351 plant extracts, as phenolic compounds present functional groups able to interact with the corresponding

352 molecules in each antioxidant assay (DPPH, ABTS, FRAP, etc.) (Xu et al., 2007; López-Romero et al.,  
353 2018).

354 Two treatments (ultrasound and agitation) were conducted to allow the release of the content of the  
355 microcapsules, and the results are presented in Table 3. In the case of MC-RmA, there were no significant  
356 differences between the treatments used for TPC and antioxidant capacity. However, for MC-RmO, the  
357 samples using only agitation presented a higher TPC and better antioxidant capacities ( $p<0.05$ ) compared  
358 with the microcapsules treated with ultrasounds. This difference might be caused by the effect of sonication  
359 on phenolic compounds, because the cavitation may cause a slight degradation generating hydroxyl  
360 radicals (Aguilar-Villalva et al., 2021; Kaderides et al., 2019; Martins Strieder et al., 2019). In addition, MC-  
361 RmA showed the higher values of TPC and the highest RSA values for DPPH and ABTS assays. As  
362 previously reported by Guía-García et al. (2021), RmA extract is composed of a more complex structure  
363 (gallic acid, p-cumaric+epicatechin, catechin, ferulic acid, ellagic acid and resveratrol) than RmO (gallic  
364 acid and ellagic acid), which could partially explain the differences in release behavior.

### 365 3.6. Antifungal activity of microcapsules

366 Phytopathogenic fungi cause important losses in fruit and vegetables. *F. oxysporum* is an important fungus  
367 involved in preharvest losses in berries, causing the *Fusarium* wilt in strawberry crops, a disease that affects  
368 the whole plant system (Henry et al., 2017). In addition, *R. stolonifer* is a fastest-growing fungus and its  
369 invasion causes the development of a cottony mycelium with characteristic black spores in many fruits and  
370 vegetables during pre and postharvest stages, including the berries (Bautista-Baños et al., 2014). Plant  
371 extracts have recently been shown to successfully inhibit the development of phytopathogenic fungi, *in vitro*  
372 (Mahdi et al., 2021; Wang et al., 2018). The inhibition percentages of the microcapsules against  
373 *F. oxysporum* and *R. stolonifer* are shown in Fig. 4. The results evidenced that the MC-RmA had the best  
374 ( $p<0.05$ ) antifungal activity against both fungi compared with MC-RmO, with inhibition percentages of  
375  $56.4\pm 4.2\%$  and  $46.3\pm 1.2\%$  against *F. oxysporum* (Fig. 5a) and *R. stolonifer* (Fig. 5b), respectively. The  
376 two highest concentrations tested (0.50 and 0.60 %, w/v) did not show a significant difference in both cases.  
377 The higher antifungal effect of MC-RmA could be attributed to their antioxidant capacity previously reported,  
378 and a major number of phenolic compounds in the extract, both characteristics associated with the

379 promotion of antifungal activity (Jasso de Rodríguez et al., 2017). Since the MC-RmA exhibited higher  
380 antioxidant and antifungal properties, it was selected to evaluate its effect on the shelf life of strawberry  
381 fruit.

### 382 3.7. Effect of microcapsules containing RmA on strawberry fruit

#### 383 3.7.1. Weight loss and firmness

384 Fruit weight loss is mainly associated with the respiration rate and the release of water into the environment  
385 (Yang et al., 2019). The effect of functionalized microcapsules on weight loss is presented in Fig. 5a. The  
386 highest **weight loss** ( $p<0.05$ ) was in control fruit (uncoated) during all storage period. **Besides, the uncoated**  
387 **fruit showed a significant difference ( $p<0.05$ ) in weight loss on each day evaluated.** At the end of the storage  
388 (14 d), the strawberries treated with 0.25 % ( $20.35\pm 1.36$  %) and 0.50 % ( $19.18\pm 0.38$  %) of MC-RmA had  
389 significantly ( $p<0.05$ ) less weight loss than uncoated strawberries ( $40.86\pm 1.64$  %). This results confirm that  
390 the use of MC-RmA treatment provided a barrier capable to reduce the water loss by acting as a coating  
391 on the fruit surface (Salas-Méndez et al., 2019). On the other hand, there was no significant difference  
392 between the amount of MC-RmA used. These results are consistent with a previous study of Guerreiro et  
393 al. (2015), in which edible coatings containing 0.1 and 0.2 % eugenol did not show significant differences,  
394 but both were an effective barrier to water loss during the storage of strawberries for 14 d at 0.5 °C. In  
395 addition, the use of HPMC as an encapsulating agent in synergy with the use of chia mucilage based  
396 coating as vehicle, could favor the formation of a semi-permeable matrix (Gol et al., 2013; Urbizo-Reyes et  
397 al., 2020). Gol et al. (2013) reported a lower weight loss in strawberries treated with an HPMC edible coating  
398 at day 12 of storage at  $11 \pm 1$  °C, associating this effect to the formation of the barrier on the surface of the  
399 fruit.

400 The strawberries' firmness is an important quality parameter for consumers, and its decrease is related to  
401 the loss of cell wall strength caused by the degradation of the middle lamella of cortical parenchyma cells,  
402 and also by the loss of turgidity due to the activity of degrading enzymes (e.g., pectinamethylesterase and  
403 polygalacturonase) (Oliveira et al., 2021). In this study, the fruits treated with MC-RmA showed a significant  
404 less decrease of their initial firmness (MC-RmA 0.25%:  $5.26\pm 1.10$ % of decrease; MC-RmA 0.50%:  
405  $6.98\pm 1.14$ % of decrease) in comparison with uncoated strawberries ( $64.17\pm 0.61$ % of decrease) at the end

406 of storage, being consistent with the results of weight loss. Similarly, Li et al. (2020) reported that the active  
407 film of microcapsules containing oregano essential oil allowed the highest firmness values in strawberries  
408 due to the decrease in the moisture content surrounding the fruit surface. In addition, the coatings act as a  
409 barrier to O<sub>2</sub> uptake and metabolic activity is slowed down (Sogvar et al., 2016). The MC-RmA showed to  
410 have a positive effect on fruit firmness, resulting in improved fruit quality by reducing their softness during  
411 the storage.

### 412 3.7.2. Color

413 Color significantly influences the acceptability of strawberry fruits, and it is related to their ripening process  
414 (Gol et al., 2013). Color change in fruits was monitored by means of chroma, Hue angle, and redness  
415 values (Fig. 6). For chroma and redness there was a significant reduction from day 0 to day 14 in all  
416 treatments ( $p < 0.05$ ), which results in a loss of fruit brightness and changes in the fruit color. Nevertheless,  
417 treated fruit showed no significant changes compared to untreated fruit in terms of  $C^*$ ,  $H^*$  and redness on  
418 each day of evaluation. Similarly, Guerreiro et al. (2015) found no differences between untreated  
419 strawberries and those treated with a pectin coating containing essential oils. This phenomenon is also  
420 reported in other studies, where slight changes in fruit color are considered a natural occurrence due to  
421 factors such as loss of freshness, oxidative processes, and microbial contamination during storage (Fan et  
422 al., 2009; Liguori et al., 2021; Pinzon et al., 2020; Valenzuela et al., 2015). These results are important  
423 because significant changes in fruit color induced by the treatments could affect consumer acceptability;  
424 and, in this study it is demonstrated that it is possible to incorporate the bioactive properties of MC-RmA  
425 without negatively altering the color of the fruit.

426

### 427 3.7.3. Fungal decay

428 Decay in strawberries is mainly caused by their high susceptibility to postharvest fungal attack, mainly by  
429 *R. stolonifer*, *Botrytis cinerea*, *Penicillium* spp., and *Colletotrichum* spp. (Feliziani & Romanazzi, 2016). The  
430 results of fungal decay are shown in Fig. 5b, and it can be observed that after day 8, the coated fruit started  
431 to show a significant less fungal decay compared with uncoated fruits and during the following days, the

432 decay was faster and more significant in the control treatment. This behavior demonstrates the particularity  
433 of MC-RmA to gradually release their content (Kittitheeranun et al., 2015). Besides, a concentration-  
434 dependent effect was observed at 14 d of storage, as the fruits treated with 0.25 % MC-RmA presented a  
435 higher ( $p < 0.05$ ) fungal decay compared to those treated with 0.50 %. Likewise, Fan et al. (2019) reported  
436 that a higher amount of lotus leaf extract incorporated in coatings significantly reduces decay in goji berries  
437 due to the presence of a higher amount of bioactive compounds.

438 Fig. 7 shows the visual evolution of the strawberry fruits during the storage period, in which it can be  
439 observed that fungal development was faster in the control group. A similar behavior was previously  
440 reported by Liu et al. (2021), as coated strawberries (containing asparagus waste extract) showed better  
441 control of *P. italicum*, than uncoated fruit after 8 d of storage at 25 °C and 80% RH. Other works have also  
442 reported interesting results on the antifungal effect of coatings or microcapsules containing plant extracts  
443 on strawberry fruits (Sangsuwan et al., 2016; Oliveira et al., 2021; Saleh & Abu-Dieyeh, 2022), which is an  
444 indicator of the potential of these technologies to extend the shelf life of these fruits. These results prove  
445 that the use of MC-RmA effectively reduces the decay of strawberry fruits due to their bioactive compounds  
446 with antifungal properties, besides, the encapsulation provides a slow release of its content, thus extending  
447 their activity.

#### 448 4. Conclusions

449 Microcapsules containing extracts from *R. microphylla* fruit were developed using electrospray technique  
450 and HPMC as encapsulating agent, which showed a spheric shape and particle size between 2.05-2.41  $\mu\text{m}$ .  
451 With both concentrations of MC-RmA evaluated (0.25 and 0.5 %, w/v), the treated fruits showed a decrease  
452 in weight loss, fungal decay, and firmness, compared with the uncoated fruits. Therefore, the effectiveness  
453 of MC-RmA in extending the shelf life of strawberry fruits under the test storage conditions (4 °C for 14 d  
454 and 85 % HR) was confirmed. The results are promising and demonstrate the positive effect of the  
455 functionalized microcapsules on the quality of strawberries. They provide a novel biorational alternative for  
456 use in the postharvest stage, where the use of synthetic product is avoided due to the proximity of the final  
457 product to the consumer. This technology could help to reduce the product losses while maintaining quality

458 attributes. However, it is important to evaluate feasible application methods, as well as to design appropriate  
459 vehicles to improve the use of this technology.

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## Figures Captions

Fig. 1. SEM images of the developed microcapsules and their particle diameter distribution. A) MC-HPMC (T2); b) MC-RmA (T4); and c) MC-RmO (T8).

Fig. 2. FTIR spectra of free extracts and encapsulated extracts. a) HPMC powder; b) MC-HPMC; c) RmA; d) MC-RmA; e) RmO; f) MC-RmO.

Fig. 3. X-ray diffractograms (A) and TGA curves (B) of developed microcapsules. a) MC-HPMC; b) MC-RmA; and c) MC-RmO.

Fig. 4. Mean inhibition percentage of microcapsules containing RmA and RmO against (a) *F. oxysporum* and (b) *R. stolonifer*. Different uppercase letters indicate statistical differences between treatments in each concentration ( $p < 0.05$ ). Different lowercase letters indicate statistical differences between concentrations in each treatment ( $p < 0.05$ ).

Fig. 5. Influence of MC-RmA at different concentrations on strawberry fruits stored at  $4 \pm 1$  °C and 85 % HR. (a) Weight loss percentage, and (b) fungal decay percentage. Different uppercase letters indicate statistical differences between days for each treatment ( $p < 0.05$ ). Different lowercase letters indicate statistical differences between treatments in each day ( $p < 0.05$ ).

Fig. 6. Changes in color parameters of control (uncoated) and treated strawberries with MC-RmA at different concentrations and stored at  $4 \pm 1$  °C and 85 % HR. a) Chroma, b) Hue angle, c) redness.

Fig. 7. Appearance changes of strawberries treated with HPMC microcapsules containing RmA (0.25 and 0.50 %, w/v) and control, stored at  $4 \pm 1$  °C and 85 % HR.

**Tables**

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**Table 1.**

Electrospraying testing conditions.

Treatment	Extract	Extract concentration (mg/mL)	Ethanol concentration (% v/v)	Voltage (kV)
T1	Blank (HPMC)	3.0 %	50	10
T2	Blank (HPMC)	3.0 %	75	10
T3	RmA	1.0	50	16
T4	RmA	1.0	75	14
T5	RmA	2.5	50	18
T6	RmA	2.5	75	15
T7	RmO	1.0	50	19
T8	RmO	1.0	75	17
T9	RmO	2.5	50	25
T10	RmO	2.5	75	**

\*\* The extract could not be solubilized.

**Table 2.**

Particle size and particle aspect ratio of selected samples.

Sample	Particle Size ( $\mu\text{m}$ )	PAR
MC-HPMC	2.31 $\pm$ 0.62 <sup>a</sup>	1.10 $\pm$ 0.08 <sup>a</sup>
MC-RmA	2.41 $\pm$ 0.57 <sup>a</sup>	1.08 $\pm$ 0.07 <sup>a</sup>
MC-RmO	2.05 $\pm$ 0.50 <sup>b</sup>	1.08 $\pm$ 0.06 <sup>a</sup>

Different letters in the same column indicate statistical differences ( $p < 0.05$ ).

**Table 3.**

Total phenolic content (TPC) and antioxidant capacity of HPMC microcapsules with and without RmA and RmO.

Assay	Treatment	Sample		
		MC-RmA	MC-RmO	MC-HPMC
TPC (mg GA/g MC)	Ultrasound	3.08±0.30 <sup>a</sup>	0.51±0.08 <sup>b</sup>	n.d.
	Agitation	2.94±0.39 <sup>a</sup>	1.45±0.21 <sup>a</sup>	n.d.
DPPH (mg/mL, %RSA)	Ultrasound	17.15±0.36 <sup>a</sup>	3.61±0.77 <sup>b</sup>	n.d.
	Agitation	16.52±0.39 <sup>a</sup>	10.46±0.62 <sup>a</sup>	n.d.
ABTS (mg/mL, %RSA)	Ultrasound	16.20±0.82 <sup>a</sup>	2.87±0.74 <sup>b</sup>	n.d.
	Agitation	15.24±1.35 <sup>a</sup>	7.23±0.93 <sup>a</sup>	n.d.
FRAP (mg AA/g MC)	Ultrasound	4.04±0.17 <sup>a</sup>	0.52±0.09 <sup>b</sup>	n.d.
	Agitation	3.99±0.92 <sup>a</sup>	1.30±0.16 <sup>a</sup>	n.d.

Different uppercase letters in the same row indicate statistical differences ( $p < 0.05$ ) between release treatments for each assay.

n.d. not detected.



**Figures**

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Fig. 1.

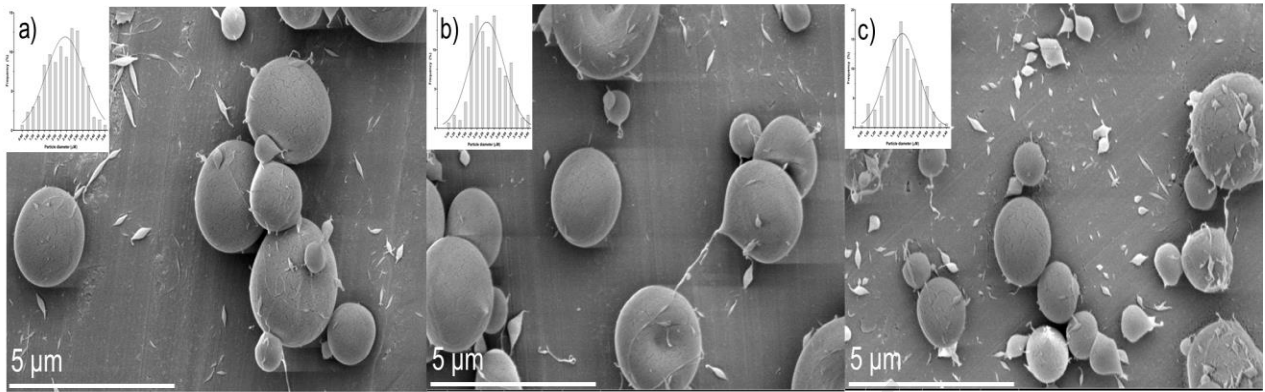


Fig. 2.

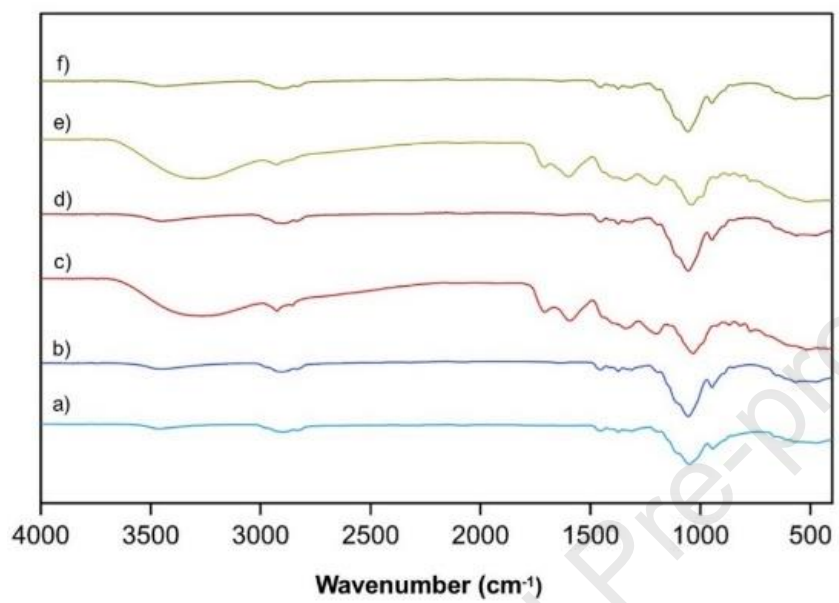


Fig. 3.

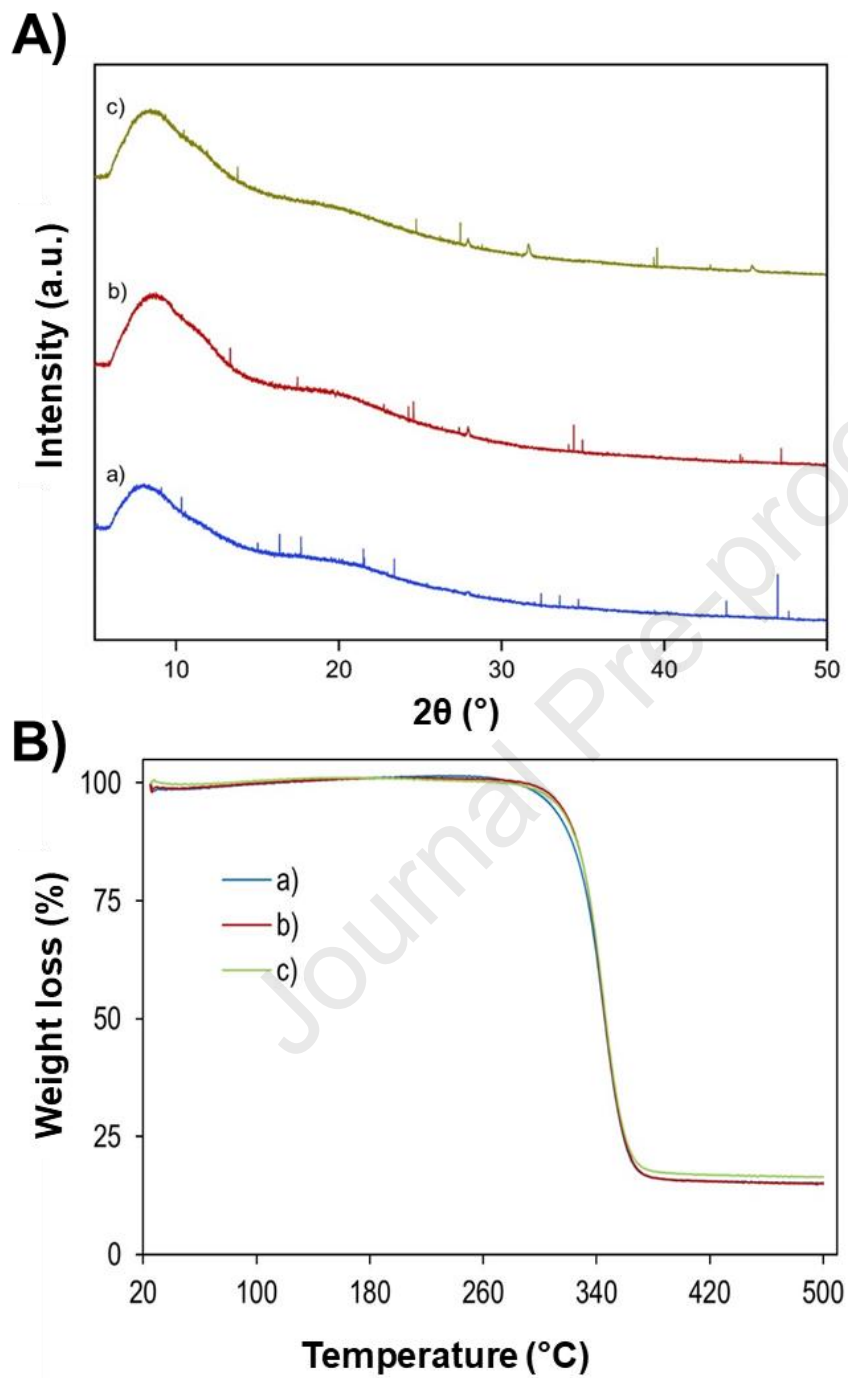


Fig. 4.

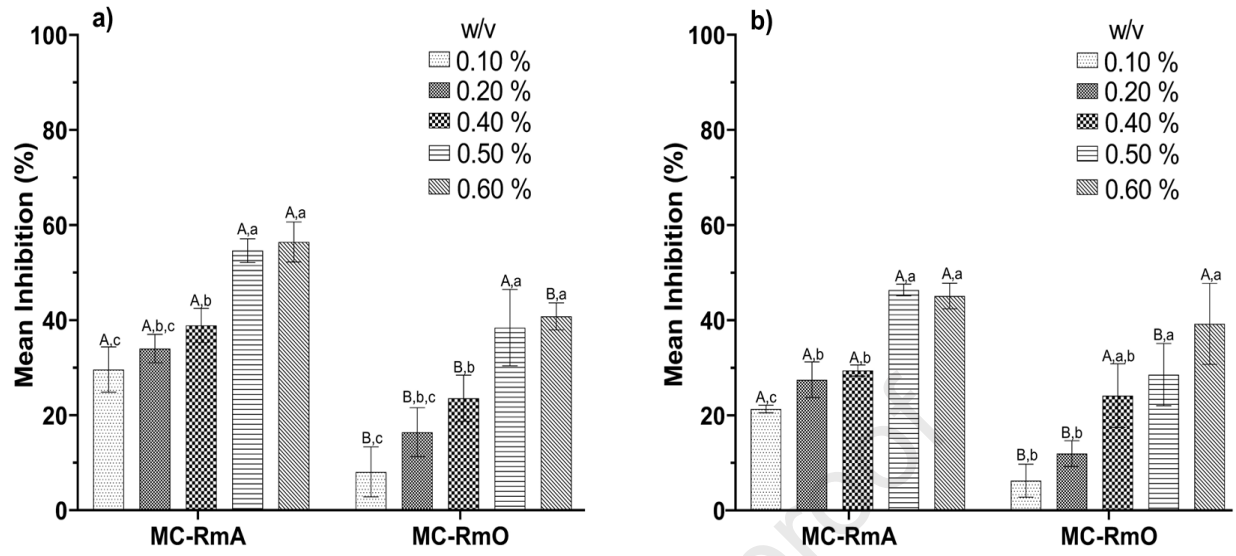


Fig. 5.

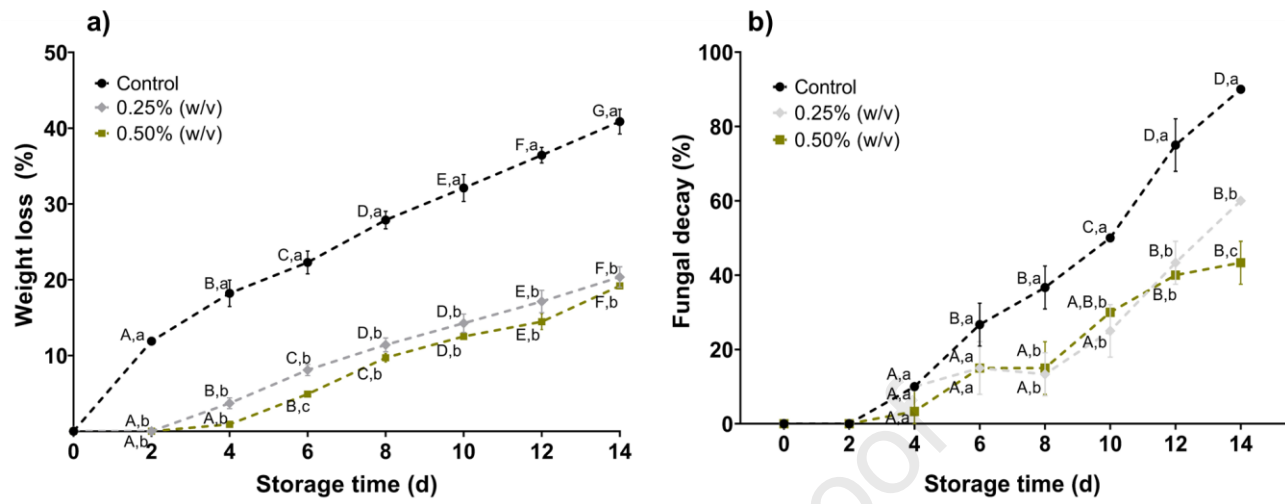


Fig. 6.

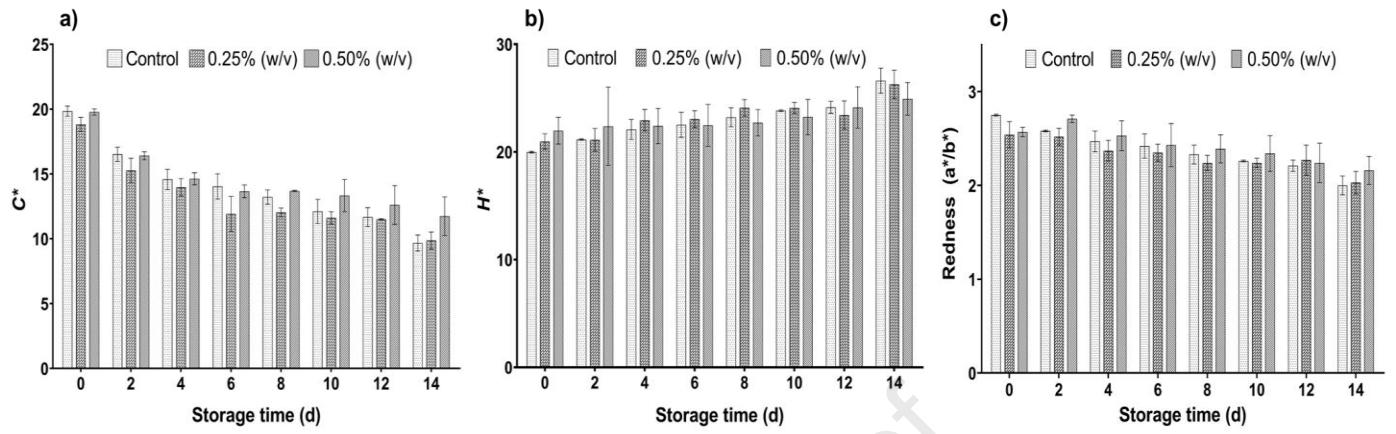
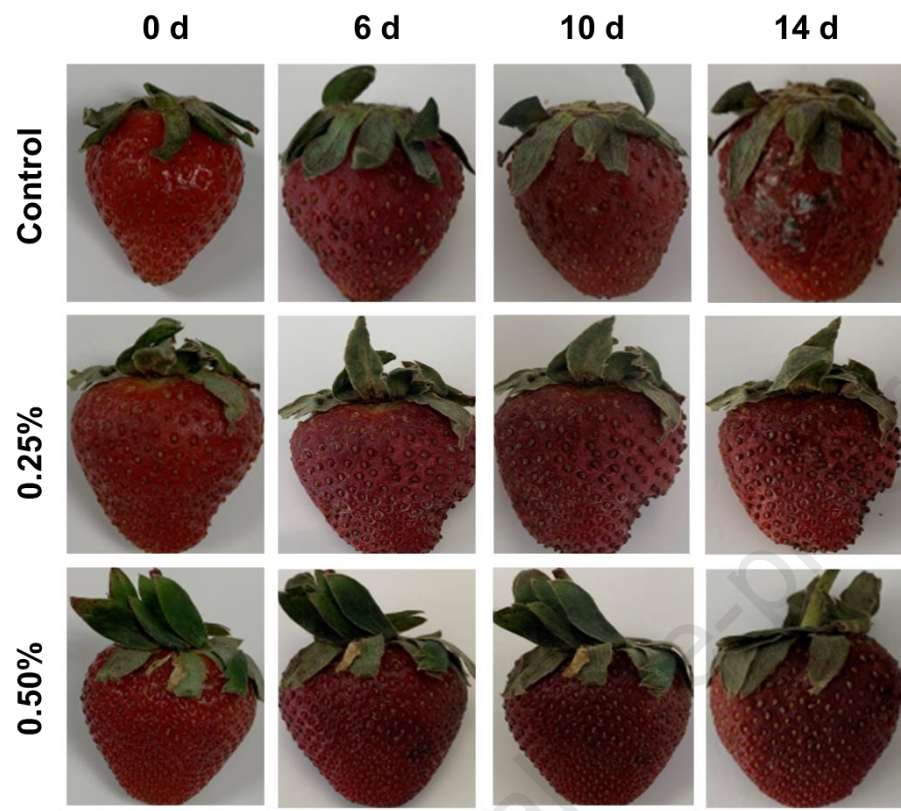


Fig. 7.





## Highlights

- Spherical-microcapsules with *R. microphylla* extracts were obtained by electrospray
- HPMC and electrospray enabled extract incorporation into microcapsules
- Functionalized microcapsules delay fungal decay and weight loss in strawberries
- Microcapsules with *R. microphylla* extracts are a novel postharvest technology

### **Conflict of Interest Statement**

The authors declare that there is no conflict of interest.

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