UNIVERSIDAD AUTÓNOMA AGRARIA ANTONIO NARRO

SUBDIRECCIÓN DE POSTGRADO

PERFIL FITOQUÍMICO DE LOS FRUTOS DE *Myrtillocactus geometrizans* Y *Rhus microphylla* PARA LA FORMACIÓN DE CÁPSULAS CON ACTIVIDAD ANTIFÚNGICA

Tesis

Que presenta JORGE LUIS GUÍA GARCÍA

como requisito parcial para obtener el Grado de

DOCTOR EN CIENCIAS EN RECURSOS FITOGENÉTICOS PARA ZONAS ÁRIDAS

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Dra. Ana Verónica Charles Rodríguez	Dra. María Liliana Flores López
(Director UAAAN)	(Co-director Externo)

Saltillo, Coahuila — Junio 2022

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Elaborada por JORGE LUIS GUÍA GARCÍA como requisito parcial para obtener el grado de Doctor en Ciencias en Recursos Fitogenéticos para Zonas Áridas con la supervisión y aprobación del Comité de Asesoría

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Saltillo, Coahuila

Junio 2022

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A **mi madre**,

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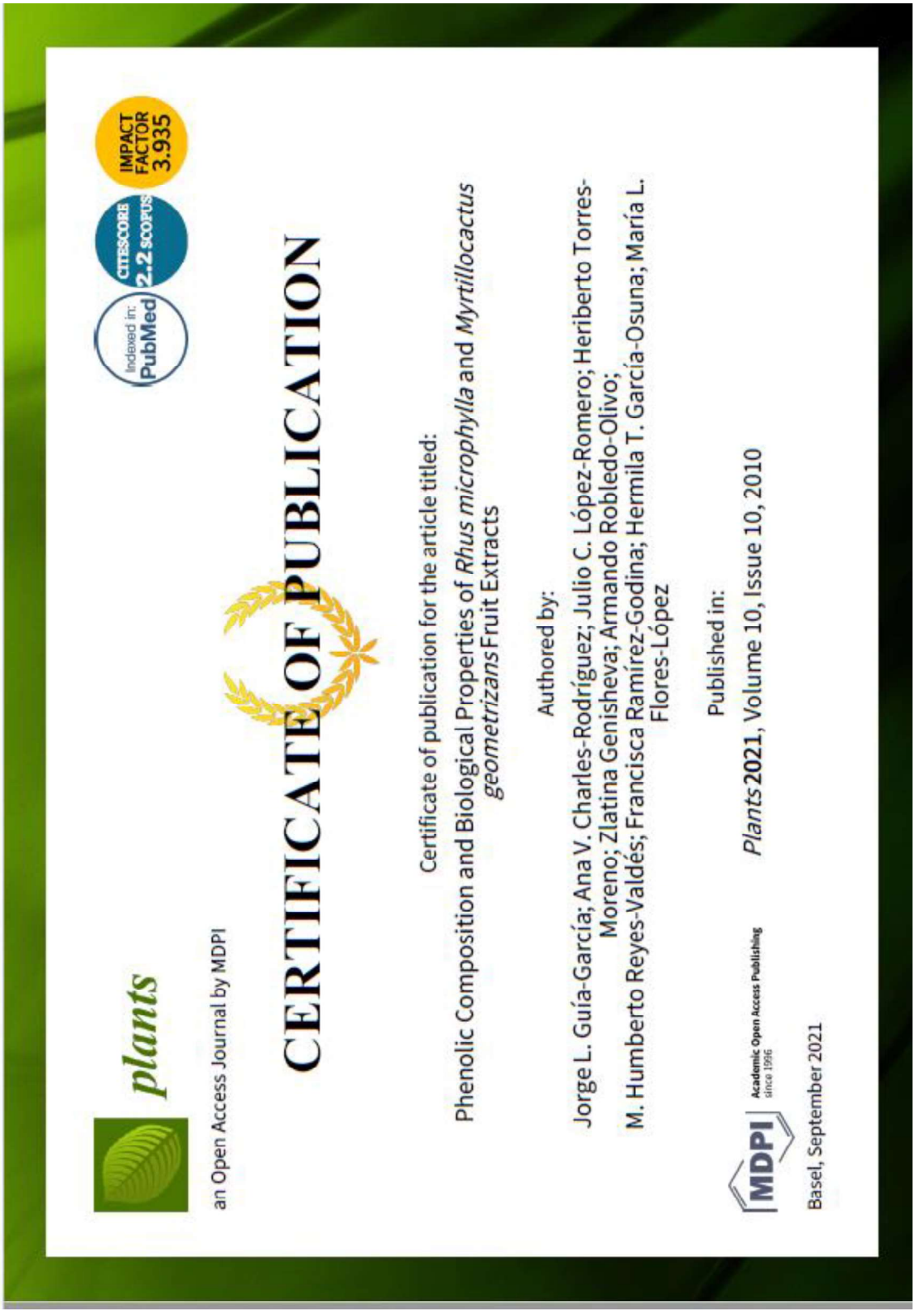

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CAPÍTULO 1.

INTRODUCCIÓN.

1. INTRODUCCIÓN.

Las zonas áridas presentan un ambiente donde la pérdida de agua por evaporación es mayor que la entrada por precipitación, lo que provoca temperaturas extremas y una baja biodisponibilidad de nutrientes y agua en el suelo (Briones et al., 2018). Las plantas que se desarrollan en estos climas han mostrado la presencia de fitoquímicos con interesantes propiedades antioxidantes, antifúngicas y anticancerígenas en sus extractos (Jasso de Rodríguez et al., 2017; López-Romero et al., 2018); los cuales pueden ser utilizados como alternativas ecológicas para el combate de hongos en las etapas de postcosecha de frutas. Por ejemplo, en fresa, las pérdidas provocadas por hongos como *Botrytis cinerea*, *Rhizopus stolonifer*, *Mucor* spp, entre otros, alcanzan el 50% de la producción total (Feliziani & Romanazzi, 2016). Dos plantas de estas zonas con prometedoras características fitoquímicas son: *Rhus microphylla (Rm*) y *Myrtillocactus geometrizans* (*Mg*), que de manera tradicional son empleadas por sus propiedades anti-inflamatorias y antioxidantes (Calderón Gómez et al., 2015; Correa-Betanzo et al., 2011).

Por otro lado, aunque los extractos de plantas tienen una gran versatilidad, su uso y aprovechamiento se ve limitado por su elevada susceptibilidad a factores ambientales (humedad, luz, temperatura, entre otros), lo que puede provocar la degradación de compuestos bioactivos presentes en éstos y la pérdida parcial o completa de sus propiedades (Al-Maqtari et al., 2021). Con la finalidad de combatir esta problemática y propiciar el uso de los extractos de plantas, se han desarrollado tecnologías que mejoren y brinden protección a los mismos, siendo la encapsulación una alternativa novedosa para el desarrollo de productos agrícolas conteniendo extractos de plantas (Ye et al., 2018).

Por estas razones, el objetivo de la presente investigación fue caracterizar y evaluar las propiedades bioactivas de los extractos de los frutos de *Rm* y *Mg*, con la finalidad de desarrollar microcápsulas con actividad antifúngica y su aplicación en fresa (*Fragaria* × *ananassa*) como fruto modelo.

CAPÍTULO 2.

REVISIÓN DE LITERATURA.

2. REVISIÓN DE LITERATURA.

Las zonas áridas y semiáridas en conjunto ocupan más de la mitad del territorio mexicano, donde su vegetación está compuesta mayoritariamente por plantas leñosas, suculentas y gramíneas formando matorrales y pastizales de gran extensión que, principalmente son utilizados como forraje para ganado o con algunos fines industriales por la presencia de sustancias que pueden ser utilizados en textiles, alimentos, etcétera (Barbosa-Briones et al., 2019; Cervantes, 2005).

2.1. *Rhus microphylla* Engelm

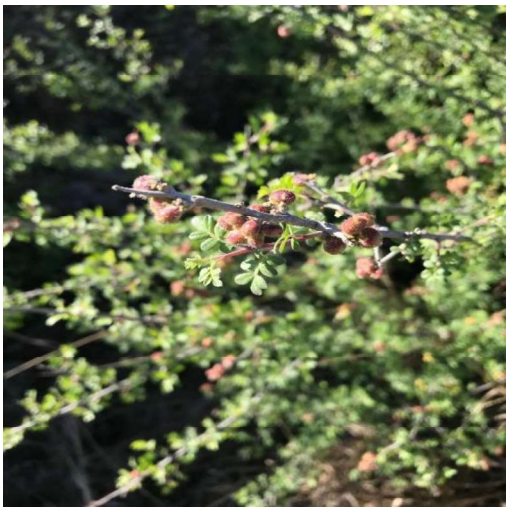

Figura 1. Frutos de *Rhus microphylla.*

El género *Rhus* de la familia Anacardiaceae presenta 35 especies, las cuales se clasifican en 2 subgéneros (*Rhus* y *Lobadium*). Para el género *Rhus* se reconocen 10 especies, que están distribuidas en el este de Europa y Asia, América del Norte y en el sureste de Europa; por otro lado, en el subgénero *Lobadium* son cerca de 25 especies distribuidas en México y el suroeste de Estados Unidos, incluyendo a *R. microphylla* (*Rm*) que tiene una altura de 1.5 a 2.0 m y se caracteriza por presentar hojas perennes, simples, imparipinnadas y unas bayas de color rojizo con tricomas glandulares (Andrés-Hernández & Terrazas, 2006). Diversas especies del género *Rhus*, han sido objeto de estudios en donde se ha demostrado que sus compuestos tienen diferentes aplicaciones

en diferentes áreas como anticancerígenos y colorantes (Calderón Cómez et al., 2015; Varela-Rodríguez, 2018). Además, existe una alta presencia de antioxidantes y fenoles en las bayas de la planta, lo cual se ha relacionado con su actividad antimicrobiana (Fang and Bhandari, 2010; Liu et al., 2019). Recientemente, Guía-García et al. (2021) reportó que los extractos hidroalcohólicos de *Rm* inhiben completamente a *Rhizopus stolonifer* a una concentración de 2500 mg/L.

2.2. *Myrtillocactus geometrizans* (Mart. ex Pfeiff.) Console

Dentro de la familia Cactaceae se encuentra el género *Myrtillocactus*, siendo 4 especies las reportadas: *M. geometrizans, M. schenkii, M. cochal* que crecen en las zonas áridas y semiáridas de México y, *M. eichlami* una especie endémica de Guatemala, con mayor producción en las zonas áridas del centro de México (San Luis Potosí, Querétaro, Oaxaca, Guanajuato) (Hernández-López et al., 2008). Estos cactus llegan a medir más de 6 m de altura y presentan ramas de color verde oscuras a verde-azulosas, de 5 a 8 costillas con un surco intercostal amplio y aréolas grandes y distantes entre sí (Martínez-Hernández et al., 2015).

Figura 2. Frutos de *Myrtillocactus geomerizans.*

M. geometrizans produce un pequeño fruto color rojo-púrpura con un diámetro aproximado de 1.5-2.0 cm con una cubierta que cubre la pulpa del fruto y es cosechado en el período de junio a agosto (Guzmán-Maldonado et al., 2010). Se

le ha atribuido un gran número de beneficios a este fruto, como son: actividad antioxidante, antiinflamatoria y auxiliar en el estrés oxidativo (Correa-Betanzo et al., 2011). Se ha identificado la presencia de fenoles, betalaínas y vitamina C en los frutos (Montiel-Sánchez et al., 2021). Además, extractos metanólicos de los tallos de *Mg* han mostrado actividad insecticida contra el gusano de la harina (*Tenebrio molitor*) y el cogollero del maíz (*Spodoptera frugiperda*), donde concentraciones de 100 a 200 mg/L es letal para las larvas de estas plagas (Céspedes et al., 2005). Mientras que, los extractos hidroalcohólicos de estos frutos han mostrado inhibir satisfactoriamente el desarrollo de *Fusarium oxysporum in vitro*, identificándose además, ácidos fenólicos (ácido elágico y ácido ferúlico) con propiedades antioxidantes (Guía-García et al., 2021).

2.3. Extractos de plantas y técnicas de extracción

En las plantas se pueden encontrar moléculas de diversa naturaleza como parte de su metabolismo primario y secundario. Los metabolitos primarios (aminoácidos, nucleótidos y fitoesteroles) son producidos para realizar roles esenciales en el desarrollo de las plantas; mientras que, los metabolitos secundarios pueden ser considerados como compuestos bioactivos, ya que proveen principalmente protección y actúan como una barrera para evitar el ataque de insectos, animales u otros predadores (Xu et al., 2021). Debido a que los compuestos bioactivos pueden variar de una planta a otra, existe un gran número de éstos, por lo que presentan una gran versatilidad en sus capacidades y su potencial uso industrial (Montiel-Sánchez et al., 2021).

Por lo general, los compuestos bioactivos pueden ser clasificados en 3 grandes categorías (Barba et al., 2016):

- Compuestos fenólicos: También conocidos como polifenoles, son un grupo de cerca de 10,000 compuestos que en su estructura presentan grupos hidroxibencenos, su rol en las plantas es como protección (resistencia a hongos, bacterias e insectos) (Manousi et al., 2019).

- Terpenos: Son estructuras de una o más unidades de isoprenos de 5 carbonos y se caracterizan por su hidrofobicidad. Además, presentan un amplio rango de propiedades bioactivas (Diniz et al., 2021)
- Compuestos nitrogenados: El mayor grupo de compuestos bioactivos de esta categoría son las betalaínas. Éstos son compuestos solubles en agua y son responsables de la coloración de frutas (variando de rojo-violeta a amarillo-naranja), además tienen una gran estabilidad en un amplio margen de pH en un rango de 3-7 (Azeredo, 2009).

El contenido de compuestos bioactivos depende mayormente de la planta y parte de la planta (E.j. raíz, hoja, fruto), el solvente y la técnica de extracción elegida para la obtención de los extractos (Garavand et al., 2019).

En general, en las técnicas de extracción existen dos categorías: técnicas convencionales y técnicas no convencionales. En la primera categoría se incluyen principalmente la extracción por Soxhlet, maceración (agitación) e hidrodestilación, siendo las técnicas más antiguas y mayormente utilizadas por su simplicidad y bajo costo (Azmir et al., 2013). Sin embargo, estas técnicas consumen una gran cantidad de tiempo y en algunos casos se usan temperaturas elevadas, lo que puede provocar la degradación de compuestos termolábiles presentes en los extractos (Lee & Wong, 2014).

Por otro lado, las técnicas de extracción no convencionales fueron desarrolladas con la finalidad de contrarrestar las desventajas de las técnicas de extracción convencionales. En esta categorías están incluidas las siguientes técnicas: extracción asistida por ultrasonido, extracción asistida por microondas, extracción enzimática, extracción por fluido supercríticos, extracción por solventes presurizados y extracción asistida por electricidad (calentamiento óhmico) (Azmir et al., 2013; Sik et al., 2020). Ésta última técnica ha llamado la atención debido a que la aplicación de la corriente eléctrica provoca la formación de poros en la pared celular por donde ingresa el solvente y extrae los compuestos bioactivos hacia el exterior, siendo los tiempos de operación muy cortos y los rendimientos mayores en comparación con técnicas de extracción convencionales (Ferreira-

Santos et al., 2020; Varghese et al., 2014). La extracción por calentamiento óhmico ha sido empleada en diversos estudios para la obtención de compuestos bioactivos, por ejemplo: El Darra et al. (2013) utilizaron calentamiento óhmico para extraer polifenoles de uvas rojas, mientras que Pereira et al. (2016) extrajeron antocianinas de *Solanum tuberosum*; y recientemente, Mojtahed Zadeh Asl et al. (2018) obtuvieron aceite esencial de *Artemisia aucheri* Boiss.

Los extractos obtenidos deben ser protegidos de las condiciones medioambientales (humedad, luz, temperatura) para evitar la degradación de su contenido, esto dificulta su aprovechamiento y aplicación en exteriores (Aguilar-Tuesta et al., 2018). Es por ello, que es importante el desarrollo de alternativas que brinden protección y puedan facilitar su uso, para así beneficiarse de las diversas propiedades bioactivas que presentan los extractos de plantas.

2.4. Micro y nanoencapsulación

La encapsulación es un proceso donde un núcleo es rodeado por un material polimérico (natural o sintético) en donde, por su tamaño, las estructuras formadas pueden catalogarse como microcápsulas y nanocápsulas. Las microcápsulas presentan tamaños que van desde 1-1000 µm, mientras que las nanocápsulas, tienen tamaños menores a 100 nm (Shishir et al., 2018). La encapsulación de extractos puede mejorar o controlar la liberación de éstos; además, brindan la protección necesaria para poder utilizarlos bajo condiciones ambientales no favorables y prolongar su actividad por más tiempo (Đorđević et al., 2015).

Para la encapsulación de compuestos bioactivos existen varios métodos, como son: emulsificación, spray-drying, liofilización, coacervación, microfluidos y electrospray (Rehman et al., 2019). Entre los métodos anteriores, destaca la encapsulación por electrospray, donde los encapsulados obtenidos presentan formas y tamaños de partícula más homogéneos (Silva et al., 2021). El fundamento de este método se basa en la aplicación de un voltaje que genera una ruptura de la tensión superficial de la solución polimérica conteniendo los extractos. Esta ruptura ocasiona la formación de una estructura llamada “cono de Taylor” en la punta de la aguja de eyección que funciona como un atomizador de

pequeñas gotas que caen completamente secas sobre un colector a una distancia previamente establecida, cabe mencionar que el voltaje aplicado debe ser el suficiente para romper la tensión superficial, en caso contrario, la atomización no sería efectiva (Niu et al., 2020). Por medio de esta tecnología se han logrado encapsular con éxito catequinas extraídas de té verde (Bhushani et al., 2017), extractos ricos en antocianinas (Atay et al., 2018) y terpenos como el β-caroteno (Rodrigues et al., 2020).

La correcta elección del material encapsulante, el método y las condiciones de encapsulación son factores que afectan significativamente la estructura y funcionalidad de los encapsulados conteniendo extractos y/o compuestos bioactivos (Papoutsis et al., 2018; Shao et al., 2019).

2.5. Aplicación de encapsulados conteniendo extractos de plantas en la industria agroalimentaria

En la actualidad, los extractos de plantas han llamado la atención de la industria agroalimentaria por sus diversas bioactividades, sobre todo su capacidad antifúngica, lo que los convierte en una alternativa ecológica para reducir las pérdidas postcosecha en frutas que pueden ser de cerca del 40% de la producción total (Flores-López et al., 2016). Para combatir esta problemática se ha estudiado el uso de recubrimientos comestibles conteniendo extractos de plantas; sin embargo, su desarrollo es complejo, se requieren grandes cantidades de extracto y materiales poliméricos, además, los recubrimientos pueden afectar la tasa de respiración de algunas frutas, afectando su maduración (Joshi & Rao, 2018). Debido a esto, el uso de encapsulados al emplear menores cantidades de extracto y de polímeros, sumado a su capacidad de brindar una liberación más controlada, ha tomado relevancia en su posible uso como agente innovador para su aplicación en postcosecha de frutas y verduras (Oliveira et al., 2021). Sin embargo, los estudios de la aplicación de encapsulados conteniendo extractos de plantas para extender la vida útil de frutas y verduras son limitados. En el Cuadro 1 se presentan algunas investigaciones relevantes sobre la aplicación de encapsulados para extender la calidad postcosecha de frutos y vegetales.

Cuadro 1. Aplicación de encapsulados conteniendo extractos de plantas en postcosecha de frutas y vegetales.

Muestra	Fruto modelo	Resultados	Referencia
Nanocápsulas con aceite de tomillo (*Thymus vulgaris*)	Aguacate (*Persea americana* var. Hass)	Redujo la incidencia de *Colletotrichum gloeosporioides* en un 60% (8 días a 27°C).	(Correa-Pacheco et al., 2017)
Nanocápsulas de β-caroteno	Melón (*Cucumis melo* L.)	Menor pérdida de firmeza (9.9%) en comparación con el control (39.0%) a 4°C por 6 días.	(Zambrano-Zaragoza et al., 2017)
Microcápsulas de aceite esencial de *Perilla frutescens*	Fresa (*Fragaria x ananassa*)	Retardó significativamente el decaimiento de las fresas (4 días a 25°C).	(Li et al., 2018)
Nanoemulsión con extracto de *Opuntia joconostle* y aceite esencial de naranja (*Citrus x sinensis*)	Aguacate (*Persea americana*)	Menor pérdida de peso (2.13%) y firmeza (39.66 N) en comparación con el control (9.81% y 6.33 N) (10 días a 6°C).	(Cenobio-Galindo et al., 2019)
Recubrimiento con nanocápsulas de extracto de *Byrsonima crassifolia*	Tomate (*Solanum lycopersicum*)	Menor presencia de microorganismos (768 UFC) en comparación con el control (1708 UFC) (19 días a 10°C).	(Gutiérrez-Molina et al., 2021)

CAPÍTULO 3. PRIMER ARTÍCULO.

PHENOLIC COMPOSITION AND BIOLOGICAL PROPERTIES OF *Rhus microphylla* AND *Myrtillocactus geometrizans* FRUIT EXTRACTS.

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Phenolic Composition and Biological Properties of *Rhus microphylla* and *Myrtillocactus geometrizans* Fruit Extracts

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Abstract: Plants from arid zones of Mexico are an interesting source of phytochemicals that exhibit a large number of biological properties. In this context, *Rhus microphylla* (Rm) and *Myrtillocactus geometrizans* (Mg) fruits have been used as folk remedies and to make traditional foods, respectively; however, studies on their composition and bioactivity are limited. Thus, the objective of this work was to evaluate the yields, phenolic composition, and bioactive properties (scavenging and reducing capacities, antiproliferative, and antifungal) of aqueous and hydroalcohol extracts of Rm and Mg fruits obtained by conventional agitation and ohmic heating (OH). The results showed that the Rm fruit extracts had the highest total phenolic content (TPC) values and the strongest scavenging and reducing capacities compared to those of Mg fruits, being characterized by the presence of gallic acid, while the composition of the Mg extracts varied with respect to the extraction conditions used. Regarding antifungal activity in vitro against two phytopathogenic fungi, *Rhizopus stolonifer* and *Fusarium oxysporum*, the hydroalcohol extracts obtained by conventional agitation of both plants (RmH-C and MgH-C) showed the best inhibitory effect, respectively. Interestingly, none of the extracts under study presented cytotoxicity against the noncancerous ARPE-19 cell line, while three extracts of Rm fruit exhibited a moderate antiproliferative activity against HeLa (cancerous) cell line. These findings reveal for the first time the potential of Rm and Mg fruits as a new source of bioactive compounds for future industrial applications.

Keywords: *Rhus microphylla*; *Myrtillocactus geometrizans*; ohmic heating; phenolic compounds; antioxidant activity; antiproliferative activity; antifungal activity

1. Introduction

Bioactive compounds (BCs) are produced by the secondary metabolism of plants, mainly as part of their defense system, as a barrier against pathogen agents and extreme climatic conditions [1]. Depending on their nature (e.g., phenolic compounds, anthocyanins, carotenoids, among others), the BCs can have different functions in the plant [2–4]. Humans have empirically used these compounds by preparing herbal infusions for treatment of dysentery, fever, diarrhea, stomach aches, and general ailments [5]. Recent studies have been directed to elucidate the potentialities of the BCs, especially as therapeutic agents in pharmaceutical and cosmetic industries [6], and as preservatives, colorants, fertilizers, and antimicrobials in the agrifood industry [7].

There are different techniques to obtain BCs, varying in the use of solvents, operating times, and temperatures [8]; and these can be divided into two types: conventional and nonconventional [9]. The first includes the most common and simple techniques, such as infusion, hydrodistillation, Soxhlet, and agitation, which have been used successfully over the years [10,11]. However, they are characterized by requiring large quantities of harmful solvents (e.g., methanol, ether, hexane, etc.) and usually long operating times, increasing the energy consumption [11,12]. Nonconventional techniques have been developed to overcome these drawbacks, among which are ohmic heating (OH), ultrasound, microwaves, and supercritical fluids [13–15]. These require lower operating times, less solvent use, in addition to allowing higher yields with better properties of the extracted BCs [15,16].

On the other hand, Mexico has a great diversity of plants, highlighting those that grow in arid areas due to their metabolic machinery [2]. Among these, wild species of *Myrtillocactus geometrizans* (Mart. ex Pfieff.) Console (known as garambullo) (Mg) and *Rhus microphylla* Engelm. (known as agrito) (Rm), develop interesting fruits composed mainly of flavonoids, phenolics acids, betacyanins, and betaxanthins [17,18]. The fruits vary in size and color, while Mg fruits are juicy, spheric, and purple, Rm fruits are small, spheric, orange, and dry drupe [19,20]. Mg fruit has been reported for its high antioxidant [1] and antihyperglycemic [21] activities, while the methanol extracts from roots and aerial parts of Mg have shown an insecticidal effect on the fall armyworm (*Spodoptera frugiperda*) [22]. Regarding Rm fruits, their ethanol and aqueous extracts were recently reported for their strong antifungal activity in vitro against two important crop pathogens, *Fusarium oxysporum* and *Corynespora cassiicola* [17]. The application of novel green extraction technologies, such as OH, represents an excellent tool for the recovery of crude extracts and BCs from these species. The OH extraction involves an electric field to promote the depolarization of the cellular wall and the consequent release of BCs from the matrix, being critical to study several parameters to optimize its performance (i.e., voltage, operational times, solute: solvent ratio, temperature, and conductivity) [23]. The OH has allowed better yields (13.2%) of clove essential oil than those with simple hydrodistillation (8.2%) [15]. In addition, the yields of hydroalcohol *Pinus pinaster* bark extracts have shown an increase of 30% when compared to conventional heating [23].

Despite the promising potential of Rm and Mg fruits, research on the possible benefits of extracts from both plants in different industrial areas is still scarce. Therefore, the objective of this work was to determine the yields and phenolic composition of aqueous and hydroalcohol extracts of *R. microphylla* and *M. geometrizans* fruits obtained by means of conventional agitation and OH; additionally, their bioactive properties as antioxidant, antiproliferative, and antifungal agents were determined.

2. Results

2.1. Yields and Total Phenolic Content of Extracts

Table 1 shows the extract yields and TPC values for Rm and Mg fruits obtained by conventional agitation and OH technique. The maximum yields ($p < 0.05$) for Rm fruits were obtained by conventional agitation, in addition these conditions allowed a higher recovery of TPC with values of 75.34 ± 6.48 and 62.00 ± 3.34 mg GA/g extract using aqueous and hydroalcohol solution as extracting agents, respectively. Significantly lower yields were observed with the application of OH, but when it was combined with hydroalcohol solution and extraction time of 10 min (RmH-OH10) presented a noteworthy value of TPC (41.37 ± 4.25 mg GA/g extract).

Table 1. Yields (%) and total phenolic content (TPC) of *R. microphylla* (Rm) and *M. geometrizans* (Mg) fruit extracts obtained by conventional agitation (C) and ohmic heating (OH).

Extract	Yield (%)	TPC (mg GA/g Extract)
	Rm fruits	
RmA-C	27.63 (± 1.12) ^{b}	75.34 (± 6.48) ^{a}
RmA-OH5	23.16 (± 1.66) ^{b,c}	25.89 (± 1.00) ^{c}
RmA-OH10	20.73 (± 1.55) ^{c}	25.16 (± 2.35) ^{c}
RmH-C	37.03 (± 1.99) ^{a}	62.00 (± 3.34) ^{a}
RmH-OH5	7.99 (± 1.95) ^{d}	21.78 (± 2.49) ^{c}
RmH-OH10	10.42 (± 0.35) ^{d}	41.37 (± 4.25) ^{b}
	Mg fruits	
MgA-C	37.63 (± 3.52) ^{a}	8.45 (± 1.04) ^{a,b}
MgA-OH5	20.82 (± 1.35) ^{b}	6.89 (± 0.74) ^{b}
MgA-OH10	40.07 (± 1.28) ^{a}	9.66 (± 1.92) ^{a,b}
MgH-C	43.96 (± 5.93) ^{a}	13.69 (± 4.88) ^{a}
MgH-OH5	18.14 (± 1.90) ^{b}	9.07 (± 1.07) ^{a,b}
MgH-OH10	20.28 (± 3.76) ^{b}	5.46 (± 0.82) ^{b}

Values are presented as mean (± standard deviation, $n = 3$), different lowercase letters in the same column indicate statistically significant differences ($p < 0.05$) for each plant. A: aqueous extract; H: hydroalcohol extract; C: conventional agitation; OH: ohmic heating; 5 and 10: correspond to operating time (min) for OH extraction.

Regarding the Mg fruits, the yields of the extracts obtained by conventional agitation did not show significant differences according to the solvent used, being in the range of 37.63 to 43.96%. The OH extracts yields were not influenced ($p > 0.05$) by the extraction conditions, only the extract MgA-OH10 stood out as it showed values similar to those obtained with conventional agitation (40.07 ± 1.28%). However, the extraction technique and conditions did not affect the recovery of TPC, which were lower than those detected in Rm fruits.

Furthermore, the experimental variables, yields and TPC, were correlated following Equation (1) for OH extraction, and the resulting equations that describe the variation in the responses and the R^2 value of each model are shown in Table 2. The R^2 values were between 0.74 and 0.97, which shows that the models used might be suitable for future estimations of the factors analyzed.

Table 2. Linear models describing the response variation of yields and total phenolic content (TPC) in function of time (A) and solvent (B) tested in experimental models for OH extraction, and their correspondent R^2 coefficients.

Model	Response	Model Regression Equation	R^2
Rm fruits	Yields	15.51 + 0.001(A) − 10.01(B) + 0.486(A*B)	0.97
	TPC	14.41 + 1.886(A) − 12.21(B) + 2.032(A*B)	0.92
Mg fruits	Yields	8.80 + 2.137(A) + 7.20(B) − 1.710(A*B)	0.96
	TPC	8.40 − 0.084(A) + 4.28(B) − 0.638(A*B)	0.74

Rm: *Rhus microphylla*, Mg: *Myrtillocactus geometrizans*.

2.2. Phenolic Profile by UHPLC

The phenolic profile was identified by means of UHPLC (Tables 3 and 4). In general, a total of six and five different phenolic compounds were identified in the Rm and Mg fruit extracts, respectively. Gallic acid was the most abundant compound in all Rm fruit extracts, mainly in those obtained by conventional agitation (RmA-C and RmH-C), where the aqueous solvent significantly influenced in the content of this compound ($p < 0.05$). For the Mg fruit extracts, the solvent and extraction technique used also influenced the yields and the presence of phenolic compounds; the occurrence of rosmarinic acid (12.36 ± 0.01 mg/L) in the MgH-C extracts (Table 4) was particularly notable.

Table 3. Phenolic profile obtained by UHPLC of the different extracts of *R. microphylla* (Rm) fruits.

Compound	RT (min)	Wavelength (nm)	Extract (mg/L)					
			RmA-C[1]	RmA-OH5	RmA-OH10	RmH-C[1]	RmH-OH5	RmH-OH10
Gallic acid	2.21	280	203.20 (± 0.70) [a]	9.18 (± 0.64) [e]	12.83 (± 1.07) [d]	98.60 (± 4.40) [b]	16.21 (± 0.40) [c]	14.69 (± 0.22) [c,d]
p-cumaric acid+epicatechin	11.54	280	7.40 (± 0.20) [b]	n.d.	n.d.	78.20 (± 1.50) [a]	n.d.	n.d.
Catechin	7.15	280	n.d.	n.d.	n.d.	10.40 (± 0.40)	n.d.	n.d.
Ellagic acid	12.75	250	n.d.	8.73 (± 0.11) [b]	8.80 (± 0.18) [b]	2.90 (± 0.10) [c]	9.43 (± 0.23) [a]	9.59 (± 0.02) [a]
Ferulic acid	13.02	320	5.70 (± 0.10) [a]	n.d.	n.d.	6.10 (± 1.30) [a]	n.d.	n.d.
Resveratrol	14.48	308	n.d.	n.d.	n.d.	2.90 (± 0.01)	n.d.	n.d.

Values are presented as mean (± standard deviation, $n = 3$), different lowercase letters in the same row indicate statistically significant differences ($p < 0.05$). A: aqueous extract; H: hydroalcohol extract; C: conventional agitation; OH: ohmic heating; 5 and 10: correspond to operating time (min) for OH extraction. n.d.: not detected. RT: retention time. [1]Adapted from Charles-Rodríguez [17].

Table 4. Phenolic profile obtained by UHPLC of the different extracts of *M. geometrizans* (Mg) fruits.

Compound	RT (min)	Wavelength (nm)	Extract (mg/L)					
			MgA-C	MgA-OH5	MgA-OH10	MgH-C	MgH-OH5	MgH-OH10
Rosmarinic acid	12.22	329	n.d.	n.d.	n.d.	12.36 (± 0.01)	n.d.	n.d.
Ellagic acid	12.75	250	4.76 (± 0.14) [c]	8.47 (± 0.03)[a]	n.d.	5.12 (± 0.01) [b]	8.62 (± 0.03) [a]	8.59 (± 0.01) [a]
Ferulic acid	13.02	320	8.30 (± 0.02) [a]	n.d.	n.d.	8.30 (± 0.00) [a]	n.d.	n.d.
o-cumaric acid	13.60	280	3.10 (± 0.01) [a]	n.d.	n.d.	3.15 (± 0.10) [a]	n.d.	n.d.
Rutin	12.74	350	n.d.	n.d.	n.d.	2.76 (± 0.07) [a]	n.d.	n.d.

Values are presented as mean (± standard deviation, $n = 3$), different lowercase letters in the same row indicate statistically significant differences ($p < 0.05$). A: aqueous extract; H: hydroalcohol extract; C: conventional agitation; OH: ohmic heating; 5 and 10: correspond to operating time (min) for OH extraction. n.d.: not detected. RT: retention time.

2.3. Biological Activity of Extracts

2.3.1. Scavenging and Reducing Properties

The relation between the antioxidant capacity of the plant extracts and their bioactivity is well known. Thus, this parameter was determined through the radical scavenging activity (RSA) measured by the DPPH and ABTS assays, as well as the ferric reducing power by the FRAP assay (Table 5). In general, the Rm fruit extracts evidenced a stronger scavenging and reducing capacities compared to those of Mg fruits in all assays. The Rm fruit extracts obtained by conventional agitation with both solvents had higher DPPH radical scavenging activity and higher ferric reducing power compared with the OH extracts ($p < 0.05$); meanwhile for the ABTS results, only the aqueous extracts showed differences ($p < 0.05$) as a function of the extraction technique employed. Furthermore, the results observed in the DPPH and FRAP assays for the OH aqueous extracts (RmA-OH5 and RmA-OH10) were significantly influenced by the operating times ($p < 0.05$). Otherwise, in the EC_{50} values obtained in ABTS assay, the operating times did not affect ($p > 0.05$) the results of scavenging capacity in OH extracts.

Table 5. Scavenging (DPPH, ABTS) and reducing (FRAP) properties of *R. microphylla* (Rm) and *M. geometrizans* (Mg) fruit extracts obtained by conventional agitation (C) and ohmic heating (OH).

Extract	DPPH EC_{50} (mg/mL)	ABTS EC_{50} (mg/mL)	FRAP µM Fe(II)/g extract
	Rm fruit		
RmA-C	0.36 (± 0.02) ^{a}	0.17 (± 0.01) ^{a}	1662.00 (± 108.30) ^{a}
RmA-OH5	0.94 (± 0.03) ^{d}	0.48 (± 0.03) ^{b}	840.23 (± 60.65) ^{b}
RmA-OH10	0.72 (± 0.09) ^{c}	0.41 (± 0.02) ^{b}	660.52 (± 12.70) ^{c}
RmH-C	0.32 (± 0.04) ^{a}	0.24 (± 0.02) ^{a}	1589.39 (± 53.02) ^{a}
RmH-OH5	0.60 (± 0.02) ^{b,c}	0.20 (± 0.11) ^{a}	840.50 (± 23.03) ^{b}
RmH-OH10	0.56 (± 0.04) ^{b}	0.21 (± 0.00) ^{a}	485.51 (± 13.92) ^{d}
	Mg fruit		
MgA-C	8.75 (± 0.92) ^{b}	5.65 (± 0.87) ^{b,c}	136.94 (± 4.92) ^{d}
MgA-OH5	21.06 (± 2.59) ^{c}	6.95 (± 1.19) ^{c}	168.18 (± 1.95) ^{c}
MgA-OH10	9.62 (± 1.34) ^{b}	6.19 (± 0.73) ^{b,c}	173.06 (± 5.73) ^{c}
MgH-C	5.25 (± 0.86) ^{a}	3.79 (± 0.37) ^{a}	255.78 (± 24.36) ^{a}
MgH-OH5	17.92 (± 0.87) ^{c}	7.04 (± 0.61) ^{c}	207.56 (± 10.65) ^{b}
MgH-OH10	16.76 (± 3.62) ^{c}	4.59 (± 0.31) ^{b}	174.14 (± 6.57) ^{c}

Values are presented as mean (± standard deviation, $n = 3$), different lowercase letters in the same column indicate statistically significant differences ($p < 0.05$) for each plant. A: aqueous extract; H: hydroalcohol extract; C: conventional agitation; OH: ohmic heating; 5 and 10: correspond to operating time (min) for OH extraction.

On the other hand, the hydroalcohol extracts of Mg fruits showed similar behavior to that of hydroalcohol extracts of Rm fruits in the three assays evaluated, highlighting the stronger capacity of the MgH-C, with EC_{50} values of 5.25 and 3.79 mg/mL for DPPH and ABTS assays, respectively, and a higher ferric reducing power (255.78 µM Fe (II)/g extract). Furthermore, the operating times significantly affected the EC_{50} values of the OH aqueous extracts (MgA-OH5 and MgA-OH10) examined with the DPPH assay; for the OH hydroalcohol extracts, minor extraction time (MgH-OH5) allowed better reducing capacity of Fe^{3+} in comparison with its counterpart with a longer extraction time (MgH-OH10).

2.3.2. Cell Viability Assay

The cytotoxicity of the extracts against cell lines was studied through the cell viability assays. The twelve extracts showed no cytotoxicity against the noncancerous cell line (ARPE-19), even at high concentrations (>800 µg/mL) (Table 6). Regarding the antiproliferative activity against HeLa (cancerous cell line), only the hydroalcohol extracts of Rm fruits (RmH-C, RmH-OH5, and RmH-OH10) showed moderate effectiveness, highlighting the RmH-C extract as it showed significant effect with an IC_{50} value of 417.73 ± 29.06 µg/mL. These results propose for the first time the antiproliferative capacity of the Rm fruit extracts, which could be selective according to the extraction conditions.

Table 6. Antiproliferative activity of *R. microphylla* (Rm) and *M. geometrizans* (Mg) fruit extracts obtained by conventional agitation (C) and ohmic heating (OH) in ARPE-19 and HeLa cell lines.

Extract	Cell lines IC_{50} (µg/mL)	
	ARPE-19	HeLa
Rm fruit		
RmA-C	> 800 [a]	> 800 [c]
RmA-OH5	> 800 [a]	> 800 [c]
RmA-OH10	> 800 [a]	> 800 [c]
RmH-C	> 800 [a]	417.73 (± 29.06) [a]
RmH-OH5	> 800 [a]	705.73 (± 21.59) [b]
RmH-OH10	> 800 [a]	615.33 (± 64.56) [b]
Mg fruit		
MgA-C	> 800 [a]	> 800 [c]
MgA-OH5	> 800 [a]	> 800 [c]
MgA-OH10	> 800 [a]	> 800 [c]
MgH-C	> 800 [a]	> 800 [c]
MgH-OH5	> 800 [a]	> 800 [c]
MgH-OH10	> 800 [a]	> 800 [c]

IC_{50} values represent a mean and standard deviation (± SD; $n = 3$) of three independent experiments. Different lowercase letters in the same column indicate statistically significant differences ($p < 0.05$). A: aqueous extract; H: hydroalcohol extract; C: conventional agitation; OH: ohmic heating; 5 and 10: correspond to operating time (min) for OH extraction.

2.3.3. Antifungal Activity In Vitro

Figure 1 shows the Rm and Mg fruit extracts with the highest antifungal effect on *F. oxysporum* and *R. stolonifer*. The MgH-C extract was more effective in inhibiting the growth of *F. oxysporum*, showing 100% inhibition at 6000 mg/L, whereas, the RmH-C extract did not exceed 50%. Regarding *R. stolonifer*, the RmH-C extract presented complete inhibition at 2000 mg/L; additionally, good inhibitions (in the range of 50–75%) were achieved at doses of 3000, 3500, and 8000 mg/L for the RmH-OH5, RMH-OH10, and MgH-C extracts, respectively. It is important to note that only some extracts (MgH-C, RmH-OH5, and MgH-C) showed a concentration-dependent effect, which is interesting for its application as antifungal agents.

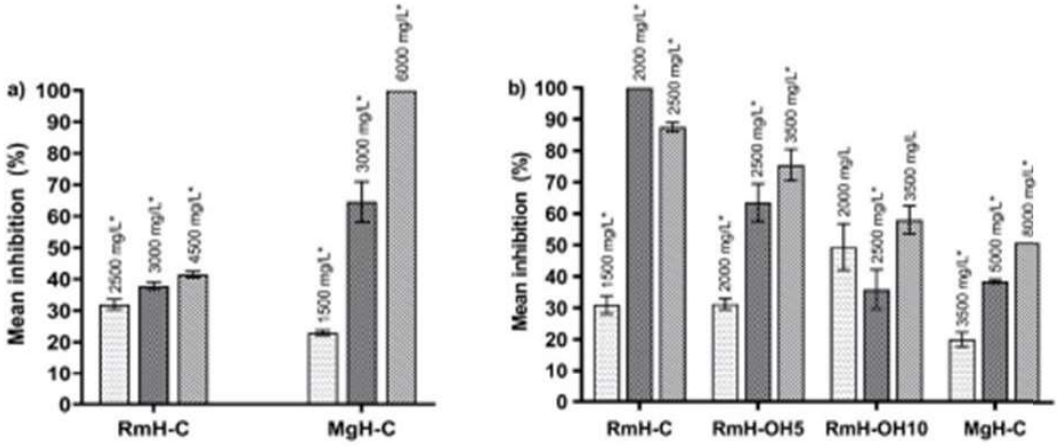

Figure 1. Mean inhibition (%) of *R. microphylla* (Rm) and *M. geometrizans* (Mg) fruit extracts against *F. oxysporum* (**a**) and *R. stolonifer* (**b**). Values are expressed as mean ± standard deviation (error bars), $n = 4$. The asterisk indicates significant difference between concentrations in each extract ($p < 0.05$). H: hydroalcohol extract; C: conventional agitation; OH: ohmic heating; 5 and 10: correspond to operating time (min) for OH extraction.

The ability of Rm and Mg fruit extracts to inhibit the growth of *R. stolonifer* and *F. oxysporum* is also reported as the minimum extract concentration required to inhibit 50 and 90% of the mycelia growth of fungi (MIC_{50} and MIC_{90}, respectively) (Table 7). The RmH-C extract showed the higher antifungal activity on *R. stolonifer*, as lower values of MICs were observed (1599 and 2219 mg/L for MIC_{50} and MIC_{90}, respectively). In addition, the Rm extracts obtained by means of OH showed MIC_{50} values in a range of 2366–2918 mg/L, whilst the MIC_{50} value for the MgH-C extract was approximately three-fold higher (8415 mg/L) for the inhibition of *R. stolonifer*. Otherwise, the MgH-C extract evidenced stronger antifungal activity against *F. oxysporum*, with MIC_{50} and MIC_{90} values of 1915 and 4881 mg/L, respectively. The other extracts only inhibited mycelia growth of *F. oxysporum* at higher concentrations (up to 5940 mg/L). These results exhibit the specificity of some Rm and Mg fruit extracts to inhibit the development of the fungi under study.

Table 7. Minimum inhibitory concentrations (MICs) of *R. microphylla* (Rm) and *M. geometrizans* (Mg) fruit extracts causing a reduction of 50 and 90% of mycelia growth of *R. stolonifer* and *F. oxysporum*.

Extract	MIC_{50} (mg/L)	95% Fiducial Limits		MIC_{90} (mg/L)	95% Fiducial Limits	
		Lower	Upper		Lower	Upper
			R. stolonifer			
RmH-C	1599	1291	1784	2219	1974	2927
RmH-OH5	2366	2164	2541	4432	3825	5819
RmH-OH10	2918	n.d.	n.d.	40912	n.d.	n.d.
MgH-C	8415	7355	10026	54621	37318	92963
			F. oxysporum			
RmH-C	5940	4551	12395	73199	24989	1653990
MgH-C	1915	1631	2256	4881	3908	6661

A: aqueous extract; H: hydroalcohol extract; C: conventional agitation; OH: ohmic heating; 5 and 10: correspond to operating time (min) for OH extraction. The MICs values of extracts with low inhibition percentages (< 40%) were not included. n.d.= not detected.

3. Discussion

In the present work, two extraction techniques (conventional agitation and OH) were evaluated on the yields, phenolic composition, and bioactive properties of Rm and Mg fruit extracts. In the OH extraction, the conductivity of the water provides a higher conductivity allowing a better dispersion of the electric current and promoting an increase in permeability within the cell wall with the subsequent appearance of pores through which the solvent enters and releases the BCs to the medium, thus increasing the extraction yields [23].

On the other hand, the highest yields obtained for RmH-C can be associated with the fact that conventional agitation is a gentler procedure that in combination with hydroalcohol solution as solvent, allowed a greater release of free phenolic compounds located inside of the cell vacuoles; in addition, the solubility of the phenolic compounds is improved owing to the solvent employed [24]. Otherwise, the OH technique can be helpful to enhance the extraction of intracellular BCs from plants (e.g., flavonoids, anthocyanins, etc.) because the applied electrical current causes a partial or total rupture of the membrane by the electroporation generated and the consequent increase in temperature [16,23]. These results agreed with the TPC values and phenolic composition, as those extracts obtained by conventional agitation and a hydroalcohol solution as an extracting agent for both plants (RmH-C and MgH-C) were those that showed the best performance for these parameters. Generally, the Rm fruit extracts obtained by conventional agitation were characterized by the presence of gallic acid and p-coumaric acid+epicatechin as main compounds, which probably affected their strong DPPH and ABTS scavenging capacities, as minor values of EC_{50} indicate higher activity [25]. Recently, extracts of *Ephedra alata* containing these phenolic compounds were reported by Benabderrahim [26] as powerful antioxidant agents. It has been reported that the antioxidant capacity of phenolic compounds results from two

mechanisms, by the donating a hydrogen atom or by acting as electron donors; additionally, there is a close relationship between the structure and the antioxidant capacity of these compounds, which is associated with the presence and number of hydroxyl groups [27]. It was also noted that according to the classification reported by Wong [28], five Rm fruit extracts can be classified as strong reducing agents (RmA-C, RmA-OH5, RmA-OH10, RmH-C, and RmH-OH5), as they presented a high ferric reducing power with values of >500 µm Fe (II)/g per extract. The lower scavenging and reducing capacities of Mg fruit extracts are consistent with their lower TPC values and their minor phenolic composition [29]. Several BCs, such as epigallocatechin, protocatechuic acid, rutin, kaempferol, among others, have been reported in other plants of the *Rhus* genus [30,31]. Recently, Montiel-Sanchez [21] reported the presence of betaxanthins such as indicaxanthin and vulgaxanthin I; betacyanins, mostly phyllocactin and betanin; and some other phenolic compounds such as rutin and quercetin derivatives in the pulp, skin, and whole fruit of *M. geometrizans*.

Some of these phenolic compounds detected in Rm and Mg fruit extracts (e.g., gallic acid, *p*-coumaric acid, and ferulic acid) have been reported for their cytotoxicity properties against cancerous cell line cultures, such as in human breast cancer MCF-7 cell line, human prostate cancer cells (PC-3), and lung cancer cells (A549) [32,33]. The anticancer activity of these compounds is caused by the promotion and generation of reactive oxygen species and the arrest of the cell cycle, which induces apoptosis; furthermore, the synergism between compounds can improve their bioactivities [34,35]. López-Romero [36] identified the presence of epicatechin in extracts of *Litsea glaucescens*, having antiproliferative effect against HeLa cancer cell line (IC_{50} of 45.80 µg/mL) and selectivity between cancerous and noncancerous cell lines. Similarly, Rm and Mg fruit extracts did not show cytotoxicity against the noncancerous ARPE-19 cell line; in addition, three extracts showed selectivity (RmH-C, RmH-OH5, and RmH-OH10) by presenting moderate activity against HeLa. However, they cannot be considered as cytotoxic extracts, according to the US National Cancer Institute, as only extracts with an IC_{50} value of < 30 µg/mL can fit into this classification [37]. It is important to note that this is the first report of the cytotoxicity of extracts from Rm and Mg fruits against ARPE-19 and HeLa cell lines.

One of the most important effects reported for plant extracts is their ability to inhibit the mycelial development of fungi, which leads to their potential use as biofungicides [38]. In this context, RmH-C extract stood out for its potent antifungal activity against *R. stolonifer*, an important phytopathogenic fungus. This extract showed growth inhibition at a lower concentration (MIC_{50}: 1599 mg/L) than those reported by Yang and Jiang [39] for tea polyphenols (mainly containing catechins) with MIC_{50} values of 2900 mg/L. For *F. oxysporum* (a very devastating crop pathogen), the MgH-C extract achieved lower MIC_{50} values (1915 mg/L) than those reported by Jasso de Rodríguez [40] for ethanol extracts of *R. muelleri* (MIC_{50} of 3363 mg/L). These results can be attributed to the interaction of phenolic acids and flavonoids present in the plant extract matrix of the Mg and Rm fruits, because although the mechanism of action of the extracts to inhibit mycelial growth is not completely elucidated, it is known that phenolic compounds can act against pathogens through enzymatic inhibition by oxidation of the fungal cell membrane [2,41,42].

Generally, the technique and conditions used can impact the nature and quantity of phenolic compounds isolated from the plants under study. Considering the demonstrated biological activities of Mg and Rm fruit extracts, they can be a novel proposal for the development of biofungicides, being an interesting alternative for synthetic products that affect the environment and health. In addition, their low cytotoxicity results indicate that they can be incorporated as a natural source of BCs in foods, having a positive impact on their properties.

This work provides the basis for future research, where the antiproliferative and antifungal potential of Mg and Rm fruit extracts is evaluated against other cell lines and fungal strains, respectively, in addition to the elucidation of the other compounds associated with their bioactivity.

4. Materials and Methods

4.1. Materials and Strain

Sodium carbonate (Na_2CO_3), gallic acid (GA), potassium persulfate (PP), 2,2-diphenyl-1-picrylhydrazil (DPPH), 2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,4,6-tri(2-pyridyl)-striazine (TPTZ), iron (III) chloride hexa-hydrate, dimethyl sulfoxide (DMSO), and all standards reagents for UHPLC analysis were purchased from Sigma (Sigma-Aldrich, Saint Louis, MO, USA). Absolute ethanol (99.9%) was obtained from Jalmek (Jalmek Científica S.A. de C.V., San Nicolás, NL, Mexico). Potato dextrose broth (PDB) was purchased from TM MEDIA (Titan Biotech Ltd., Delhi, India). The Folin–Ciocalteu (FC) reagent was from Merck (Merck KGaA, Darmstadt, Germany). All standards, samples, and eluents were prepared using Milli-Q water (Millipore, Bedford, MA, USA).

Rhizopus stolonifer (CDBB accession no. 1384) was supplied from CINVESTAV (Centro de Investigación y de Estudios Avanzados del IPN, CDMX, Mexico), and *Fusarium oxysporum* (National Center for Biotechnology Information, NCBI, accession no. MT001892) was acquired from CICY (Yucatan Center for Scientific Research, Yucatan, Mexico).

HeLa (human cervix carcinoma) and ARPE-19 (human retinal pigmented epithelium) cell line cultures were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 5% fetal bovine serum (FBS) (Sigma-Aldrich, Saint Louis, MO, USA).

4.2. Plant Material

Rm fruits were randomly collected in wild areas located in the city of Saltillo, in Coahuila State, Mexico (25°20′44.5′′N 101°01′48.7′′W), and Mg fruits were collected in Pozo Hondo, Guanajuato State, Mexico (21°24′33.9′′N 100°36′27.8′′W) from April to May 2019. The samples were transported in plastic bags to the Fermentation Laboratory at the Universidad Autónoma Agraria Antonio Narro (UAAAN); the collected fruits were washed with distilled water and dried in a stove (Biobase Biodustry Shandong Co., Ltd., Jinan, SHG, China) at 60 °C for 48 h [38]. Subsequently, the fruits were ground to obtain a particle size equivalent to mesh no. 20; then the samples were stored in bags in a dark place until further use.

4.3. Preparation of R. microphylla (Rm) and M. geometrizans (Mg) Fruit Extracts

4.3.1. Extraction by Ohmic Heating (OH)

The laboratory-built OH system consisted of a power supply (Voltage Autotransformer, NAPEE, Mexico), two iron electrodes, a circulating water bath, and a three-neck flask. The extraction was conducted by evaluating two parameters (solvent and operational times) using a 2^2 factorial design (Table 8). For the experiment, a dried sample (20 g) was placed in the three-neck flask containing 400 mL of solvent, water, and a hydroalcohol solution (50:50), the electrodes were placed in the flask applying a voltage of 70 V in all of the experiments (conditions were chosen from a preliminary study, data not shown), and the temperatures were maintained below the boiling points of the solvents (99 °C for water and 75 °C for the hydroalcohol solution, respectively). The extracts obtained were filtered using a vacuum pump, and subsequently concentrated using a rotary evaporator (IKA RV 10 basic, IKA Werke GmbH and Co, KG, Staufen, Germany). Finally, the extracts were stored in the dark at 5 °C until further analyses were performed.

Table 8. Factorial design for ohmic heating (OH) extraction.

Run	Solvent	Time (min)
1	Water	5
2	Water	10
3	Hydroalcohol solution (50:50)	5
4	Hydroalcohol solution (50:50)	10

The experimental data (yields and TPC) for extracts obtained by means of OH extraction were analyzed with a linear first-order regression model with the following general regression equation:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{12} x_1 x_2 \quad (1)$$

where y is the response (dependent variable); β_0, β_1, β_2 and β_{12} are regression coefficients calculated from experimental data; and x_1 and x_2 correspond to independent variables.

4.3.2. Extraction by Conventional Agitation

Conventional agitation was carried out following the method previously described by Charles-Rodríguez [17]. Initially, 11.5 g of dried sample was placed in a flask containing 125 mL of water and a hydroalcohol solution (50:50) and extracted at 150 rpm for 24 h at room temperature (Innova 44 Incubator, New Brunswick Scientific Co., Inc., Edison, NJ, USA). The aqueous and hydroalcohol extracts were concentrated and stored as previously described for samples obtained by OH extraction (Section 4.3.1).

4.4. Analytical Methods

4.4.1. Determination of Extraction Yields

The yield percentage for each extraction was determined as follows:

$$Yield\ (\%) = \left(\frac{M_{EC}}{M_{ES}}\right) \times 100 \quad (2)$$

where M_{EC} is the mass of extract obtained at the end of the extraction process and M_{ES} is the initial mass of dried sample used for the test.

4.4.2. Total Phenolic Content (TPC)

The TPC was made by FC method adapted to microplate [38]. Initially, 5 µL of diluted sample (20 mg of extract in 5 mL of its respective solvent, water and a hydroalcohol solution) were placed in a 96-well microplate; 60 µL of FC reagent was then added and mixed for 2 min, followed by the addition of 15 µL of Na_2CO_3 solution (7.5% w/v) and 200 µL of distilled water. Finally, the reaction mixture was incubated at 60 °C for 5 min and the absorbance was measured at 750 nm in a fully automatic microplate lector (BIOBASE-EL 10A, Jinan, SHG, China). The values were compared with a calibration curve of GA at 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 mg/L (R^2 = 0.9973). The results were expressed as mg equivalents of GA per gram of extract. All experiments were made in triplicate.

4.4.3. Phenolic Profile by Ultra High-Performance Liquid Chromatography (UHPLC)

It is crucial to perform a chromatographic analysis for the correct elucidation of the phenol composition as the presence of compounds in the extracts, such as reducing sugars (glucose and fructose), vitamin C, among others, can interfere with the accuracy of the Folin–Ciocalteu method [43]. The identification and quantification of phenolic compounds in the extracts were evaluated using Shimatzu Nexpera X2 equipment coupled with a diode array detector (Shimadzu, SPD-M20A, Tokyo, Japan). For this purpose, a reversed-phase Acquity UPLC BEH C18 column of 2.1 mm × 100 mm, 1.7 µm (Waters) and a precolumn filled with the same material. The temperature and the flow rate were 40 °C and 0.4 mL/min, respectively. The elution gradient used was in accordance with the previous report [44], where solvent A was a water/formic acid (0.1%) and acetonitrile as

solvent B. For solvent B, the elution gradient was as follows: from 0.0 to 5.5 min eluent B at 5%, from 5.5 to 17 min was linearly increasing from 5 to 60%, from 17.0 to 18.5 min a linear increase to 100%; and, lastly, from 18.5 to 30.0 min, the column was equilibrated at 5%. The identification was conducted by comparing the ultraviolet spectra and retention times of samples with those of the standards. For each compound, calibration curves were conducted with concentrations between 2.5 and 250 mg/mL ($R^2 > 0.99$); the compounds were identified and quantified at wavelengths between 209 and 370 nm. All measurements were performed in triplicate.

4.5. Bioactivity of Extracts

4.5.1. Scavenging Properties

2,2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay

The capacity of free radical capture of extracts was determined by the DPPH assay [17]. The DPPH microplate-adapted assay was conducted using 25 µL of each diluted sample at 20 to 2500 mg/L, dissolved in its respective extraction solvent, then the samples were mixed with a 200 µL of DPPH solution (150 µM, dissolved in absolute ethanol) in a 96-well microplate. The reaction was incubated under dark conditions at room temperature for 30 min. The absorbance was measured at 520 nm in a fully automatic microplate lector (BIOBASE-EL 10A, Jinan, SHG, China), using absolute ethanol as a control. The scavenging activity was expressed as DPPH radical scavenging activity percentage (*% RSA*), determined using the following equation:

$$RSA\ (\%) = \left(\frac{A_{control} - A_{sample}}{A_{control}}\right) \times 100 \quad (3)$$

where $A_{control}$= control absorbance and A_{sample}= sample absorbance. The scavenging activity was reported as EC_{50} (half-effective concentration) values, which expresses the concentration that gives the 50% maximal response of radical scavenging activity and was calculated from the regression equation given by the concentration–%RSA curve. All measurements were performed in triplicate.

2,2′-Azino-di-[3-ethylbenzthiazoline Sulfonate] (ABTS) Radical Scavenging Assay

The scavenging capacity by the cation radical discoloration test (ABTS) of the extracts was conducted by the microplate-adapted assay as described by Jasso de Rodríguez [38] with minor modifications. The solution of ABTS was prepared at concentration of 7 mM in distilled water and mixed with a solution of potassium persulfate (2.45 mM); the mixture was kept at 4 °C during 14–16 h under dark conditions to ensure a stable oxidative state. The work solution was adjusted with ethanol at 20% to an absorbance of 0.700 ± 0.010 at 750 nm. In order to determine the scavenging activity, 10 µL of diluted samples were added to a 96-well microplate and mixed with 200 µL of work solution of ABTS, the reaction mixture was maintained for 10 min under dark conditions, and then the absorbance was measured at 750 nm, using the respective solvent as a control (i.e., water and a hydroalcohol solution). The results were calculated using the same Equation (3) described for the DPPH assay. The results were expressed as EC_{50} values. All experiments were carried out in triplicate.

4.5.2. Reducing Properties

Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay involves the ability of extract to reduce ferric ions (Fe^{3+}) [45]; the ferric reducing ability was evaluated according to the microplate-adapted methodology described by Lopéz-Romero [36]. The working solution of FRAP was made mixing 10 volumes of 300 mM acetate buffer (pH 3.6), 1 volume of 20 mM aqueous ferric chloride, and 1 volume of 40 mM TPTZ (HCl 40 mM as solvent). To evaluate the reducing activity, 280 µL of working solution was mixed with 20 µL of the extracts (0.5 mg/mL) in a 96-well microplate and put under dark conditions for 30 min to complete the reaction. The

absorbance was read at 630 nm in a microplate reader (BMG Labtech, Ortenberg, Germany). Results were reported as μM Fe(ll)/g extract. All experiments were carried out in triplicate.

4.5.3. Cell Viability Assay

Cell lines HeLa (human cervix carcinoma) and ARPE-19 (human retinal pigmented epithelium) were obtained from the American Type Culture Collection (ATCC; Rockville, MD, USA). Cells were cultured in DMEM supplemented with 5% FBS (Sigma, St. Louis, MO, USA). The evaluation of the cell viability was conducted by the MTT assay against ARPE-19 and HeLa cell line cultures, where metabolically active cells reduce the tetrazolium salt to colored formazan crystals and the amount of formazan produced is directly proportional to the number of viable cells [46]. The procedure was made according to Hernandez [47]. Initially, 50 μL (at 1×10^4 cells) was incubated in a 96-well microplate for 24 h at 37 °C, with a 5% CO_2 atmosphere. Then, 50 μL of medium containing different concentrations of extracts (previously dissolved in DMSO) were added and incubated for 48 h under the same conditions. Each well was washed with a PBS solution and refilled with fresh culture medium in the last 4 h. Finally, 10 μL of an MTT solution (5 mg/mL) was added to each well of the 96-well microplate and read at 570 and 650 nm in a microplate reader (iMark, Bio-Rad Laboratories, D.F., Mexico). The results were expressed as IC_{50} values that corresponded to the required concentration to inhibit 50% of the viable cells proliferation. All experiments were carried out in triplicate.

4.5.4. Antifungal Activity in vitro

The antifungal activity was made by a microdilution technique according to a previous report [48], with some modifications. Briefly, extracts were diluted with 100 μL of sterile PDB to obtain different doses and were added into a sterile 96-well microplate. Then, 100 μL of the spore's suspension of *R. stolonifer* or *F. oxysporum* at a concentration of 10^4 spores/mL, was added. Fungal sporulation was monitored by changes in the optical density (OD) in fully automatic microplate lector (BIOBASE-EL 10A, Jinan, SHG, China) at 530 nm during 36 h (12 h intervals, at an incubation temperature of 25 ± 2 °C). A positive control was prepared by mixing 100 μL of sterile PDB with 100 μL of spore suspension. The percentage of growth inhibition (%) was calculated by the following equation:

$$Inhibition\ (\%) = \left(\frac{OD_{control} - OD_{sample}}{OD_{control}}\right) \times 100 \quad (4)$$

where OD_{sample} represents the optical density of each treatment and $OD_{control}$ represents the optical density of the control. All treatments were replicated four times. The inhibition results were used to estimate the minimum inhibitory concentration (MIC) of extract that causes a 50% and 90% of reduction in fungal growth (MIC_{50} and MIC_{90}, respectively).

4.6. Statistical Analysis

Statistical analyses of the data were performed by one-way analyses of variance (ANOVA) to detect significant differences and the Tukey mean comparison test ($p < 0.05$) using Minitab software version 17.0 (State College, PA, USA). The Probit analysis (SAS Program Version 9.1) was used to calculate the minimum inhibitory concentration of extract causing a 50% and 90% (MIC_{50} and MIC_{90}, respectively) of reduction in fungal growth at $p < 0.05$ significant level.

5. Conclusions

The conditions and type of extraction technique can influence the properties and nature of compounds isolated from plant extracts. In this work, the phenolic composition of Rm and Mg fruit extracts was characterized by the presence of phenolic acids and flavonoids, showing that hydroalcohol extracts of both fruits exhibited the highest scavenging and reducing properties and antifungal activity. Among these, the RmH-C and MgH-C extracts are highlighted for their antifungal efficacy to inhibit the growth of

R. stolonifer and *F. oxysporum*, respectively. Additionally, low cytotoxicity was observed against HeLa line cell in some treatments (RmH-C, RmH-OH5, and RmH-H10) while there was no cytotoxic effect for noncancerous ARPE-19 cell line.

The use of plants as a source of BCs is an area of relevance for the development of novel products to meet regional needs, and it is necessary to choose the appropriate extraction technique to guarantee their properties. This study reveals the potential of Rm and Mg fruits as novel sources of BCs for future applications in various areas, such as agrifood and pharmaceutical industries.

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CAPÍTULO 4. SEGUNDO ARTÍCULO.

ELECTROSPRAYED HYDROXYPROPYL METHYLCELLULOSE MICROCAPSULES CONTAINING *Rhus microphylla* FRUIT EXTRACTS AND THEIR USE IN STRAWBERRY (*Fragaria x ananassa*) PRESERVATION.

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

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

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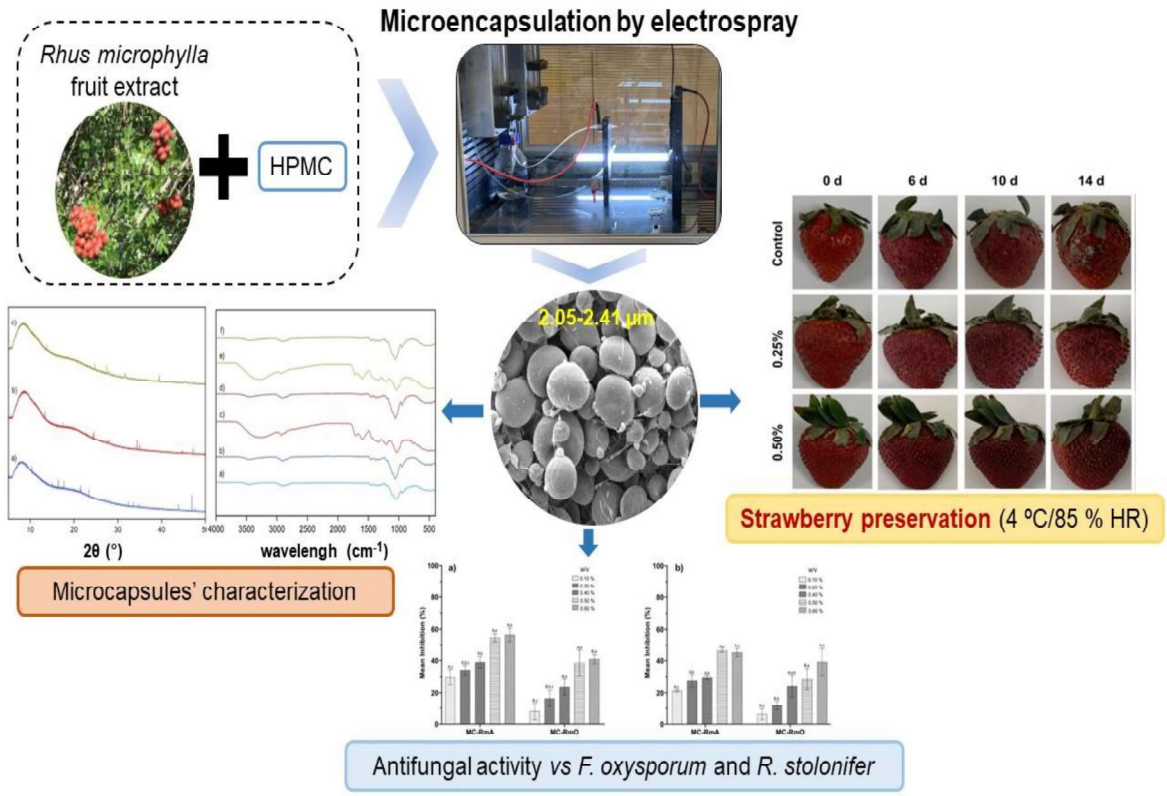
Graphical abstract



Abstract

Encapsulation technology is used for the incorporation of a great variety of compounds, being helpful to protect and improve the bioactivity of plant extracts. In this study, microcapsules (MC) containing two different extracts from *Rhus microphylla* fruit were prepared by electrohydrodynamic processing, and then characterized in terms of Scanning Electron Microscopy (SEM), ATR-Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), and thermogravimetric analysis (TGA). Microcapsules with spherical shape and particle size between 2.05-2.41 µm were obtained, and according to the FTIR results, there was a correct incorporation of the extracts. The microcapsules containing RmA extract (MC-RmA) showed the best antioxidant and antifungal activities *in vitro*; therefore, their effectiveness in extending the quality of strawberry fruits during storage at 4±1 °C and 85 % of relative humidity (RH), was evaluated at concentrations of 0.25 and 0.50 % (w/v). After 14 d of storage, the fruits treated with MC-RmA demonstrated lower weight loss, higher firmness, whereas the color of strawberry fruits was not affected. Furthermore, the MC-RmA showed a slow release, as the antifungal activity was gradual, suggesting that it could be used as a novel alternative to reduce postharvest losses of strawberry fruits.

Keywords: *Rhus microphylla; electrospraying; microcapsules; antifungal activity; strawberry; shelf life*

1. Introduction

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

Electrohydrodynamic processing is a novel technique to produce capsules and fibers in micro and nanoscale and that can be used in two modes, electrospinning for the production of fibers and electrospraying to produce particles (Silva et al., 2022). Electrospraying presents some advantages comparing to others methods; for example, it is possible to encapsulate thermolabile compounds without affecting their integrity, it has a lower energy consumption, and it also provides greater homogeneity in the shape and particle size of the structures produced (Gómez-Mascaraque et al., 2017). During electrospraying process, the polymer solution containing the compounds of interest is atomized into a collector through

a capillary employing a high electric field, where the electric energy promotes the atomization by a deformation of the droplet at the tip of the capillary nozzle, forming a structure known as Taylor cone (Silva et al., 2021). Proper atomization and formation of the Taylor cone is ensured by employing electrical forces higher than the surface tension forces of the encapsulating solution (Nikoo et al., 2018). Electrospraying has been used for the microencapsulation of bioactive compounds such as anthocyanins (Atay et al., 2018), β-carotene (Gómez-Mascaraque et al., 2017), and curcumin (Gómez-Estaca et al., 2017), demonstrating its effectiveness to produce homogeneous structures using different wall materials and concentrations.

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

pepper (González-Saucedo et al., 2019), tomato (Gutiérrez-Molina et al., 2021), and strawberry (Hesami et al., 2021), among others.

Strawberry (*Fragaria* × *ananassa*) is a widely consumed and appreciated worldwide fruit for its flavor and multiple nutritional benefits (e.g., antioxidant, anti-aging, and anti-tumor properties), representing a valuable economic market, with Mexico being the third largest exporter of fresh strawberries (Müller et al., 2010; Morales-Mora et al., 2019; Li et al., 2020). However, strawberries are highly perishable during postharvest due to their sensitivity to injuries and fungal infections, which affect their quality (e.g., firmness, color, flavor), thus causing important product losses (Chu et al., 2020). The application of new technologies, such as the development of encapsulates containing bioactive plant extracts through electrospraying, emerges as an alternative to improve the postharvest quality of fruits and vegetables. Therefore, the aims of the present study were to develop and characterize microcapsules containing *R. microphylla* fruit extracts using food-grade hydroxypropyl-methylcellulose (HPMC), using electrospraying, and to evaluate their effect on the postharvest decay of strawberries, as model fruit. It is noteworthy that this is the first report about the development of HPMC microcapsules through electrospray containing *R. microphylla* fruit extract and the study of their effect on strawberry preservation.

2. Materials and methods

2.1. Materials and reagents

Hydroxypropyl-methylcellulose (methoxyl 28-30 %, hydroxypropyl 7-12 %, viscosity 2 % aqueous solution, viscosity range of 40-60 mPa/s, at 20 °C, 90kDa) was purchased from Alfa Aesar GmbH & Co KG (Germany). Folin-Ciocalteu reagent (FC), 2,2-Diphenyl-1-picryl hydrazyl (DPPH), 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,4,6-tri(2-pyridyl)-striazine (TPTZ), iron (III) chloride hexa-hydrate, ascorbic acid (AA), potassium persulfate ($K_2S_2O_8$), sodium carbonate (Na_2CO_3) and gallic acid monohydrate (GA) were purchased from Sigma-Aldrich (Germany). Absolute

ethanol (>99.5 %) was purchased from Honeywell (USA) and Sabouraud dextrose broth (SDB) was purchased from PanReac AppliChem (Germany).

Strawberries were obtained from local market (Coahuila, Mexico), twelve hours after harvesting and immediately transported to the laboratory of the Universidad Autónoma Agraria Antonio Narro (UAAAN). Fruit with uniform color and size, without physical damage or fungal infection were selected.

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

2.2. Preparation of polymer solutions containing Rm extracts and electrospraying conditions

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

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

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.

2.3. Microcapsules characterization and bioactivity

2.3.1. Morphology and particle size of microcapsules

To select the best encapsulation conditions, the surface morphology of the particles obtained was examined by Scanning Electron Microscope (SEM) (Quanta FEG 650, FEI, USA). Briefly, 1.0-2.0 mg of sample were deposited on a double-sided conductive carbon tape, then analyzed at an acceleration voltage of 3.0 kV with a working distance of ~10 mm. After selecting the best treatment for each extract, 1.0-2.0 mg of specific samples were coated with gold under vacuum for 1 min (Leica EM ACE200, Germany) and analyzed in the SEM, with a voltage

of 5 kV and the same working distance. The morphology of at least 150 microcapsules was analyzed using ImageJ software (version 1.53k, USA), and the particle size and the particle aspect ratio (PAR) were determined. PAR was calculated with the following equation:

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

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

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

2.3.3. X-Ray diffraction analysis (XRD)

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

2.3.4. Thermogravimetric analysis (TGA)

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

2.3.5. Extract release from microcapsules

To determine the extract release from the microcapsules (MC-RmA and MC-RmO), samples were treated using two treatments: ultrasound (U) or agitation (A). For the ultrasonic bath (VWR, Spain) release, the methodology of Šturm et al. (2019) was followed with some modifications. Firstly, 10 mg of microcapsules were placed in 0.5 mL of mili-Q water and sonicated for 5 min. Then, the solutions were centrifuged at 13,300 rpm for 15 min. In the second method, the same concentration was used, but the solutions were kept in agitation for 1 h. The supernatant of the solutions was used for TPC, DPPH, ABTS, and FRAP assays.

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

The TPC released from the microcapsules was determined using the Folin-Ciocalteu (FC) method, following the methodology of Müller et al. (2010) with minor modifications. Twenty microliters of the supernatant were mixed with 100 µL of diluted FC solution (1:10 v/v, in water) for 5 min in a 96-well microplate, and 75 µL of Na_2CO_3 (7.5 % w/v) were added. The reaction was incubated for 5 min at 40 °C, cooled and kept at room temperature for 30 min more under dark conditions. The absorbance was measured at 750 nm on a Sinergy H1 Hybrid Reader microplate equipment (Biotek, USA), and the values were compared with a GA calibration curve (2.5-200 mg/L, R^2=0.9994). The results were expressed as mg GA equivalents per gram of microcapsules (mg GA/g MC). All experiments were performed in quadruplicate.

2.3.7. Radical scavenging capacity

2.3.7.1. DPPH radical scavenging activity

The scavenging capacity for DPPH was measured according to the method described by Guía-García et al. (2021), with minor modifications. Twenty-five microliters of the supernatant were placed in a 96-well microplate and mixed with 200 µL of DPPH solution (150 µM, dissolved in absolute ethanol). The reaction was incubated at room temperature for 30 min under dark conditions. The absorbance was measured at 520 nm in a Sinergy H1 Hybrid Reader microplate

equipment (Biotek, USA), using absolute ethanol as control. The scavenging capacity was expressed as percentage of Radical Scavenging Activity (%RSA), using the following equation:

$$RSA\ (\%) = \left(\frac{A_{control} - A_{sample}}{A_{control}}\right) x\ 100 \qquad (2)$$

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

2.3.7.2. ABTS radical scavenging activity

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

2.3.7.3. Ferric reducing capacity by FRAP assay

The ferric reducing capacity of the microcapsules content was evaluated following the method described by Guo & Jauregi (2018), with minor modifications. In a microcentrifuge tube was added 5 µL of the supernatant and mixed for 15 s with 150 µL of FRAP reagent (83.33 % of acetate buffer (300 mM), 8.33 % of TPTZ (10 mM) in HCl 40 mM, and 8.33 % of ferric chloride hexahydrate aqueous solution (20 mM)). Then, 100 µL were transferred to a 96-well microplate and the absorbance was measured at 595 nm in a Sinergy H1 Hybrid Reader microplate equipment (Biotek, USA). The results were expressed as ascorbic acid

equivalents (AA), using an ascorbic acid standard curve (1.5-400 mg/L, R^2=0.9992). All the assays were made by quadruplicate.

2.3.8. Antifungal properties

2.3.8.1. Fungal strains

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

2.3.8.2. Microdilution assay

The antifungal activity was made following the method report by Flores-López et al. (2016) with minor modifications. Different amounts of microcapsules (0.10, 0.20, 0.30, 0.40, 0.50, and 0.60 %, w/v) were diluted with 100 µL of SDB and placed in a sterile 96-well microplate, followed by the addition of 100 µL of a spore's suspension at 10^4 spores/mL of each strain. A positive control of 100 µL of SDB and 100 µL of spore's suspension was used. The fungal growth was measured during 36 h at 25 ± 2 °C by changes in the optical density (OD) at 530 nm in a Sinergy H1 Hybrid Reader microplate equipment (Biotek, USA). The percentage of growth inhibition (%) was calculated through Equation 3:

$$Inhibition\ (\%) = \left(\frac{ODcontrol - ODsample}{ODcontrol}\right) x100 \qquad (3)$$

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

2.4. Effect of microencapsulated extracts on strawberry fruit decay

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

A coating containing microcapsules (RmA) was prepared using a structured water vehicle, previously optimized for application in berries: 0.24 % (w/v) of lyophilized chia mucilage, 0.15 % (w/v) $CaCl_2$ and 0.05 % (w/v) glycerol (Charles-Rodríguez et al., 2021). Three treatments were evaluated: uncoated (control); coating with 0.25 % (w/v) and coating with 0.50 % (w/v) of microcapsules containing RmA. The treatments were applied on strawberry fruit by aspersion and left to dry in a convection oven at 25 °C for 25 min (Biobase Biodustry Shandong Co, Ltd., Jinan, SHG, China). For each treatment, three repetitions of 10 strawberries were evaluated (n=30), the fruits were placed in performed polypropylene plastic trays and stored at 4 ± 1 °C and 85 % RH for 14 d. Physicochemical and decay evaluations were analyzed at regular intervals (0, 2, 4, 6, 8, 10, 12, and 14 d).

2.5. Physicochemical analyses

2.5.1. Weight loss

Weight loss of strawberries (n=30) during storage was evaluated by means of the mass changing every two days using an analytical balance (Ohaus, USA), and the results were expressed as percentage using the following equation:

$$Weight\ loss\ (\%) = \frac{W_0 - W_d}{W_0} x100 \qquad (4)$$

Where W_0 is the initial weight, and W_d is the respective weight of every test day.

2.5.2. Texture analyses

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

2.5.3. Color

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

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

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

$$Redness = \left(\frac{a*}{b*}\right) \qquad (7)$$

2.5.4. Fungal decay

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

$$Fungal\ decay\ (\%) = \frac{Number\ of\ decay\ fruit}{Total\ number\ of\ fruit} x100 \qquad (8)$$

2.6. Statistical analysis

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

3. Results and discussion

3.1. Morphology and particle size of microcapsules

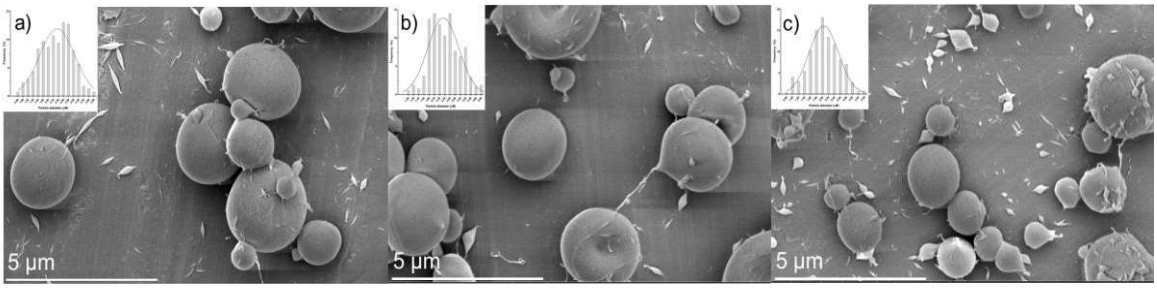


Fig. 1. SEM images of the developed microcapsules and their particle diameter distribution. a) MC-HPMC (T2); b) MC-RmA (T4); and c) MC-RmO (T8).

The ethanol and the extract amount were determining factors in the selection of the encapsulation conditions; the use of 50 % ethanol did not allow a correct evaporation of the solvent, leading to droplets, and the use of higher extract concentrations increased the presence of droplets in the samples (data not shown). On the other hand, with a concentration of 75 % ethanol and 1 mg/mL of extract and voltage of 14 and 17 kV, the best structures were obtained for both the RmA and RmO extracts (T4 and T8, respectively). Fig. 1 shows that these processing conditions allowed to produce homogeneous, spherical, structures with a smooth surface. Besides, the particle diameter followed a normal distribution, with a range between 0.80-4.00 µm. The spherical shape of the samples was confirmed by means of the PAR results (Table 2), where structures with values closer to 1 are more closely related to spherical shapes (Silva et al., 2021). The average particle sizes, shown in Table 2, allows the samples to be classified as microcapsules, considering that the sizes are in the range of 1-1000 µm (Shishir et al., 2018). The significative differences in the particle size between MC-RmA and MC-RmO could be explained by their components which can influence in the conductivity of the solutions and affect the particle size (Bhushani et al., 2017). These results are in agreement with those obtained in the encapsulation of ferulic acid, where spherical microcapsules were obtained

through spray-drying using HPMC as wall material, having a suitable incorporation of phenolic acid within the structures (Yu et al., 2021).

Table 2.

Particle size and particle aspect ratio of selected samples.

Sample	Particle Size (μm)	PAR
MC-HPMC	2.31±0.62[a]	1.10±0.08[a]
MC-RmA	2.41±0.57[a]	1.08±0.07[a]
MC-RmO	2.05±0.50[b]	1.08±0.06[a]

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

3.2. ATR-FTIR analyses

In Fig. 2 is shown the FTIR spectra of HPMC powder, empty MC-HPMC, and the free (RmA and RmO) and encapsulated extracts (MC-RmA and MC-RmO). For the HPMC powder and MC-HPMC, the peak around 3460 cm^{-1} corresponds to the —OH stretching vibration, and the presence of —CH aliphatic stretching vibrations was confirmed by the absorption peak at 2908 cm^{-1}, while the two absorption peaks of 1454 and 1371 cm^{-1} could be attributed to the —CH_3 asymmetric vibrations (Sheng et al., 2021). Besides, it was observed a strong peak around 1060-1020 cm^{-1} corresponding to the —CO stretching vibrations in all the samples (Wang et al., 2021). On the other hand, for unencapsulated extracts (RmA and RmO), the region around 3270-3300 cm^{-1} indicated the —OH stretching vibrations from phenolic compounds and ethanol (extraction solvent), whereas the absorption peak of 2926 cm^{-1} corresponds to the symmetric aliphatic stretching vibrations (—CH_2) (Hu et al., 2019). The absorption peak at 1709 cm^{-1} is related to the —C=O stretching, and in the region of 1590 cm^{-1} the absorption peak corresponds to the aromatic ring stretching (Zhao et al., 2022). However, in

the microcapsules containing RmA and RmO some minor changes in the spectra occurred, as the absorption peaks (1590-1700 cm^{-1}) of extracts were covered, indicating that extracts were correctly incorporated within the microcapsules (Sheng et al., 2021). Moreover, the intensity of the absorption peak around 3270-3330 cm^{-1} (presented in the extracts) decreased, and it was displaced to the spectral area of 3460 cm^{-1}. This could be explained by the hydrophobic interactions or hydrogen bonds formation between the polymer and the phenolic compounds from the extracts (Moreno et al., 2018). The FTIR results confirm the correct encapsulation of extracts inside the HPMC microcapsules produced by electrospray, providing protection, and reducing their susceptibility to environmental conditions.

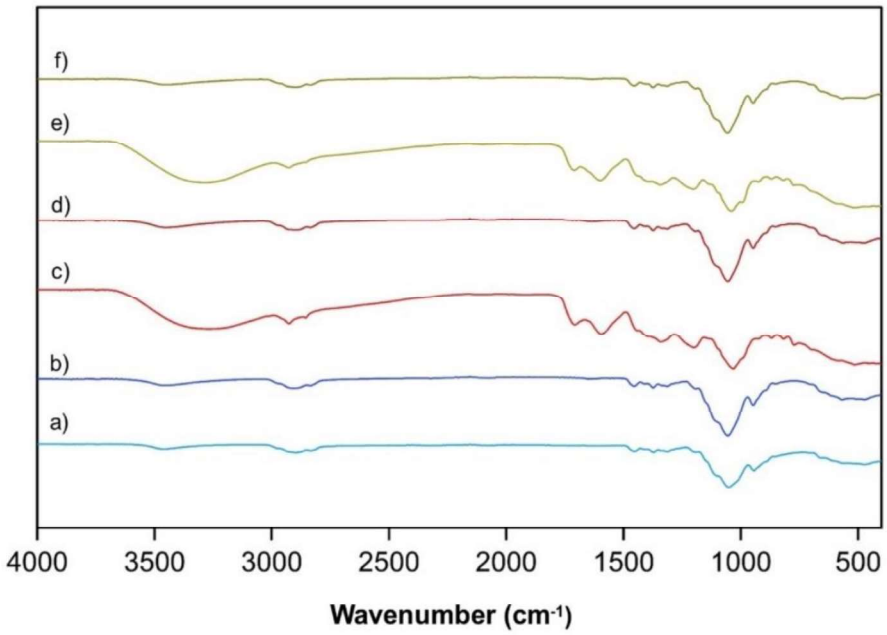


Fig. 2. FTIR spectra of free extracts and encapsulated extracts. a) HPMC powder; b) MC-HPMC; c) RmA; d) MC-RmA; e) RmO; f) MC-RmO.

3.3. XRD analyses

The XRD analysis is useful to identify the degree of crystallinity in samples, where a crystalline material exhibits specific and well-defined peaks in the diffractogram, while an amorphous material shows a rounded and diffuse peak (Papoutsis et al.,

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

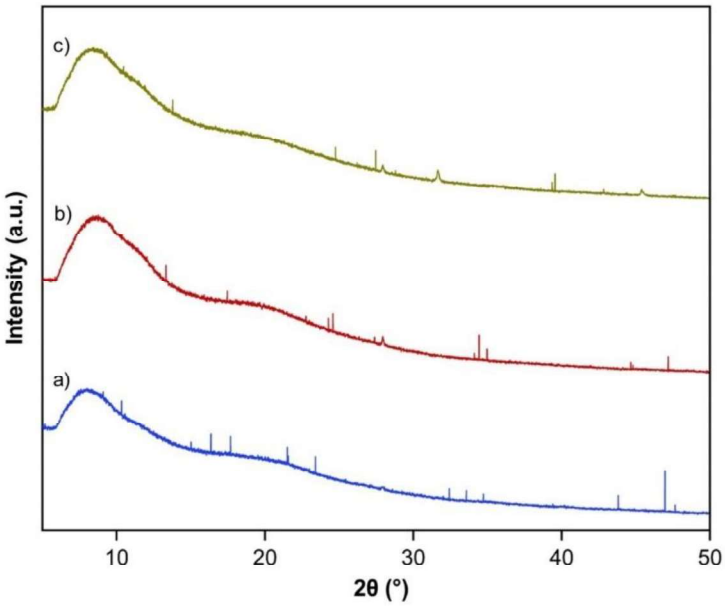


Fig. 3. X-ray diffractograms of the developed microcapsules. a) MC-HPMC; b) MC-RmA; and c) MC-RmO.

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

3.4. *TGA analyses*

Commonly, the bioactive compounds present in plant extracts are thermally unstable; in specific, polyphenols are very sensitive to high temperatures, which can cause the breakdown of the glucosyl moiety of the aglycone present in these

compounds, altering the bioactivity and bioavailability of the natural compound (Bedrníček et al., 2020).

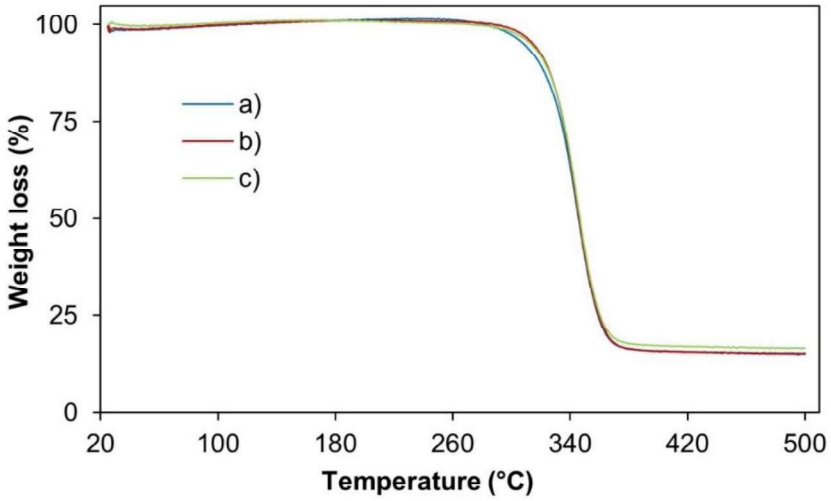


Fig. 4. TGA curves of microcapsules. a) MC-HPMC; b) MC-RmA; and c) MC-RmO.

Thermal analysis can provide information about the thermal stability of the samples and shows the amount of moisture and volatile compounds present in microparticles; and also, the thermal breakdown of the wall polymers (İnan and Özçimen, 2021). In Fig. 4, the stage of major degradation occurred between 300-360 °C, and it is associated with the depolymerization and thermal breakdown of the polymer (Cho et al., 2019). The results demonstrated the capability of HPMC to brings thermal protection to the Rm extracts.

3.5. TPC and antioxidant capacity of microcapsules

The different bioactive properties of microcapsules containing Rm extract depend on the effective release of the compounds from the polymeric matrix, since an unsuccessful release could cause a decrease in their bioactivity. Several works have reported the correlation between the TPC and the antioxidant capacity of plant extracts, as phenolic compounds present functional groups able to interact with the corresponding molecules in each antioxidant assay (DPPH, ABTS, FRAP, etc.) (Xu et al., 2007; López-Romero et al., 2018).

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[a]	0.51±0.08[b]	n.d.
	Agitation	2.94±0.39[a]	1.45±0.21[a]	n.d.
DPPH (mg/mL, %RSA)	Ultrasound	17.15±0.36[a]	3.61±0.77[b]	n.d.
	Agitation	16.52±0.39[a]	10.46±0.62[a]	n.d.
ABTS (mg/mL, %RSA)	Ultrasound	16.20±0.82[a]	2.87±0.74[b]	n.d.
	Agitation	15.24±1.35[a]	7.23±0.93[a]	n.d.
FRAP (mg AA/g MC)	Ultrasound	4.04±0.17[a]	0.52±0.09[b]	n.d.
	Agitation	3.99±0.92[a]	1.30±0.16[a]	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.

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

RmO (gallic acid and ellagic acid), which could partially explain the differences in release behavior.

3.6. Antifungal activity of microcapsules

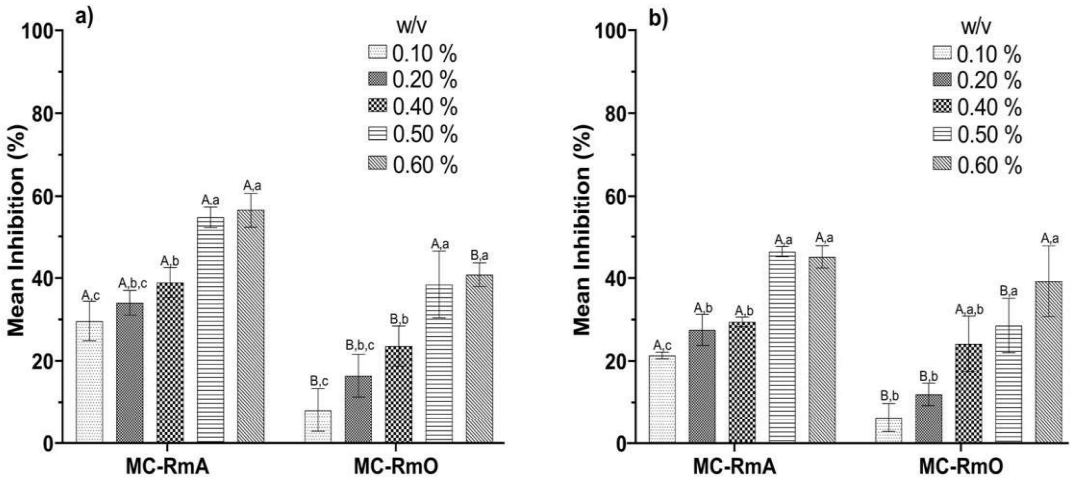


Fig. 5. Mean inhibition percentage of microcapsules containing RmA and RmO against (a) *F. oxysporum* and (b) *R. stolonifer*. Different uppercase letters indicate statistical differences between treatments in each concentration (p<0.05). Different lowercase letters indicate statistical differences between concentrations in each treatment (p<0.05).

Phytopathogenic fungi cause important losses in fruit and vegetables. *F. oxysporum* is an important fungus involved in preharvest losses in berries, causing the *Fusarium* wilt in strawberry crops, a disease that affects the whole plant system (Henry et al., 2017). In addition, *R. stolonifer* is a fastest-growing fungus and its invasion causes the development of a cottony mycelium with characteristic black spores in many fruits and vegetables during pre and postharvest stages, including the berries (Bautista-Baños et al., 2014). Plant extracts have recently been shown to successfully inhibit the development of phytopathogenic fungi, *in vitro* (Mahdi et al., 2021; Wang et al., 2018). The inhibition percentages of the microcapsules against *F. oxysporum* and *R. stolonifer* are shown in Fig. 5. The results evidenced that the MC-RmA had the best antifungal activity against both fungi compared with MC-RmO (p<0.05), with inhibition percentages of 56.4±4.2 % and 46.3±1.2 % against *F. oxysporum* (Fig.

5a) and *R. stolonifer* (Fig. 5b), respectively. The two highest concentrations tested (0.50 and 0.60 %, w/v) did not show a significant difference in both cases. The higher antifungal effect of MC-RmA could be attributed to their antioxidant capacity previously reported, and a major number of phenolic compounds in the extract, both characteristics associated with the promotion of antifungal activity (Jasso de Rodríguez et al., 2017).

3.7. Effect of microcapsules containing RmA on strawberry fruit

3.7.1. Weight loss and firmness

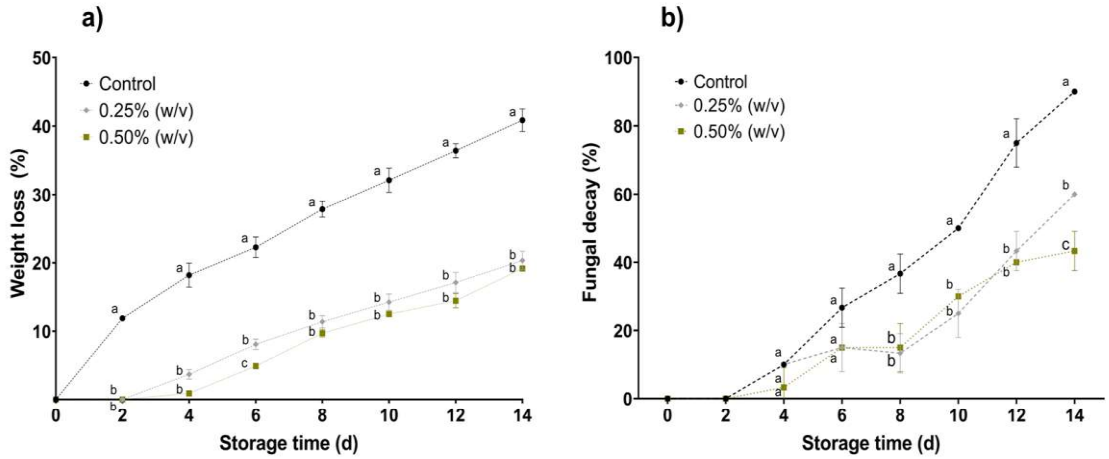


Fig. 6. A) Influence of MC-RmA at different concentrations on strawberry fruits stored at 4±1 °C and 85 % of relative humidity. (a) Weight loss percentage, and (b) fungal decay percentage. Different lowercase letters indicate statistical differences between treatments in each day (p<0.05).

Fruit weight loss is mainly associated with the respiration rate and the release of water into the environment (Yang et al., 2019). The effect of functionalized microcapsules on the weight loss is presented in Fig. 6a. The highest decrease (p<0.05) in weight was in control fruit (uncoated) during all storage period. At the end of the storage (14 d), the strawberries treated with 0.25 % (20.35±1.36 %) and 0.50 % (19.18±0.38 %) of MC-RmA had significantly less weight loss than uncoated strawberries (40.86±1.64 %). This results confirm that the use of MC-RmA treatment provided a barrier capable to reduce the water loss by acting as

a coating on the fruit surface (Salas-Méndez et al., 2019). On the other hand, there was no significant difference between the amount of MC-RmA used. These results are consistent with a previous study of Guerreiro et al. (2015), in which edible coatings containing 0.1 and 0.2 % eugenol did not show significant differences, but both were an effective barrier to water loss during the storage of strawberries for 14 d at 0.5 °C. In addition, the use of HPMC as an encapsulating agent in synergy with the use of chia mucilage based coating as vehicle, could favor the formation of a semi-permeable matrix (Gol et al., 2013; Urbizo-Reyes et al., 2020). The authors reported a lower weight loss in strawberries treated with an HPMC edible coating at day 12 of storage at 11 ± 1 °C, associating this effect to the formation of the barrier on the surface of the fruit (Gol et al., 2013).

The strawberries' firmness is an important quality parameter for consumers, and its decrease is related to the loss of cell wall strength caused by the degradation of the middle lamella of cortical parenchyma cells, and also by the loss of turgidity due to the activity of degrading enzymes (e.g., pectinamethylesterase and polygalacturonase) (Oliveira et al., 2021). In this study, the fruits treated with MC-RmA showed a significant less decrease of their initial firmness (MC-RmA 0.25%: 5.26±1.10% of decrease; MC-RmA 0.50%: 6.98±1.14% of decrease) in comparison with uncoated strawberries (64.17±0.61% of decrease) at the end of storage, being consistent with the results of weight loss. Similarly, Li et al. (2020) reported that the active film of microcapsules containing oregano essential oil allowed the highest firmness values in strawberries due to the decrease in the moisture content surrounding the fruit surface. In addition, the coatings act as a barrier to O_2 uptake and metabolic activity is slowed down (Sogvar et al., 2016). The MC-RmA showed to have a positive effect on fruit firmness, resulting in improved fruit quality by reducing their softness during the storage.

3.7.2. Color

Color significantly influences the acceptability of strawberry fruits, and it is related to their ripening process (Gol et al., 2013). Color change in fruits was monitored by means of chroma, Hue angle, and redness values (Fig. 7). For chroma, there

was a significant reduction from day 0 to day 14 in all treatments, which results in a loss of fruit brightness. On the other hand, Hue angle and redness values significantly increased and decrease, respectively, but there were no significant differences between the different treatments for both parameters. Likewise, the use of functionalized coatings has shown to not affect the color of strawberry fruits in other studies (Fan et al., 2009; Guerreiro et al., 2015; Valenzuela et al., 2015). These results are important because significant changes in fruit color induced by the treatments could affect consumer acceptability; and, in this study it is demonstrated that it is possible to incorporate the bioactive properties of MC-RmA without negatively altering the color of the fruit.

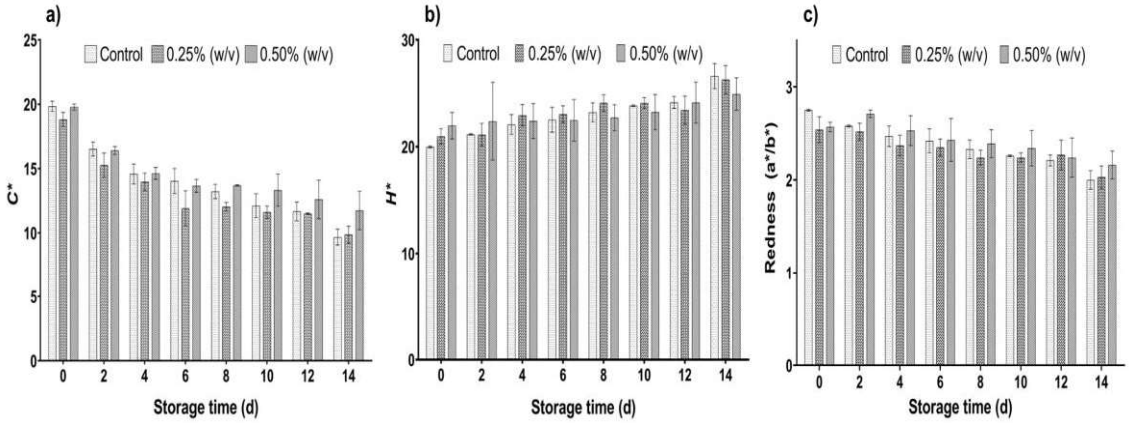


Fig. 7. Changes in color parameters of control (uncoated) and treated strawberries with MC-RmA at different concentrations and stored at 4±1 °C and 85 % HR. a) Chroma, b) Hue angle, c) redness.

3.7.3. Fungal decay

Decay in strawberries is mainly caused by their high susceptibility to postharvest fungal attack, mainly by *R. stolonifer*, *Botrytis cinerea*, *Penicillium* spp., and *Colletotrichum* spp. (Feliziani and Romanazzi, 2016). The results of fungal decay are shown in Fig. 6b, and it can be observed that after day 8, the coated fruit started to show a significant less fungal decay compared with uncoated fruits. This behavior demonstrates the particularity of MC-RmA to gradually release their content (Kittitheeranun et al., 2015). Besides, a concentration-dependent effect was observed at 14 d of storage, as the fruits treated with 0.25 % MC-RmA

presented a higher (p<0.05) fungal decay compared to those treated with 0.50 %. Likewise, Fan et al. (2019) reported that a higher amount of lotus leaf extract incorporated in coatings significantly reduces decay in goji berries due to the presence of a higher amount of bioactive compounds.

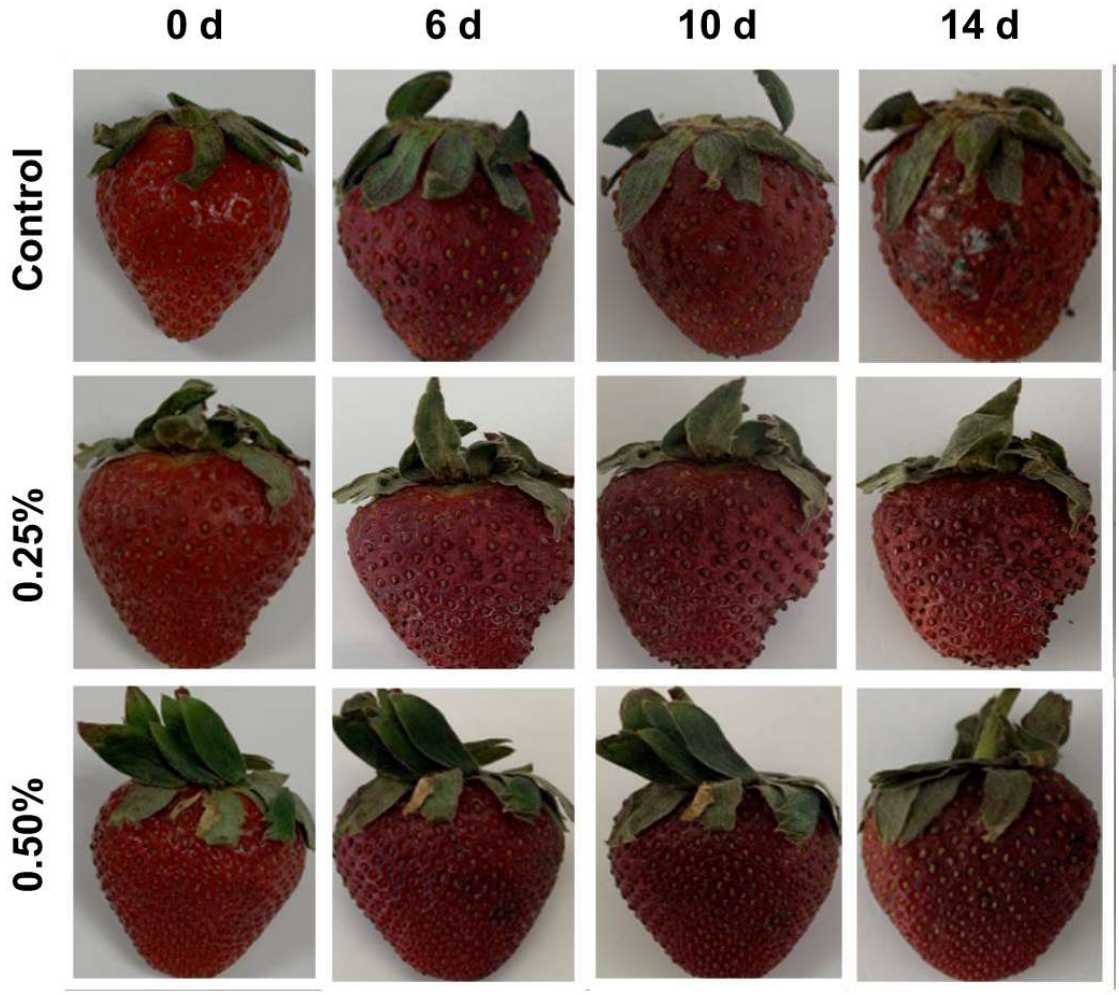


Fig. 8. Appearance changes of strawberries treated with HPMC microcapsules containing RmA (0.25 and 0.50 %, w/v) and control, stored at 4±1 °C and 85 % HR.

Fig. 8 shows the visual evolution of the strawberry fruits during the storage period, in which it can be observed that fungal development was faster in the control group. A similar behavior was previously reported by Liu et al. (2021), as coated strawberries (containing asparagus waste extract) showed better control of *P.*

italicum, than uncoated fruit after 8 d of storage at 25 °C and 80% RH. Other works have also reported interesting results on the antifungal effect of coatings or microcapsules containing plant extracts on strawberry fruits (Sangsuwan et al., 2016; Oliveira et al., 2021; Saleh & Abu-Dieyeh, 2022), which is an indicator of the potential of these technologies to extend the shelf life of these fruits. These results prove that the use of MC-RmA effectively reduces the decay of strawberry fruits due to their bioactive compounds with antifungal properties, besides, the encapsulation provides a slow release of its content, thus extending their activity.

4. Conclusions

Microcapsules containing extracts from *R. microphylla* fruit were developed using electrospray technique and HPMC as encapsulating agent, which showed a spheric shape and particle size between 2.05-2.41 µm. With both concentrations of MC-RmA evaluated (0.25 and 0.5 %, w/v), the treated fruits showed a decrease in weight loss, fungal decay, and firmness, compared with the control treatment. Therefore, the effectiveness of MC-RmA in extending the shelf life of strawberry fruits under the test storage conditions (4 °C for 14 d and 85 % HR) was confirmed. The results are promising and demonstrate the positive effect of the functionalized microcapsules on the quality of strawberries, besides to be a novel biorational alternative for use in postharvest stage where, due to proximity to the final consumer, the use of synthetic products is avoided. This technology could help to reduce the product losses while maintaining quality attributes. However, it is important to evaluate feasible application methods, as well as to design appropriate vehicles to improve the use of this technology.

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CAPÍTULO 5.

CONCLUSIONES GENERALES.

5. CONCLUSIONES GENERALES.

- La extracción por calentamiento óhmico permite reducir los tiempos de operación en comparación con la extracción convencional por agitación; sin embargo, los extractos obtenidos por calentamiento óhmico presentaron menor bioactividad en los ensayos antioxidantes y antifúngicos que los extractos obtenidos por agitación.
- En los frutos de *Rhus microphylla* se identificaron compuestos fenólicos con elevada capacidad antioxidante (ácido gálico, ácido elágico, ácido ferúlico y catequina).
- Los extractos hidroalcohólicos convencionales de los frutos de *Rhus microphylla* y *Myrtillocactus geometrizans* mostraron una fuerte actividad antifúngica *in vitro* en contra de *Rhizopus stolonifer* y *Fusarium oxysporum.*
- De acuerdo con los resultados de SEM, FTIR y TGA, el método de electrospray sumado al uso de HPMC como material de pared permiten obtener microcápsulas termorresistentes (30-300°C) con formas esféricas y una correcta incorporación de los extractos.
- Las microcápsulas conteniendo el extracto hidroalcohólico convencional de *Rhus microphylla* presentaron las mejores propiedades antioxidantes y antifúngicas *in vitro*.
- La aplicación de microcápsulas conteniendo el extracto hidroalcohólico convencional de agrito fueron efectivas para lograr reducir la pérdida de peso y el porcentaje de decaimiento en fresas almacenadas 14 días a 4°C y 85% de HR.
- La aplicación de microcápsulas funcionalizadas con extractos de *R. microphylla* son una alternativa novedosa para la extensión de la calidad postcosecha de fresa.

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