Application of essential oils and polyphenols as natural antimicrobial agents in postharvest treatments: Advances and challenges

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Abstract
The use of natural antimicrobial agents is an attractive ecological alternative to the synthetic fungicides applied to control pathogens during postharvest. In order to improve industrial production systems, postharvest research has evolved toward integration with science and technology aspects. Thus, the present review aims to draw attention to the achieved advances and challenges must be overcome, to promote application of essential oils and polyphenols as antimicrobial agents, against phytopathogens and foodborne microorganisms during postharvest. Besides that, it attempts to highlight the use of coating and encapsulation techniques as emerging methods that improve their effectiveness. The integral knowledge about the vegetable systems, molecular mechanisms of pathogens and mechanisms of these substances would ensure more efficient in vitro and in vivo experiences. Finally, the cost-benefit, toxicity, and ecotoxicity evaluation will be guaranteed the successful implementation and commercialization of these technologies, as a sustainable alternative to minimize production losses of vegetable commodities.

KEYWORDS
essential oil, natural antimicrobial agents, polyphenols, postharvest

1 | INTRODUCTION
The sustainability of the agroalimentary industry it is a global need, and in developing countries, it faces more risks, due to technological disadvantages and climate change phenomena. Particularly, in the Latin America and the Caribbean area great attention is drawn to the food losses and waste dimension, mainly in postharvest processes. Only for fresh fruit and vegetables, the estimated losses are around 55% (FAO, 2019; Eguíllo, 2019). The pathogenic fungi and bacteria are the fundamental responsible of the quality and nutrient composition deterioration and mycotoxin contamination of products, affecting their conservation, useful life, and commercial value (Arias & Toledo, 2018; Cortés-Higareda, Ramos-García, Correa-Pacheco, & Rio, 2019; Sandoval-Contreras, Villarruel- López, Sierra-Beltrán, & Torres-Vitela, 2017; Sanzani, Reverberi, & Geisen, 2016).

Considering this background, many efforts are currently dedicated to develop research works related to the control of postharvest diseases. Besides the influence of the environmental factors (temperature, relative humidity, light, oxygen concentration, etc.), the attention has been focused to study the metabolic processes of pathogens and those that take place in fruit or vegetables.
during respiration and transpiration (Cortés-Higareda et al., 2019; Wenchao, Zhang, & Bhandari, 2019).

Taking into account, that postharvest process is focused on food safety and food properties conservation (nutritional, taste, aroma, and good appearance), the study about disinfection of foodborne bacteria, which threaten the health of consumers, is among the aspects that must be considered during postharvest research also (Gómez-López, 2012; Leyva et al., 2017; Meireles, Giaouris, & Simes, 2016). The application of chemical microbiocides continues to be the main form of pathogens control in different contexts. However, in the last decade, their intensive use has caused great concern about important issues related to environmental and human health (Arias & Toledo, 2018; García-Robles, Medina-Rodríguez, Mercado-Ruiz, & Báez-Sañudo, 2017; Mari, Bautista-Baños, & Sivakumar, 2016; Palou, Ali, Fallik, & Romanazzi, 2016). This situation is inducing to reinforce the regulations regarding the allowed maximum residue limit in the products (Regulation (EU) No 528/2012).

In order to ensure safe and quality food, different physical, chemical, and biological methods are being developed (De la Vega, Cañarejo, & Pinto, 2017; Dobry, Wisniewski, Teixido, Spadaro, & Jijakli, 2016; Franco-Vega, Palou, & López-Malo, 2012; Gómez-López, 2012; Romanazzi et al., 2016; Sivakumar & Bautista-Baños, 2014). Among these, the biological methods have received great attention as a viable and safer alternative. In this sense, the antagonistic microorganisms as biological control agents have been investigated in laboratory, semi-commercial, and commercial settings (Bautista-Baños, 2006; Droby et al., 2016; González-Estrada, Carvajal-Millán, Ragazzo-Sánchez, Bautista-Rosales, & Calderón-Santoyo, 2017; Parafati, Vitale, Reststucia, & Cirvirelly, 2016).

By the other hand, plant extracts containing compounds with antimicrobial properties aroused the most interest also (Mari et al., 2016; Palou et al., 2016; Pino, Sánchez, & Rojas, 2013). Thus, some vegetable volatile organic compounds (VOCs), such as aldehydes (acetaldehyde, 2-E-hexenal and benzaldehyde), alcohols, acetic acid, isothiocyanates, and mainly essential oils (EOs) have received consideration (Kumar & Kudachikar, 2018; Mari et al., 2016; Palou et al., 2016; Sivakumar & Bautista-Baños, 2014; Torrenegra, Nerlis, Pájaro, & León-Méndez, 2017; Yilmaz, Ermis, & Boyraz, 2016). In addition, other nonvolatile compounds, including plant extracts, as peptides and small proteins (Mari et al., 2016; Palou et al., 2016), chitosan and Aloe vera biopolymers (Bautista-Baños, Hernández-López, Bósquez-Molina, & Wilson, 2003; Palou et al., 2016) and phenolic compounds (PPhs) have also been studied (Cushnie & Lamb, 2005; Loizzo et al., 2010; Pagnussatt et al., 2013; Pino et al., 2013; Rodríguez-Maturino et al., 2015; Ruiz-Barbaj, Rios-Sánchez, Fedrani-Irisol, Oliasi, & Rios, l., Jiménez-Díaz, R., 1990; Sarkar & Shetty, 2014). In this sense, the PPhs must be considered very attractive compounds because in fact they have been reported as responsible of the antimicrobial activity of several EOs (Guarda, Rubilar, Miltz, & Galotto, 2011).

Taking into account the importance of the research and technological developments, related with implementation of postharvest conservation technologies, the present work aims to update the state of the art about the study of EOs and PPhs as antimicrobial agents with potential use in postharvest field. Besides that, some challenges to be overcome for the industrial applications and commercialization of these antimicrobials in postharvest treatment should be evaluated.

2 | ESSENTIAL OILS AND POLYPHENOLS AS NATURAL ANTIMICROBIAL AGENTS

Among the plant extracts that favor the development of efficient management strategies against microbial contamination, EOs have been the most investigated compounds among other antimicrobials (Kumar & Kudachikar, 2018; Mari et al., 2016; Rao, Chen, & McClement, 2019).

3 | ESSENTIAL OILS

The EOs are included among VOCs that are produced as secondary metabolites by plants and microorganisms. They have been extensively studied as aromas and flavoring substances, appreciated for food applications (Bosquez-Molina, Bautista-Baños, Morales-López et al., 2009). Furthermore, they have received a great attention for their antimicrobial action, useful during postharvest process (Kumar & Kudachikar, 2018; Mari et al., 2016). As GRAS (Generally Considered as Safe) substances, they have been applied in the agro-food market, and some of them such as thyme (Thymus vulgaris L.), clove (Syzygium aromaticum L. Merr. & L.M.Perry), and mint oil (Mentha rotundifolia L. Huds) have included in the pesticide database by the European Commission (http://ec.europa.eu/food/plant/plagucid/pesticidas-database-redirect/index_en.htm) (Mari et al., 2016).

The extraction of EOs is possible from the plants that belong to several typical botanical orders, using different parts of the tree (bark and wood, flowers, fruit, peel, leaves, roots, and exudates) (Kumari et al., 2014; Mari et al., 2016). EOs mainly contain volatile terpenoids (monoterpenoids and sesquiterpenoids) with different characteristic functional groups (Rao, Chen, & McClement, 2019). At least 100 different compounds (aldehydes, phenols, oxides, esters, ketones, alcohols, and terpenes) may be present in each EO. They are unstable compounds, because of their volatility, photo-, and temperature sensitivity (Troncoso-Rojas et al., 2013), properties that must be considered during extraction procedures by classical or emerging techniques (Al-Mamoori & Al-Janabi, 2018; Azmir et al., 2013; Bosquez-Molina et al., 2009; Chen, Hu, Yan-Dong, & Hanhua-Liang, Y., 2016; Fernández, Minutil, Visinoni, Cravotto, & Chemat, 2013).

Many reports have demonstrated that EOs and their chemical constituents have a broad spectrum of antimicrobial activities mainly against foodborne pathogens (Bosquez-Molina, Ronquillo–de Jesús, Bautista–Baños, Verde–Calvo, & Morales–López, 2010; Pino et al., 2013). For instance, the inhibitory action of EOs obtained from different Citrus species as mandarine (Citrus reticulata Blanco), lemon (Citrus aurantifolia (Christm.) Swingle), and orange (Citrus sinensis L.),
against some bacterial strains (Escherichia coli, Staphylococcus aureus, Bacillus cereus) and yeast (Candida albicans) was demonstrated by Fisher and Phillips (2008). In this sense, Espina et al. (2011) showed their antimicrobial activity also, against Enterococcus faecium, Pseudomonas aeruginosa, and Salmonella enterica. A low minimal inhibitory concentration (MIC) of 0.2 μg/ml, in combination with a mild heat treatment (54 °C/10 min) showed synergistic lethal effect. Besides that, Torrenegra et al. (2017) during in vitro evaluation of these EOs against ATCC strains of Staphylococcus aureus ATCC, showed a strong antibacterial activity with minimal bactericidal concentration (MBC) and MIC of 0.25 and 0.12 mg/ml, respectively, which causes serious losses to tomato crops. Six of them were selected against the bacterial subspecies, which affect Pectobacterium carotovorum, especially where they fulfill various functions related to growth and reproduction, allelopathy, and protection against pathogens, predators, diseases, and UV radiation. Usually, they do not have a significant toxic effect depending on their concentration and gallic acid content (Paredes-López, Cervantes-Ceja, Vigna-Pérez, & Hernández-Pérez, 2010).

Recently, research has been focused on characterization of the specific major EOs extracts components present in different oils in relation to their antibacterial and antioxidant properties. Indeed, most of the constituents of common EOs that exhibit high antimicrobial efficacy are phenols, followed by oxygenated terpenoids. Thymol, eugenol, and mainly carvacrol, from thyme, clove, cinnamon, and oregano oils, respectively, ensure a broad spectrum of antimicrobial efficacy against Gram-negative, Gram-positive bacteria, and some pathogenic fungi; stronger than terpenes and other compounds (Guarda et al., 2011; Rao et al., 2019).

### 4 | POLYPHENOLS

The PPHs are secondary metabolites widely distributed in plants, where they fulfill various functions related to growth and reproduction, allelopathy, and protection against pathogens, predators, diseases, and UV radiation. Usually, they do not have a significant toxic effect depending on their concentration and gallic acid content (Paredes-López, Cervantes-Ceja, Vigna-Pérez, & Hernández-Pérez, 2010).

PPHs refer to a group of chemical compounds that consist of hydroxyl groups bonded directly to an aromatic carbon. They have different structural and physicochemical properties and possess a diverse and heterogeneous reactivity and liability to different environmental factors such as pH, temperature, oxygen, light, etc. (Bakowska-Barczak & Kołodziejczyk, 2011; Manchón, 2013). Usually, they are classified as flavonoids and nonflavonoids compounds. Dozens of different flavonoids may be present in the same plant.
species. Some of them are conjugated with various sugars and further distinguished by the number and arrangement of the hydroxyl groups, and degree of alkylation and glycosylation (Manchón, 2013).

Many investigations about the PPhs from different sources have been oriented to the study of their antioxidant properties, attractive for food industry applications (Paredes-López et al., 2010; Ruiz-Barbá et al., 1990). However, in last decade an increased interest has been shown in relation to their antimicrobial properties. They are obtained not only from the fruit, but also from other parts of the plant: root, stem, leaves, bark, etc. (Azmir et al., 2013; Ojwang, Muge, Mbatta, Mwanza, & Ogoyi, 2017; Sarkar & Shetty, 2014). Interestingly, many research works related to PPhs and other phytochemicals have recently been carried out from leaves of different specimens belonging to the Moraceae family: Artocarpus L. (Loizzo et al., 2010; Jagtap & Bapat, 2010; Ojwang et al., 2017), Mulberry (Morus spp.), as Morus alba L. (Khan et al., 2013), and from different Ficus spp. (Awolola, Chenia, Koorbanaly, & Bainjath, 2014; Salem, Salem, Camacho, & Hayssam, 2013; Usman, Abdulrahman, & Usman, 2009) (Table 1).

Phenolic extracts of these plants have been shown a broad spectrum of antimicrobial activity mainly against foodborne microorganisms (Table 1). For instance, Ficus thommingii Blume extracts inhibited the growth of P. aeruginosa and Streptococcus spp. among other pathogens (Usman et al., 2009), while Ficus sansibarica Warb. subsp. sansibarica extracts, rich in terpenes and flavonoids components, showed antibacterial action against S. aureus and antibiofilm action (Awolola et al., 2014) (Table 1). In general, a broad range of concentrations of different extracts has been used but in the majority of papers the authors report values of growth inhibition less than 50%, results that are must be improved in order to obtain a good efficient during in vivo tests.

Regarding jackfruit tree, Loizzo et al. (2010) reported antibacterial activity against E. coli, L. monocytogenes, Salmonella typhimurium, Salmonella enterica, B. cereus, Enterococcus faecalis, and S. aureus and antioxidant capacity of PPhs from leaves, extracted by different solvents. Aqueous extracts with concentration of 237–1,000 µg/ml caused 7.5–15.0 mm growth inhibition haloes.

Concerning phytopathogenic bacteria, Ramirez-Reyes et al. (2015) observed a growth inhibition of Pseudomonas carotovorum by the action of floral ethanolic extracts of Magnolia schiedean, which was attributed to the presence of phenols among other active compounds.

For phytopathogenic fungi, satisfactory effects have been reported with some natural extracts mainly for Alternaria alternata and Fusarium sp. among other species. Results also show a high variability in relation to concentration and inhibitory capacity of evaluated substances (Table 2). Among various plant species tested, aqueous extracts of leaves of papaya (Carica papaya L.) and custard apple (Annona reticulate L.) showed important fungistic effect against Rhizopus stolonifer and C. gloeosporioides in ciruela (Spondias purpurea L.) and mango (Mangifera indica L.) during fruit storage (Bautista-Baños et al., 2003). Similarly, effect of bee propolis on different fungal species: A. alternata, Aspergillus niger, Aspergillus parasiticus, B. cinerea, Fusarium oxysporum f. sp. melonis, and Penicillium digitatum has been reported. After that, Ojeda-Contreras, Hernández-Martínez, Domínguez, and Mercado (2008) evaluated the effect of caffeic acid phenethyl ester, a component of propolis, to control A. alternata infecting tomato fruit. They concluded that it has a superior inhibitory action than commercial fungicide without negative effect on fruit quality.

On the other hand, Pagnussatt et al. (2013) assessed Spirulina LEB-18 phenolic extract for its antifungal activity on strains of Fusarium graminearum, isolated from barley and wheat. They demonstrated that this extract reduced the growth rate of the toxigenic species investigated (Table 2). In this sense, during in vitro evaluation of antifungal activity of extracts of cherimoya (Annona cherimola Mill.), and cinnamon (C. zeylanicum J.Presl) both extracts showed inhibitory effects on the mycelial growth and sporulation of Fusarium oxysporum, Fusarium culmorum, and Fusarium solani strains also. Cinnamon extracts were effective to control Fusarium species in doses of 330–539 ppm, while the best control of the cherimoya extract was at 593 ppm against F. culmorum, and at higher doses from 2,060 to 2,571 ppm for other species (Ochoa-Fuentes, Cerna-Chávez, Landeros-Flores, Hernández-Camacho & y Delgado-Ortiz J.C., 2012).

Therefore, interesting antifungal effect was showed by phenolic and carotenoid extracts of “chiltepín” (Capsicum annuum var. Glabriusculum) against F. oxysporum and A. alternata strains in relation to mycelial growth inhibition and conidial germination inhibition (Rodríguez-Maturino et al., 2015) (Table 2).

### 5 | MECHANISMS OF ACTION

Regarding the action mechanisms of EOs and PPhs as antimicrobial agents, it is important to consider that their effectiveness will depend not only on the concentration and chemical nature, but also on the susceptibility and concentration of the pathogen, and even of the microbial strain characteristics. This capacity will vary depending on the composition and nature of the microbial cell surface (Melgarejo & Postilla, 2011; Rao et al., 2019). Furthermore, it is important to know and understand the mechanisms of action of pathogens, depending on the characteristics of the fruit and environmental conditions (Barkai-Golan, 2001; Basak & Guha, 2018; Rao et al., 2019; Sanzani et al., 2016; Sarkar & Shetty, 2014).

Four possible mechanisms of action have been defined for antimicrobial substances: interference in the cellular structure; interference in cellular biosynthesis; inhibition of the energy mechanism and multisite activity (Calvo & Martínez-Martínez, 2009; Melgarejo & Postilla, 2011; Rodríguez-Maturino et al., 2015). Besides that, the environmental factors such as temperature, pH, medium composition, etc. and contact time will play an important role, as well (Barkai-Golan, 2001; Sandöval-Contreras et al., 2017). In the case of EOs, due to their hydrophobic nature, they could penetrate through bacterial cell outer membranes and cytoplasmic membranes into the interior of cell and thus disintegrate its structures. It renders them more permeable, causing the leakage of cellular components or inactivating the enzymes.
tryptophan and decreases the production of phenylalanine or tyrosine, rose-4-phosphate, and shikimic acid. This favors the production of benzoic acid by interference in the reactions of phosphoenolpyruvate, erythrose-4-phosphate, and reduction of H$_2$O$_2$, which affect many pathogens (Zhao & Drlica, 2014). It has been proved that some tree EOs inhibit respiration and scavenging free radicals (Rodríguez-García et al., 2016). In the same manner, these structural modifications take place in fungi and would cause morphological variations of the hyphae and inhibition of conidial germination (Cushnie & Lamb, 2005; Rao et al., 2019; Rodríguez-García et al., 2016).

In general, EOs and PPhs may provoke a physical, chemical, or biochemical change in the microorganisms, and different constituents may operate by different mechanisms and may target different kinds of microbes, such as Gram-positive and Gram-negative bacteria, yeasts, or molds, because they differ in the composition of their cell membranes. Therefore, it is difficult to predict how susceptible pathogens are and why the susceptibility varies from strain to strain after applying these compounds.

It has been proved that some tree EOs inhibit respiration and also causing potassium ion leakage in both Gram-positive bacteria and Gram-negative bacteria, and the most of their constituents are phenols (thymol, eugenol, and carvacrol), followed by oxygenated terpenoids (Rao et al., 2019).

Regarding PPhs some mechanisms are reported: formation of complexes with soluble and extracellular proteins, generating a disruption of the fungal cell wall (Rodriguez-Maturino et al., 2015); reduction of the decomposition of H$_2$O$_2$, which affect many pathogens since it becomes highly reactive oxygen species promoting oxidative damage; action as a proton exchanger, and the resulting collapse of proton motive force and depletion of adenosine triphosphate (ATP) eventually lead to cell death (Rao et al., 2019).

PPhs could inactivate the essential amino acid synthesis caused by interference in the reactions of phosphoenolpyruvate, erythrose-4-phosphate, and shikimic acid. This favors the production of tryptophan and decreases the production of phenylalanine or tyrosine, modifying the structure of some proteins essential for the formation of fungal appressorium structure (Pagnussatt et al., 2013).

It has been shown that PPhs act at the membrane level by modifying the polar heads of the lipid molecules. Some results demonstrated that their antimicrobial activity is associated with the presence and position of a free hydroxyl group bonded directly to a C6 aromatic ring as a system for electron delocalization, that favor their ability to modify the microbial cell membrane integrity. Furthermore, the hydroxyl group has a key role in the inactivation of microbial enzymes such as ATPase, histidine decarboxylase, amylase, and protease. Inhibition of ATPase may be important for cell death due to disturbed cellular respiration (Rao et al., 2019).

For instance, tannins are able to block the activity of catalase, provoking a lethal effect on some pathogens (Zhao & Drlica, 2014). Also, they could inhibit enzymes involved in ergosterol synthesis, the main component of the fungal cell membrane, reducing its intracellular content (Campoy & Adrio, 2017; Rao et al., 2019). Besides that, it is important to take into consideration that EOs and PPhs compounds also could induce fruit resistance processes to pathogenic microorganisms, as other natural compounds do (chitosan, fructooligosaccharides, and vegetable hormones), provoking a sequential reaction by activation of particular genes and biosynthesis of antimicrobial substances (Romanazzi et al., 2016).

### USE OF COATINGS AND FILMS FOR NATURAL ANTIMICROBIALS AGENTS’ APPLICATION

In order to prevent postharvest diseases, the natural antimicrobial agents can be used in vapor or liquid systems, mixed with surfactants in immersion tanks during the packaging process or in wax formulations. In the practice, these compounds have been incorporated...
separately, in sachets, in packaging systems, during storage, transport or even marketing, depending on the fruit (Mari et al., 2016). But many reasons difficult their incorporation to the biological formulations: fast release, low solubility, low bioactivity, and lability against environment stresses (temperature, moisture, pH, oxygen light, etc.). The oxidative degradation may deteriorate these compounds leading to the generation of free radicals and development of unpleasant tastes and off-odors. It results in an undesirable effect on shelf stability, sensory characteristics, and consumer acceptability of the products (Ariyarathna & Karunaratne, 2016; Gómez-Mascaraque et al., 2016; Shishir, Xie, Sun, Zheng, & Chen, 2018; Sánchez et al., 2017; Yuan et al., 2016).

Therefore, in order to guarantee effective applications of natural antimicrobial agents, it must be considered that their stability and bioactivity will depend on their physicochemical properties (structure, molecular mass, solubility, and reactivity) and agroindustrial application conditions (Kamil, Chen, & Blumberg, 2015; Rodríguez, Martín, Ruiz, & Clares, 2016; Shaaban, El-Ghorab, & Shimamoto, 2012). Thus, during the last decades their application has been diversifed through edible coatings and films development, because these technologies represents an opportunity of mitigation of environmental risks and improving of the food quality (Fernández, Echeverría, Mosquera, & Paz, 2017; Marín, Atarés, & Chiralt, 2017; Ponce, Roura, Valle, & Moreira, 2008; Ramos-García, Bautista-Baños, & Estrada-Carrillo, 2010; Yuan, Chen, & Li, 2016).

These techniques combine the principles of physical, chemical, and biological methods and include the use of GRAS ingredients of different nature such as salts, peptides, small proteins, EOs, PPhs and other plant extracts (Aloui et al., 2014; Mari et al., 2016; Ponce et al., 2008), antagonistic microorganisms (González-Estrada et al., 2017; Parafati et al., 2016), etc.

An edible coating creates a modified atmosphere by constituting a barrier to gases (O₂, CO₂, and water vapor), reduces the speed of breathing and the weight loss during storage and transport, delaying the process of fruit senescence (Ramos–García et al., 2010). Furthermore, many biological materials including polysaccharides, proteins, lipids, and their mixtures have been employed, considering also the morphological and physiological characteristics of the products, their condition as climacteric or nonclimacteric fruit. Films properties (transparency, brightness, resistance, etc.) and effectiveness of antimicrobial agent action will depend on biopolymers and high biological value compounds (HBVC) characteristics (Marín et al., 2017; Yuan et al., 2016).

For instance, antifungal activity of some natural additives added to hydroxypropyl methylcellulose-lipid edible coatings against B. cinerea and A. alternata was exhibited in vitro on cherry tomato (Fagundes, Pérez-Gago, Monteiro, & Palou, 2013), while grapefruit EO or grapefruit seed extract containing in alginate coatings have been successfully used for grapes preservation (Aloui et al., 2014).

In the same way, chitosan edible films and coatings are promising systems to be used as EOs and PPhs carriers in postharvest applications, owing to their antioxidant and antimicrobial activities. This combination can provide improved additive or synergistic interactions, showing greater effectiveness against fungi and foodborne bacteria in food systems than pure films and coatings (Ávila-Sosa et al., 2012; Yuan et al., 2016).

For instance, Bautista-Baños et al. (2003) evaluated the fungicidal effect of chitosan (2.5%) and aqueous extracts of custard apple leaves, papaya leaves, and papaya seeds on the growth of

### Table 2: Antimicrobial effect of phenolic extracts of different origin on phytopathogenic fungi (in vitro)

| Compound               | Source                          | Pathogen                          | Concentration | Inhibition (%) | Authors                      |
|------------------------|---------------------------------|-----------------------------------|---------------|---------------|-----------------------------|
| Caffeic acid phenethyl ester | Propolis                       | Alternaria alternate (Solanum lycopersicum L., Mill.) | 80 µM         | 32.5          | Ojeda–Contreras et al. (2008) |
|                        |                                 |                                   | 90 µM         | 30.6          |                             |
|                        |                                 |                                   | 100 µM        | 30.0          |                             |
| Methanolic extracts    | Cherimoya (Annona cherimola Mill.) | Fusarium oxysporum                | 2000–10,000 ppm | 53–89.55     | Ochoa–Fuentes et al. (2012) |
|                        |                                 | Fusarium culmorum                 | 2000–10,000 ppm | 78.35–89.62   |                             |
|                        |                                 | Fusarium solani                   | 2,000–10,000 ppm | 45.25–62.5    |                             |
|                        | Cinnamon (Cinnamomum zeylanicum J.Presl) | Fusarium oxysporum                | 300 ppm       | 43.15         |                             |
|                        |                                 | Fusarium culmorum                 | 300 ppm       | 31.81         |                             |
|                        |                                 | Fusarium solani                   | 300 ppm       | 45.58         |                             |
| Phenolic extract       | Spirulina LEB–18                | Fusarium graminearum              | 3%–8% (p/p)   | 50            | Pagnussatt et al. (2013)    |
| Phenolic extracts      | Chiletepin (Capsicum annum var. glabriusculum) | Alternaria alternate (Fusarium oxysporum) | 100 mg/ml     | 38.5³         | Rodríguez–Maturino et al. (2015) |
| Carotenoids            |                                 | A. alternate (Fusarium oxysporum)  |               |               |                             |

³% inhibition of mycelial growth.

b% inhibition of conidial germination.
C. gloeosporioides in papaya fruit. During in vitro test, the combina-
tion of 2.5% chitosan with all the extracts had an improved fungi-
static effect, while in vivo studies, control of anthracnose disease
was obtained with 1.5% chitosan. Furthermore, alterations in the
hyphae and spore development of F. oxysporum sp. gladioli and
R. stolonifera, and lyzes of A. alternata cells after treatment with
chitosan and natural product mix, were observed (Bautista-Ánhos
et al., 2012). Besides that, Ávila-Sosa et al. (2012) revealed the an-
tifungal effects of edible films of chitosan, amaranth, and starch with
Mexican oregano oil (0.5%) against A. niger and P. digitatum.

In the complex host/antimicrobial compound/pathogen system,
many biochemical processes can occur with different effects on
biological activity of the antimicrobial compound. The pathogens
would be less likely to develop resistance against the mixture of
compounds. Furthermore, compounds present in the coatings
may act as elicitors of resistance through different mechanisms
mediated by the host tissue (Yuan et al., 2016). For instance, the
combined coating could control the EO vapor diffusion and re-
duce the structural changes or loss of HBVC. The coatings, films,
and microcapsules are more profitable because small volumes
are applied, product contamination is avoided, and the prolonged
action is guaranteed. The use of small volumes ensure effective
conservation of the products without affecting their sensory prop-
erties (Cortés-Higareda et al., 2019; Ezhilarasi, Karthik, Chhanwal,
& Anandharamakrishnan, 2013; Kamil et al., 2015; Parafati et al.,
2016; Wenchoo et al., 2019).

Furthermore, from the commercial point of view, the imple-
mentation of new coating technologies does not generally require
the acquisition of new equipment or space, since many fresh pro-
duce packinghouses already employ waxing equipment (Fernández
et al., 2017; Mari et al., 2016). Besides that, a greater emphasis has
been placed on the currently films development from new natural
biopolymers, due to the growing demand for sustainable food pro-
duction and opportunities to open new markets from nontraditional
agricultural products and wastes. Recently, the use of conventional
raw materials (tubers, rice, amaranth, and quinoa) has allowed to ob-
tain films and coatings with good mechanical and barrier properties
(Mari et al., 2016).

7 | ENCAPSULATION OF NATURAL ANTIMICROBIAL COMPOUNDS

In postharvest processes as in food practice, applications of encap-
sulation methods have been linked with ensuring controlled releas-
ing and avoiding undesirable structural and bioactivity changes of
HBVC. Micro- and nano-encapsulation are two major ways of this
technology, able to improve product functionality. Recently, there
have been found remarkable interest in development of these
nano-scale delivery systems for this compounds due to their ad-
vantages—high encapsulation efficiency and loading capacity, en-
hanced bioavailability, improved stability, sustained release profile,
and masking undesirable flavors (Esfanjani & Jafari, 2016; Fathi,
Martin, & McClements, 2014; Kamil et al., 2015; Liang et al., 2017;
Maes, Bouquillon, & Fauconnier, 2019; Shishir et al., 2018; Yu et al.,
2018).

Micro- and nanocapsules have been used as a functional ingre-
dient in coatings, edible films, and formulations. Depending on com-
pounds and their final application, different physical and chemical
methods have been applying, using biocompatible and biodegrad-
able polymeric materials, natural or synthetic, food grade or GRAS.
They allow improving the physicochemical properties of the cap-
sules (microstructure, thermal stability, solubility, hydrophobicity,
zeta potential, etc.) in order to guarantee an adequate release behav-
ior of the HBVC and their biological mechanism (Kamil et al., 2015;
Kothalawala & Sivakumaran, 2018; Maes et al., 2019).

Among the used methods, reports mainly have been focused to
coprecipitation (emulsion/solvent evaporation), freeze drying and
spray drying (Ballesteros, Ramírez, Orrego, Teixeira, & Mussatto,
2017; Çam, Içyer, & Erdoğan, 2014; Guarda et al., 2011; Ribeiro &
Pedreira, 2018). During last years, satisfactory results with electro-
dynamic techniques have been reported. Nano-capsules and natural
nano-carriers for phenolics have been developed using cyclodex-
trins, nano-caseins, nano-crystals, among other matrixes by elec-
trospraying, electrospinning, and nano-spraying (Drosou, Magdalini,
Krokida, & Biladeris, 2010; Esfanjani & Jafari, 2016; Hernández
et al., 2015). It has shown that the technique, nature of the coating
material and HBVC also greatly influenced the encapsulation capa-
city (Ballesteros et al., 2017).

Concerning the antimicrobial effectiveness of micro- and
nanoparticles, some attractive results have been obtained (Table 3).
An improvement in the antimicrobial action of monoterpenoid phe-
nols, carvacrol, and thymol was observed when they were added as
microcapsules in flexible plastic film coating (Guarda et al., 2011).
In this case, microcapsules were prepared using emulsion of soybean
oil in aqueous solution of arabic gum and tween 20. They showed a
strong activity against several foodborne microorganisms: E. coli
O157: H7, S. aureus, L. innocua, S. cerevisiae, and A. niger, but inhibited
mainly the growth of mycelium. A significant activity for thymol and
carvacrol with MIC of 125–250 ppm and 75–375 ppm, respectively,
was observed, and highest synergism at 1:1 relation.

Among synthetic polymers, polylactates (PLA) and their poly-
ethylene glycol derivatives (PLGA) have been used (Table 3). In this
sense, nanoencapsulation and delivery of phytochemicals of tropical
fruit by-products was studied by Silva, Hill, Figueredo, and Gomes
(2014). They demonstrated the advantages of the use of this ma-
trix for antioxidant and antimicrobial applications. The same man-
ner, nanoencapsulation of hydrophobic phytochemicals and PPhs of
guaiiroba fruit (Campomanesia xanthocarpa O. Berg) was achieved
by Pereira, Hill, and Zambiasi (2015) and Pereira et al. (2018).
Evaluation of capsules with these compounds against the high-re-
sistant Gram-positive bacterium, L. innocua, showed a significantly
greater growth inhibition in comparison with nonencapsulated com-
pounds. Furthermore, capsules of polyphenolic extracts of passion
fruit (Passiflora edulis Sims) in PLGA were obtained using the coprecipitation method (Oliveira, Ferreira, & Gomes, 2017). These authors observed 23.8%–79% efficiency and a greater antimicrobial action in comparison with the whole extracts (Table 3).

Therefore, β-cyclodextrin (CD) and their derivate hydroxypropyl-β-cyclodextrin have been used successfully also for encapsulation of PPs as eugenol and (+)-catechin and (–)-epicatechin, improving their stability (Table 3). In vitro assays showed inhibitory effect of complex CD–eugenol on of Peronospora litchi colony growth in a concentration- and time-dependent manner (MIC = 0.2 g), while in vivo assays showed its impact on reduction of the decay index of treated fresh litchi fruit. After exposure to CD–EG, the surface of fungal hyphae and/or sporangiophores became wrinkled, with folds and breakage. Besides that, damage to cell walls and membrane structures was confirmed (Gong, Li, Chen et al., 2016). On the other hand, investigation about complexation of two isomers, (+)-catechin and (–)-epicatechin, with hydroxypropyl-β-cyclodextrin in Tris–HCl buffer solutions at pH 6.8–8.0 using isothermal titration calorimetry, was carried out. Stability study indicated that a complex with CD showed a stronger but different protection effect on isomers, depending on their molecular structure (Liu et al., 2016).

Besides that, Anaya-Castro et al. (2017) evaluated encapsulation efficiency of clove and Mexican oregano EOs in β-cyclodextrin matrix by determination of eugenol and carvacrol, respectively. The 4:96 ratios (clove EO/CD) gave the highest eugenol content and greatest microencapsulation efficiency; and the 8:92 and 12:88 ratios (Mexican oregano EO/CD) the highest carvacrol content. After that, antimicrobial activity of the complexes was tested against L. monocytogenes ATCC 19114 and E. coli ATCC 25922. Inclusion of the majority of biologically active clove and Mexican oregano EOs molecules, providing increased stability by reducing their volatility and preserving their biological properties was demonstrated.

All these results show the potentialities of nanoencapsulation of EOs and PPs compounds (Liang et al., 2017). However, they have been developed mainly against foodborne microorganisms and for pharmaceutical and food applications. Concerning postharvest treatments, fewer works have been reported. For instance, a study of a nanoemulsion of xoconostle (Opuntia oligacantha C. F. Först) with EO of C. sinensis, demonstrated that it is an excellent source of phenolic acids and flavonoids, appropriated to significantly affect the C. gloesporioides growth. Nonencapsulated compounds showed minor values of the cell lysis haloes diameter in solid medium, in comparison with encapsulated ones (17.1 ± 21.25 mm respectively) (Solís-Silva et al., 2018). Respecting these results, emulsions are a probable approach to guarantee appropriate delivery systems of HBVC with maximum efficacy in the food systems. Nanoemulsions could contribute to increase their dispersibility improving their bioavailability and bioactivity (Basak & Guha, 2018; Donsi & Ferrari, 2016).

On the other hand, characterized nanostructured chitosan/propolis formulations demonstrated their inhibitory effect on the growth of A. flavus, its spore germination (97%), and aflatoxin production (100%) (Cortés-Higareda et al., 2019).

### 8 | CHALLENGES IN RESEARCH AND APPLICATION OF NATURAL ANTIMICROBIALS IN POSTHARVEST SYSTEMS

According to ethnobotanical studies in our context, there is great tropical plant biodiversity with possibilities of being used in postharvest and other agroindustrial processes, because they are sources of HBVC. However, the possibilities of their commercial scale production and practical applications are limited, first because of the current availability of highly effective, and cheaper conventional fungicides. Thus, this technology presents a major challenge in the coming years, because all the aspects related to it have not yet been clarified and the development capacities, knowhow about in situ processing good alternatives have not been guaranteed for local development (Mari et al., 2016).

Inconsistent results are often reported, depending on the type of crop or nature of disease or storage conditions. In some research studies, biological evaluation does not always correlate with chemical composition and identification of the main compounds (Pino et al., 2013). This has wide implications in terms of developing and commercializing this technology.

In order to standardize the commercial production of natural antimicrobials, multiple factors causing their high variability must be considered. They depend on (a) variety and cultivation conditions, influenced by the climatic factors (photoperiod, shade, temperature, etc.); the plant age (maturity) and agronomic practices (soils, time of planting and time of harvest, application of fertilizers, irrigation, etc.) (Kumari et al., 2014), (b) part of plant used in the biological evaluation (Loizzo et al., 2010; Ojwang et al., 2017), (c) experimental procedures for extraction and conservation (Manchón, 2013), and (d) methods of chemical characterization (Azmir et al., 2013).

On the other hand, it is indispensable to increase in vivo practices, under semi-controlled or real production conditions. For instance, in developed studies about the antifungal action of EOs against the anthracnose pathogens in tropical fruits, in vivo tests practically are not included (Kumar & Kudachikar, 2018). They are needed in order to evidence the individual and synergistic effects of HBVC more clearly and show their true effectiveness against the disease (Bautista–Baños et al., 2003; Nikkhah, Hashemi, Habibi Najafi, & Farhoosh, 2017).

For these investigations, the type of treatment and conditions must be clearly defined depending on the aims. To conceive a treatment against superficial contamination of the products, it is easier than to develop a treatment to avoid pathogens inside the fruit. For this, several physical barriers (plant cuticle, subcuticular cells, and pathogen membranes) and processes (fruit and pathogen metabolism, internal absorption mechanisms during translocation) have to be overcome. Therefore, integrated knowledge about mechanisms of pathogens and antimicrobial compounds action and respiration and transpiration processes in fruit and vegetables must be considered in order to conceive any research project it includes. More knowledge about the antimicrobial mechanisms of
action of EOs and PPhs at the molecular level, rather than just at the cellular level is required (Basak & Guha, 2018; Gómez-López, 2012; Rao et al., 2019; Romanazzi et al., 2016; Wenchao et al., 2019). Thus, this information may be useful to develop a new paradigm in postharvest biocontrol and in strategies of use of natural antimicrobials also. Therefore, more knowledge about the antimicrobial mechanisms of action of EOs and PPhs at the molecular level, rather than just at the cellular level is required (Rao et al., 2019).

Besides that, considering the value of nanotechnology-related combined preservation strategies and nanotechnology-related intelligent labeling system, modeling studies, legal aspects, safety concerns, and technical optimization need to be taken into account in future researches (Kothalawala & Sivakumaran, 2018; Maes et al., 2019).

From the commercial point of view, it is recognized that the most important limitation related with natural antimicrobial agents is the registration process. Some components of EOs with antimicrobial activity are registered by USA as flavoring agents for foods (carvacrol, carvone, cinnamaldehyde, citral, p-cymene, eugenol, limonene, menthol, and thymol), while others such as estragole and methyl eugenol are not allowed because of their genotoxicity (Mari et al., 2016). Then, there is a need to develop more detailed toxicological studies to evaluate the health risks of postharvest treatments and microorganisms on products and its dynamics (Droby et al., 2016). Thus, this information may be useful to develop a new paradigm in postharvest biocontrol and in strategies of use of natural antimicrobials also. Therefore, more knowledge about the antimicrobial mechanisms of action of EOs and PPhs at the molecular level, rather than just at the cellular level is required (Rao et al., 2019).

Natural alternatives would have a better chance of success if they have been applied at optimum timing, and thus, the early and rapid detection of pathogens is important. Consequently, smart technologies should be used to characterize the composition of microbiota on products and its dynamics (Droby et al., 2016).
to understand the decomposition mechanisms of these substances in organism (Mari et al., 2016). Among other relevant aspects, the organoleptic properties of the fruit are very important since the taste should not be affected by the treatment or by other quality attributes, related to the appearance, the nutrient concentration, etc. (Mari et al., 2016; Palou et al., 2016).

Finally, besides the satisfactory results at laboratory or semi-industrial level, a techno-economic feasibility study it is indispensable, in order to glimpse the cost-benefit relationship of the proposed production process. It will depend on infrastructure, raw material availability, local needs and technical facilities, among other factors. The integral approach, must be consider for costeffectiveness control programs of preharvest, harvest, and post-harvest processes.

For successfully development of this technology will be indispensable to attend the cost and energy sustainability, applying strategies related with circular economy. Mass and energy integration are necessary for reducing the costs and environmental impact. The use of the biorefinery-based platform concept will enhance the efficiency of the plant residues utilization and to develop technological and marketing approaches for production of other HBVC and bioenergy (Moncada, Tamayo, & Cardona, 2014). Recently, a biorefinery model for PPhs, ethanol, and xylitol coproduction from spent blackberry pulp was developed by Davila, Rosenberg, and Cardona (2017). According to the sale-to-total-production-cost ratio, they demonstrated the importance of the mass and heat integration. The productivity values of 193.4, 6,912, and 452.2 kg/day of polyphenols, ethanol, and xylitol and yields of 3.88, 352.9, and 18.37 kg/ton of spent blackberry pulp were obtained, respectively.

On the other hand, it is necessary to develop cheaper technological alternatives, on the basis of efficient emergent extraction methods, and mainly drying techniques used during the raw material pretreatment, extracts concentration, and encapsulates formation. In this sense, some studies about spray drying conditions were carried out in order to avoid high energy consumption. For instance, the best conditions for the energy required, production costs, and physicochemical characteristics of the cheese whey were determined by Domínguez-Niño, Cantú-Lozano, Ragazzo-Sánchez, Andrade-González, and Luna-Solano (2018). A dried product of 0.2165 kg/hr was obtained, with a moisture content of 2.08%, cost of 17.06 $/kg, and energy consumption of 2.0490 kW·hr/kg of dry product. Besides that, great interest to use and optimization of the renewable energies for production processes has been observed (Costales, 2010).

However, there are still many limitations that make difficult the implementation of this technology as a control strategy. Among some, challenges must be overcome for the industrial application, is necessary to consider the variability in quantity and quality of natural products, depending on the source, extraction conditions, and characterization methods. It is needed to show their true effectiveness against the disease in order to evidence the individual and synergistic effects.

Concerning methods, smart innovative tools are needed to improve the accuracy and promptness in diagnosing plant pathogens, foodborne microorganisms, and their toxic metabolites. On the other hand, it is indispensable to increase experimental tests in vivo, in order to confirm the laboratory in vitro results, and to apply a holistic method of analysis of postharvest process for improving the efficiency of treatments. Finally, genomic knowledge will give information about gene functions, molecular receptors, and metabolic reactions, allowing a better understanding of the physiological changes in fruit and vegetables and favoring the optimization of treatments also. Besides that, more studies about the microbiome diversity and composition on harvested products and its variability after harvest and during storage are necessary.

Therefore, attention must be paid to their toxicological properties, mechanisms of action against the pathogens and the effect on the organoleptic properties of the products. All these aspects will favor the successful implementation and commercialization of natural antimicrobial postharvest technology, but a cost-benefit relationship, toxicity and ecotoxicity analysis will be indispensable in order to comply the sustainability of this technology.

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CONFLICT OF INTEREST
The authors declare that they do not have any conflict of interest and written informed consent was obtained from all of them.

ETHICAL STATEMENTS
This study does not involve any human or animal testing.

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