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Plant antimicrobial polyphenols as potential natural food preservatives

Lynda Bouarab Chibane,[a] Pascal Degraeve,[a] Hicham Ferhout,[b] Jalloul Bouajila[c] and Nadia Oulahal[a]*

Abstract

BACKGROUND: The growing demand for natural food preservatives in the last decade has promoted investigations on their application for preserving perishable foods. In this context, the present review is focused on discussing the prospective application of plant extracts containing phenolics or isolated plant phenolics as natural antimicrobials in foods. Plant essential oilsare outside the scope ofthis review since utilization of their antimicrobial activity forfood preservation has been extensively reviewed.

RESULTS: Although the exact antimicrobial mechanisms of action of phenolic compounds are not yet fully understood, it is commonly acknowledged that they have diverse sites of action at the cellular level. Antimicrobial phenolics can be added directly to the formulation of perishable food products or incorporated into food-contact materials to release them in the immediate zone of perishable foods. Edible coatings or active food packaging materials can thus be used as carriers of plant bioactive compounds.

CONCLUSION: These materials could be an interesting delivery system to improve the stability of phenolics in foods and to improve the shelf life of perishable foods. This review will thus provide an overview of current knowledge of the antimicrobial activity of phenolic-rich plant extracts and of the promises and limits of their exploitation for the preservation of perishable foods.

Keywords: plant extracts; phenolic compounds; antimicrobial activity; food preservation; edible coatings

INTRODUCTION

The use of natural antimicrobials for food preservation is a trend that is followed by both consumers and food manufacturers. In the future, their use is expected to increase gradually because of the rising demand for minimally processed products, preferably those containing natural additives.

Despite considerable efforts to improve production technologies, distribution, hygiene standards, and consumer education, spoilage and foodborne pathogenic microorganisms still lead to huge economic losses and unacceptable human costs. Due to the increase in the consumption of fresh, minimally processed, and ready-to-eat foods, new ecological routes for microbial growth have emerged. To ensure the microbial safety of their food, consumers demand 'healthier' and more environmentally friendly food production systems, which promote the development of innovative biopreservation concepts based on the use of natural antimicrobial agents rather than synthetic preservatives.

Such concerns and the growing demand for organic foods are driving a growing interest in natural antimicrobials, which exhibit an effective antagonistic effect against a wide range of unwanted microorganisms in foods. The present knowledge shows that the growth of pathogenic and spoilage microorganisms may be strongly reduced or inhibited by several plant extracts. Their effective antimicrobial activities make them potentially an interesting alternative to synthetic preservatives. Antimicrobial plant extracts or molecules, such as an extract of moso bamboo (Takeguard™) launched by Takex Labo (Osaka, Japan) or a mixture of different natural antimicrobial extracts (Biovia™ YM10) including green tea extract launched by Danisco DuPont, have been proposed as alternatives to chemical ones. Thousands of antimicrobial plant molecules have been listed in the botanical literature and represent a renewable source of bioactive constituents. Natural antimicrobials traditionally used and recorded by individuals are a small part of the preservatives available in nature. Currently, there is a need to expand the list of natural antimicrobial molecules that could be used as food preservatives.

Most of the natural alternatives to synthetic food preservatives investigated in recent studies are plant extracts in raw or purified form, namely, essential oils or pure compounds, most of which were used by our ancestors. They have become the center of interest for *in situ* application in food products.[1] In this context,

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essential oils are the plant extracts that have been the most studied for their antimicrobial activity. However, in addition to their higher cost than other plant extracts (due to their lower extractability and yield), essential oils contain volatile compounds (frequently unpleasant or generating off-odors) and have low water solubility. These drawbacks often limit their use for food preservation. Since applications of essential oils for improving food safety have been widely reviewed by Burt[2] and more recently by Hyldgaard *et al.*,[3] essential oils are outside the scope of this review, which will instead be focused on other plant extracts.

Plant extracts play an important role because of their nutritional, visual (color), and taste properties; polyphenols are thus considered relevant due to their qualities.[4] Most of the applications of phenolic-rich plant extracts for food safety purposes are related to their antioxidant activity, including the prominent example of rosemary extract,[5] which has food additive status in the European Union (E 392). Antioxidant rosemary extract contains more than 900 g kg^{-1} of carnosic acid and carnosol, both of which are phenolics.

In addition to these antioxidant properties, polyphenols have also been shown to extend the shelf life of some food products through their antimicrobial activities, and they may also act as inhibitors of pathogenic microorganisms.[6]

This review will thus help the reader to identify (i) phenolic-rich plant extracts with potential as antimicrobials, (ii) their mechanism of action, (iii) the factors known to affect their *in situ* antimicrobial activity in real food systems, and (iv) most of their potential applications for perishable food preservation reported up to now.

NATURAL EXTRACTS/MOLECULES FOR FOOD PRESERVATION

Edible plants: a potential source of antimicrobial molecules

Controlling microbial growth in food products has always been a major concern for the different stakeholders in the agri-food sector. A double challenge must be considered: ensuring both food safety and food waste reduction. In fact, microbes causing infectious diseases are frequently the cause of morbidity and mortality across the world. In addition, microbial spoilage induces the loss of approximately a quarter of the world's food supply, and more than 40% of food damage occurs at the retail and consumer levels in developed countries.[7] This context has induced a rise in biocide and antibiotic application in order to guarantee efficient control of the microbial contamination of foods.

The emergence and spread of antibiotic resistance among human pathogenic microorganisms are a critical challenge. Indeed, the appearance of resistance or even multiresistance in a large bacterial community can be induced by the routine use of antibiotics.[8] In addition to this phenomenon of antibiotic resistance, the presence of antimicrobial agent residues in the environment has attracted much attention from modern consumers. Thus, the search for natural antimicrobials that are effective against both pathogenic and spoilage microorganisms is crucial.

Natural preservatives are considered healthier and to have an added value arising from their bioactivity and nutritional value. Therefore, an increasing number of food companies have made an effort to meet the increasing consumer demand for natural food preservatives. However, a soft transition from chemical additives to natural alternatives is expected, particularly because of economic and antimicrobial efficacy issues that still have to be solved.[1]

Plant-derived antimicrobials are promising in this context. Indeed, plant extracts are generally considered edible based on their traditional human consumption. In addition, plant secondary metabolites (PSMs) account for the greatest diversity of structures (e.g. there are more than 12 000 known alkaloids, more than 10 000 phenolic compounds and over 25 000 different terpenoids). Different antimicrobial polyphenol subgroups, their chemical structures and examples are presented in Fig. 1.

Antimicrobial PSMs were thus proposed as potential alternatives to synthetic preservatives. Nevertheless, plant-derived antimicrobials have not been frequently applied until now. To expand the use of plant extracts as natural preservatives, their bioactive compounds could be extracted and purified by developing economic processes preserving their activities.

Typically, extraction of phenolic compounds as a mixture and their purification are simple to perform. The edible plant-derived compounds remain the most favored for food use to limit concern

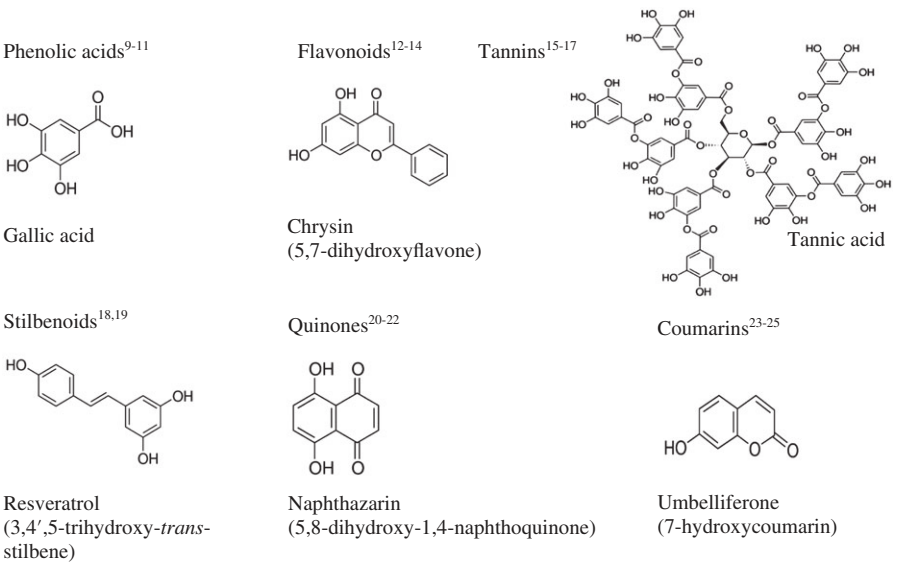

Figure 1. Major groups of plant-derived antimicrobial phenolics, chemical structures and examples. Phenolic acids,[9–11] flavonoids,[12–14] tannins,[15–17] stilbenoids,[18,19] quinones[20–22] and coumarins.[23–25]

regarding toxicity. Therefore, several recent studies have explored these plants as a potential source of antimicrobial molecules. The *in vitro* antimicrobial activity of the most studied edible plants byproducts in the past decade is described in Table 1.

Polyphenols: a diversity of antimicrobial mechanisms of action

The OH groups of phenolic compounds interact with the cell membrane of bacteria by hydrogen bonding. Importantly, the presence of OH functional groups is relevant to the antibacterial activity of many phenolics.[57–60] Indeed, the interaction of phytochemicals with bacterial cell membranes usually causes either the disruption of the membrane structure, which induces loss of cellular content,[58,61] or the delocalization of electrons (because of the double bonds of the aromatic nucleus), which results in depolarization of bacteria (acting as proton exchangers) and thus affects the proton motive force, reducing the pH gradient across the membrane and the level of the ATP pool.[60] This series of mechanisms initiated by the active hydroxyl groups of phytochemicals such as phenolics can lead to cell death. Furthermore, the relative position of the OH group on the phenolic nucleus has been reported to influence the antibacterial efficacy of phenolic compounds.[62]

The presence of alkyl groups in the aromatic nucleus generates phenoxyl radicals[63] reported to enhance the antibacterial efficacy of phenolics and alter their distribution ratio between aqueous and non-aqueous phases, including bacterial phases.[64] The presence of an acetate moiety in the molecular structure appeared to increase the activity of parent phenolic compounds by either the alcohol groups as protein denaturing agents[64] or by an increase in their electronegativity due to the aldehyde groups promoting electron transfer and reactions with membrane proteins.[65] The galloyl moiety has been reported to induce important damage to the membrane structure, thereby enhancing the antibacterial activity of epigallocatechin gallate against Gram-positive bacteria.[66] In addition to their chemical composition and structure, the lipophilic properties of phytochemicals are also involved in their antibacterial activity.[63]

The antimicrobial activity of many phenolics has been observed to increase with the elevation of their lipophilic character; this may be directly related to their potential interactions with the cell membrane.[66] The ability to penetrate the cell membrane and interact with cell compounds induces irreversible damage to the cell membrane and coagulation of the cell content, affecting both membrane and intracellular enzymes. Hydrogen-bonding descriptors (H-bond donors, H-bond acceptors), polar surface area, log *P* (octanol/water partition coefficient) and HOMO (highest occupied molecular orbital) and LUMO (lowest unoccupied molecular orbital) energy levels are the physicochemical parameters involved in the capacity of bioactive molecules to permeate through lipid membranes.[67,68] Van der Waals bonding can be established when the electron bonding energy of phenolics (acting as hydrogen bond donors) is higher than that of membrane lipids. A high number of hydrogen bond donors leads to higher interactions with the membrane of bacteria. Furthermore, molecules with a polar surface area of greater than 140 Å squared (higher than acyl chains) tend to interact strongly with the choline head groups of membrane phospholipids.[69] Strong interactions with the Caco-2 cell model membrane have been observed for molecules with log *P* values higher than 0 and less than 3,[68] allowing them to get closer to membrane surfaces and interact with them; however, such data regarding bacterial membranes are lacking. Attraction between two different molecules can be induced by the interaction between their HOMO and LUMO. The difference in the HOMO and LUMO energy levels between phenolics and lipids constitutes a key element for bacterial membrane and phenol interactions.

The main antimicrobial mechanisms of action of polyphenols cited in this review are summarized in Fig. 2.

Not all information on the mechanisms of the antimicrobial action of phenolics has been acquired yet. However, these components are considered to have many sites of action at the cellular level.[70] There are at least three mechanisms on which several authors agree: (i) modification of the permeability of cell membranes, formation of cytoplasmic granules and rupture of the cytoplasmic membrane; (ii) changes in various intracellular functions induced by hydrogen bonding of the phenolic compounds to enzymes through their OH groups; and (iii) modification of fungal morphology (cell wall rigidity and integrity losses) induced by different interactions with cell membranes.[71]

In general, Gram-negative bacteria are more resistant to PSMs, including phenolics, than Gram-positive bacteria.[2,72] This difference is likely because Gram-negative bacteria possess a cell wall linked to an outer complex membrane,[73] namely, the lipopolysaccharide envelope, which slows down the passage of phytochemicals.[74,75] Nevertheless, the appearance of lipopolysaccharides released from the outer membrane provides evidence that some polyphenols may also affect the outer membrane of Gram-negative bacteria.[76–78]

Additional impacts were reported when Gram-positive bacteria and fungi were considered. The first one is the modification of intracellular pH (due to variations in the flow of ions such as H^+ and K^+ influencing the proton motive force), and the second one is the blocking of energy production (interference with the energy (ATP)-generating system).[79]

Condensed phenylpropanoids – tannins – may induce damage at the cell membrane level and even inactivate metabolism by binding to enzymes,[80,81] while phenolic acids have been shown to disrupt membrane integrity, as they cause consequent leakage of essential intracellular constituents.[10]

Flavonoids are considered able to promote complex formation by linking with soluble proteins located outside the cells and within the cell walls of bacteria.[82,83] Quercetin was reported to have a significant effect on the bacterial membrane by increasing the membrane permeability and disturbing its potential.[84] Investigations on the membrane action of several flavonoids ((−)-epigallocatechin gallate,[71] (−)-epicate chin gallate and 3-*O*-octanoyl-(+)-catechin,[85] as well as 2,4,2′-trihidroxy-5′-methylchalcone[86]) showed that these compounds induce a reduction in membrane fluidity.

Furthermore, some flavonoids may act by inhibiting both energy metabolism and DNA synthesis, as observed by Haraguchi *et al.*,[87] and affect protein and RNA syntheses to a lesser extent. In addition to their versatile activities, catechins exert antibacterial effects via DNA gyrase inhibition.[88]

Other flavonoids, such as apigenin, have been proven to have an inhibitory effect on the activity of DNA gyrase and hydroxyacyl-acyl carrier protein dehydratase.[83,89]

Naphthoquinones (e.g. plumbagin) were found to inhibit potential efflux pumps. Interestingly, they have been shown to have a significant antibacterial effect against Gram-negative bacteria, whose resistance to most natural antimicrobial products is related to efflux pumps,[90] while coumarins have been reported to cause a reduction in cell respiration.[16]

Recent advances in multiparameter flow cytometry offer the opportunity to obtain high-speed information on the

Table 1. *In vitro* antimicrobial effect of aqueous extracts from the most studied edible plants byproducts in the past decade (from 2007 to 2017)

Byproducts	Major component	Target organisms	References
Coffee pulp extract	Polyphenols such as flavan-3-ols, hydroxycinnamic acids, flavonols, and anthocyanidins	*Pseudomonas fluorescens*, *Staphylococcus aureus*, *Aspergillus flavus*	26
Spent coffee extract	Flavan-3-ols, hydroxycinnamic acids, flavonols, and anthocyanidins	*Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus subtilis*, *Candida albicans*	27
Green tea waste	Tannins	*Staphylococcus aureus*, *Escherichia coli*, *Listeria monocytogenes*, *Bacillus coagulans*, *Shigella flexneri*	28
Green, white and black tea extracts	Tannins	*Salmonella typhimurium*, *Listeria monocytogenes*	29
Olive pomace	Phenolic compounds including oleocanthal, deoxyloganic acid lauryl ester	*Escherichia coli* O157:H7, *Salmonella enteritidis*, *Listeria monocytogenes*, and *Staphylococcus aureus*	30
Olive leaf extract	Phenolics and flavonoids	*Listeria monocytogenes*, *Escherichia coli* O157:H7, *Salmonella enteritidis*, *Candida albicans*	31,32
Pomegranate fruit peel extract	Phenolics and flavonoids	*Salmonella* spp., *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli*, *Yersinia enterocolitica*, and *Pseudomonas fluorescens*	33,34,35,36,37,38,39
		Pseudomonas stutzeri	
		Gram-negative bacteria, Gram-positive bacteria, and fungi	
Pomegranate aril and peel extracts	Phenolics and flavonoids	*Staphylococcus aureus*, *Escherichia coli*	40
Winery products	Phenolic acids, flavonoids, stilbenes	*Bacillus cereus*, *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella enterica*, *Staphylococcus aureus*, *Yersinia enterocolitica*	41
Grape pomace	Phenolic acids, flavonoids, stilbenes	*Staphylococcus aureus*, *Salmonella*, *Enterococci*, total aerobic mesophilic and psychrotrophic bacteria	42 43 44 45
		Escherichia coli, *Salmonella enteritidis*, *Salmonella typhimurium*, *Staphylococcus aureus*, and *Yersinia enterocolitica*	
		yeasts, and molds	
		Campylobacter jejuni	
Grape fruit seed extract	Flavonols, phenolic acids, catechins, proanthocyanidins and anthocyanins	*Campylobacter jejuni*	46 47 48
		Pseudomonas spp.	
		Listeria monocytogenes	
Mango seed kernel extract	Phenolic compounds, saturated fatty acids, monounsaturated oleic acid, tocopherols, squalene, and different sterol fractions	Total bacterial count, coliforms, and *Escherichia coli*	49
Myrtle berries seeds extract	Phenolic acids and flavonoids	*Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhimurium*, and *Bacillus cereus*	50
Date extract	Phenolic acids and flavonoids	*Escherichia coli*, *Bacillus subtilis*, *Enterococcus faecalis*, and *Salmonella* spp.	51
Walnut green husk extract	Phenolic compounds	*Staphylococcus aureus*, *Bacillus cereus*, *Bacillus subtilis*	52
Almond skin extract	Polyphenols	*Staphylococcus aureus*, *Listeria monocytogenes*	53
Tomato seeds extract	Metabolites such as fatty acids, carotenoids, saponins, and phenolic compounds	*Staphylococcus aureus*, *Salmonella epidermidis*, *Micrococcus luteus*, *Enterococcus faecalis*, *Bacillus cereus*, and *Candida albicans*	54
Buckwheat hull extract	Phenolics, flavonoids, antioxidants comprising tocopherols, rutin, and quercetin derivatives	Gram-positive (*Bacillus cereus*, *Staphylococcus aureus*, *Enterococcus faecalis*) and Gram-negative bacteria (*Salmonella choleraesuis*, *Escherichia coli*, and *Proteus mirabilis*)	55
Pummelo peel extract	Flavonoids	*Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus*, *Chromobacterium violaceum*, and *Vibrio anguillarum*	56

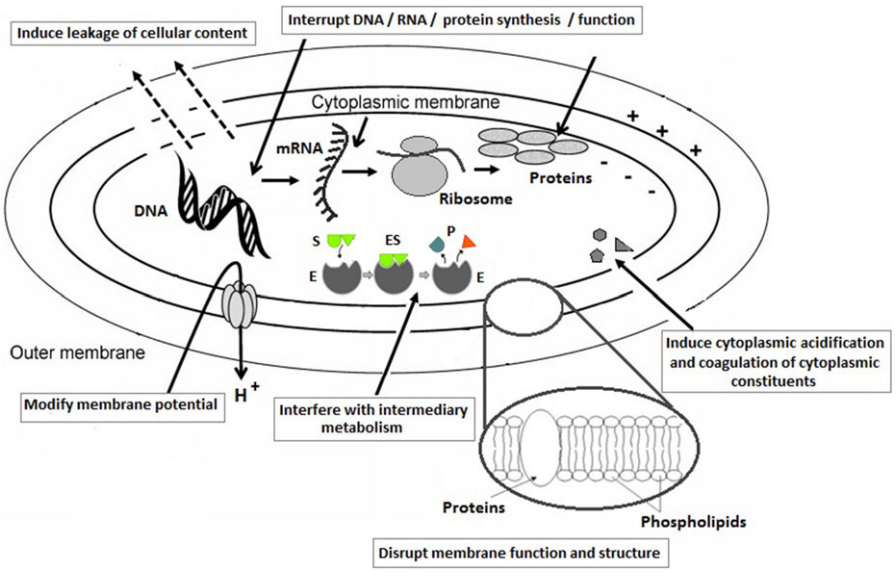

Figure 2. Different sites of action of antimicrobial polyphenols at the cellular level.

mechanism of action of antimicrobials at real time at the single-cell level.[91] Flow cytometric analysis revealed that resveratrol-*trans*-dihydrodimer disturbs the membrane potential and hinders DNA synthesis of microorganisms.[92] Paulo *et al.*[93] used microscopic analysis combined with flow cytometry to determine the bacteriostatic effect of 200 mg L^{-1} resveratrol (4 × minimal inhibitory concentration (MIC) for *Bacillus cereus* and 2 × MIC for *Staphylococcus aureus* at 1–2 × 10^8 CFU mL^{-1}) against Gram-positive bacteria. Modifications in cell morphology and DNA were observed in the presence of resveratrol; it can thus be assumed that it interferes with the cell cycle of bacteria. Duggirala *et al.*[94] screened 11 natural phenolics for the inhibition of the bacterial division protein FtsZ and identified coumarins as promising candidates. Recently, Liu *et al.*[31] observed that sublethal treatments of *Listeria monocytogenes* cells with olive leaf extract abolished flagella and thereby reduced their motility. Other antibacterial mechanisms of action of phenolic-rich plant extracts, such as inhibition of quorum sensing, which is involved in pathogenesis of many food bacteria, cannot be excluded; such an antibacterial mechanism of action was recently reported for oregano essential oil.[95] The modes of action of polyphenols discussed earlier are diverse, and this can be advantageous for their application as natural antimicrobial agents. However, further studies in this field are needed to obtain the rationale for their utilization as antimicrobial food additives. For instance, transcriptomic analysis of microbial cells treated with sublethal doses of plant phenolics or plant extracts should be performed to identify genes whose expression would be modified. For instance, quantitative polymerase chain reaction (qPCR) analysis of the expression of genes in *Escherichia coli* O157:H7 following treatment with cranberry concentrate allowed observation of the downregulation of genes coding for bacterial membrane and cell wall constituent synthesis.[96]

USES OF ANTIMICROBIAL PLANT POLYPHENOLS IN FOODS

Antimicrobial activity of plant polyphenols in foods

The food use of plant extracts has been shown to be promising, and some of these extracts already possess a generally recognized as safe (GRAS) status. A comprehensive document regarding the use of phytochemicals as food preservatives has been developed by the International Life Sciences Institute-Europe:[97] it is focused on their effective identification and characterization. However, the recent increase in natural preservative use has induced changes in European legislation (EC/1334/2008)[98] implemented in January 2011. These changes include new statements for natural extracts and treatments used for their preparation. At the same time, there is a need to highlight processes that are more environmentally friendly than the usual methods. In the Code of Federal Regulations, Title 21, the Food and Drug Administration (FDA)[99] defines safe natural additives for food use according to the following statements: (i) they are used in the minimum quantity required to produce their intended physical or technical effect and in accordance with all the good manufacturing practice principles; (ii) in the appropriate forms (plant parts, fluid and solid extracts, concentrates, absolutes, oils, gums, balsams, resins, oleoresins, waxes, and distillates), they can be used alone or in combination with flavoring substances and GRAS adjuvants in food. Many plant-derived products have thus been proposed as food ingredients or supplements, and they take an interesting place in the market among other healthy products.

Beverages (water- and tea-based drinks, yogurts and smoothies) are the most common foods fortified with polyphenols.[100]

In addition to their antioxidant activity, a great deal of effort has recently been made to include polyphenols in natural functional ingredients as food antimicrobial preservatives.[101]

Plants synthesize polyphenols in response to stress because of their self-defense from diseases mainly induced by microorganisms. That is why they are considered a promising source of antimicrobials with healthy features. Many natural phenolics that are widespread in nature, especially those that are extracted from edible plants and that have proven antimicrobial activities, could be used as potential food preservatives.[9,19,102–104]

The antibacterial activity of three pure compounds naturally occurring in plants, caffeic acid, *p*-coumaric acid, and rutin, in different food products was tested by Stojkovic *et al.*[104] Amounts of *p*-coumaric and caffeic acids above 0.1 g L^{-1} completely inhibited *Staphylococcus aureus* growth in chicken soup, and after 72 h, no cell survival was observed in samples treated with greater than

0.9 g L^{-1} rutin and stored at either 25 or 4 °C. Phenolic compounds have a relevant role in the visual appearance (pigmentation and browning), taste (astringency) and odor (aromas) of plant-derived products.[105,106] Interestingly, sensory evaluation for overall acceptance revealed that compared to those of the control samples, the sensory features of chicken soup and pork meat exposed to *p*-coumaric acid and caffeic acid were well appreciated.[104]

The antibacterial effect of gallic acid combined with caffeic acid, rutin and quercetin against *Escherichia coli* was investigated in a meat model system at 4 °C. This combination of phenolics at a concentration of 100 or 200 mg L^{-1} was bactericidal after 14 or 21 days of incubation. Such a synergistic effect makes it possible to enhance the activity of the polyphenols and reduce their effective concentration.[107]

The antimicrobial potency of pinosylvin was evaluated in different food systems:[19] 25–200 mg kg^{-1} pinosylvin caused a decrease of 2–4 log of *Enterobacteriaceae* in fermenting sauerkraut. The antimicrobial activity of pinosylvin (140 mg kg^{-1}) against *Listeria monocytogenes* inoculated in fresh gravlax was higher at 8 °C than at 20 °C. *Saccharomyces cerevisiae* inoculated in strawberry jam was completely eliminated by 300 mg kg^{-1} pinosylvin. However, 75 mg L^{-1} pinosylvin was sufficient to completely inhibit *Staphylococcus aureus* growth in culture media, while 200 mg L^{-1} pinosylvin had no effect in milk.

Numerous *in vitro* studies have been performed in microbiological culture media to assess the antimicrobial activity of plant extracts, but far fewer studies have addressed their application to food products. The lower antimicrobial efficacy of plant extracts in real foods may be the reason for this imbalance of information.[72] The presence of the glycosyl groups of flavonoids contained in the crude extracts is partially responsible for the reduction in their activity against a wide range of bacteria reported in several studies.[90–92,108,109,110]

In situ evaluation of the antimicrobial activity of plant extracts is crucial for food use because of interactions of their bioactive compounds with food components, most likely reducing their effectiveness. However, *in vitro* screening remains a first step to identify the antimicrobial potential of plants. An antimicrobial efficacy similar to that in *in vitro* cultures could be achieved by adding higher amounts of plant extract to foods.[58] Two-fold, ten-fold, 50-fold, and 25–100-fold higher plant extract concentrations were necessary to have the same antimicrobial effect in skimmed milk (from 0.6 g kg^{-1} to 10 g kg^{-1} for rosemary extract),[111] in pork liver sausage (from 5 to 50 mL kg^{-1} for rosemary extract against *Listeria monocytogenes*),[112] and in soft cheese (from 0.04 to 2.5 mL kg^{-1} for a mixture of rosemary, sage and citrus extracts against *Listeria monocytogenes*),[113] respectively, as in *in vitro* trials.[2,114] For instance, Miceli *et al.*[115] observed that a ten-fold increase in the quantity of *Borago officinalis* (from 10 to 100 g L^{-1}) and *Brassica juncea* (from 3.1 to 31 g L^{-1}) aqueous extracts was necessary to achieve an antimicrobial effect in meat, fish, and vegetables. This variance can result from the interactions that occur in food systems between hydrophobic bioactive constituents of plant extracts and major food ingredients such as fat and proteins. Based on their hydrophilic character, other phytochemicals behave differently in food products. The dose of phenolics applied for food preservation should be set with sensory considerations and not based solely on *in situ* antimicrobial efficacy. To be accepted as food preservatives, phenolic-rich plant extracts should not strongly impart their typical color and flavor to foods. Ideally, the plant extract is chosen according to culinary associations already existing in consumer behaviors (e.g. rosemary for meat,[112] thyme for vegetable and chicken soups[60]).

In food matrices rich in fat, a lipid coating that wraps the microorganisms and protects them from antimicrobials can form.[71] Uhart *et al.*[116] reported that spices inactivate *Salmonella* Typhimurium DT104 under *in vitro* conditions, whereas a decrease in their inhibition efficacy was observed when the spices were included in complex food matrices (e.g. ground beef). Similarly, green and jasmine tea did not significantly reduce *Listeria monocytogenes*, *Staphylococcus aureus* or total bacterial counts in ground beef.[117]

In addition, compared to culture media, many foodstuffs have a reduced water content, which may limit the transport of antimicrobials into the microbial cells.[71] Other potential causes include modifications in the solubility and charge of phenolics and variations in the cell envelope of target bacteria. The known interaction of many polyphenols with proteins might result in polyphenol–protein complexation (as reviewed by Papadopoulou and Frazier[118]) and thus limit the action of active polyphenolic compounds against microbial cells. Food-mimicking matrices prepared by dispersing proteins and/or fat in liquid media can help estimate the minimum concentrations inhibiting or killing microorganisms (minimum inhibitory concentrations (MICs) or minimum bactericidal concentrations (MBCs) for bacteria, respectively) in food systems.

The physiological state of target microorganisms in foods is also likely an important factor affecting the *in situ* efficiency of many antimicrobials: most *in vitro* antimicrobial activity assays are performed with microorganisms in an optimal environment without any limiting substrates to allow their exponential growth, which is not the case in real foods.

Some phenolic-containing aqueous plant extracts that have exhibited a broad antimicrobial spectrum (among those that were non-toxic and had a relatively limited odor and taste) and that have already been used for direct incorporation in foods or in food packaging materials in the past 15 years are listed in Table 2.

Stability of plant polyphenols

Polyphenol stability is a crucial property for application in food systems and is a function of several factors, such as size, chemical structure, water solubility and polarity. Recently, nutrition has become a tool to promote human health, and maximal knowledge of the effects of treatment processes is essential for maintaining the functions of plant biomolecules not only as food preservatives but also as compounds of nutritional interest.[72]

For the application of phenolic compounds for food preservation, they have to be stable until the expiration date of the product to which they were added. However, polyphenols are relatively unstable when directly applied in foods. The stability of such compounds in food systems can be attributed to a series of stabilities: physical, chemical, colloidal, and biological, which are correlated with each other.[159]

Co-extrusion of a linear low density polyethylene (LLDPE)-based film blended with grape seed extract (10 g kg^{-1}) in a twin-screw extruder with a barrel temperature ranging from 160 to 190 °C resulted in a strong reduction in the antimicrobial activity of the extract.[160] Conversely, a polyethylene-based film blended with pomegranate peel extract (15 g kg^{-1}) and produced by the same process demonstrated good antimicrobial activity in another work.[161]

Polyphenol stability in solution depends on environmental factors (e.g. pH, electrolyte composition, and presence of oxidants). The instability of phenolic compounds can occur at pH 1–11,

Table 2. Some studies regarding the application of aqueous phenolic-rich plant extracts to food preservation in the past 15 years

Food group	Plant extract	Microorganisms	References
Meat and poultry			
Raw beef	*Capsicum* extract	*Salmonella typhimurium*	119 120
	Lemon/cherry/vinegar extract	*Pseudomonas aeruginosa*	
		Listeria monocytogenes	
Meat products	Rosemary/oregano extracts	Total viable counts	121 122 123
		Psychrotrophic bacterial counts	
		Pseudomonas spp.	
		Lactic acid bacteria	
Beef slices	Oregano/cranberry extracts	Total viable counts	124
Beef meat balls	Rosemary/orange/ lemon extracts	Bacterial spoilage	125
Fried meat	Oregano and thyme, oregano with marjoram and thyme with sage	*Bacillus cereus*	126
		Pseudomonas aeruginosa	
		Escherichia coli O157:H7	
		Listeria monocytogenes	
Chilled steak	Water spice extracts: clove, cinnamon, star anise, pricklyash peel and common fennel	Spoilage microorganisms: *Staphylococcus aureus*, *Lactobacillus* spp., *Brochothrix thermosphacta*, *Pseudomonas* spp., *Escherichia coli*	127
Ground beef	Grape seed extract pine bark, rosemary oleoresin extract	*Escherichia coli* O157:H7	128,129 130 131 132 96
	Oregano/cranberry (50:50) extract	*Listeria monocytogenes* Scott A	
	Water-soluble arrowroot tea extract	*Salmonella typhimurium*	
	Dried plum puree	*Listeria monocytogenes* Scott A, *Salmonella typhimurium*	
	Cranberry concentrate	*Salmonella enteritidis*, *Listeria monocytogenes*	
		Escherichia coli, *Salmonella*	
		Listeria monocytogenes	
Beef patties	Grape pomace extract	*Enterobacteriaceae* and coliforms, lipolytic bacteria,	43 36
	Pomegranate peel extract	*Salmonella*, *Staphylococcus aureus*, yeasts and molds	
		Listeria monocytogenes	
Meat surfaces	Oregano/pimento extract	*Escherichia coli* O157:H7	133,134 135
		Pseudomonas spp.	
Cured cooked meat model system	Cranberry/cherry/lime/grape seed extracts	*Listeria monocytogenes*	136
Raw pork	Cinnamon stick/oregano/clove/pomegranate peel/grape seed extracts	*Listeria monocytogenes*	137
		Staphylococcus aureus	
		Salmonella enterica	
Fresh pork sausages	Rosemary extract/chitosan/alpha-tocopherol	*Enterobacteriaceae*, *Pseudomonas* spp., yeasts and molds, lactic acid bacteria	138
Ham	Rosemary extract	*Listeria monocytogenes*	139 120
	Lemon/cherry/vinegar extract	*Listeria monocytogenes*	
Chicken meat	Pomegranate peel extract	Total bacterial count, Coliforms	140 141 142
	Oregano extract	*Staphylococcus aureus*	
	Rosemary extract	*Campylobacter jejuni*	
Chicken meat juice	Rosemary extract	*Campylobacter jejuni*	142
Chicken sausage	Fresh garlic powder	Aerobic plate count	143
Chicken liver patties	Pomegranate (*Punica granatum*) peel extract	*Listeria monocytogenes*	36

Table 2. Continued

Food group	Plant extract	Microorganisms	References
Raw turkey meat balls	Sage extract Lemon/cherry/vinegar extract	Mesophilic bacteria Coliforms *Listeria monocytogenes*	144 120
Ready-to-eat vacuum-packaged diced turkey	Rosemary extract	*Listeria monocytogenes*	139
Fish			
Fresh chilled fish	Pomegranate peel extract	*Listeria monocytogenes*	33
Cold fish filet	Oregano and cranberry	*Listeria monocytogenes*	124
Smoked fish	Coffee pulp smoke	Coliforms and fungal counts	145
Salami	*Eremophila duttonii* and *Eremophila alternifolia* extracts	*Listeria monocytogenes*	146
Cooked shrimp and raw tuna	Pomegranate peel extract	*Vibrio parahaemolyticus*	147
Dairy			
Full cream milk, skim milk	*Eremophila duttonii* and *Eremophila alternifolia* extracts	*Listeria monocytogenes*	146
Pasteurized cow milk	Extract of mango seed kernel	Coliforms, *Escherichia coli*	49
Yogurt stew	Citrus flowers extract	*Escherichia coli* O157:H7, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*	148
Kalari cheese	Pomegranate rind extract	Total plate count, psychrophilic count, yeast and mold counts, coliforms	149
Pate and brie cheese	*Eremophila duttonii* and *Eremophila alternifolia* extracts	*Listeria monocytogenes*	146
Vegetables			
Tomatoes	Grape seed extract	*Listeria monocytogenes* Scott A *Listeria innocua* ATCC 33090	48
Carrots	Hydrosol of thyme, black cumin, sage, rosemary and bay leaf extracts	*Salmonella typhimurium Escherichia coli* O157:H7	150
Salad vegetables	Unripe grape juice	*Salmonella typhimurium*	151
Vegetable soup	Grape pomace	*Staphylococcus aureus*, *Escherichia coli*	152
Rice			
Rice cakes	Green tea/rosemary extract	*Bacillus cereus*, *Staphylococcus aureus*	153
Fruits			
Murcott tangor fruits	*Anadenanthera colubrine* extract	*Alternaria alternata*	154
'*Rocha*' pears	*Origanum vulgare* extract	*Botrytis cinerea*, *Penicillium expansum*	155
Apple	Hydrosol of thyme, black cumin, sage, rosemary and bay leaf extract	*Salmonella typhimurium*, *Escherichia coli* O157:H7	150
Fresh-cut apples	Ethanolic extract of cinnamon bark	*Escherichia coli* O157:H7, *Listeria innocua*	156
Juice			
Fresh-squeezed tomato juice	Extract of pine needles	Total counts of viable bacteria, *Escherichia coli*, *Proteus vulgaris*, *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*	157
Wine	Extracts from eucalyptus leaves and almond skins	Lactic acid bacteria	158

favorable for oxidative degradation, complex formation and reactions with other phenols, amino acids, proteins, and metal ions.[162,163] However, pH changes may induce new structures and colors of phenolics (the red wine pigment malvidin 3-glucoside may change from red at pH 1 to colorless at pH 4–5, to purple at pH 6–7, and to yellow at pH 7–8 [163]).

The OH groups located on the benzene ring of phenolic compounds are often involved in responses to pH variations. Ultraviolet spectral monitoring allowed Friedman and Jurgens[163] to observe that in contrast to caffeic, chlorogenic and gallic acids (with two or three OH groups attached to the benzene ring), conjugated non-phenolic aromatic acids such as *trans*-cinnamic acid without any OH groups are stable at high pH (pH 11).

Other structural criteria play a role in promoting the complexation of phenolics with other solutes at high pH values and thus induce their instability. For instance, more complex phenolics with multiring aromatic structures such as catechin, epigallocatechin, and rutin have ionized and resonance forms that are more resistant to degradation by pH than monocyclic compounds.[163] The number of OH groups located on the benzene ring can indicate the ability of phenolics to form quinone oxidation products. Ferulic acid with no more than one OH group is stable at high pH (pH 7–11), whereas caffeic acid is unstable to pH variations because of the two adjacent phenolic OH groups on the benzene ring.[163] Not only the number of OH groups but also their presence on the same or separate aromatic rings and their position (meta- or ortho-position) affect the ability of phenolics to interact with each other via conjugation or quinone formation. The spatial arrangement of OH groups is reported to influence the stabilities to pH variations.[163,164]

The phenolic acids caffeic acid, chlorogenic acid, and gallic acid have been found to be irreversibly affected by high pH values.[163] Conversely, an acidic pH had no effect on chlorogenic acid stability after inclusion in apple juice. Other phenolics, such as (−)-catechin, (−)-epigallocatechin, ferulic acid, rutin, and *trans*-cinnamic acid, may resist pH-induced degradation.[163] Concerning anthocyanins, it is well known that variations in pH significantly influence their stability and color.[163]

Curcumin is primarily used as a food additive (coloring agent: E100 (i)), but it is increasingly considered a multifunctional bioactive molecule. Interestingly, glycosylated[165] or amino acid-conjugated[166] curcumin have shown similar antibacterial, antioxidant and antimutagenic activities as pure curcumin, so such chemical modifications did not affect the earlier mentioned bioactive properties of curcumin. However, microcapsules of curcumin (prepared with gelatin and porous starch by the spray-drying method) have better solubility and stability than free curcumin along with similar antibacterial and antifungal activities.[167]

Colloidal stability is defined as the ability of polyphenols to maintain a homogeneous dispersion in food matrices under various storage conditions. The stability of polyphenols added to complex food matrices can be predicted based on information on various interactions that can occur with surrounding components present in foods. Repulsive forces among charged groups may prevent polymerization and aggregation of these active compounds. Through the choice of the appropriate formulation of phenolic extracts, the formed electrostatic repulsive forces may thus increase the stability of the system once incorporated into the foods.

Biological stability includes the ability of polyphenols to preserve their antioxidant and antimicrobial properties after processing and for long-term storage under the same conditions. Room or refrigerated temperatures are the environmental conditions in which polyphenol activities are usually evaluated. To preserve their bioactivity, polyphenols can be freeze-dried. Many bioactive molecules can undergo chemical degradation, isomerization or polymerization during harsh food processes such as baking, steaming and extrusion, thereby possibly inducing a loss of their activities.[101]

When incorporated into food matrices, phenolic compounds may be subjected to temperature variations. The matrix nature is the most influential factor in the thermal stability of botanical compounds in foods. Normally, phenolic compounds with higher melting temperatures are more stable to heat processing, but the effect of heat can be more pronounced in the presence of other food ingredients. A pure aqueous solution of chlorogenic acid (207–209 °C melting temperature) has been found to be stable to heat treatment (1 h at 90 °C)[163], while the loss (leaching out or decomposition) of chlorogenic acid heated (30 min at 100 °C or 18 min at 121 and 204 °C) in the presence of food constituents was reported by other authors.[168,169] Heat-treated (100 °C, 15 min) drumstick leaf extracts showed a significant decrease in their antioxidant activity compared to that of untreated samples. In contrast, the antioxidant activity of carrot tuber extract was not affected by the same heat treatment.[170] In some cases, heat treatment (105 °C, 20 min) induced the formation of new molecules, which either reduce, preserve or even improve the antimicrobial activity of different plant extracts.[171,172] For instance, new phenolics with low molecular weights were found in heated grape seed extracts.[173] However, it was reported that the polyphenol content of foods decreases in response to thermal processing and long-term storage.[174,175] The thermal stability of polyphenols in apple juice was studied by Spanos *et al.*[176] and van der Sluis *et al.*[177] Cinnamic acid, procyanidin and quercetin contents have been found to decrease when apple juice was stored at room temperature. Compared to freeze-dried grape seeds, grape seeds that were heat-dried at 100 and 140 °C exhibited 18.6% and 32.6% decreases in total polyphenols, respectively.[178] Heating at 60 °C or above for 8 h dramatically reduced the procyanidin and anthocyanin contents in freeze-dried grape pomace.[179]

Some studies have suggested heat-stable plant extracts that could be used as food preservatives, such as cinnamon in cookies;[180] *Garcinia* extract;[181] grape, amla, and drumstick leaf extracts[182] in biscuits; and mango fiber concentrate (with 16.1 mg g^{-1} of soluble polyphenols) in bread and cookies.[183]

Successful food product development is deeply based on the nature of the interactions that may occur between the ingredients of food.[101] A strong interaction between grape seed procyanidins and proteins leads to the formation of protein–tannin aggregates. The molecular weight and polymerization degree of procyanidins increase this aggregation.[184] Carvalho *et al.*[185] indicated that this type of binding is influenced by the protein properties (molecular size, hydrophobicity and structural flexibility), the polyphenol properties (degree of polymerization, extent of galloylation, structural flexibility) and environmental factors (temperature, pH, ionic strength, presence of organic solvents and presence of carbohydrates). Since the astringent taste of polyphenol-rich fruits and vegetables,[106] haze in beverages[186] and the bioavailability reduction in both food protein and polyphenols[118,187] depend on tannin–protein interactions, these interactions are the most studied polyphenol–protein interactions. Some studies have used bovine serum albumin, while others used α-lactalbumin.[188]

The storage of polyphenols for a long time under specific environmental conditions such as high temperature and light

exposure could seriously affect their chemical and physical stabilities. With respect to long-term storage, two phenomena must be considered in food systems: (i) the polyphenol components may be altered by oxidation, and (ii) the physical structure of polyphenols may be influenced by polymerization. Normally, oxidative damage is hardly a problem in practice, but it can be minimized by protection from light and air using an inert atmosphere as a preventive measure to maintain the effective biological activity of compounds.

Reducing sugars[189] or different carbohydrates[190] (e.g. trehalose) are commonly used as antioxidants in foods. Komes *et al.*[190] showed that due to its glass transition property, trehalose can retain and preserve hydrophobic phenolic compounds of fresh fruits during dehydration processes.

Other phytochemicals and antioxidants intentionally introduced to a food system may help to stabilize polyphenols.[72] Vitamin C added to processed yellow passion fruit exerted a protective effect on plant chemicals.[191] Red clover leaf extracts were demonstrated to make anthocyanins more stable when added to muscadine wines during storage (20 and 37 °C for 9 weeks).[192] Lecithin addition to tea catechin solution at acidic pH and room temperature has the ability to protect tea catechins from oxidative damage.[193]

High-performance liquid chromatography (HPLC) coupled with mass spectrometry (MS) analysis is usually used to assess the stability of polyphenols (e.g. molecular structure and quantity).[194]

Quantitative structure–activity relationship (QSAR) studies can be used to predict and determine the extract formulations that would be more stable in different food matrices. Indeed, the QSAR approach has already been used to determine the structure–reactivity and structure–antimicrobial activity relationships of phenols under different conditions by evaluating different parameters.[195] In this way, QSAR analysis can provide information on how interactions between phenolics or with other molecules (proteins, lipids, oxygen, etc.) modulate their antimicrobial activity.

Release of plant polyphenols from active edible coatings or packaging materials to foods in direct contact

To alleviate the deficiencies in using plant phenolic compounds in foods, polyphenols can be added to the immediate zone of foods in direct contact through slow release from edible coatings or packaging materials. Such systems could maintain an efficient concentration of antimicrobial plant phenolics in the superficial zone of foods over time. This approach could be advantageous for foods such as raw muscle foods (fish fillets, meat pieces) or some fruit and vegetables for which most microbial contamination occurs in their superficial zone. One advantage of edible coatings or food-contact packaging materials with antimicrobial plant phenolics over their direct spraying is their controlled release over time. Various studies on the use of active films and edible coatings to deliver antimicrobial agents to the surface of a wide range of foods in contact (fruit, vegetables, and meat products) have been conducted.[196,197] The delivery of phenolic compounds from edible coatings is mainly described as a sequence of material transfer movement starting with diffusion, followed by desorption from the film's or coating's surface, sorption of the compounds at the interface and finally sorption into the food.[198–203] It has been acknowledged that the delivery rate of bioactive compounds from films or edible coatings to food is faster when the release is a consequence of their swelling or dissolution, which is conditioned by the nature of the food matrix in direct contact and the polymer matrix of film or coating.[200] Furthermore, the time and temperature of contact,[204] the polymer matrix (promoting or no interaction) with phenolics via functional groups,[199,201,203] the properties (chemical structure and polarity)[200] of the phenolic compounds (migrating substances), and their contents in the edible films have been reported to affect the migration rate through coatings to foods in contact.[198,199,202,205] Additionally, the microstructure of the polymer matrix[206] and the way in which the phenolic compounds are oriented with respect to the food based on their hydrophilic or hydrophobic properties strongly affect their migration and thus their effectiveness in protecting foods in contact. Edible coating or active packaging design should thus exploit the possibility of tuning the physicochemical interactions between antimicrobial plant phenolics and the polymers, which are the main components of edible coatings/packaging materials, to control their release kinetics. Another potential advantage of edible coatings/packaging materials incorporating antimicrobial plant phenolics is their increased stability to oxidation in these polymeric matrices. Packaging materials made of edible biopolymers combined with natural antimicrobials are favorable for making foods safer and of higher quality.[201] The association of antimicrobial agents with edible coatings has thus increasingly been considered a favorable approach to increase the shelf life and/or enhance the safety of perishable foods in recent decades. This trend is illustrated by the increase in the proportion of articles on edible coatings that also consider antimicrobials in the Web of Science® database from 0% before 1994 to 25% since 2010.

Edible coatings/films incorporating antimicrobial plant polyphenols

Edible coating/film formulations are based on only food ingredients and additives. On the one hand, an edible coating is described as a thin layer of edible material formed directly on the superficial zone of a food that can be consumed with the food product. To prepare edible coatings, film-forming suspensions can be applied to foods by various processes, as reviewed by Andrade *et al.*[207] (e.g. panning, fluidized bed, dipping, spraying). A subsequent draining of excess film-forming suspension and drying are necessary following the dipping of food products. On the other hand, stand-alone edible films may be prepared by either solvent-casting or extrusion-blowing methods. Cast films can be prepared by pouring a film-forming suspension on a flat substratum, which is subsequently dried. Most edible films are prepared using this versatile technology at the laboratory scale. However, at the industrial scale, most food packaging plastic films are prepared by extrusion-blow molding. Edible films can also be prepared with this technology.[208]

Edible coatings/films provide a barrier to gaseous exchange as well as the transmission of moisture, flavors and other soluble constituents of processed products when manipulated and stored, thereby enhancing their shelf life.[209] The use of edible coatings/films as vectors of bioactive molecules can ensure their availability to act effectively at their site of action.[210] Incorporation into edible coatings/films is a good alternative to preserve bioactive compounds such as antimicrobial phenolics in foods. Bioactive compounds can be incorporated (i) on the external surface of the film, (ii) on the internal surface of the film, (iii) in the multilayers of the edible coatings, or (iv) in different parts of the film.[26]

Zein, whey proteins, caseinates, soy proteins, chitosan, alginate, carrageenan, pullulan, pectin, cellulose, and its derivatives are examples of biopolymers that have been used to prepare edible films and coatings.[211–219] These coatings and films can delay food spoilage due to their gas barrier properties, their intrinsic activity

(e.g. the antifungal activity of chitosan) or the antimicrobial compounds added to their formulation. Therefore, in addition to the biodegradability of these natural biopolymers, such films and coatings are considered environmentally friendly since they could contribute to food waste reduction.

Recent studies dealing with the incorporation of antimicrobial plant extracts into edible films and coatings are listed in Table 3. The incorporation of such compounds into films may enhance their antimicrobial activity;[231] the uptake of a sufficient quantity of phytochemicals on the surface of foods over time could be made possible by their gradual release through the film.[58]

In the literature, antimicrobial packaging acts primarily on foodborne pathogenic bacteria such as *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli* O157:H7, and *Salmonella* spp.[232–234] However, food spoilage microorganisms such as *Bacillus* spp. and *Lactobacillus* spp. could also be targeted[228] to increase perishable food shelf life and/or contribute to food waste reduction.

The release of antimicrobials from the edible coatings depends on many attributes, such as electrostatic (ionic and hydrogen bonds) and hydrophobic interactions between antimicrobials and polymers, osmosis, structural modifications of the polymeric matrix resulting from the presence of the antimicrobial agents, and the surrounding conditions (temperature, pH).[71]

Coatings may be used as a system for releasing antimicrobial phytochemicals over time on a wide range of food surfaces (e.g. vegetables, fruit, and meat products).[71] Rapeseed protein/gelatin coatings containing grape seed extract have been used to inhibit *Escherichia coli* O157:H7 and *Listeria monocytogenes* growth in strawberries.[235] Edible zein coatings applied to fruit and vegetables have been found to have a protective effect during storage (by controlling their respiration, ripening, and senescence).[201] Some phenolic acids (gallic, vanillic, and cinnamic acids) and extracts from clove, oregano, artichoke stems and walnut shells were assessed as antimicrobial zein film additives against four plant pathogenic bacteria.[201] The incorporation of phenolic compounds ($10-40\ g\ m^{-2}$) into zein films was shown to improve the porosity of films, which became more flexible and lost their brittleness.

Chitosan-coated films incorporated with green tea extract ($40\ g\ kg^{-1}$) had bactericidal activity against *Listeria monocytogenes* in ham steak for 8 weeks of storage at 4 °C.[236] In a more recent study, incorporation of green tea extract ($5-10\ g\ L^{-1}$) considerably enhanced the antifungal activity of chitosan coatings on fresh walnut kernel; fungal growth was not detected during 18 weeks of storage at room temperature.[237]

Food packaging materials incorporating antimicrobial plant polyphenols

Although conventional plastic packaging raises environmental concerns and its current use in direct contact with foods is highly regulated, many studies regarding active food packaging consider polyethylene or polypropylene and their derivatives due to their excellent physical and chemical characteristics.[161,238,239] Such active packaging falls under the scope of the EC 450/2009 regulation.[240]

At the industrial scale, there are two possible processes for including antimicrobials in packaging materials: (i) direct incorporation into the polymers during extrusion by melt-blending[241] and (ii) coating of the antimicrobial agents onto polymer surfaces.[242,243] Extrusion is preferred by manufacturers because of the high cost of the coating process (additional steps and technical changes).[244] However, additional costs may also result from the degradation of actives during extrusion processes operating at high temperature, which would have to be compensated for by the addition of a higher amount of active ingredients.[241] Moreover, the fact that antimicrobials are equally spread throughout the entire thickness of films made by extrusion can lead to limitations in their release from the packaged material and therefore lower *in situ* activity.[241] Therefore, in addition to the question of the stability of antimicrobial plant phenolics to the conditions prevailing during melt blending with the polymer (high temperature and shear stress), the transport properties of the plant phenolics once incorporated into the polymeric matrix of the packaging material also must be considered. The amount of plant phenolics released and their release kinetics should effectively inhibit the multiplication of unwanted microorganisms in the superficial zone of food.

Adequate analytical methods to assay plant phenolics in active food packaging materials and monitor their release in food matrices in direct contact should thus be developed. Therefore, Colon and Nerin[245] developed a method to quantify tea compounds released from a polyethylene terephthalate (PET) film with an internal coating layer containing green tea extract to IV gamma nectarines placed in a tray covered by this active film. Solid-phase extraction (SPE) combined with ultra-performance liquid chromatography (UPLC)-MS was used to check that the amounts of catechins and caffeine from the green tea extract delivered by the packaging material were below the migration limits of 10 μg per kg of nectarine. In agreement with the European Union (EU) regulations for food-contact materials (EU 10/2011[98] and EC 450/2009[240]), all components not available in the positive lists of the regulations introduced and not specifically recognized as a food additive, such as catechins and caffeine, must be below 10 μg per kg of food or food simulant. The ability of packaging films composed of cast polypropylene/polyvinyl alcohol incorporated with rhubarb ethanolic extracts and cinnamon essential oil to preserve the quality of fresh beef was investigated. Interestingly, all the experimental cast films used significantly decreased the total viable counts (TVCs).[246] However, as stated earlier, preparation of cast films by solvent evaporation, despite likely limiting essential oil loss by evaporation during the preparation of stand-alone films, is not the preferred method. In another study, $15\ g\ kg^{-1}$ pomegranate peel extract was blended with polyethylene resin by twin-screw extrusion at 160–190 °C to obtain active films.[161] A decrease in total volatile basic nitrogen (TVB-N) was observed during refrigerated storage of fresh pork meat packaged in these active films. The shelf life of the fresh pork meat was thus extended by 3 days. Interestingly, this result suggests that pomegranate peel extract still had significant antimicrobial and antioxidant activities following its incorporation by extrusion in polyethylene films. Despite its promise, this type of practice is still in its infancy.[247,248]

CONCLUSION

The *in vitro* antibacterial activity of many phenolic-rich plant extracts and pure plant phenolics has been reported in this review. Although their activity in perishable foods is often reduced or even lost, numerous examples of plant extracts or phenolics effectively preventing microbial contamination or degradation of foods are given. Since no pure plant phenolics are authorized as food preservatives, only direct incorporation of edible plant extracts (i.e. food ingredients) into perishable foods can be considered currently. In addition to direct incorporation into perishable food matrices, incorporation into the polymeric matrix of either food packaging materials or edible coatings is a promising approach to deliver

Table 3. Examples of edible coatings incorporated with antimicrobial plant extracts

Antimicrobial plant extract	Biopolymer	Target microorganisms	Coated food	References
Grape seed extract	Sodium alginate	*Micrococcus luteus*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella enteritidis*	-	220
Grape seed extract	Soy protein isolate	*Listeria monocytogenes*, *Escherichia coli* O157:H7		221
Wine grape pomace water extract	Low-methoxyl pectin, sodium alginate, Ticafilm®	*Listeria innocua*, *Escherichia coli*	-	222
Apple skin extract	Acai	*Listeria monocytogenes*	-	223
Guarana seed ethanolic extract	Gelatin and chitosan	*Staphylococcus aureus*, *Escherichia coli*	-	224
Boldo-do-chile (*Peumus boldus* Molina) leaf ethanolic extract	Gelatin and chitosan	*Staphylococcus aureus*, *Escherichia coli*	-	224
Cinnamon bark ethanolic extract	Gelatin and chitosan	*Staphylococcus aureus*, *Escherichia coli*	-	224
Rosemary leaves ethanolic extract	Gelatin and chitosan	*Staphylococcus aureus*, *Escherichia coli*	-	224
Oregano aqueous extract	Porcine skin gelatin	Aerobic plate counts (15 °C)	Cold-smoked sardine muscle	225
Rosemary aqueous extract	Porcine skin gelatin	Aerobic plate counts (15 °C)	Cold-smoked sardine muscle	225
Satureja thymbra ethyl acetate extract	Carboxymethyl cellulose (CMC)	Total viable count, *Pseudomonas* spp., *Enterobacteriaceae* spp.	Fresh gilthead seabream (*Sparus aurata*) fillets	226
Sweet basil hydroalcoholic extract	Pullulan	*Rhizopus arrhizus*	Jonagored apples	227
Satureja hortensis L. water extract	Pullulan	*Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella enteritidis*, *Escherichia coli*, *Penicillium expansum*	Pepper, apples	228
Quillaja saponaria Mol. hydroalcoholic extract	Milk proteins (calcium caseinate and whey protein isolate)	*Botrytis cinereal*	Fresh strawberries	229
Moringa plant extract	CMC	*Colletotrichum gloeosporioides*, *Alternaria alternata*, *Lasiodiplodia theobromae*	Avocado fruit	230

active plant phenolics to the immediate zone of foods in direct contact. However, again, due to the absence of active plant phenolics from the positive list of food preservatives and the legislation regarding active food packaging, only the application of edible coatings made of biopolymers with a food ingredient or additive status and edible plant extracts with a food ingredient status can be considered today. In addition to evaluation of some plant phenolics as new food preservatives, future research on building a rationale for the application of phenolic-rich plant extracts or phenolics for food preservation should specifically focus on (i) identifying the molecular mechanisms underlying their ability to control unwanted microorganisms, (ii) understanding the effect of food microstructure and composition on their antimicrobial activity and (iii) designing innovative and sustainable systems of delivery of active phenolics preserving their stability before use and favoring their controlled release in the superficial zone of perishable foods where postprocessing microbial contamination mainly occurs.

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DECLARATION OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTHOR CONTRIBUTIONS

All authors listed have made substantial, direct and intellectual contribution to the work and approved it for publication.

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