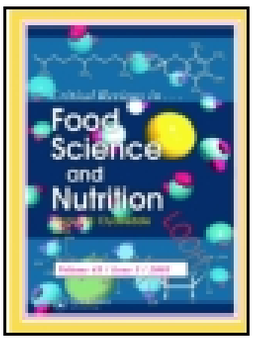

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Critical Reviews in Food Science and Nutrition

ISSN: 1040-8398 (Print) 1549-7852 (Online) Journal homepage: http://www.tandfonline.com/loi/bfsn20

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To cite this article: Susana Ribes, Ana Fuentes, Pau Talens & Jose Manuel Barat (2017): Prevention of fungal spoilage in food products using natural compounds: a review, Critical Reviews in Food Science and Nutrition, DOI: 10.1080/10408398.2017.1295017

To link to this article: http://dx.doi.org/10.1080/10408398.2017.1295017

Accepted author version posted online: 10 Apr 2017.

Prevention of fungal spoilage in food products using natural compounds: a review

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Abstract

The kingdom Fungi is the most important group of microorganism contaminating food commodities, and chemical additives are commonly used in the food industry to prevent fungal spoilage. However, the increasing consumer concern about synthetic additives has led to their substitution by natural compounds in foods. The current review provides an overview of using natural agents isolated from different sources (plants, animals and microorganisms) as promising antifungal compounds, including information about their mechanism of action and their use in foods to preserve and prolong shelf life. Compounds derived from plants, chitosan, lactoferrin, and biocontrol agents (lactic acid bacteria, antagonistic yeast and their metabolites) are able to control the decay caused by fungi in a wide variety of foods. Several strategies are employed to reduce the drawbacks of some antifungal agents, like their incorporation into oil-in-water emulsions and nanoemulsions, edible films and active packaging, and their combination with other natural preservatives. These strategies facilitate the addition of volatile agents into food

products and, improve their antifungal effectiveness. Moreover, biological agents have been investigated as one of the most promising options in the control of postharvest decay. Numerous mechanisms of action have been elucidated and different approaches have been studied to enhance their antifungal effectiveness.

Keywords

Plant secondary metabolite, essential oil, chitosan, lactic acid bacteria, antagonistic yeast, antifungal protection

ACCEPTED MANUSCRIPT

1. Introduction

The kingdom Fungi is a group of eukaryotic organisms that includes unicellular microorganisms such as yeasts and moulds. They are the most important group of organisms to contaminate fruits and vegetables, and other food commodities like wine, juice, fruit puree, jams, meat and cheddar cheese, among other food products (Nieminen et al., 2008; Gammariello et al., 2014). Furthermore, postharvest spoilage of fruits, vegetables, and cereals by phytopathogens, particularly fungal pathogens, produces significant economic losses (Liu et al., 2017).

The food industry has resorted to several techniques to prevent fungi growth and spoilage (Davidson and Taylor, 2007). Even though the most effective methods to control food spoilage are achieved by chemical additives, their negative consumer perception and the more severe regulations on the use of fungicides (Wisniewski et al., 2016; Calvo et al., 2017) have increased an interest in new alternatives to protect food products by replacing synthetic agents with natural compounds (Parafati et al., 2015; Russo et al., 2017).

Natural antifungals can be obtained from different sources, including plants, animals and microorganisms (Table 1). Plant secondary metabolites are an important source of antifungal bioactive substances, and include essential oils, phenolic compounds, flavonoids and alkaloids among others (Ciocan and Băra, 2007). Among the natural antifungals of animal origin, chitin, chitosan and lactoferrin are reported to possess antifungal activity against a wide range of fungi (Perdones et al., 2012; Wang et al., 2013). Furthermore, lactic acid bacteria (LAB) produces a wide variety of products with antifungal activity, among them, proteinaceous compounds called bacteriocins have shown to inhibit the growth and development of fungi (Hondrodimou et al., 2011). Recently, the use of antagonistic yeasts has attracted more interest since their inhibitory

activity is not related with the production of toxic metabolites, which occurs with antibiotics that derive from bacteria, fungi, plants and animals (Vardanyan and Hruby, 2016).

The purpose of this review is to highlight the different sources of natural compounds of plant, animal and microbiological origins that can be used to control food spoilage caused by moulds and yeasts, and to explain their biological mode of action. Hence, this paper focuses on analysing the potential application of these natural compounds in food products to improve their shelf life.

2. Antifungals of plant origin

Plant antifungals are usually compounds that belong to their secondary metabolisms. Essential oils (EOs), phenolic compounds, glucosinolates, hexanal and hexanol, among others, are antifungals that derive from plants.

2.1 Essential oils

Essential oils (EOs) are highly complex mixtures of often hundreds of individual aroma compounds that are poorly soluble in water and have a pleasant odour and taste. Moreover, EOs have been recognised as GRAS (Generally Recognised as Safe) by the U.S. Food and Drug Administration (CFR, 2014).

Plant EOs have been used for many years in food and pharmaceutical products for their antifungal, antimycotic and pest control properties (Bajpai et al., 2012). In fact, their antifungal effectiveness has attracted the growing interest of researchers for being used as food preservatives (Vitoratos et al., 2013). The most widely employed EOs as natural food preservatives are cinnamon, clove, lemongrass, oregano, thyme, nutmeg and basil. Figure 1 shows the main compounds of these EOs.

2.2.1 Mode of action

Several hypotheses have been put forward to explain EOs' antifungal activity: i) direct effects on enzymes and intracellular functions modification due to the presence of OH groups that can form hydrogen bonds (Soylu et al., 2006; da Cruz-Cabral et al., 2013); ii) changes in the morphology of different species of moulds and yeasts as a result of the interactions with membrane enzymes, which diminish cell wall firmness and integrity (Soylu et al., 2006; da Cruz-Cabral et al., 2013); iii) accumulation of EO compounds in the cell membrane because of their molecular structure and the position of functional groups, which leads to cell membrane destabilisation and damage (Rao et al., 2010); and iv) variations in membrane permeability, granulation of the cytoplasm and cytoplasmic membrane disruption (Bennis et al., 2004; Tao et al., 2014).

2.1.2 Incorporation into food systems

The strong flavour of EOs makes their incorporation into food products at high doses difficult given the changes in their sensory profile (Perdones et al., 2012). Emulsions, nanoemulsions and edible coatings have exhibited several advantages, such as the encapsulation of functional lipophilic substances, which allow the preservation and lower the concentrations of antifungal agents, diminishing the impact on the sensory profile of food commodities while maintaining their effectiveness (Salvia-Trujillo et al., 2015). In these sense, emulsions incorporating EOs have been successfully applied in jam preservation and tomato plant. Clove and cinnamon leaf emulsions containing a final EO concentration of 0.34 and 0.39 mg/g, respectively, were incorporated into strawberry jams to prevent fungal spoilage (Ribes et al., 2016). The jam samples with the emulsions were inoculated with two moulds of the *Aspergillus*

genus and one mould of the *Penicillium* genus and were stored for 63 days at 4 °C and 25°C. No mould development was noticed at day 49 and 28 for samples stored at cold and ambient temperature, respectively. Soylu et al. (2010) sprayed tomato plants with oil-in-water (O/W) emulsions prepared with different EOs (oregano, lavender and rosemary) to control fungal development. The obtained results showed that oregano EO proved the most effective against *Botrytis cinerea* (77% of protection by using 75 mg/L of oregano EO; and 0% of protection in control samples) and no signs of phytotoxicity were found on the plants treated at the maximum concentration used.

Several studies have dealt with the use of coatings to extend the shelf life of fruit products that are susceptible to fungi contamination due to their composition (high water and fructose content). Fuji apples were coated by dipping them in solutions prepared by mixing apple puree and an alginate solution with EOs (Rojas-Graü et al., 2007). An alginate-apple puree coating that contained 1% and 1.5% of lemongrass, and 0.5% of oregano, inhibited fungi growth during a 21-day period. In the same context, fresh-cut melon was dipped into an alginate edible coating with EOs (Raybaudi-Massilia et al., 2008) in order to prevent fungi development. The incorporation of EOs into edible coatings reduced the natural fungi population of fresh-cut melon for 21 days, and cinnamon EO and eugenol displayed the most marked antifungal action (<3 log CFU/g). In another study, cinnamon leaf coatings with pectin have also been employed to prevent grape spoilage by *B. cinerea* (Melgarejo-Flores et al., 2013) revealing that samples treated with coatings, which contained 5 g/L of cinnamon leaf EO, presented 100% fungal decay. Recently, Arancibia et al. (2014) formulated biodegradable bilayer films with soya protein isolate, lignin and formaldehyde that contained citronellal and geraniol to control fungi growth in banana

samples during 6 days at 15°C. The incorporation of the films coatings with EO reduced fungi counts; especially at the end of the storage period, when mould and yeast counts were below 0.1 log CFU/g in treated bananas.

In addition to emulsions, nanoemulsions and edible coatings, active packaging by incorporating EOs is another possibility to reduce the impact of EOs on the sensory properties of foods and to extend the shelf life of food products. The use of active packaging systems with EOs has been employed in sliced bread, cheese and apples (Balaguer et al., 2013). In this context, cinnamon EO incorporated into paraffin as a bioactive coating has been tested against *Rhizopus stolonifer* in sliced bread, where 80% and 90% of inhibition was achieved with 4% and 6% of cinnamon leaf on the coating (Rodriguez et al., 2008). Similarly, Balaguer et al. (2013) evaluated gliadin films that contained 5% of cinnamaldehyde as active packaging of bread and cheese spread. The results revealed that the active packaging of bread slices was effective for delaying fungal growth. The antifungal assays with cheese spread were carried out at 4°C and the results showed no fungal growth for 30 days. Recently, apples inoculated with a mixture of twelve *Penicillium* spp. strains were treated in a chamber with oregano, cinnamon and clove EO. Apparently, 14% of the control apples did not present signs of infection compared with 39-42% of the samples treated with EOs, where oregano EO was still the most effective after 21 storage days, followed by clove and cinnamon EOs (Frankova et al., 2016).

2.2 Phenolic compounds

Phenolic compounds constitute the main class of plant secondary metabolites with more than 8,000 identified phenolic structures. They are present in fruits, legumes, vegetables and whole

grains (Pulido et al., 2000). Figure 2 shows a simplified classification of phenolic compounds that possess biological activity.

2.2.1 Mode of action

The mode of action of phenolic compounds is related to: i) membrane dysfunction and disruption, which leads to the dissipation of the pH gradient and electrical potential, interference with the ATP-system in the cell, inhibition of enzymes, inhibition of germination, suppression of mycelia development and germ tub elongation (El-Mogy and Alsanius, 2012; da Cruz-Cabral et al., 2013); and ii) the interaction with membrane proteins, whose conformation and functionality change, increasing concentrations of reactive oxygen species (da Rocha Neto et al., 2015).

2.2.2 Incorporation into food systems

Some researchers have reported the effectiveness of different phenolic compounds as protective natural preservatives for inhibiting spoilage fungi in multiple food systems. To this extend, phenolic compounds from edible herbaceous species have been used to prevent growth of *Monilinia laxa* on nectarines and apricots; *Penicillium digitatum* on oranges; and *B. cinerea* on table grapes. Brown rot due to *M. laxa* growth in apricots and nectarines was inhibited by using phenolic compounds after 6 days. Moreover, on post-treatment day 25, a reduction of 92% in *P. digitatum* growth was observed in oranges by the authors, and a reduction of 53% in *B. cinerea* growth was obtained after 6 days of treating table grapes (Gatto et al., 2011).

Effectiveness of esculetin, ferulic acid, quercetin, resveratrol, scopoletin, scoparone and umbelliferone to control *Penicillium expansum* on Golden Delicious and Granny Smith apples was evaluated by Sanzani et al. (2009). Quercetin and umbelliferone proved to be the most effective compounds, and independently of the application methodology (wound or dipping).

These phenolic compounds, either alone or combined, prevented Golden Delicious apples from decaying by 86-92% compared to the control samples by wound application. Differently, the combination of quercetin and umbelliferone by dipping, highlighted that only 33% of apples showed fungal decay after 8 days of treatment compared with 63% of the control samples (Sanzani et al., 2009). Furthermore, Quaglia et al. (2016) reported the effect of spraying pomegranates with phenolic compounds from olive-mill wastewater after inoculation with *Penicillium* sp. The treatment of pomegranate fruits with phenolic compounds at 4 mg/mL and 8 mg/mL lowered the percentage disease index of *Penicillium adametzioides*, and the disease index was 30% and 15%, respectively.

2.3 Glucosinolates

Glucosinolates are secondary metabolites present in plants in the *Brassicaceae*, *Capparaceae* and *Caricaceae* families. They are found in grains, roots, peduncles and leaves of plants, and their amounts depend on the vital part of plants and maturation stage (Brown et al., 2003). When plants are wounded, glucosinolates are released from vacuoles and hydrolysed by the enzyme myrosinase to produce isothiocyanates (Grubb and Abel, 2006), which are characterised by their high volatility. Numerous reports focused on the antifungal action of glucosinolates, and isothiocyanates have been found (Troncoso et al., 2005).

2.3.1 Mode of action

The hypotheses formulated to elucidate the mechanism of action of glucosinolates include: i) inhibition of oxygen uptake by fungi through the uncouple action of oxidative phosphorylation in the mitochondria of fungi by inhibiting coupling between electron transport and phosphorylation reactions, and thus hindering ATP synthesis (Kojima and Oawa, 1971); ii) formation of reactive

oxygen species which leads to an intolerable level of oxidative stress in fungal cells, and irreparable damage (Wang et al., 2010); iii) the non-specific and irreversible interaction of isothiocyanates with the sulfhydryl groups, disulphide bonds and amino groups of protein and amino acid residues (Kojima and Oawa, 1971; Banks et al., 1986); and iv) the reaction of some glucosinolates, such as butenyl-isothiocyanate, with some enzymes present at the plasma membrane level to produce fungal growth inhibition or cell death (Sikkema et al., 1995).

2.3.2 Incorporation into food systems

Treatments based on atmospheres enriched with glucosinolates have been demonstrated as a good alternative to control fungi spoilage in fruits and vegetables. Troncoso-Rojas et al. (2005) investigated the use of benzyl-isothiocyanate to control *Alternaria alternata* growth on tomatoes. The results indicated that this compound reduced fruit disease compared with the control samples.

Other works have suggested the use of different glucosinolates to control fungi growth on peppers, blueberries, apples and strawberries. Green bell peppers were exposed to isothiocyanates to investigate their antifungal potential against *A. alternata*. To this end, a sterile filter paper was soaked with the glucosinolates solution and placed inside the bag with the samples. The results showed that the effect of 0.56 mg/mL of isothiocyanate was determinant in reducing fungi rot (Troncoso et al., 2005). Furthermore, vaporisation with allyl-isothiocyanates on blueberries has proven effective against fungi by showing a 3% fungal decay after 21 days of treatment (Wang et al., 2010). Wu et al. (2011) treated apples with isothiocyanate vapours to control *B. cinerea* and *P. expansum* spoilage. The combination of different isothiocyanates reduced fungal growth incidence by up to 85%. Likewise, Ugolini et al. (2014) treated

strawberries infected naturally with *B. cinerea* by using an atmosphere enriched with pure allyl-isothiocyanate or one derived from defatted seed meals of *Brassica carinata*. This treatment significantly reduced the decay produced by *B. cinerea* by over 47% and up to 91% for 2 strawberry varieties.

2.4 Other compounds

Some natural constituents, such as hexanal, hexanol, 2-(E)-hexenal, trans-2-hexenal, and 2-nonanone, which are responsible for the aroma of some vegetables and fruits, protect damaged areas from fungi proliferation (Gardini et al., 2002). Their use also provides changes in the flavour and quality of food products owing to the presence of volatiles (Utama et al., 2002).

2.4.1 Mode of action

Even though the mechanism of action of some volatile compounds like antifungals is not clear, most authors agree that: i) the interaction of these compounds with protein sulfhydryl and amino groups causes severe damage to fungal membranes and cell walls, which results in the collapse and deterioration of hyphae (Andersen et al., 1994; Fallik et al., 1998); and ii) membrane disruption, which in turn produces leakage of electrolytes, reduces sugar and, amino acids from cells (Song et al., 2007).

2.4.2 Incorporation into food systems

The application of natural extracts, such as hexanal, trans-2-hexenal, and 2-nonanone, into active packages improves the release of bioactive compounds during storage and diminishes the development of undesirable flavours in foodstuffs (Soares et al., 2009).

Numerous works have reported the use of volatile compounds to prevent fungi development in fruits. Pears, apples, tomatoes, peaches, raspberries and strawberries are some fruits that have

been studied by different authors to test the effect of volatile compounds to control fungal growth. Neri et al. (2006a) evaluated trans-2-hexenal antifungal activity on pears and apples. According to the authors, efficacy ranged from 53.6% to 97.8% in pears and apples, respectively. In another study, Neri et al. (2006b) investigated the effectiveness of trans-2-hexanal against *P. expansum* in pears wounded with a conidial suspension. In this case, the most marked reduction in infection compared with the control samples took place in pears which were treated 24 h after pathogen inoculation (Effectiveness Index: 96.2%).

In the same way, hexanal fumigations have been used to prevent mould growth in apples, tomatoes, raspberries and peaches. Apple treatments with hexanal vapours to control fungi development and lesions were evaluated by Fan et al. (2006). Apples were exposed to different concentrations of hexanal vapours at several temperatures. At 4°C and with 5-7 µmol/L, only 52% of the apples developed lesions, whereas 98% of those treated with 8-12 µmol/L at 22°C exhibited lesions. Similarly, the incidence obtained from natural raspberry decay caused by *B. cinerea*, after exposure at different concentrations of hexanal vapours, was evaluated (Song et al., 2007). The results highlighted that the incidence of decay lowered by 30% for all the raspberry varieties. Furthermore, Utto et al. (2008) treated tomatoes with hexanal vapours to study their antifungal activity. The tomatoes treated with 200-270 µL/L exhibited 40% of fungal growth. Finally, the use of 2- nonanone to prevent *B. cinerea* growth on strawberries has been investigated by Almenar et al. (2007). Treated fruit presented neither visible injuries nor fungi development, whereas 10% of the control strawberries showed *B. cinerea* development.

3. Antifungals of animal origin

Antifungals from animal sources involve compounds that are isolated from animals or are animal-derived. Chitin, chitosan and lactoferrin are the most widely used antifungals that derive from animals.

3.1 Chitin and chitosan

Chitin is an abundant biopolymer found in the exoskeleton of arthropods and crustaceans, fungal cell walls, and other biological materials. Crustacean shells, like those from carbs and shrimps, are the most widely used chitin sources for commercial applications given the availability of waste from seafood processing industries (Hamed et al., 2016).

Chitosan is derived from chitin by deacetylation in alkaline media. It is a copolymer which consists in β-(1--4)-2-acetamido-D-glucose and β-(1--4)-2-amino-D-glucose units. Chitosan exhibits different biological properties, including antifungal, antibacterial and antiviral activities (Chirkov, 2002). Because of its wide biological spectrum, an increasing interest for applications of coatings for perishable foods has been observed in the last few years (Raafat et al., 2008).

3.1.1 Mode of action

Different mechanisms of action of chitosan have been reported in the literature: i) interferences with uptake of minerals, such as Ca^{2+} or other nutrients, that delay the spore germination process (Plascencia-Jatomea et al., 2003); ii) interaction on the spore wall that inhibits spore germination (Plascencia-Jatomea et al., 2003); iii) induction of cell leakage by stress (Zakrzewska et al., 2005); iv) membrane permeabilisation through specific interactions with high-affinity binding sites on the fungal surface (Zakrzewska et al., 2005; Park et al., 2008;

Galván Márquez et al., 2013); and v) interactions with DNA and/or RNA which, in turn, inhibit protein synthesis (Galván Márquez et al., 2013).

3.1.2 Incorporation into food systems

Chitosan has demonstrated its great antifungal activity in various food commodities. Chitosan has been approved by the FDA as a GRAS food additive (USFDA, 2013), and its application in the industry is safe for both consumers and the environment (Romanazzi et al., 2017). Table 2 summarises the relevant antifungal effect of chitosan applications on different plants, seeds and food commodities.

Chitosan has been used to prevent grey mould provoked by *B. cinerea* in cucumber plants (Ben-Shalom et al., 2003) by spraying solutions of chitosan or a chitin oligomers mixture. Treatment proves effective as it lowers the disease index compared with the control plants. Moreover, the antifungal effectiveness of chitosan on the development of *Fusarium oxysporum* f. sp. *albedinis (Foa)*, the agent responsible for Bayoud disorder, in date palm roots was elucidated (El Hassni et al., 2004). In this case, when seedling roots of date palm were treated with the chitosan solution and inoculated with the mould, the seedling mortality lowered.

Rodríguez et al. (2007) investigated the efficacy of chitosan and hydrolysed chitosan to induce enzymatic activities against *Pyricularia grisea* when applied to rice seeds (*Oryza sativa L.*), and also its results on leaf blast intensity in seedlings. For both chitosan types, the greatest disease defence in rice seedlings was obtained using 1,000 mg/L after 14 days of inoculation. Nevertheless, when applying 500 mg/L of chitosan and hydrolysed chitosan, severity was evidenced 14 days after treatment.

Several authors have highlight that grey mould on grapes is one of the most economically important disorders of grapevine and table grapes worldwide (Reglinski et al., 2010; Vasconcelos de Oliveira et al., 2014). The antifungal activity of chitosan against *B. cinera* on Chardonnay grape leaves was studied by Reglinski et al. (2010). Chardonnay leaves treated with chitosan proved more resistant to infection. Moreover, the effectiveness of chitosan on postharvest fungus infection by *B. cinerea* and *P. expansum* on grapes was elucidated (Vasconcelos de Oliveira et al., 2014). Chitosan treatment reduced the number of infected plants compared with untreated samples.

Some studies have employed chitosan in combination with other biopolymers or antifungal substances used by the food industry as edible coatings or packaging materials to control fungal spoilage in different fruits. Vu et al. (2011) coated berries with modified-chitosan films that contained limonene and peppermint. Strawberries were sprayed, dried and stored for 14 days at 4°C. On day 8, 45% of the fruits displayed some decay when chitosan-limonene films were applied, whereas the percentage of decay lowered by up to 60% when chitosan alone was applied or chitosan-peppermint was employed.

Some authors have also coated strawberry fruits by dipping them into chitosan solutions. Different combinations of chitosan and calcium gluconate solutions were prepared by Hernández-Muñoz et al. (2008) to coat strawberries. The combination of chitosan and calcium gluconate at a ratio of 1:0.5 has inhibited fungal fruit decay during storage. Conversely, fungal decay was observed in samples coated with chitosan solution. The results showed that only 35% of strawberries were infected after 6 days of treatment when 1% of chitosan was employed. Similarly, strawberries were treated with film-forming dispersions of the chitosan-lemon EO.

The results showed that pure chitosan coatings reduced the percentage of samples that displayed visual mould growth compared with the control strawberries. These results confirmed that chitosan antifungal action improved when the lemon EO was incorporated (Perdones et al., 2012).

Sánchez-González et al. (2011) dipped grapes in film-forming dispersions prepared with chitosan, hydroxypropylmethylcellulose and the bergamot EO to prevent their spoilage. The development of moulds and yeast after 18 days were 0.25 log CFU/g and 0.1 log CFU/g for the control and coated samples with the chitosan and bergamot EO, respectively.

The effect of alginate, chitosan, and their combinations, on *Colletotrichum musae* growth has been studied in bananas (Maqbool et al., 2010) by simulating marketing conditions (5 days/25°C/60% RH). The authors suggested that the bananas coated with alginate would show no fungicidal activity. In fact, the fruits with alginate and chitosan delayed anthracnose for 28 days and food freshness was maintained. Chitosan has been also employed on dry fruit. Walnut kernels were immersed in a coating solution that contained chitosan and green tee extracts (Sabaghi et al., 2015). As a result, chitosan coatings reduced yeast and mould growth on samples. In some samples with green tea and chitosan (10 g/L), no yeast and mould growth was detected throughout the storage period.

3.2 Lactoferrin

Lactoferrin is an iron-binding glycoprotein present in milk and often employed as an antimicrobial agent in human medicine and food preservation. The protein is folded into two homologous globular lobes connected by a short α-helix peptide (Berlutti et al., 2011). Among

its protective effects, several authors have reported antifungal activity (Wei et al., 2008; González-Chávez et al., 2009).

3.2.1 Mode of action

The mechanism of action of lactoferrin appears to be complex. Some researchers have suggested that the antifungal mode of action of lactoferrin might be related to: i) membrane rupture and leakage of intracellular proteins and sugars, which inhibit fungal growth (Wang et al., 2013; González-Chávez et al., 2009); ii) reduced ATP production as a result of inhibited mitochondrial respiration (Wang et al., 2013); iii) oxidative injuries (Wang et al., 2013); and iv) Fe^{3+} chelation (Zarember et al., 2007).

3.2.2 Incorporation into food systems

Very few studies are available in the literature that have reported antifungal effects of lactoferrin on food products. In one, Wang et al. (2013) sprayed tomato plants with a solution prepared with different lactoferrin concentrations and Tween 80. Treatment lasted 24 h before *B. cinerea* inoculation took place. The index of the samples that displayed visual mould growth lowered as the lactoferrin dose increased. Therefore, it can be stated that lactoferrin solution was able to protect more than 50% of samples when 100 mg/L of lactoferrin solution was used.

4. Antifungals of microbiological origin

Biopreservation or use of microorganisms and/or their metabolites to prevent fungi spoilage and to extend the shelf life of foodstuffs has attracted growing interest in the scientific and industry areas owing to changes in consumer opinions and demand (Le Lay et al., 2016). For these reasons, alternative methodologies to control postharvest loss caused by fungi have been investigated. Among natural biological agents, attention has been paid to lactic acid bacteria

(LAB) and antagonistic yeasts as a result of their excellent effectiveness as antifungal agents (Leroy and De Vuyst, 2004; Gerez et al., 2013) (Figure 3).

4.1 LABs

LABs are Gram (+), non-sporulating, catalase-negative, acid-resistant and anaerobic aerotolerant microorganisms. Eleven genera have been associated with food products: *Carnobacterium, Enterococcus, Lactococcus, Lactobacillus*, *Lactosphaera, Leuconostoc, Oenococcus, Pediococcus, Streptococcus, Vagococcus* and *Weissella* (Vries et al., 2006).

LABs have obtained the GRAS and Qualified Presumption of Safety (QPS) status by the FDA and EU, respectively. They are used in the food industry for their capacity to control fungi spoilage and/or pathogen microorganisms by producing different antimicrobial compounds (Martinez et al., 2013). The main identified antifungal metabolites include organic acids, proteinaceous compounds and fatty acids: i) the organic acids present in food commodities are additives or carbohydrate end-products produced by LABs, which include acetic, lactic and propionic acids as the main compounds originated by carbohydrate LABs fermentation (Ross et al., 2002); ii) bacteriocins, are a kind of ribosomal synthesised antifungal peptides or proteins (Nes et al., 1996). Various studies describe the production of these compounds originated by *Lactococcus, Streptococcus, Lactobacillus* and *Pediococcus* (Crowley et al., 2013); and iii) fatty acids perform an important function in antifungal activity and require at least one hydroxyl group and one double bond along the carbon backbone (Crowley et al., 2013).

4.1.1 Mode of action

The mode of action of LABs could be related to their stationary phase. There is a possibility that cell lyses could contribute to fungal toxicity. Likewise, other mechanisms that could explain

the antifungal effect of LABs include their competition for nutrients, space and exclusion of pathogens from entry sites in the matrix, and alteration of spore membrane, viscosity and permeability (Pawlowska et al., 2012).

Regarding the mechanism of action of LAB products, some authors have put forward the following hypothesis: i) organic acids defuse through the membrane and cause its dissociation by releasing hydrogen ions which, in turn, cause pH to drop. Organic acids also increase membrane permeability and neutralise the electrochemical proton gradient (Oliveira et al., 2014); ii) bacteriocins provoke fungal cell membrane disruption, changes in their permeability to small monovalent cations (K^+) and a large macromolecule like ATP (Sharma and Srivastava, 2014); and iii) fatty acids contribute to high membrane fluidity due to a low sterol content and a high degree of phospholipid fatty acid instauration (Benyagoub et al., 1996).

4.1.2 Incorporation into food systems

Utilisation of LABs to prevent fungi spoilage has been studied in different foodstuffs, which include fruits and vegetables, bakery and dairy products (Table 3).

4.1.2.1 Fruits and vegetables

To prevent fungal spoilage on fruits and vegetables, LABs have been employed as promising antifungal agents. Sathe et al. (2007) evaluated the antifungal activity of *Lactobacillus plantarum* CUK501 to prevent fungal spoilage of cucumber provoked by the inoculation of *Aspergillus flavus, Fusarium graminearum, R. stolonifer* and *B. cinerea*. This treatment effectively avoided the lesions provoked by all the evaluated moulds. In another study, apples were used as models to investigate the antifungal action of *Pediococcus pentosaceus* R47 against *P. expansum* (Rouse et al., 2008). The authors pointed out that no mould growth was detected

during the 14-day study. Similarly, Crowley et al. (2012) studied the antifungal effect of *Lb. plantarum* 16 and 62 in orange juice against *Rhodotorula mucilaginosa* and *P. expansum*. The authors highlighted that *R. mucilaginosa* counts were under 10^1 CFU/mL from days 8 to 25 and 8 to 14 for *Lb. plantarum* 16 and 62, respectively.

Grapes have been also employed as a model to determine the antifungal potential of LABs (*Weissella cibaria* 861006 and *Weisella paramesenteroides* 860509) against *Penicillium oxalicum* (Lan et al., 2012). After 2 days, hyphal development was perceived on the surface of the control samples, but no fungal hyphae were detected on the surface of the grapes treated with *W. cibaria* 861006 until day 6 of treatment.

In order to investigate the antifungal bio-preservation potential of *Lb. plantarum* IMAU10014 and a shuffled mutant strain F3A3, kumquats were infected with *P. digitatum* by Wang et al. (2013). The results revealed mycelia and spores on the control samples 2 treatment days. However, no mould growth was observed on the kumquats sprayed with the F3A3 strain, which is a promising bio-preservative agent. Gosh et al. (2015) employed jackfruit to study the antifungal activity of LAB against *R. stolonifer*. To this end, harvested jackfruits were treated with *Lactococcus lactis* subsp. *lactis* followed by an application of fungal spores. Only 4-27% rot was observed on jackfruits after 15 days of treatment with the LAB.

4.1.2.2 Bakery products

Bakery products are a problem for the food industry since fungal development provokes high economic loss and health costs (Garofalo et al., 2012; Crowley et al., 2013). For this reason, Gerez et al. (2009) evaluated the ability of LABs to inhibit *Aspergillus*, *Fusarium*, and *Penicillium* in bread. In this case, LABs were used in the formulation of a mixed starter culture

with *Saccharomyces cerevisiae* in bread making. Loaves of bread were surface-sprayed with a conidial suspension of *Aspergillus niger*. When the LAB was used, fungal growth was delayed for 5 days compared to the control samples. A similar methodology was employed with slices of bread to determine the antifungal activity of *Lactobacillus amylovorus* strains. The capacity of both *Lb. amylovorus* strains to inhibit the environmental mould outgrowth in an industrial bakery was studied (Ryan et al., 2011). Therefore, *Lb. amylovorus* sourdough bread delayed the outgrowth of the environmental fungi. Certainly, the maximum shelf life was obtained for the *Lb. amylovorus* sourdough bread tested against the mould flora obtained in the bakery (Table 3). Furthermore, bread slices of sourdough fermented wheat germ bread with *Lb. plantarum* LB1 and *Lactobacillus rossiae* LB5 were nebulised with *Penicillium roqueforti* DPPMAF1 to study their antifungal activity (Rizello et al., 2011). After 21 days of inoculation, mycelial growth was visible in slices of sourdough-fermented wheat germ bread. The contamination score proposed by the authors suggested 10% contamination. Garofalo et al. (2012) investigated the antifungal effectiveness of *Lb. rossiae* LD108 and *Lactobacillus paralimentarius* PB127 against *Aspergillus japonicus* M1 on biologically acidified dough prepared with sourdoughs to make bread and panettone. The sourdoughs inoculated with *Lb. rossiae* LD 108 were able to prolong the shelf life of this product by around 30 days compared to baker's yeast bread (11 days). On the contrary, the sourdoughs inoculated with *Lb. paralimentarius* PB127 prolonged shelf life by only 19 days compared with baker's yeast bread which prolonged the shelf life of the sample 39 days. The results revealed that the antifungal activity of *Lb. rossiae* LD108 sourdough allowed fungal growth to be inhibited for over 4 months in panettone cakes contaminated with *A. japonicus* M1 spores.

The antifungal activity of *Pediococcus pentosaceus* KTU05-9 used as a starter on wheat bread samples that contained sourdough, and the antifungal activity of *Pediococcus acidilactici* KTU05-7, *P. pentosaceus* KTU05-8 and KTU05-10, sprayed on the bread surface against moulds, was evaluated by Cizeikiene et al. (2013). Their results highlighted that the addition of sourdough prepared with *P. acidilactici* KTU05-7, *P. pentosaceus* KTU05-8 and KTU05-10 reduced fungal spoilage of bread better than the control samples. The *P. acidilactici* KTU05-7, *P. pentosaceus* KTUO5-8 and KTU05-10 single cell suspensions, sprayed on bread surfaces, inhibited the growth of fungi over an 8-day storage period, whereas the control bread exhibited visual fungi colonies. Recently, Axel et al. (2015) tested *Lb. amylovorus* DSM19280 antifungal activity as a starter culture in sourdough. Therefore, slices of bread were exposed to the bakery environment for 5 min on each side and were then packed in sterile bags. The addition of *Lb. amylovorus* DSM19280 prolonged the bread shelf life by 4 days compared to the control samples, where moulds were visible after 2 days.

4.1.2.3 Dairy products

Dairy products are also susceptible to fungal attack and LABs could be used to prevent fungi contamination in cheese and yoghurt products (Table 3). On this view, Schwenninger and Meile (2004) tested the antifungal activity of *Propionibacterium jensenii* SM11 and *Lactobacillus paracasei* subsp. *paracasei* strain SM20, SM29 or SM63 against yeasts, on yoghurt and cheese surfaces. Yoghurts prepared with *P. jensenii* SM11 and *Lb. paracasei* subsp. *paracasei* strains SM20, SM29 or SM63 were contaminated with *Candida pulcherrima*, *Candida magnoliae*, *Candida parapsilosis* and *Zygosaccharomyces bailii*. According to the authors, the samples treated with lactobacilli and propionibacteria at the 10^8 CFU/ mL concentration showed a

constant number of yeasts during the evaluation period at levels of 10^2 CFU/mL. Moreover, cheese samples, were permeated in a protective culture (*P. jensenii* SM11 and *Lb. paracasei* subsp. *paracasei* SM20, SM29 or SM63). Afterwards, samples were contaminated with *C. pulcherrima*, *C. magnoliae*, *C. parapsilosis* and *Z. bailii*. The cheeses formulated with *P. jensenii* SM11 and *Lb. paracasei* subsp. *paracasei* SM20 presented yeast development (4.11 log CFU/g on treatment day 21). Likewise, Delavenne et al. (2013), employed yoghurt formulated with *Lactobacillus harbinensis* K.V9.3.1Np, *Lactobacillus rhamnosus* K.C8.3.1I and *Lb. paracasei* K.C8.3.1Hc1 and *Lactobacillus zeae* K.V9.3.1Ng to evaluate their antifungal activity against *Debaryomyces hansenii, Rhodotorula mucilaginosa, Yarrowia lipolytica, Penicillium brevicompactum, Kluyveromyces lactis* and *Kluyveromyces marxianus.* Hence, the surface of yoghurts was contaminated by inoculating the target fungi. Only the *Lb. rhamnosus* K.C8.3.1I strain was able to inhibit *R. mucilaginosa* growth (<2 log CFU/g). Conversely, fungi strains were completely inhibited (<2 log CFU/g) in the samples formulated with *Lb. harbinensis* K.V9.3.1Np. Similarly, Li et al. (2013) studied the antifungal potential of *Lactobacillus casei* AST18 to control spoilage of yoghurts from *Penicillium* sp. In this case, no fungal development was noted in the samples maintained at 4°C, whereas the samples stored at 30°C exhibited mould development after 10 treatment days for the 2% and 4% AST18-added yoghurt samples. The samples that contain 6% and 8% of *Lb. casei* AST18 presented mould growth after 14 days. Indeed, 18 days after beginning treatment, all the samples presented mould development.

Cheong et al. (2014) tested the antifungal effect of *Lb. plantarum* isolates on cottage cheese against *Penicillium commune*. To this end, cheese samples were inoculated with LAB and incubated for 2 days at 24°C. Cottage cheese samples treated with *Lb. plantarum* isolates started

to show mould development 18 days after treatment, while the control samples displayed deterioration after 4 days. Another work focused on preventing the cheese spoilage produced by *P. expansum* FST 4.22, which was carried out by Lynch et al. (2014). Cheese was formulated using a starter mixed with *Lb. amylovorus* DSM 19280 as an adjunct culture. *Lb. amylovorus* DSM 19280 delayed fungi development compared to the control samples (from 8 to 12 days).

Finally, Aunsbjerg et al. (2015) investigated the antifungal action of *Lb. paracasei* DGCC 2132 against *Penicillium* sp. nov. DCS 1541 and *Penicillium solitum* DCS 302. For this purpose, yoghurt samples were inoculated with the target fungi. The results revealed the antifungal properties of LAB against *Penicillium* sp. nov. DCS 1541 and *P. solitum* DCS 302 compared with the control samples after 4 days of treatment at 25°C.

4.2 Antagonistic yeasts

The use of antagonistic yeasts as biopreservative microorganisms has been studied in depth because they possess some important features that increase their suitability as biocontrol agents. Many have simple nutritional requirements and are able to colonise dry surfaces and to grow on inexpensive substrates in bioreactors (Chanchaichaovivat et al., 2007). Yeast present on fruit surfaces represents the main yeast group used to manage postharvest diseases (Liu et al., 2013).

4.2.1 Mode of action

Numerous authors have suggested different modes of action of antagonistic yeasts against fungi, which seem to be related to: i) antifungal hydrolases (El Ghaouth et al., 2003); ii) production of pigments, which causes iron depletion in the cell environment (Sipiczki et al., 2006); iii) induction of some defence-related proteins attributed to the metabolism of proteins, defence response, transcription, energy metabolism and cell structure (Chan et al., 2007); iv)

presence of enzymes associated with sugar metabolism (Chan et al., 2007); v) production of volatile organic compounds (Parafati et al., 2015); vi) tolerance to reactive oxygen species (ROS) (Liu et al., 2012); vii) induction of ROS production in host (Marcarisin et al., 2010); and viii) biofilm formation (Parafati et al., 2015).

4.2.2 Incorporation into food systems

Utilisation of antagonistic yeasts as biocontrol agents has been studied by different authors in apples, grapes, peaches, pears, and strawberries. Table 4 presents the *in vivo* applications of antagonistic yeasts.

El Ghaouth et al. (2003) determined the prevention of *B. cinerea* disease on apples treated with *Candida saitoana*. No effect on lesion development was noted in the samples treated with the antagonistic yeast 1 day before *B. cinerea* contamination. Conversely, lesion development effectively reduced on the samples to which *C. saitoana* was applied 2 and 3 days prior to mould contamination. In addition, the efficacy of *Candida guillermondii* and *S. cerevisiae* M25 on preserving apples from *P. expansum* was evaluated by Scherm et al. (2003). The lesion diameter was lower than 55% for the *C. guillermondii*-treated samples, but was under 30% for the *S. cerevisiae* M25-treated apples after 7 days of storage. However, all the control samples showed a lesion diameter of 100%.

Li et al. (2011) studied the antagonistic activity of *R. mucilaginosa* against *P. expansum* and *B. cinerea*, which cause grey and blue mould in apples. For this purpose, apples were wounded and pipetted with different yeast suspensions. The authors revealed that *R. mucilaginosa* inhibited *P. expansum* growth completely, while the disease incidence of the control samples was 97.2%. Indeed, *B. cinerea* decay and lesion diameter decreased by 97.1% and 56.1% in

comparison with control samples. Likewise, the efficacy of *Pichia guilliermondii* strain M8 against *B. cinerea* using apples as a model was investigated by Zhang et al. (2011). These authors determined that *P. guilliermondii* M8 was able to lower *B. cinerea* incidence from 45% to 20% compared to the control samples.

Grapes have also been employed as models to describe the antifungal potential of different antagonistic yeasts. *S. cerevisiae* and *Schizosaccharomyces pombe* strains were employed to prevent grey mould on grapes after harvesting (Nally et al., 2012). *S. cerevisiae* and *Sch. pombe* showed a reduction over 70%, whereas 100% of the control samples presented mould development. Calvo-Garrido et al. (2013a) investigated the effectiveness of *Candida sake* CPA-1 combined with Fungicover® against botrytis bunch decay in organic vineyards. The control samples had an incidence and severity of 90% and 22%, respectively. Similarly, the sour rot of grapes to be controlled by biological agents was described by Calvo-Garrido et al. (2013b). The combination of *C. sake* CPA-1 with Fungicover®, *Ulocladium oudemansii*, and chitosan was applied to organic vineyards, which reduced the severity of sour rot from 47% to 70%, compared with the control samples.

Lutz et al. (2013) tested *Cryptoccocus albidus* NPCC 1248, *Pichia membranifaciens* NPCC 1250, *Cryptoccocus victoriae* NPCC 1263 and NPCC 1259 to prevent *P. expansum* and *B. cinerea* growth on pears. The results showed that *C. albidus* NPCC 1248, *P. membranifaciens* NPCC 1250 and *C. victoriae* NPCC 1263 were able to reduce *P. expansum* disease and lesion diameter was ≥ 50%, but was ≥ 30% for *B. cinerea*. The control samples had a disease incidence of 100% for both mould strains, while lesion diameter was 35% and 80% for *P. expansum* and *B. cinerea*, respectively.

Peaches are easily contaminated by *R. stolonifer* in some countries like China, where it is one of the most relevant postharvest problems. For this reason, Xu et al. (2013) studied the effectiveness of *Pichia caribbica* (JSU-1) against *R. stolonifer* on peaches. The highest concentration of the antagonistic yeast employed in tests, showed the lower incidence (4%) and lesion diameter (26 mm) compared with the control samples (100% and 58 mm, respectively). Recently, the influence of strawberry preharvest spraying with *Cryptococcus laurentii* on the postharvest decay of fruits was tested against *B. cinerea* by Wei et al. (2014). The disease incidence of grey mould decay was higher than 70% at 4°C and 20°C in the control samples. On the contrary, the application on *C. laurentii* at 6, 3 and 0 days prior to harvesting gave the best results, and disease incidence was lower than 22% at both temperatures.

Even though a wide variety of yeasts has been reported to be good postharvest biocontrol agents, very few yeasts that are considered to act as biocontrol products are available on the market (Shemer^{TM}, Candifruit^{TM} and Boni-Protect^{TM}). Shemer^{TM} (AgroGreen, Asgdod) is a fungicide based on *Metschnikovia fructicola*. It is registered for postharvest use in Israel, but not in Europe. Candifruit^{TM} (SIPCAM INAGRA, S.A., Valencia, Spain) is based on *C. sake*, which has been commercially available only in Spain from 2008 for pome fruits against postharvest pathogens (Mari et al., 2010). Boni-Protect^{TM} (Bio-ferm, Germany) contains two strains of *Aureobasidium pullulans*, isolated from untreated apple trees. Both strains act competitively against fungal pathogens, which is why the development of resistances is not possible. Since 2002, Boni-Protect^{TM} has been used in field trials and can be incorporated without any pre-harvest interval before harvest, or even between picking dates.

5. FUTURE TRENDS

Despite the promising potential of preventing fungal contamination of natural compounds such as EOs, phenolic compounds and glucosinolates among others, they all have present some important limitations when applied to food products given their impact on the final product's sensory profile (Perdones et al., 2012; Aloui and Khwaldia, 2016). The incorporation of these compounds into O/W emulsions, nanoemulsions, edible coatings and active packaging could overcome these drawbacks, but these techniques do not allow to completely mask the flavour of these antifungal agents. New studies now focus on studying the immobilisation of EOs on the surface of different materials. Immobilisation guarantees microbial action, while the natural agent's volatility is suppressed (Chen et al., 2009; Gharbi et al., 2015; Higueras et al., 2015). The immobilisation process has being used to develop antimicrobial materials that contain peptides and enzymes (Yala et al., 2011; Hanušová et al., 2013), but further research should centre on the practical applications of these innovative systems, particularly on testing the fungicidal effect on packaged foodstuffs during the completely storage period. Toxicity studies should also be carried out to confirm the safety of these materials before being employed in food commodities.

Recently, filters impregnated with silver nanoparticles have been used as antimicrobial agents, and have suggested that this technique can be further used by the food industry in a vast variety of liquid products thanks to its low cost and high efficiency (van Halem et al., 2009; van Erven Cabala et al., 2015; Fernández et al., 2016). These promising results offer new uses of antifungals such as EOs or glucosinolates immobilised on different materials and impregnated on biodegradable filters to preserve liquid food products.

The persistence of antifungal activity during storage periods is another problem to solve. The efficacy of emulsions, edible coatings, and active packaging materials that contain active compounds diminishes with time when applied to food systems (Sung et al., 2013; Nguyen Van Long et al., 2016). To this end, further studies that focus on developing new antifungal systems which can be restored against losses of active compounds are required.

Regarding the use of biocontrol agents, only some antagonistic yeasts are commercially available. Many present poor performance and inconsistency in commercial circumstances as knowledge about their mode of action is scarce (Spadaro and Droby, 2016; Romanazzi et al., 2016). Future trends should focus on understanding the interactions among antagonistic yeasts, fungi, fruits and the microenvironment in order to develop economical and great formulations and operation processes. Antagonistic yeasts should be incorporated before pathogenic fungi establish on fruits to thus avoid their infection as a result of preventing propagules of pathogenic fungi on the host superficies (Romanazzi et al., 2016). Epiphytic microflora studies should be also considered since microbial communities present on fruit surfaces could affect disease control through their interaction with host fruits, the pathogenic fungi, and biocontrol agents (Massart et al., 2015).

Interactions between plant or animal antifungals with the macromolecules present in food products and external factors should also be studied. This information will provide reliable data to producers and consumers about their advantages compared with chemical additives and fungicides.

In addition, despite the number of works conducted on the laboratory, very few have been done on a large-scale. Hence, further studies conducted in industrial trials and field or

greenhouse trials to test different antifungal emulsions, nanoemulsions, coatings and biocontrol agents are needed.

6. CONCLUSIONS

This review shows the main advantages of using different natural antifungals to preserve food products from fungi spoilage. In general, plant EOs, chitosan and biocontrol agents have attracted growing interest of researchers for being used as food preservatives given their antifungal, antimycotic and pest control properties. New strategies are being studied to overcome the drawbacks of using some natural antifungals, such as sensory modifications, which essential oils, phenolic compounds, glucosinolates or other volatile compounds cause in food products. The protection or encapsulation of these natural agents into oil-in-water emulsions and nanoemulsions, their incorporation into edible films and active packaging systems, and their combination with other natural compounds, such as chitosan or phenolic extracts, improve their antifungal properties while reducing their doses. In the same way, use of microorganisms and/or their metabolites to control fungi spoilage is a good alternative to chemical additives for a wide variety of food products.

Acknowledgements

Author S. Ribes is grateful to the Universitat Politècnica de València (UPV) for a FPI grant.

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Table 1. Depiction of representative natural antifungals of different sources: plant, animal and microorganisms.

NATURAL ANTIFUNGALS		
PLANT ORIGIN	Essential oils (EOs)	
	Phenolic compounds	Phenolic acids
		Flavonoids
	Glucosinolates	
	Other compounds	
ANIMAL ORIGIN	Chitin	
	Chitosan	
	Lactoferrin	
MICROBIOLOGICAL ORIGIN	Lactic acid bacteria (LAB)	
	Antagonistic yeasts	

Table 2. Relevant chitosan applications on plants, seeds and food products (Adapted from Kashyap et al.,2015).

Plan/harvest	**Organisms**	**Effect of chitosan application**	**References**
Cucumber plant	*Botrytis cinerea*	Control the grey mould caused by *Botrytis cinerea*.	(Ben-Shalom et al., 2003)
Roots and seedlings of date palm	*Fusarium oxysporum* f. sp. *albedinis (Foa)*	Reduction of the growth of *Foa* and production of morphological changes in *Foa* mycelium.	(El Hassni et al., 2004)
Seeds of rice	*Pyricularia grisea*	Defense response induction associated with the concentration and type of chitosan. Symptoms of resistance were observed.	(Rodríguez et al., 2007)
Strawberries	Fungi	The combination with calcium gluconate inhibited strawberries decay.	(Hernández-Muñoz et al., 2008)
Banana	*Colletotrichum musae*	Chitosan and alginate combinations inhibited *C. musae* growth.	(Maqbool et al., 2010)
Grapevine leaves	*Botrytis cinerea*	Suppression of mould development on detached grapevine leaves.	(Reglinski et al., 2010)
Grapes	Moulds and yeasts	The combination with hydroxypropylmethylcellulose and bergamot oil reduced mould and yeasts development on grapes.	(Sánchez-González et al., 2011)

Strawberries	Moulds	Chitosan films alone or combined with limonene and peppermint reduced fruit decay.	(Vu et al., 2011)
Strawberries	*Botrytis cinerea*	Reduction of the percentage of infected fruits and; chitosan coatings with lemon oil presented great anti-*Botrytis* action.	(Perdones et al., 2012)
Grape	*Botrytis cinerea and Penicillium expansum*	Fungal growth was delayed when chitosan was applied as a coating on table grapes artificially contaminated with fungi spores.	(Vasconcelos de Oliveira et al., 2014)
Walnut kernels	Moulds and yeasts	Reduction of mould and yeasts growth in chitosan coatings and inhibition of fungal growth in chitosan-green tea coatings.	(Sabaghi et al., 2015)

Table 3. LABs in fruit and vegetables, bakery products and dairy products (Adapted from Crowley et al., 2013).

Food products	**Antifungal LAB**	**Organism**	**Reference**
Fruits and vegetables			
Cucumber	*Lactobacillus plantarum* CUK501	*Aspergillus flavus, Fusarium graminearum, Rhizopus stolonifer, Botrytis cinerea* and *Sclerotinia minor*	(Sathe et al., 2007)
Apples	*Pediococcus pentosaceus* R47	*Penicillium expansum*	(Rouse et al., 2008)
Orange juice	*Lactobacillus plantarum* 16 and 62	*Rhodotorula mucilaginosa Penicillium expansum*	(Crowley et al., 2012)
Grapes	*Weissella cibaria* 860106 and *Weissella paramesenteroides* 860509	*Penicillium oxalicum*	(Lan et al., 2012)
Kumquats	*Lactobacillus plantarum* IMAU10014 and a shuffled mutant strain (F3A3)	*Penicillium digitatum*	(Wang et al., 2013)
Jackfruit	*Lactococcus lactis* subsp. *lactis*	*Rhizopus stolonifer*	(Ghosh et al., 2015)
Bakery products			
Bread	*Lactobacillus plantarum* CRL 778, *Lactobacillus reuteri* CRL 1100, *Lactobacillus brevis* CRL	*Aspergillus*, *Fusarium*, and *Penicillium* species	(Gerez et al., 2009)

	772 and CRL 796		
Bread	*Lactobacillus amylovorus* DSM 19280	*Fusarium culmorum* FST 4.05, *Aspergillus niger* FST4.21, *Penicillium expansum* FST 4.22, and *Penicillium roqueforti* FST 4.11	(Ryan et al., 2011)
Bread	*Lactobacillus plantarum* LB1 *Lactobacillus rossiae* LB5	*Penicillium roqueforti* DPPMAF1	(Rizzello et al., 2011)
Bread Panettone	*Lactobacillus rossiae* LD108, and *Lactobacillus paralimentarius* PB12	*Aspergillus japonicus*, *Eurotium repens* and *Penicillium roseopurpureum*	(Garofalo et al., 2012)
Bread	*Lactobacillus sakei* KTU05-6, *Pediococcus acidilactici* KTU05-7, *Pediococcus pentosaceus* KTU05-8, *Pediococcus pentosaceus* KTU05-9 and, *Pediococcus pentosaceus* KTU05-10	Moulds	(Cizeikiene et al., 2013)
Bread	*Lactobacillus amylovorus* DSM19280	Moulds	(Axel et al., 2015)
Dairy products			
Yoghurt and Cheese surface	*Propionibacterium jensenii* SM11, *Lactobacillus paracasei* subsp. *paracasei* SM20, *Lactobacillus paracasei* subsp. *paracasei* SM29, and *Lactobacillus paracasei* subsp.	*Candida pulcherrima*, *Candida magnoliae*, *Candida parapsilosis* and *Zygosaccharomyces bailii*	(Schwenninger & Meile, 2004)

	paracasei SM63		
Yoghurt	*Lactobacillus rhamnosus* K.C8.3.1I, *Lactobacillus paracasei* K.C8.3.1Hc1, *Lactobacillus zeae* K.V9.3.1Ng and *Lactobacillus harbinensis* K.V9.3.1Np	*Debaryomyces hansenii, Kluyveromyces lactis, Kluyveromyces marxianus, Penicillium brevicompactum, Rhodotorula mucilaginosa* and *Yarrowia lipolytica*	(Delavenne et al., 2013)
Yoghurt	*Lactobacillus casei* AST18	*Penicillium* sp.	(Li et al., 2013)
Cottage cheese	*Lactobacillus plantarum* isolates	*Penicillium commune*	(Cheong et al., 2014)
Cheddar cheese	*Lactobacillus amylovorus* DSM 19280	*Penicillium expansum* FST 4.22	(Lynch et al., 2014)
Yoghurt	*Lactobacillus paracasei* DGCC 2132	*Penicillium* sp. nov. DCS 1541 and, *Penicillium solitum* DCS 302	(Aunsbjerg et al., 2015)

Table 4. *In vivo* applications of antagonistic yeasts.

Food products	Antagonistic yeast	Organism	Reference
Apple	*Candida saitoana*	*Botrytis cinerea*	(El Ghaouth et al., 2003)
Apple	*Candida guillermondii Saccharomyces cerevisiae* M25	*Penicillium expansum*	(Scherm et al., 2003)
Apple	*Rhodotorula mucilaginosa*	*Penicillium expansum* and *Botrytis cinerea*	(Li et al., 2011)
Apple	*Pichia guilliermondii* M8	*Botrytis cinerea*	(Zhang et al., 2011)
Grapes	*Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*	*Botrytis cinerea*	(Nally et al., 2012)
Grapes	*Candida sake* CPA-1	*Botrytis cinerea*	(Calvo-Garrido et al., 2013a)
Grapes	*Candida sake* CPA-1 and *Ulocladium oudemansii*	*Botrytis cinerea*	(Calvo-Garrido et al., 2013b)
Pear	*Cryptoccocus albidus* NPCC 1248, *Pichia membranifaciens* NPCC 1250, *Cryptoccocus victoriae* NPCC 1263 and *Cryptoccocus victoriae* NPCC 1259	*Penicillium expansum* and *Botrytis cinerea*	(Lutz et al., 2013)
Peaches	*Pichia caribbica* JSU-1	*Rhizopus stolonifer*	(Xu et al., 2013)

Strawberries	*Cryptococcus laurentii* 2.3803	*Botrytis cinerea*	(Wei et al., 2014)

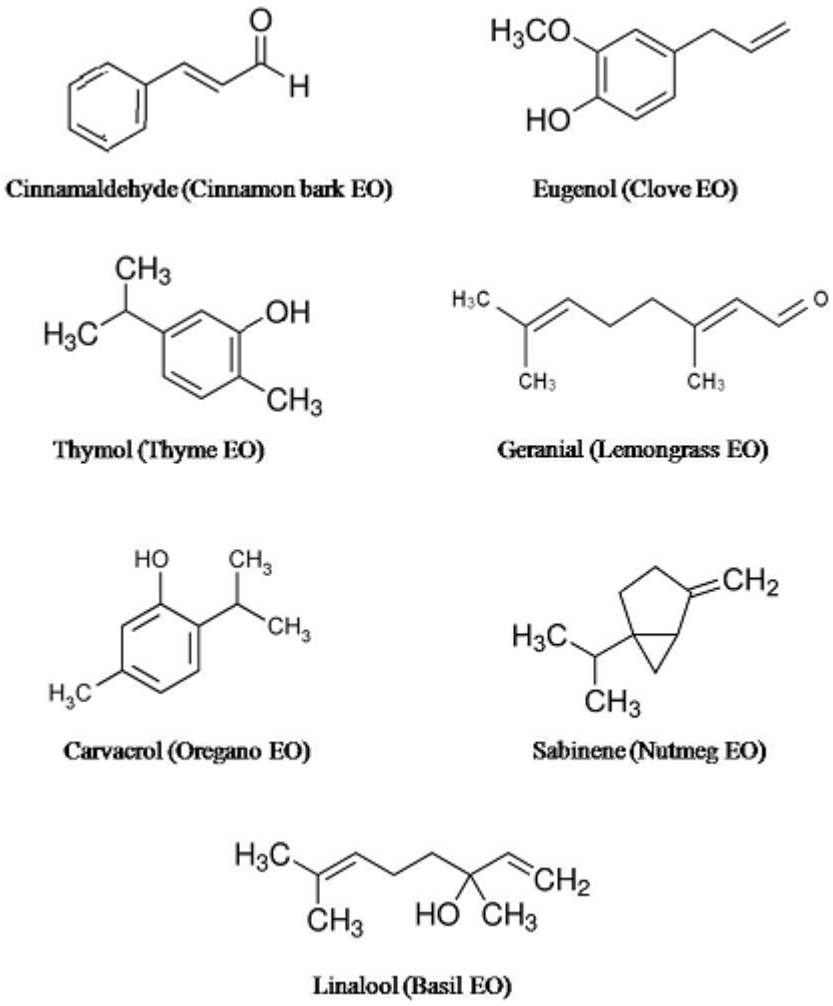

Figure 1. Main compounds of cinnamon, clove, lemongrass, oregano, thyme, nutmeg and basil EOs.

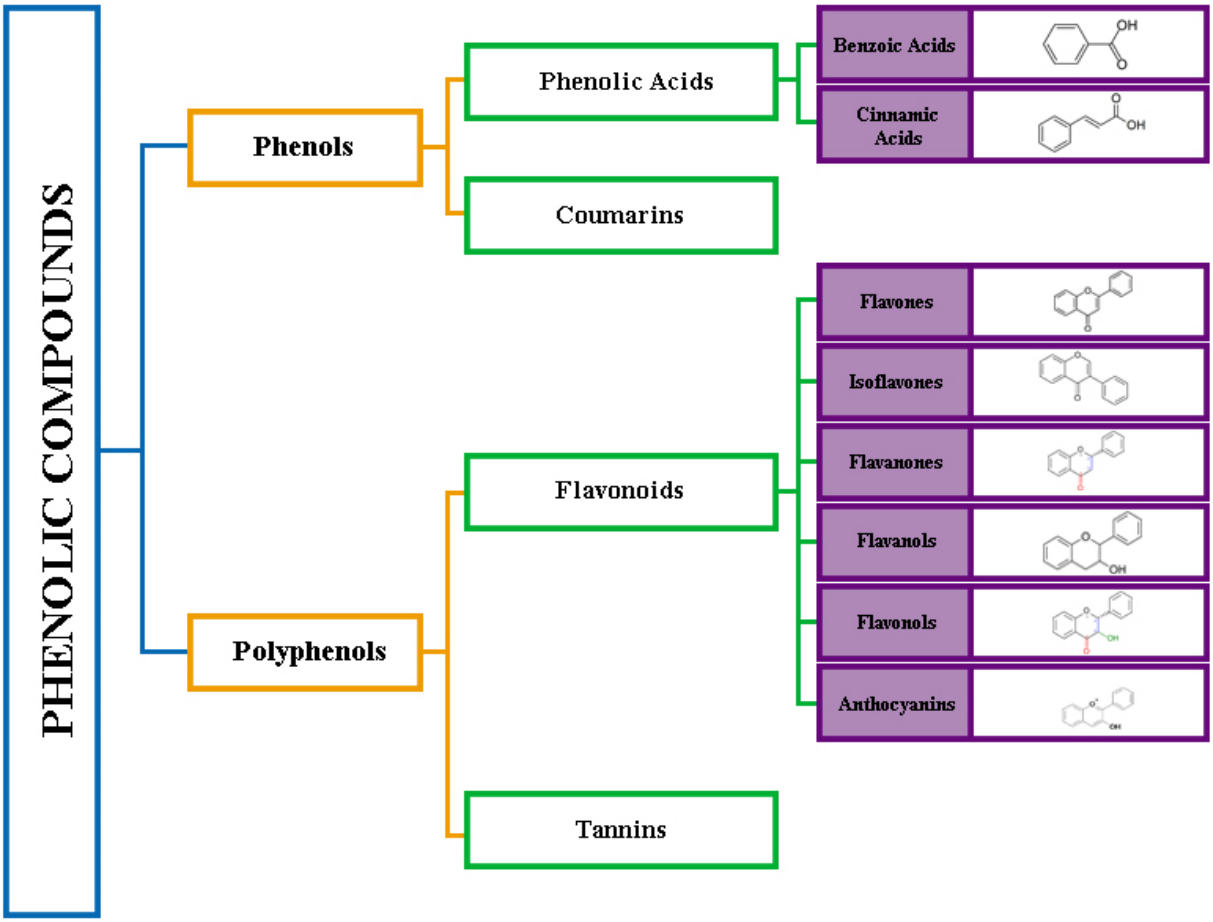

Figure 2. Simplified classification of phenolic compounds with biological activity (Adapted from Hurtado-Fernández et al., 2010).

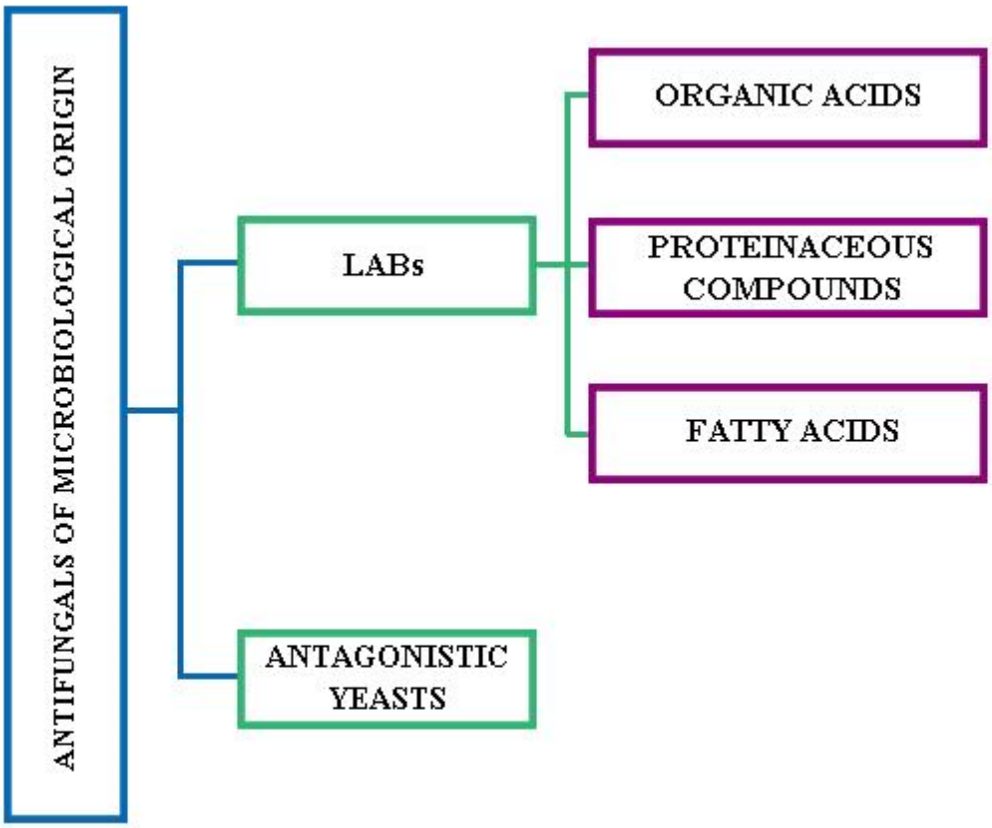

Figure 3. Classification of the antifungals of microbiological origin.