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Natural antimicrobial-coated supports as filter aids for the microbiological stabilisation of drinks

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ABSTRACT

The strategy of applying surface modifications to inert supports to improve their physicochemical, biological and functional properties can be adopted to develop novel filtering materials by modifying surfaces of widely used materials with biocompatible antimicrobial molecules. This approach would enable overcoming the limitations of conventional filtration methodologies in water treatment and the microbial stabilisation of drinks. The covalent immobilisation of naturally-occurring antimicrobial compounds on filter surfaces allows the antimicrobial effectiveness of the anchored molecule to be preserved by conferring the support material antimicrobial and antifouling properties. This review focuses on describing the naturally-occurring antimicrobials used in the food industry to either prevent growth or inhibit microorganisms, and defines the most established immobilisation methodologies by reviewing the different materials that can be used as grafting supports, and providing details of some examples of naturally-occurring antimicrobial compounds immobilised on filter aids applied to microbiologically stabilise beverages. Besides overviewing the most recent research, this work discusses the advantages, limitations and challenges of developing natural antimicrobial-coated filter aids, and it points out the innovation potential beyond current research on different supports and applications. Before such aids are applied to a real environment, more knowledge on safety and manufacturing is needed on the industrial scale. Therefore, the information in this review may be valuable to extend new emerging preservation technologies to control the food and drink microbial contamination that results in minimally processed products.

1. Introduction

The covalent immobilisation of antimicrobial molecules onto substrate surfaces is an approach that allows materials with additional functionalities to be developed, while maintaining bulk properties, which can be used in many technical fields (Treccani et al., 2013). In the food industry, antimicrobial-coated materials can be applied to different food processing stages because immobilised antimicrobials exert their biocidal properties after grafting and do not significant affect food properties because of their immobilisation (Ruiz-Rico et al., 2017). Indeed the use of natural antimicrobial-coated supports as filtering materials can potentially be applied as a food preservation technique (Cappannella et al., 2016; Peña-Gómez, Ruiz-Rico, Fernández-Segovia, & Barat, 2019; Zhang et al., 2021).

Filtration as a separation technique is applied to remove suspended particles from a liquid by passing them through a filter medium. Different filtration processes, including depth filtration and membrane filtration, have been used as beverage stabilisation techniques. Despite their removal properties, they involve clogging problems, require regeneration techniques, and their ability to eliminate certain pathogens is limited (Gibert et al., 2013). Therefore, enhancing the effectiveness of the common filters used in water and food treatments by developing antimicrobial-coated supports can address the challenges that current technologies face, and confer the capability to stabilise and eliminate microorganisms from drinks without affecting food properties.

This review focuses on describing naturally-occurring antimicrobials covalently immobilised on materials' surfaces to develop antimicrobial-coated supports to be used as filter aids to microbiologically stabilise beverages. A literature search in the databases Web of Science® and Scopus® was conducted with the following keywords: natural antimicrobial, immobilisation, coating, functionalisation, filtration, water, drink. Combinations of these keywords identified relevant studies in the field. Over a hundred articles were collected, but selection criteria were applied to focus this review on research on the immobilisation of natural biocidal molecules and the application of natural antimicrobial-coated supports in the drink industry.

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2. The naturally-occurring antimicrobial compounds to be immobilised

Natural antimicrobials are defined as biomolecules with inhibitory activity that are obtained from various sources in nature, including those of plant, animal and microbial origins (Lucera, Costa, Conte, & Del Nobile, 2012). Increasing attention has been paid to the use of natural antimicrobials for food biopreservation purposes thanks to their ample biocidal activity (Pisoschi et al., 2018). Details of their origin and mode of action are summarised in Fig. 1. Their direct application is limited by interaction with food components, instability and impact on food properties (Abdelhamid & El-Dougdoug, 2020). This is why their covalent immobilisation on supports has been proposed to overcome current limitations with their use in free forms, but it allows the resulting antimicrobial-coated materials to be applied in new technical fields. To better understand the potential immobilisation techniques that can be applied to the different natural antimicrobials, the next subsections describe their main properties.

2.1. Essential oils and other phenolic compounds

Essential oils and other aqueous extracts are antimicrobials of plant origin generated by plants as a protection strategy (Burt, 2004; Daglia, 2012). Although their mechanism of action is not altogether clear, it is related to their chemical structure, particularly to the presence of hydrophilic functional groups like hydroxyl groups and substituents on the aromatic ring, the number of double bonds and/or lipophilicity (Lucera et al., 2012). The interaction of these molecules with lipids of microbial membranes enhances their permeability, which causes cytoplasmic content to leak and leads to cell death. They can also interfere with membrane proteins by inducing their alteration, which affects electron transport, nutrient uptake, synthesis of proteins and nucleic acids, and enzyme activity (Pisoschi et al., 2018).

Their direct application to food can affect food properties, mainly sensory properties, and diminish efficacy due to food matrix factors, such as fat content, proteins, water activity, pH and enzymes (Lucera et al., 2012). They can also present environmental instability and low water solubility, which make their dispersion in food difficult (Burt, 2004; Vico et al., 2016).

2.2. Fatty acids

Medium-chain saturated and long-chain unsaturated fatty acids are powerful biocidal agents. Lipids inactivate microorganisms primarily by disrupting the bacterial cell membrane or inhibiting other intracellular targets like hindering DNA replication (Pisoschi et al., 2018).

The disadvantages of using free fatty acids are instability and interaction with food components, which can reduce their availability, along with their impact on food organoleptic and functional properties (Desbois & Smith, 2010).

2.3. Polysaccharides, proteins, peptides and enzymes

Chitosan is a biodegradable and non-toxic biopolymer with antimicrobial properties. Its mechanism of action is related to its polycationic structure, which acts as a water-binding agent, an enzyme inhibitor and a bioabsorbant of nutrients, and improves membrane permeability and hampers mRNA and protein synthesis (Pisoschi et al., 2018). Application to food is limited given its scarce solubility at neutral and alkaline pH values.

Lactoferrin is an iron-binding glycoprotein present in milk or other secretions (saliva or tears) with good antimicrobial activity. Lactoferrin binds two atoms of iron, which has been related to the antibacterial effect due to iron sequestration. Other mechanisms might also be involved, such as the blocking microbial metabolism of carbohydrates or destabilising the bacterial cell wall (Harouna et al., 2015).

Other antimicrobial proteins, like avidin and ovotransferrin, and peptides like pleurocidin, defensins, protamine, magainin, and casocidin, present a wide range of antibacterial activities (Quinto et al., 2019). The mechanism of action of these peptides is related mainly to their amphipathicity, which allows the electrostatic interaction with the microbial membrane to result in membrane permeabilisation and bacterial osmoregulation alteration (Li et al., 2021). Antimicrobial peptides have some interesting features such as biocompatibility, low toxicity, broad antimicrobial spectrum and selectivity towards specific multi-drug resistant species. However, they also present some limitations mostly limited efficacy *in vivo* due to reduced bioavailability and high production costs (Hazam, Goyal, & Ramakrishnan, 2019).

Lysozyme is a lytic enzyme found in foods (milk and eggs) or human

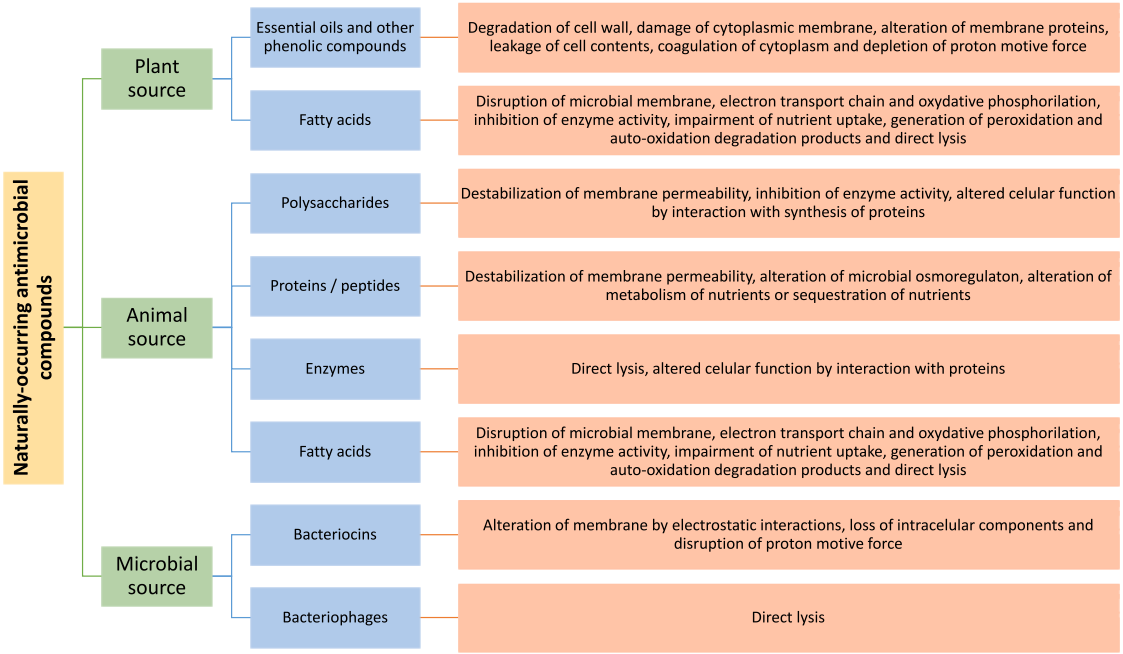

Fig. 1. Classification and mode of action of naturally-occurring antimicrobial compounds according to their source in nature.

secretions. It presents antimicrobial properties mainly against Gram-positive bacteria because their cell wall consists of peptidoglycans that are hydrolysable by this enzyme, which results in cell lysis (Pisoschi et al., 2018). Enzymes are sensitive to production and storage processes, and can interact undesirably with food components, which can decrease their antimicrobial efficacy (Lopes, Barreto Pinilla, & Brandelli, 2019).

2.4. Bacteriocins

Bacteriocins, such as nisin, natamycin or pediocin, are small bacterial peptides with excellent antimicrobial activity. Their antimicrobial activity is due to an alteration to membranes by electrostatic interactions with negatively charged membrane phospholipids, which results in both the loss of intracellular components and in proton motive force disruption (Pisoschi et al., 2018).

Their effectiveness is conditioned by external factors like pH, temperature, composition, structure and microbiota of food. Interaction with food components or microbial load present in food can result in precipitation, degradation or inactivation of bacteriocins (Gálvez, Abriouel, López, & Omar, 2007).

2.5. Bacteriophages

Lytic bacteriophages have been proposed as natural antimicrobials to control foodborne pathogens in food. These viruses only infect bacteria and can inhibit specific pathogenic bacteria. However, the ability of microorganisms to develop immunity to phages can create resistance by undermining their application (Quinto et al., 2019).

3. Natural antimicrobial-coated filtering materials to stabilise drinks

Surface coating strategies have been developed to immobilise bioactive compounds on materials that can be potentially applied to diverse industrial areas (Treccani et al., 2013). The aim of applying this approach is to modify the properties of bulk materials, and can be applied to common filters to confer them new properties. There are many examples in the literature of such that describe the immobilisation of silver or titanium dioxide nanoparticles, often used as water disinfectants (Hossain, Perales-Perez, Hwang, & Román, 2014), on filters applied to water treatment (Lalley et al., 2014). However, they are not natural antimicrobials and are not, therefore, the aim of this review. In contrast, some examples exist of natural antimicrobial-coated filters applied to microbiologically stabilise water or other beverages. So apart from describing and discussing these natural antimicrobial-coated filters, some examples of covalent immobilisation of natural antimicrobials on diverse supports with preserved biocidal properties are included in this review to provide a more complete picture of the enormous possibilities of covalent immobilisation of natural biomolecules that may be applied to develop filtering materials. This work also briefly discusses the main properties that support materials should have and the principal immobilisation techniques.

3.1. Properties of support materials used for immobilisation

Biomolecule immobilisation depends on the features and properties of the material used as a support. This support should meet some requirements to make it suitable for immobilisation: (i) relatively inexpensive and environmentally harmless; (ii) good thermal, chemical and mechanical stability; (iii) microbial attack resistance; (iv) inert to immobilised biomolecules; (v) a large surface area and a proper particle size; (vi) presence of enough functional groups for biomolecule grafting (Zucca & Sanjust, 2014).

Supports can be classified as organic and inorganic substrates according to their nature. Of organic materials, cellulose is considered to be non-toxic, cost-effective and biocompatible material (Zdarta, Meyer, Jesionowski, & Pinelo, 2018). Cellulose is a linear polymer of glucose units in which hydroxyl groups are responsible for its chemical activity. However, backbone hydroxyl groups are non-reactive for specific reactions and most cellulose supports need to be activated to make surface functional groups suitable for a subsequent bioconjugation reaction. Chitosan is a linear polysaccharide with several functional groups. The presence of many hydroxyl and amine groups in its structure facilitates the effective grafting of biomolecules without involving any modifying or cross-linking agents (Zdarta et al., 2018). Besides natural organic materials, synthetic polymers like polystyrene, polyethylene, polyamide or ethylene vinyl alcohol, among others, are also used as substrates for biomolecule immobilisation (Goddard & Hotchkiss, 2007).

Ceramic materials are suitable inorganic substrates for biomolecule immobilisation thanks to their excellent mechanical and chemical stability, large surface area, porous structure, controllable shape and size, and the functional moieties (hydroxyl, carbonyl and carboxyl groups) present on their surface (Zdarta et al., 2018). Silica is the most widely used inorganic material for biomolecule immobilisation given its surface hydrophilic character and the presence of many hydroxyl groups that come in the form of silanol groups (Zucca & Sanjust, 2014). Other mineral substrates can also be used for biomolecule immobilisation, including glass, sand, halloysite, kaolin, clays or bentonite (Zdarta et al., 2018).

Metallic materials are inorganic supports with excellent structural, physico-chemical, thermal and mechanical properties, biocompatibility, good durability and high conductivity (Su et al., 2018). Titanium, zinc oxide, alumina, magnetic iron oxide or stainless steel can be used for biomolecule immobilisation (Zdarta et al., 2018). Some of these inert metal surfaces need to be pre-activated to modify their surface prior to immobilisation.

3.2. Techniques used for immobilisation

Immobilisation consists of entrapping or anchoring a biomolecule to a support, preserving the bioactive properties of grafted molecules, conferring the support material new properties, and preventing them from continuous circulation in the environment. Immobilisation can be divided into reversible and irreversible techniques according to the interaction between the biomolecule and support. Reversible immobilisation examples include physical entrapment, adsorption or ionic binding, whereas covalent immobilisation is an irreversible methodology.

Covalent immobilisation offers remarkable advantages over reversible immobilisation techniques, including greater stability, longer shelf life, biomolecule metabolism prevention and biomolecule migration inhibition, all because the covalent bond between the functional groups present in the biomolecule and on the support surface does not allow the biomolecule to be released. This review focuses on the covalent immobilisation of natural antimicrobials and, therefore, the fundamentals of this technique are herein reviewed.

Covalent immobilisation is a multi-step process that generally requires the activation of the support's surface, followed by surface functionalisation to add functional reactive groups, and then the activation of reactive moieties and reaction with the biomolecule.

The support has to have functional groups on its surface because otherwise it may be necessary to modify its surface for immobilisation. Surface functionalisation involves introducing a functionalising agent into a surface to tailor the surface properties for a specific purpose. Different techniques, including wet chemical, silanisation and dry oxidation, are used to introduce reactive groups into material surfaces, which serve as an anchorage for immobilisation (Goddard & Hotchkiss, 2007).

Wet chemical modification consists of treating a material with liquid agents to create reactive functional groups on its surface (Goddard & Hotchkiss, 2007). This is an easy technique with excellent penetration capacity. However, it is an irregular functionalisation process, and is not

efficient and fast enough due to low reaction kinetics and multiple-step approaches, and it generates hazardous chemical waste (Nayak, Rahaman, & Giri, 2019).

Silanisation involves covering the support's surface with alkoxysilane moieties, which form a coating of covalently bonded silanes on the surface with an organic reactive functional group to enable them to immobilise biomolecules. Selecting the most appropriate organosilane must be done according to the chemistry of the biomolecule that needs to be immobilised. Organosilanes terminated with amine, thiol, isocyanate or carboxyl functional groups are often used in silanisation (Goddard & Hotchkiss, 2007).

Dry oxidation using ozone or reactive plasma provides a highly reactive chemical environment that can change the surface properties of inert materials. Ozone oxidation is an industrially cost-effective technique for generating a variety of oxygen-bearing polar groups (Nayak et al., 2019). Plasma can be used as a precursor to other surface modification techniques like silanisation or UV irradiation. Surfaces exposed to UV light can generate reactive sites for functional groups upon exposure to gas, or can be utilised to initiate UV-induced graft polymerisation (Goddard & Hotchkiss, 2007).

Activation is the final step prior to biomolecule immobilisation when the support's surface is functionalised. This step consists of generating both reactive groups on supports and the biomolecule compatible for the coupling reaction (Zucca & Sanjust, 2014). To convert one functional group into another, or to assist the bioconjugation process, different activation and cross-linking agents (i.e. glutaraldehyde, carbodiimides, etc.) can be used to gain the common functional groups employed in bioconjugation chemistry, such as thiols, aldehydes, carboxylic acids, hydroxyls and primary amines (Goddard & Hotchkiss, 2007).

3.3. Covalent immobilisation of naturally-occurring antimicrobial compounds

The covalent immobilisation of natural antimicrobials should result in antimicrobial-coated supports with a contact-active mode of action, and not in the release of a biocidal agent. The literature describes the covalent immobilisation of natural antimicrobials on different supports which, despite most not being applied as filtering materials, are included in this review as examples of possible strategies to develop new antimicrobial-coated filters for beverage microbial stabilisation.

The most important examples of the covalent immobilisation of natural antimicrobials reported in recent years are found in Table 1. It is highlighted that the mechanism of action of the developed antimicrobial supports is due to the immobilised biomolecule's inherent inhibitory properties after microbial cells have come into contact with the antimicrobial, which affects bacterial viability and metabolic activity (Lee & Je, 2013; Muriel-Galet, Talbert, Hernandez-Munoz, Gavara, & Goddard, 2013; Nogueira et al., 2017; Ribes et al., 2017; Yuan et al., 2013). Examples of the covalent immobilisation of natural antimicrobials and their biocidal mode of action are shown in Figs. 2 and 3. Nevertheless, the covalent grafting of these biomolecules on inert supports has distinct advantages over their free-form counterparts.

The irreversible immobilisation of natural antimicrobials can result in systems with enhanced antimicrobial activity. The use of phenolic compounds, such as vanillin or gallic acid immobilised on inorganic supports, has shown that the inhibitory effect of immobilised biomolecules is preserved, and even enhanced (Ruiz-Rico et al., 2017; Vico et al., 2016; Polo et al., 2018). Improved antimicrobial activity is probably due to the presence of high local antimicrobial concentrations on particles' surfaces and, therefore, in the cell's surroundings, which can easily disrupt cell membrane and induce cell death (Fig. 2C) (Ruiz-Rico, Moreno, & Barat, 2020; Vico et al., 2016).

Another advantage of the covalent immobilisation of natural antimicrobials is grafted biomolecules' increased stability. Liburdi, Benucci, Palumbo, and Esti (2016) reported the immobilisation of lysozyme on chitosan beads and showed a reduction in the lysozyme's cell lysis velocity and antimicrobial activity after immobilisation, probably due to diffusion limitation, steric hindrance and coupling via amino acid residues essential for catalytic activity. However, antimicrobial supports presented greater stability than the free lysozyme after being incorporated into wine because enzymatic activity was less affected by wine components. Tavakolian et al. (2018) developed lysozyme and nisin-coated nanocrystalline cellulose supports in which immobilisation reduced the possible spatial orientations of these proteins by restricting their interaction with the microbial membrane and, therefore, reducing inhibitory properties. In contrast, immobilisation allowed the antimicrobials' activity to continue longer. Besides longer stability, covalent grafting allowed resistant antimicrobial-coated supports for several cleaning conditions to be obtained (Duday et al., 2013; Nogueira et al., 2017).

One crucial drawback of some natural antimicrobials like essential oil components (EOCs) is their strong odour and flavour, which limit their use in the food industry. However, their covalent immobilisation on a support can mask the sensory impact of antimicrobials. Ruiz-Rico et al. (2017) and Ribes et al. (2017) evaluated the effect of incorporating EOCs-coated silica particles into different food matrices by sensory analyses. These authors confirmed that sensory perception significantly decreases when antimicrobials are covalently immobilised because covalent grafting avoids the release and volatilisation of biomolecules.

The biocompatibility and safety of natural antimicrobials can also be modified after their covalent immobilisation. Chen, Shi, Neoh, and Kang (2009) reported a lower cytotoxicity of carvacrol and eugenol after grafting them onto chitosan nanoparticles using fibroblast cells. In contrast Verdú et al. (2020) reported potential alterations of the toxicological parameters of gallic acid on the *in vivo Caenorhabditis elegans* system. Immobilised antimicrobial increased nematode mortality, affected locomotion behaviour and disturbed its response to thermal stress compared to the equivalent free gallic acid concentration. In this case, immobilisation was a disadvantage because it blocked gallic acid internalisation, which has a protective effect on nematode viability because of its antioxidant properties.

Some of the antimicrobial supports described in this section were designed to be applied as food additives (Lee & Je, 2013; Ribes et al., 2017; Ruiz-Rico et al., 2017), food processing aids (Liburdi et al., 2016), food packaging (Calce et al., 2014; Muriel-Galet et al., 2013) or food contact surfaces (Duday et al., 2013). Nevertheless, the immobilisation approaches described in Table 1 offer remarkable advantages and can be an inspiration to develop new antimicrobial-coated filters for the microbial stabilisation of liquid matrices.

3.4. Application of antimicrobial-coated supports as filter aids

The antimicrobial-coated filter aids described in this section (Table 2) are diverse, but share one common feature: the removal of microbial cells is achieved by combining cell retention on the support surface (partial or complete according to the particle or pore size of filters) and the immobilised molecule's biocidal activity, which confers filters: (i) antimicrobial activity based on contact killing and/or; (ii) antifouling activity based on adhesion resistance.

A first approach can be found in the literature: coated filters with antimicrobial activity. The simplest reported design is the adsorption a natural cationic protein of *Moringa oleifera* seeds onto sand as an antimicrobial flocculant for water clarification (Jerri et al., 2012; Samineni et al., 2019). Antimicrobial sand was able to capture bacteria and virus (7–8 log reduction values (LRVs)) by an electrostatic interaction between the cationic protein and the negatively charged bacteria or virus capsid proteins. These filters showed an extraordinary removal capacity, which can be applied to treat irrigation and drinking water. However, the antimicrobial is not covalently immobilised on the filter and will, therefore, be released to the treated matrix, which can limit its long-term use, safety and applicability.

Apart from adsorption, different filters with antimicrobial activity

Table 1
Examples of naturally-occurring antimicrobial compounds covalently immobilised on the surfaces of different support materials.

Immobilised molecule	Support	Immobilisation technique	Antimicrobial efficiency	Reference
Carvacrol and eugenol	Chitosan nanoparticles	Schiff base reaction via imine bonding between the amino groups of chitosan and the aldehyde group of previously modified eugenol and carvacrol.	Coated nanoparticles preserved the antimicrobial properties of the grafted antimicrobials and showed improved antibacterial efficacy after immobilisation compared to bare chitosan against *Escherichia coli* and *Staphylococcus aureus*.	Chen et al. (2009)
Carvacrol, eugenol, thymol and vanillin	Silica supports (MCM-41 microparticles, amorphous silica and fumed silica)	Schiff base reaction via imine bonding between the amino group of 3-aminopropyltriethoxysilane grafted on the surface of silica particles and the aldehyde group of pure vanillin or previously modified carvacrol, eugenol and thymol.	Antimicrobials preserved or even enhanced inhibitory properties after immobilisation according to *in vitro* and *in situ* assays against *Listeria innocua*, *E. coli*, *Aspergillus niger* and *Zygosaccharomyces bailii.*	Ruiz-Rico et al. (2017) Ribes et al. (2017)
Vanillin	Calcium phosphate microparticles and blocks	Phosphoramide bonding between the amino groups of vanillin previously derivatised with 1,5-pentanediamine and the phosphoric acid groups of the supports using carbodiimide compound as a coupling agent.	Preservation and enhancement of the antibacterial activity of vanillin against *E. coli*.	Polo et al. (2018)
Gallic acid	Chitosan particles	Radical-induced grafting reaction between hydroxyl radicals previously generated by an interaction between redox pair components (ascorbic acid/hydrogen peroxide) on the surface of chitosan particles and the aromatic ring of gallic acid.	Coated particles showed higher antimicrobial activity than unmodified chitosan against *S. aureus, Bacillus subtilis, Bacillus cereus, Enterococcus faecalis, Listeria monocytogenes, E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium* and *Shigella flexneri*.	Lee and Je (2013)
Gallic acid	Fumed silica particles	Immobilisation by (1) condensation of silanol groups with the phenolic hydroxyls of gallic acid, or (2) amide bonding between the amino groups of 3-aminopropyltriethoxysilane grafted on the surface of silica nanoparticles and the carboxyl group of gallic acid.	Both antimicrobial-coated particles showed higher antimicrobial activity than free gallic acid against *Paenibacillus larvae*.	Vico et al. (2016)
Gallic acid	MCM-41 silica microparticles	Amide bonding between the amino group of 3-aminopropyltriethoxysilane grafted on the surface of the silica particles and the carboxyl group of gallic acid.	Antimicrobial-coated particles showed enhanced inhibitory effect against *L. innocua*. The effect of free and immobilised GA on the *in vivo* system *Caenorhabditis elegans* revealing alterations of the toxicological parameters of the biomolecule.	Verdú et al. (2020)
Linoleic acid, oleic acid and palmitic acid	Pectin	Anhydrides of fatty acids were synthesised and then immobilised by acylation of the alcoholic functions of pectin with fatty acid anhydrides by grinding in the presence of the K_2CO_3 catalyst and ethanol with microwave irradiation.	Fatty acid-coated pectin partially inhibited *S. aureus* and *E. coli*. Coatings of pectin derivatives on polyethylene films were applied as an active packaging displaying oxygen scavenging function.	Calce et al. (2014)
Methyl undecenoic and methyl oleate	SBA-15 silica particles	Synthesis of silylated fatty acid by hydrometallation and introduction of a germanium moiety. Synthesis of functionalised mesoporous silicates by co-condensation of the silylated fatty acids with tetraethyl orthosilicate using pluronic P123 as a structure directing agent.	Fatty acid-functionalised silicates showed a moderate antimicrobial effect against *E. coli, Staphylococcus epidermidis, Proteus vulgaris, P. aeruginosa* and *S. aureus* in accordance with the fatty acid precursor nature and presence/absence of germanium.	Pędziwiatr-Werbicka et al. (2014)
Lysozyme	Ethylene vinyl alcohol (EVOH) films	Amide bonding between the carboxylate groups on EVOH film surface previously generated by UV irradiation and the amino groups of the lysozyme using carbodiimide as a coupling agent.	Immobilised enzyme generally preserved the antimicrobial properties against *L. monocytogenes.*	Muriel-Galet et al. (2013)
Chitosan and lysozyme	Stainless steel	Immobilisation in three steps: (1) generation of hydroxyl groups on stainless steel surface by piranha solution (H_2SO_4:H_2O_2, 3:1), and activation by biomimetic dopamine to generate active amino groups, (2) chitosan immobilisation using glutaraldehyde as a coupling agent, (3) lysozyme grafting to chitosan by carbamate bond using 1,1′-carbonyldimidiazole as a bifunctional linker.	Both antimicrobials preserved their inhibitory properties after immobilisation against *S. aureus* reducing bacterial adhesion and viability. Immobilised stability was evaluated resulting in zero lysozyme release and preserved antimicrobial properties.	Yuan et al. (2013)
Lysozyme	Chitosan beads	Lysozyme immobilisation using glutaraldehyde as crosslinker between the enzyme and the chitosan surface.	Kinetic characterization of lytic reaction and antimicrobial activity of free and immobilised lysozyme using model wine and white wine inoculated with *O. oeni* showed the reduction of cell lysis velocity and antimicrobial activity after immobilisation. However, the enzymatic activity was less affected by wine components when lysozyme was immobilised.	Liburdi et al. (2016)
Lysozyme and nisin	Nanocrystalline cellulose	Schiff base reaction via imine bonding between the amino groups of lysozyme or nisin and the aldehyde	Lower antimicrobial activity of immobilised compounds than free	Tavakolian et al. (2018)

(*continued on next page*)

Table 1 (*continued*)

Immobilised molecule	Support	Immobilisation technique	Antimicrobial efficiency	Reference
		groups of previously oxidized nanocrystalline cellulose.	compounds against *B. subtilis* and *S. aureus*. Antimicrobial-coated cellulose was able to partially inhibit biofilm formation and kill a previously formed biofilm. The antimicrobial activity of immobilised compounds was preserved over longer time than free molecules.	
Nisin	Multi-walled carbon nanotubes	Immobilisation in three steps: (1) activation of multi-walled carbon nanotubes by wet chemistry to add carboxyl acid groups, (2) grafting of poly (ethylene glycol) (PEG) via esterification reaction, and (3) linking of nisin to PEG-coated carbon nanotubes using hexamethylene diisocyanate as a coupling agent and dibutyltin dilaurate as a catalyst.	Immobilisation preserved the antimicrobial activity of nisin against *E. coli, P. aeruginosa, S. aureus* and *B. subtilis*. Nisin-coated supports showed strong anti-adhesion and anti-biofilm activity with excellent stability against leaching.	Qi et al. (2011)
Nisin	Stainless steel	Amide bonding between the carboxyl group of nisin, pre-activated by carbodiimide and succinimide , and the amine groups on stainless steel previously deposited with plasma using (3-aminopropyl)trimethoxysilane.	Immobilisation preserved the antimicrobial activity of nisin against *B. subtilis*, resulting in a surface with antibacterial and anti-adhesion properties resistant to several cleaning conditions.	Duday et al. (2013)
Bacteriophages	Glass disks	Immobilisation in three steps: (1) activation of glass disks by wet chemistry, (2) functionalisation with 3-aminopropyltriethoxysilane, and (3) immobilisation of bacteriophages by cross-linking to couple carboxyl groups in phage protein coat to amine groups on glass surface using carbodiimide and succinimide.	Immobilisation preserved the antimicrobial activity of bacteriophages against *E. coli* and *S. typhimurium*, according to bacteriophage size and shape and to the positioning of its specific binding proteins.	Hosseinidoust, Van De Ven, and Tufenkji (2011)
Bacteriophage	Polycaprolactone nanofibers	Amide bonding between the amino groups of the bacteriophage and the carboxylic acids of the nanofibers' surface previously activated in acidic solution.	Immobilisation preserved the antimicrobial activity of bacteriophage against *P. aeruginosa*. The immobilisation resulted in high stability after repeated washing cycles.	Nogueira et al. (2017)

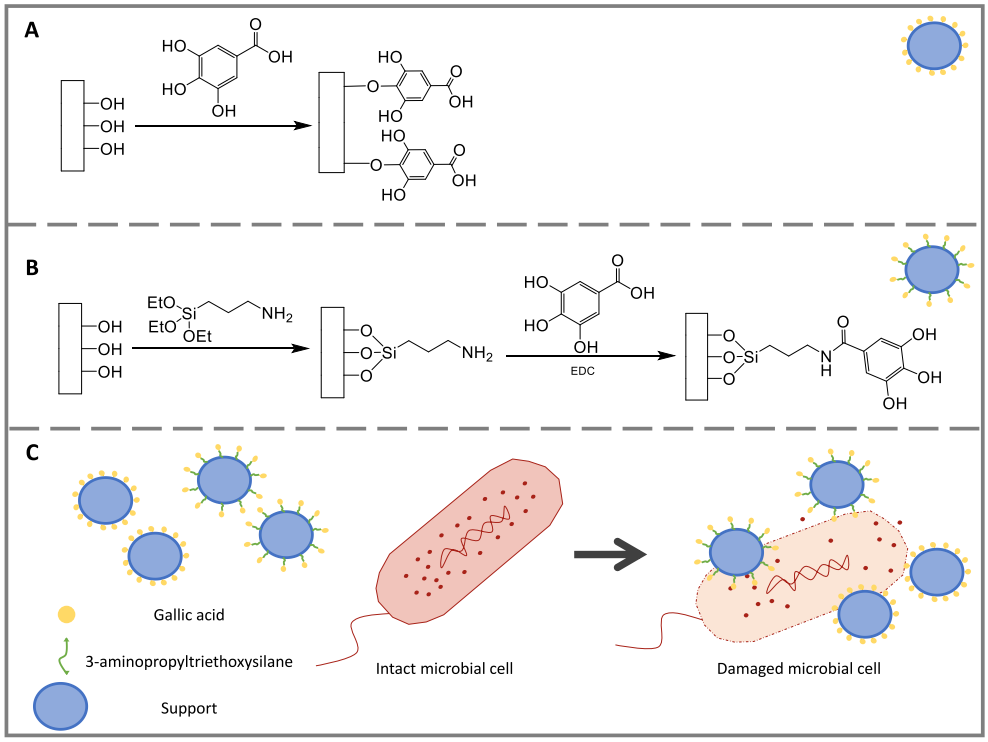

Fig. 2. (A, B) Schematic procedure of gallic acid immobilisation on fumed silica nanoparticles according to Vico et al. (2016). (C) Mode of inactivation of gallic acid-coated silica nanoparticles.

due to natural antimicrobials covalently immobilised on their surfaces have recently been reported (Cappannella et al., 2016; Kroll et al., 2012; Ribes et al., 2020; Wang et al., 2017; Zhang et al., 2021). The most important parameter to be evaluated with these antimicrobial-coated filters is their removal capability and, consequently, their mode of action. Microbial contamination elimination is based on: (i) the retention

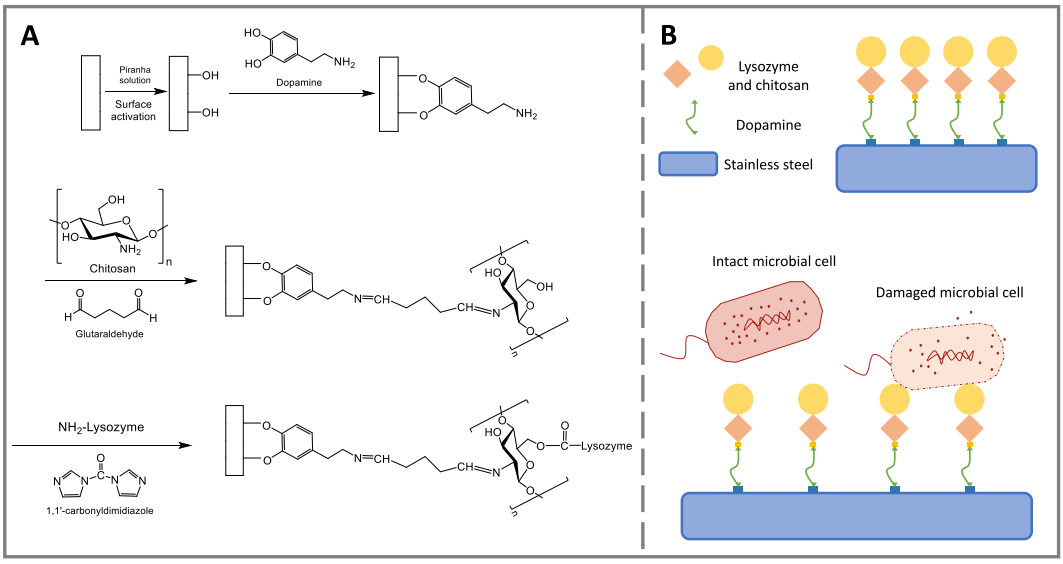

Fig. 3. (A) Schematic procedure of lysozyme and chitosan immobilisation on stainless steel surfaces according to Yuan et al. (2013). (B) Mode of inactivation of microbial contamination after coming into contact with immobilised antimicrobials.

or exclusion of microbial cells by the supports' structure; (ii) inactivation by the immobilised antimicrobial that comes into contact with microbial cells. Kroll et al. (2012) developed ceramic membranes covalently coated with lysozyme with good removal capacity thanks to the ceramic membrane's microtube structure (3 LRVs) and the immobilised enzyme's antimicrobial activity (Fig. 4). Peña-Gómez et al. (2019b) and Ribes et al. (2020) evaluated the mode of action of EOC-coated silica microparticles (Fig. 5) after filtering water inoculated with waterborne bacteria by different bacterial viability and metabolic activity techniques. They revealed the irreversible effect of immobilised antimicrobials on the filtered cells retained by physical adsorption on supports with 3–5 LRVs. Zang et al. (2021) reported the use of nisin-coated polyvinylidene difluoride microfiltration membranes to remove microbial contamination from apple juice. Nisin immobilisation conferred membranes antimicrobial activity with 5.8 LRVs which, according to microscopic analyses, resulted in simultaneous bacterial exclusion and inactivation.

After establishing filters' removal capability, relevant operating conditions should be evaluated to validate their applicability. The main limitation with this approach is filters' limited lifetime and coating stability during working operations. Some of the discussed studies have evaluated stability parameters under different conditions and reached ambiguous conclusions. The stability assays of the lysozyme-functionalised membranes reported by Kroll et al. (2012) showed that enzyme activity is affected by flow conditions and results in: (i) diminished removal at high flow rates because the contact time between the immobilised lysozyme and the bacterial cells in solution substantially shortens; (ii) immobilised molecule leaching because of the enhanced shear stress near the microtube surface. Peña-Gómez et al. (2019a, 2019b, 2020) determined the potential leaching of immobilised EOCs from silica supports after single filtration or pre-conditioning. According to the immobilised biomolecule, they found diverse wash-out effects, most of which were probably related to the immobilisation procedure rather to than the operating conditions. Therefore, a key parameter to be addressed in future works is to optimise the immobilisation procedure to ensure antimicrobial activity maintenance and zero wash-out in long-term uses. Zhang et al. (2021) assessed the stability of nisin-coated microfiltration membranes with different cleaning protocols (0.02 M HCl, deionised water, 0.001 M NaOH and 0.02% dodecyl sodium sulphate), which resulted in acceptable membrane stability. However, long-term use and successive cleaning cycles should be evaluated after establishing suitable cleaning/regeneration procedures.

The impact of the matrix composition on filters' removal capacity is another important parameter that determines shelf life, and this highlights the need to test the developed materials under conditions relevant to the final application (site-, condition- and matrix-specific). Cappannella et al. (2016) developed lysozyme-functionalised chitosan spherical supports to produce an effective fluidised bed system for wine microbial stabilisation. Covalent immobilisation significantly reduced lysozyme antimicrobial activity, probably because of the blockage of some active sites during coupling (Liburdi et al., 2016), but enzyme stability increased, which prolonged the useful life for industrial uses. The fluidised bed system was able to lyse the bacteria inoculated in wine more efficiently than the free lysozyme. This represents the notion that covalent immobilisation prevents enzyme inactivation in the presence of wine components. Other studied coated filtering materials with different liquid matrices (water, beer, apple juice) are silica microparticles covalently coated with EOCs for depth filtration (Peña-Gómez et al., 2019a, 2019b, 2020; Ribes et al., 2020). These coated silica filters presented a removal capability of 4–5 LRVs against different microorganisms naturally present or inoculated in diverse matrices, and their removal capability was maintained after filtering multiple samples and pre-conditioning (washing with water) (Peña-Gómez et al., 2019b, 2020). The removal capability of the developed supports on real drink matrices is equivalent to other preservation techniques, which confirms their potential use as an alternative methodology to microbiologically stabilise beverages.

However, the filtration impact on drink properties should also be evaluated to validate its applicability as a preservation technique. No reported study has assessed this effect, except that by Peña-Gómez et al. (2019a), who examined the filtration impact through EOC-functionalised filters on apple juice properties and shelf life. The use of these filters for juice cold pasteurisation allowed drink properties to remain or only had a slight effect if partial antimicrobial leaching occurred.

In addition to the considered parameters, one major current limitation of the developed materials is that no studies have worked under optimised process conditions to simulate real environments, which limits results being extrapolated to the drink industry. Besides, natural antimicrobial-coated filters should be tested with different liquid matrices and against several microorganisms (spoilage and pathogen microorganisms, vegetative cells, spores) to validate their efficacy.

As a second approach, natural antimicrobial-coated filter aids with both antifouling activity and antimicrobial activity can be found. Small

Table 2
Summary of recent examples of applications of natural antimicrobial-coated supports as filter aids in water and drink matrices.

Support	Immobilised molecule	Immobilisation technique	Matrix	Filtration removal efficiency	Reference
Sand	Natural cationic proteins of *Moringa oleifera* seeds	Adsorption	Water	Antimicrobial sand was able to capture *E. coli* and MS2 bacteriophage (pathogenic virus surrogate) by an electrostatic interaction between the cationic proteins and the negatively charged bacteria or the virus capsid proteins. The coated filter demonstrated 7–8 LRVs of bacteria and virus compared to <0.1 log removal for bare sand.	Jerri, Adolfsen, McCullough, Velegol, and Velegol (2012) Samineni et al. (2019)
Tubular zirconia microfiltration membranes	Lysozyme	Zirconia membranes were pre-treated with piranha solution to generate terminal hydroxyl groups. The surface was activated with (3-aminopropyl) triethoxysilane and lysozyme was covalently immobilised on the amino-modified membranes by previously activating carboxylic acid residues of the enzyme with carbodiimide.	Water	Lysozyme-coated microtubes preserved the antimicrobial activity of the immobilised lysozyme. An increase of the flow rate leads to a decrease of lysozyme activity, because the contact time between immobilised lysozyme and bacteria cells in solution was strongly reduced, and higher amount of washed-out lysozyme, because of enhanced shear stress near to the microtube surface.	Kroll et al. (2012)
Polysulfone reverse osmosis membranes	Lysozyme	The polysulfone membranes were modified with *m*-phenylenediamine and 1,3,5-benzenetricarbonyl-trichloride to form a polyamide layer on the membrane. The polyamide surface was modified with and 6-amino caproic acid by interfacial polymerisation. Lysozyme was covalently immobilised onto modified polyamide by an amine coupling reaction using carbodiimide and succinimide compounds.	Water	Lysozyme immobilisation resulted in reverse osmosis membranes with persistent antibacterial activity against *Micrococcus lysodeikticus* and *B. subtilis* that prevented the formation of bacteria biofilms, according to biofouling experiments. The antibacterial activity was stable after months of storage. The immobilisation of lysozyme onto the membranes resulted in a decrease in the water flux but maintained the salt rejection ratio.	Saeki et al. (2013)
Polyacrylonitrile membranes	Lysozyme	The membranes were modified by wet chemistry and ethylenediamine to incorporate amino moieties on the support's surface and lysozyme was covalently immobilised using glutaraldehyde as coupling agent.	Water	Lysozyme-coated membranes exhibited antibacterial performance against *S. aureus* by the inhibitory activity of immobilised lysozyme resulting in membranes with anti-fouling properties.	Liu et al. (2015)
Chitosan spherical supports	Lysozyme	Lysozyme was covalently grafted on the chitosan beads by using a coupling agent containing aldehyde groups that produced the cross linking between the aldehyde groups of the coupling agent with the amino groups of the chitosan surface and the amino groups of the lysozyme by Schiff base reaction.	White and red wine	The use of the coated supports in a fluidised bed system for wine microbial stabilisation allowed the continuous lysis of *O. oeni* inoculated in white and red wines in a more efficiently way than the free lysozyme and without leaching on the immobilised enzyme. Immobilisation increased the enzyme stability in batch, increasing the useful life for industrial use.	Cappannella et al. (2016)
Ordered mesoporous carbon composite	Lysozyme	The membranes were modified by wet chemistry to introduce carboxyl groups on the surface. Lysozyme was covalently coupled to the membranes by reaction between the amino group of lysozyme and the previously activated ester of the carbon composite by using carbodiimide as a coupling agent in presence of succinimide.	Water	Lysozyme-coated membranes showed good removal performance of *S. aureus*, due to the combination of preserved antibacterial activity of the immobilised antimicrobial and promoted adsorption of bacteria on the carbon membrane surface, thereby increasing contact with the antimicrobial. The operational stability efficiency persisted after reuse without leaching of lysozyme.	Wang et al. (2017)
Amorphous silica microparticles	Carvacrol, eugenol, thymol and vanillin	Covalent amine bonding between the amino group of 3-aminopropyltriethoxysilane previously grafted to the silica particles' surface and the aldehyde group of pure vanillin or previously modified carvacrol, eugenol and thymol. Schiff base reaction via imine bonding and reduction of the generated imine bond to yield a secondary amine increasing the stability of the covalent bond and preventing the leaching of the immobilised molecules.	Water, craft beer and apple juice	The use of coated silica microparticles in depth filtration resulted in 4–5 LRVs of *E. coli, Helicobacter pylori, Legionella pneumophila* or *P. aeruginosa* and native flora present in water, craft beer and juice. The removal capability was maintained after filtering multiple samples and pre-conditioning (washing with water). Viability and metabolic microbial activity were irreversibly affected by the treatment with filtering materials due to a	Peña-Gómez et al. (2019a) Peña-Gómez et al. (2019b) Peña-Gómez et al. (2020) Ribes et al. (2020)

(*continued on next page*)

Table 2 (*continued*)

Support	Immobilised molecule	Immobilisation technique	Matrix	Filtration removal efficiency	Reference
				combined process of physical adsorption on supports and inactivation after coming into contact with immobilised antimicrobials. Partial leaching of immobilised antimicrobials was found in some of the treated drinks.	
Polyamide reverse osmosis membrane	Arginine-Tryptophan antimicrobial peptides	Covalent grafting of antimicrobial peptides on membrane surfaces by incorporation of the photoreactive 3-(4-benzoylphenyl)alanine into the peptides sequence RWRWRWA-(Bpa), concentration of the peptide on the surface by filtration, and subsequent irradiation with UV light.	Water	The antimicrobial grafting endowed the membranes with antibacterial as well as antibiofilm activity against *P. aeruginosa*, while water permeability and salt rejection were only moderately affected.	Mao et al. (2018)
Polyvinylidene difluoride microfiltration membrane	Nisin	The membrane surface was modified with dopamine resulting in a polymerized coating. Nisin was covalently grafted to the polydopamine-coated membrane by Schiff base and Michael reactions.	Apple juice	Nisin immobilisation endowed the membrane with antimicrobial activity against *Alicyclobacillus acidoterrestris* resulting in simultaneous exclusion (by the membrane pore size) and inactivation (by contact with the immobilised antimicrobial) with LRVs of 5.8. Coating modified the hydrophilicity of the microfiltration membrane surface enhancing the water flux. The modified membrane exhibited reliable stability under different membrane cleaning procedures.	Zhang et al. (2021)

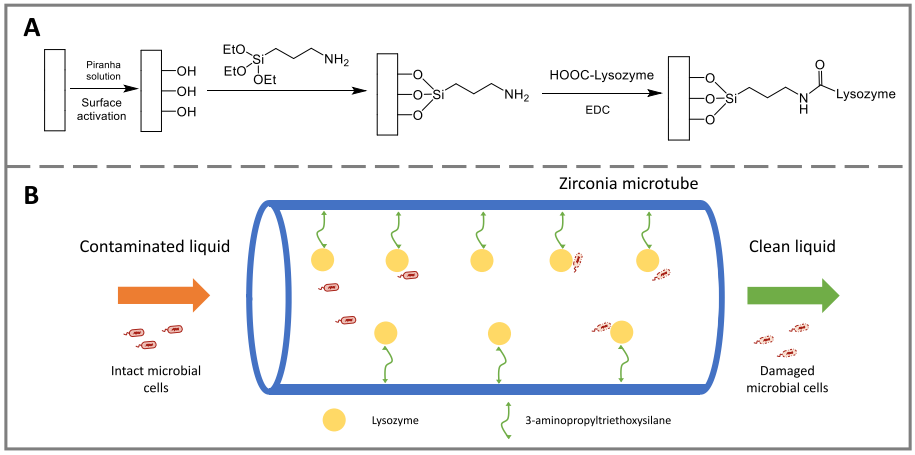

Fig. 4. (A) Schematic lysozyme immobilisation procedure applied to zirconia membranes according to Kroll et al. (2012). (B) Lysozyme-coated membranes were used in microfiltration to enhance microbial removal by inactivation after coming into contact with the immobilised antimicrobial.

pore-sized filters used, for instance in reverse osmosis, present fouling problems via undesired microbial growth through membrane pores during long filtration times. Therefore, the design of self-cleaning filters based on adhesion-resistant surfaces focuses on inhibiting or reducing microbial adhesion, which hinders biofilm formation because the initial microbial attachment is blocked. Examples of antimicrobial-coated membranes with these properties for crossflow filtration or reverse osmosis have been described by Saeki et al. (2013), Liu et al. (2015) and Mao, Mohanraj, Kandiyote, Kasher, and Arnusch (2018). As previously explained, the aim of the immobilisation of natural antimicrobials on such filters is to incorporate an antifouling agent to design self-cleaning filters. Therefore, the removal capability parameter is not often evaluated in these filters, while permeate flux and salt rejection are essential factors for reverse osmosis membranes. The antimicrobial-coating moderately affected these parameters, but enhanced their long-term use by avoiding biofilm formation (Mao et al., 2018; Saeki et al., 2013). These antifouling membranes could address the persistent challenge of biofilm blocking in water purification technology and may be applicable to other filtration systems to increase the efficiency and useful life of filters. However, performance under the operating conditions of a real environment, the possible interaction of matrix components in adhesion-resistance capacity and potential leaching should be evaluated before real applications.

The antimicrobial-coated filters described in this section correspond to a laboratory development stage, and provide very little insight into practical aspects, such as scaling-up production, long-term stability or performance under real operating conditions. Only after evaluating these parameters can realistic production cost estimations be made. Therefore, before being applied to a real scenario, it is necessary to perform additional semi-pilot and pilot scale studies closely related to final industry applications to eliminate contradictory results and to overcome current limitations. Last but not least, the safety of the developed materials should be particularly considered. The use of covalent immobilised antimicrobial-coated supports limits these materials' toxicity risk, but the impact of potential bond destabilisation leaching should be thoroughly evaluated. The immobilisation procedure

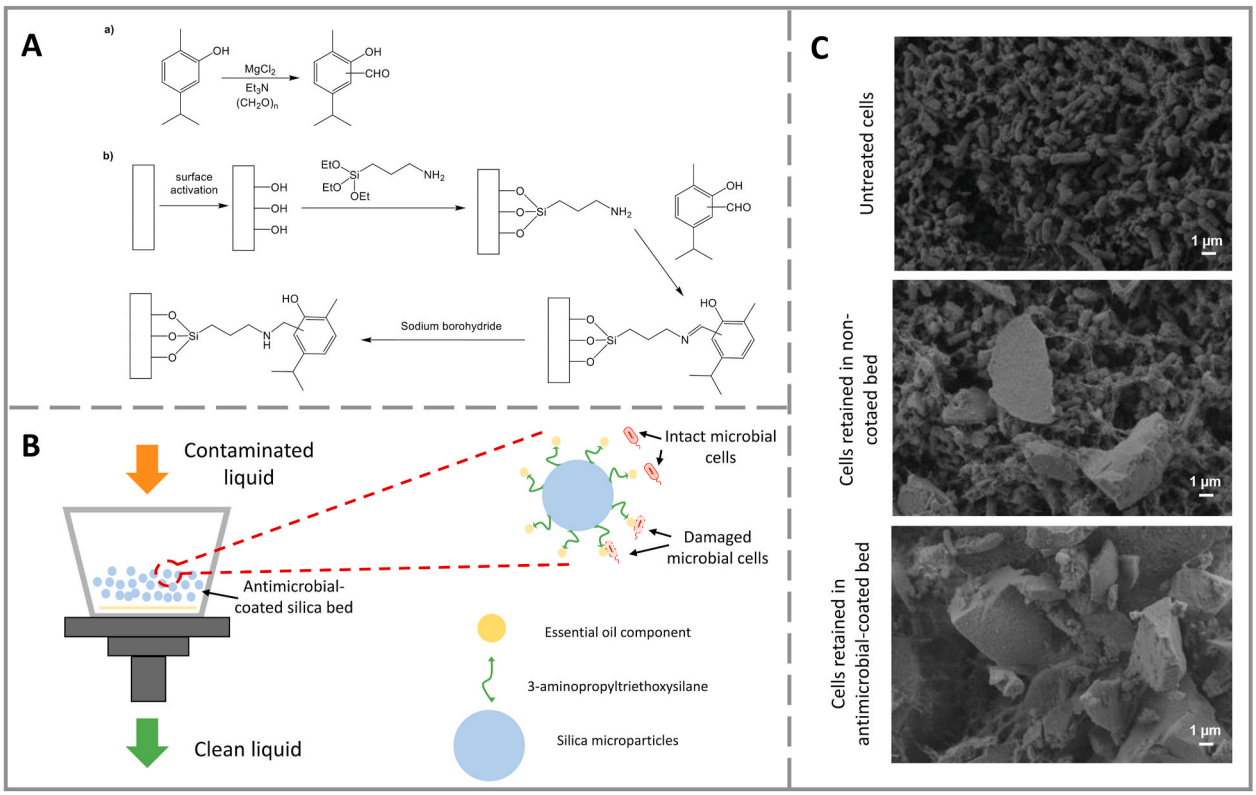

Fig. 5. (A) Schematic carvacrol immobilisation procedure applied to amorphous silica microparticles according to Peña-Gómez et al. (2019b). (B) Essential oil components-coated silica microparticles (5–50 µm) were used as filters in bed filtration to enable superior microbial removal by a combination of physical adsorption on supports and inactivation after coming into contact with immobilised antimicrobials. (C) SEM images of *Escherichia coli* after filtering through the bed of non-coated and antimicrobial-coated particles.

should be investigated in-depth for maximum effectiveness and zero leaching purposes. Besides, regulations that deal with the handling and discharge of these new materials should be established to estimate possible risks and to minimise environmental impacts.

4. Concluding remarks and future perspectives

Despite the effectiveness of today's preservation methodologies, huge efforts are being made to find suitable non-thermal methodologies capable of preserving food properties and delivering microbiological safe products. Of them all, the use of natural antimicrobials immobilised on supports as processing aids (filters) has the potential to stabilise and eliminate microorganisms from beverages.

By tailoring the filter surface with natural antimicrobials, a wide portfolio of supports can be developed to be applied to diverse liquid matrices. The covalent immobilisation of antimicrobials on filters allows bioactive properties to be maintained without releasing the compound to the environment and conferring filters antimicrobial and/or antifouling properties. Therefore, antimicrobial-coated filters can increase not only the existing technological efficiency of current filtration methodologies, but also the useful life of filters, which cut drink treatment costs. Although surface modification techniques are expensive, their advantages may surpass production costs if the design of efficient natural antimicrobial-coated filters with long-term stability is possible.

Although major knowledge gaps for the technical development and safety of natural antimicrobial-coated filters remain, their remarkable properties indicate that they can play a key role in drinks preservation in the future. They can be used as supports in different filtration techniques (standard filtration, microfiltration, nanofiltration, ultrafiltration, reverse osmosis) by modifying material properties like thickness, particle/pore size, geometry or dimensions, which extend potential applications for different matrices and in distinct areas of interest (food, biotechnology, chemistry, environmental remediation, etc.). The combination of more than one immobilised antimicrobial on the same surface also extends their application range, and this technique can be combined with other existing mild-thermal and non-thermal preservation methods to open up new pasteurisation and sterilisation possibilities. Finally, immobilisation techniques followed to develop coated filters can be applied to create antimicrobial-coated surfaces with inhibitory properties by contact or antifouling activity, which can be used in many fields (food, biomedical, textile, etc.).

Declaration of competing interest

The authors declare no conflict of interest.

CRediT authorship contribution statement

María Ruiz-Rico: Conceptualization, Writing – original draft, Writing – review & editing. **José M. Barat:** Funding acquisition, Supervision, Writing – review & editing.

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References

Abdelhamid, A. G., & El-Dougdoug, N. K. (2020). Controlling foodborne pathogens with natural antimicrobials by biological control and antivirulence strategies. *Heliyon, 6* (9), E05020. https://doi.org/10.1016/j.heliyon.2020.e05020

Burt, S. (2004). Essential oils: Their antibacterial properties and potential applications in foods—a review. *International Journal of Food Microbiology, 94*(3), 223–253. https://doi.org/10.1016/J.IJFOODMICRO.2004.03.022

Calce, E., Mignogna, E., Bugatti, V., Galdiero, M., Vittoria, V., & De Luca, S. (2014). Pectin functionalized with natural fatty acids as antimicrobial agent. *International Journal of Biological Macromolecules, 68*, 28–32. https://doi.org/10.1016/j.ijbiomac.2014.04.011

Cappannella, E., Benucci, I., Lombardelli, C., Liburdi, K., Bavaro, T., & Esti, M. (2016). Immobilized lysozyme for the continuous lysis of lactic bacteria in wine: Bench-scale fluidized-bed reactor study. *Food Chemistry, 210*, 49–55. https://doi.org/10.1016/J.FOODCHEM.2016.04.089

Chen, F., Shi, Z., Neoh, K. G., & Kang, E. T. (2009). Antioxidant and antibacterial activities of eugenol and carvacrol-grafted chitosan nanoparticles. *Biotechnology and Bioengineering, 104*(1), 30–39. https://doi.org/10.1002/bit.22363

Daglia, M. (2012). Polyphenols as antimicrobial agents. *Current Opinion in Biotechnology, 23*(2), 174–181. https://doi.org/10.1016/J.COPBIO.2011.08.007

Desbois, A. P., & Smith, V. J. (2010). Antibacterial free fatty acids: Activities, mechanisms of action and biotechnological potential. *Applied Microbiology and Biotechnology, 85*(6), 1629–1642. https://doi.org/10.1007/s00253-009-2355-3

Duday, D., Vreuls, C., Moreno, M., Frache, G., Boscher, N. D., Zocchi, G., et al. (2013). Atmospheric pressure plasma modified surfaces for immobilization of antimicrobial nisin peptides. *Surface and Coatings Technology, 218*(1), 152–161. https://doi.org/10.1016/j.surfcoat.2012.12.045

Gálvez, A., Abriouel, H., López, R. L., & Omar, N. B. (2007). Bacteriocin-based strategies for food biopreservation. *International Journal of Food Microbiology, 120*(1–2), 51–70. https://doi.org/10.1016/j.ijfoodmicro.2007.06.001

Gibert, O., Lefèvre, B., Fernández, M., Bernat, X., Paraira, M., Calderer, M., et al. (2013). Characterising biofilm development on granular activated carbon used for drinking water production. *Water Research, 47*(3), 1101–1110. https://doi.org/10.1016/j.watres.2012.11.026

*1 Goddard, J. M., & Hotchkiss, J. H. (2007). Polymer surface modification for the attachment of bioactive compounds. *Progress in Polymer Science, 32*(7), 698–725. https://doi.org/10.1016/J.PROGPOLYMSCI.2007.04.002.Reference focused on the description of different surface modification techniques for the immobilisation of bioactive compounds. Surface modification techniques are essential to introduce a specific functional group to graft a target biomolecule.

Harouna, S., Carramiñana, J. J., Navarro, F., Pérez, M. D., Calvo, M., & Sánchez, L. (2015). Antibacterial activity of bovine milk lactoferrin on the emerging foodborne pathogen *Cronobacter sakazakii*: Effect of media and heat treatment. *Food Control, 47*, 520–525. https://doi.org/10.1016/j.foodcont.2014.07.061

Hazam, P. K., Goyal, R., & Ramakrishnan, V. (2019). Peptide based antimicrobials: Design strategies and therapeutic potential. *Progress in Biophysics and Molecular Biology, 142*, 10–22. https://doi.org/10.1016/j.pbiomolbio.2018.08.006

Hossain, F., Perales-Perez, O. J., Hwang, S., & Román, F. (2014). Antimicrobial nanomaterials as water disinfectant: Applications, limitations and future perspectives. *The Science of the Total Environment, 466*, 1047–1059. https://doi.org/10.1016/j.scitotenv.2013.08.009

Hosseinidoust, Z., Van De Ven, T. G. M., & Tufenkji, N. (2011). Bacterial capture efficiency and antimicrobial activity of phage-functionalized model surfaces. *Langmuir, 27*(9), 5472–5480. https://doi.org/10.1021/la200102z

Jerri, H. A., Adolfsen, K. J., McCullough, L. R., Velegol, D., & Velegol, S. B. (2012). Antimicrobial sand via adsorption of cationic *Moringa oleifera* protein. *Langmuir, 28* (4), 2262–2268. https://doi.org/10.1021/la2038262

*2 Kroll, S., Brandes, C., Wehling, J., Treccani, L., Grathwohl, G., & Rezwan, K. (2012). Highly efficient enzyme-functionalized porous zirconia microtubes for bacteria filtration. *Environmental Science & Technology, 46*(16), 8739–8747. https://doi.org/10.1021/es3006496.Reference focused on the design and application of lysozyme-coated zirconia membranes for the removal and inactivation of microbial load from water. This reference is a relevant example of covalent immobilisation of a natural antimicrobial on a filter surface, which confers antimicrobial properties to microfiltration membranes enhancing microbial removal by inactivation after coming into contact with the immobilised antimicrobial.

Lalley, J., Dionysiou, D. D., Varma, R. S., Shankara, S., Yang, D. J., & Nadagouda, M. N. (2014). Silver-based antibacterial surfaces for drinking water disinfection—an overview. *Current Opinion in Chemical Engineering, 3*, 25–29. https://doi.org/10.1016/j.coche.2013.09.004

Lee, D.-S., & Je, J.-Y. (2013). Gallic Acid- *grafted* -chitosan inhibits foodborne pathogens by a membrane damage mechanism. *Journal of Agricultural and Food Chemistry, 61* (26), 6574–6579. https://doi.org/10.1021/jf401254g

Liburdi, K., Benucci, I., Palumbo, F., & Esti, M. (2016). Lysozyme immobilized on chitosan beads: Kinetic characterization and antimicrobial activity in white wines. *Food Control, 63*, 46–52. https://doi.org/10.1016/j.foodcont.2015.11.015

Liu, W., Cai, M., He, Y., Wang, S., Zheng, J., & Xu, X. (2015). Development of antibacterial polyacrylonitrile membrane modified with a covalently immobilized lysozyme. *RSC Advances, 5*(103), 84432–84438. https://doi.org/10.1039/c5ra14867g

Li, S., Wang, Y., Xue, Z., Jia, Y., Li, R., He, C., et al. (2021). The structure-mechanism relationship and mode of actions of antimicrobial peptides: A review. *Trends in Food Science & Technology, 109*, 103–115. https://doi.org/10.1016/j.tifs.2021.01.005

Lopes, N. A., Barreto Pinilla, C. M., & Brandelli, A. (2019). Antimicrobial activity of lysozyme-nisin co-encapsulated in liposomes coated with polysaccharides. *Food Hydrocolloids, 93*, 1–9. https://doi.org/10.1016/j.foodhyd.2019.02.009

Lucera, A., Costa, C., Conte, A., & Del Nobile, M. A. (2012). Food applications of natural antimicrobial compounds. *Frontiers in Microbiology, 3*, 287. https://doi.org/10.3389/fmicb.2012.00287

Mao, C., Mohanraj, G., Kandiyote, N. S., Kasher, R., & Arnusch, C. J. (2018). UV mediated attachment of short Arginine-Tryptophan antimicrobial peptides on reverse osmosis membrane surfaces inhibit *Pseudomonas aeruginosa* biofilm. *Desalination, 431*, 73–79. https://doi.org/10.1016/j.desal.2017.12.027

Muriel-Galet, V., Talbert, J. N., Hernandez-Munoz, P., Gavara, R., & Goddard, J. M. (2013). Covalent immobilization of lysozyme on ethylene vinyl alcohol films for nonmigrating antimicrobial packaging applications. *Journal of Agricultural and Food Chemistry, 61*(27), 6720–6727. https://doi.org/10.1021/jf401818u

Nayak, L., Rahaman, M., & Giri, R. (2019). Surface modification/functionalization of carbon materials by different techniques: An overview. In M. Rahaman, D. Khastgir, & A. Aldalbahi (Eds.), *Carbon-containing polymer composites* (pp. 65–98). Springer. https://doi.org/10.1007/978-981-13-2688-2_2.

Nogueira, F., Karumidze, N., Kusradze, I., Goderdzishvili, M., Teixeira, P., & Gouveia, I. C. (2017). Immobilization of bacteriophage in wound-dressing nanostructure. *Nanomedicine: Nanotechnology, Biology and Medicine, 13*(8), 2475–2484. https://doi.org/10.1016/j.nano.2017.08.008

Pędziwiatr-Werbicka, E., Miłowska, K., Podlas, M., Marcinkowska, M., Ferenc, M., Brahmi, Y., et al. (2014). Oleochemical-tethered SBA-15-type silicates with tunable nanoscopic order, carboxylic surface, and hydrophobic framework: Cellular toxicity, hemolysis, and antibacterial activity. *Chemistry - A European Journal, 20*(31), 9596–9606. https://doi.org/10.1002/chem.201402583

Peña-Gómez, N., Ruiz-Rico, M., Fernández-Segovia, I., & Barat, J. M. (2019a). Study of apple juice preservation by filtration through silica microparticles functionalised with essential oil components. *Food Control, 106*, 106749. https://doi.org/10.1016/j.foodcont.2019.106749

*3 Peña-Gómez, N., Ruiz-Rico, M., Pérez-Esteve, É., Fernández-Segovia, I., & Barat, J. M. (2019b). Novel antimicrobial filtering materials based on carvacrol, eugenol, thymol and vanillin immobilized on silica microparticles for water treatment. *Innovative Food Science & Emerging Technologies, 58*, 102228. https://doi.org/10.1016/j.ifset.2019.102228.Reference focused on the design and application of essential oil components-coated silica microparticles for the removal and inactivation of microbial load from water. This reference is a relevant example of covalent immobilisation of natural antimicrobials on a filter surface, which confers antimicrobial properties to filtering materials enhancing microbial removal by inactivation after coming into contact with the immobilised antimicrobial.

Peña-Gómez, N., Ruiz-Rico, M., Pérez-Esteve, É., Fernández-Segovia, I., & Barat, J. M. (2020). Microbial stabilization of craft beer by filtration through silica supports functionalized with essential oil components. *Lebensmittel-Wissenschaft & Technologie, 117*, 108626. https://doi.org/10.1016/j.lwt.2019.108626

*4 Pisoschi, A. M., Pop, A., Georgescu, C., Turcuş, V., Olah, N. K., & Mathe, E. (2018). An overview of natural antimicrobials role in food. *European Journal of Medicinal Chemistry, 143*, 922–935. https://doi.org/10.1016/j.ejmech.2017.11.095.Reference centered on the description of the main characteristics of naturally-occurring antimicrobial compounds. The features and properties of the biomolecules should be reviewed to establish the appropriate immobilisation methodology.

Polo, L., de Greñu, B. D., Della Bella, E., Pagani, S., Torricelli, P., Vivancos, J. L., et al. (2018). Antimicrobial activity of commercial calcium phosphate based materials functionalized with vanillin. *Acta Biomaterialia, 81*, 293–303. https://doi.org/10.1016/j.actbio.2018.09.033

Qi, X., Poernomo, G., Wang, K., Chen, Y., Chan-Park, M. B., Xu, R., et al. (2011). Covalent immobilization of nisin on multi-walled carbon nanotubes: Superior antimicrobial and anti-biofilm properties. *Nanoscale, 3*(4), 1874–1880. https://doi.org/10.1039/C1NR10024F

Quinto, E. J., Caro, I., Villalobos-Delgado, L. H., Mateo, J., De-Mateo-silleras, B., & Redondo-Del-río, M. P. (2019). Food safety through natural antimicrobials. *Antibiotics, 8*(4), 208. https://doi.org/10.3390/antibiotics8040208

Ribes, S., Ruiz-Rico, M., Moreno-Mesonero, L., Moreno, Y., & Barat, J. M. (2020). Natural antimicrobial compounds immobilised on silica microparticles as filtering materials: Impact on the metabolic activity and bacterial viability of waterborne microorganisms. *Environmental Technology & Innovation*, 101219. https://doi.org/10.1016/j.eti.2020.101219

Ribes, S., Ruiz-Rico, M., Pérez-Esteve, É., Fuentes, A., Talens, P., Martínez-Máñez, R., et al. (2017). Eugenol and thymol immobilised on mesoporous silica-based material as an innovative antifungal system: Application in strawberry jam. *Food Control, 81*, 181–188. https://doi.org/10.1016/j.foodcont.2017.06.006

Ruiz-Rico, M., Moreno, Y., & Barat, J. M. (2020). In vitro antimicrobial activity of immobilised essential oil components against Helicobacter pylori. *World Journal of Microbiology and Biotechnology, 36*(1), 1–9. https://doi.org/10.1007/s11274-019-2782-y

Ruiz-Rico, M., Pérez-Esteve, É., Bernardos, A., Sancenón, F., Martínez-Máñez, R., Marcos, M. D., et al. (2017). Enhanced antimicrobial activity of essential oil components immobilized on silica particles. *Food Chemistry, 233*, 228–236. https://doi.org/10.1016/j.foodchem.2017.04.118

Saeki, D., Nagao, S., Sawada, I., Ohmukai, Y., Maruyama, T., & Matsuyama, H. (2013). Development of antibacterial polyamide reverse osmosis membrane modified with a covalently immobilized enzyme. *Journal of Membrane Science, 428*, 403–409. https://doi.org/10.1016/j.memsci.2012.10.038

Samineni, L., Xiong, B., Chowdhury, R., Pei, A., Kuehster, L., Wang, H., et al. (2019). 7 log virus removal in a simple functionalized sand filter. *Environmental Science & Technology, 53*(21), 12706–12714. https://doi.org/10.1021/acs.est.9b03734

Su, Y., Luo, C., Zhang, Z., Hermawan, H., Zhu, D., Huang, J., et al. (2018). Bioinspired surface functionalization of metallic biomaterials. *Journal of the Mechanical Behavior of Biomedical Materials, 77*, 90–105. https://doi.org/10.1016/j.jmbbm.2017.08.035

Tavakolian, M., Okshevsky, M., van de Ven, T. G., & Tufenkji, N. (2018). Developing antibacterial nanocrystalline cellulose using natural antibacterial agents. *ACS Applied Materials & Interfaces, 10*(40), 33827–33838. https://doi.org/10.1021/acsami.8b08770

Treccani, L., Klein, T. Y., Meder, F., Pardun, K., & Rezwan, K. (2013). Functionalized ceramics for biomedical, biotechnological and environmental applications. *Acta Biomaterialia, 9*(7), 7115–7150. https://doi.org/10.1016/j.actbio.2013.03.036

Verdú, S., Ruiz-Rico, M., Perez, A. J., Barat, J. M., Talens, P., & Grau, R. (2020). Toxicological implications of amplifying the antibacterial activity of gallic acid by immobilisation on silica particles: A study on *C. elegans*. *Environmental Toxicology and Pharmacology, 80*, 103492. https://doi.org/10.1016/j.etap.2020.103492

Vico, T. A., Arce, V. B., Fangio, M. F., Gende, L. B., Bertran, C. A., Mártire, D. O., et al. (2016). Two choices for the functionalization of silica nanoparticles with gallic acid: Characterization of the nanomaterials and their antimicrobial activity against *Paenibacillus larvae*. *Journal of Nanoparticle Research, 18*(11), 1–13. https://doi.org/10.1007/s11051-016-3652-2

Wang, J., Tang, L., Somasundaran, P., Fan, W., Zeng, G., Deng, Y., et al. (2017). Highly effective antibacterial activity by the synergistic effect of three dimensional ordered mesoporous carbon-lysozyme composite. *Journal of Colloid and Interface Science, 503*, 131–141. https://doi.org/10.1016/j.jcis.2017.05.014

Yuan, S., Yin, J., Jiang, W., Liang, B., Pehkonen, S. O., & Choong, C. (2013). Enhancing antibacterial activity of surface-grafted chitosan with immobilized lysozyme on bioinspired stainless steel substrates. *Colloids and Surfaces B: Biointerfaces, 106*, 11–21. https://doi.org/10.1016/j.colsurfb.2012.12.048

*5 Zdarta, J., Meyer, A. S., Jesionowski, T., & Pinelo, M. (2018). Developments in support materials for immobilization of oxidoreductases: A comprehensive review. *Advances in Colloid and Interface Science, 258*, 1–20. https://doi.org/10.1016/j.cis.2018.07.004.Reference dedicated to the description of the main support materials and immobilisation methodologies. The characteristics, advantages and disadvantages of the different immobilisation techniques should be reviewed in order to choose the most suitable methodology for the immobilisation of a target biomolecule.

Zhang, J., Li, S., Wang, W., Pei, J., Zhang, J., Yue, T., et al. (2021). Bacteriocin assisted food functional membrane for simultaneous exclusion and inactivation of *Alicyclobacillus acidoterrestris* in apple juice. *Journal of Membrane Science, 618*, 118741. https://doi.org/10.1016/j.memsci.2020.118741

Zucca, P., & Sanjust, E. (2014). Inorganic materials as supports for covalent enzyme immobilization: Methods and mechanisms. *Molecules, 19*(9), 14139–14194. https://doi.org/10.3390/molecules190914139