Poly(lactic acid)/carvacrol-based materials: preparation, physicochemical properties, and antimicrobial activity

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ABSTRACT

The current demand for new antimicrobial systems has stimulated research for the development of poly(lactic acid)/carvacrol (PLA/CAR) based materials able to hinder the growth and spread of microorganisms. The eco-friendly characteristics of PLA and cytocompatibility make it very promising in the perspective of green chemistry applications as material for food and biomedical employments. The broad-spectrum biological and pharmacological properties, including antimicrobial activity of CAR, make it an interesting bioactive molecule that can be easily compounded with PLA by adopting the same techniques as those commonly used for PLA manufacturing. This review critically discusses the most common methods to incorporate CAR into a PLA matrix and their interference on the morphomechanical properties, release behavior and antimicrobial activity of systems. The high potential of PLA/CAR materials in terms of physical-chemical and antimicrobial properties can be exploited for the future development of food packaging, coated medical devices or drug delivery systems.

Keywords: PLA, essential oil, drug delivery, antimicrobial activity, food and biomedical application

Introduction

The development of antimicrobial polymer-based systems is gaining a rising attention, in the perspective of limiting microbial contamination of medical and food-related environments, thus hindering the spread of illnesses (Gazzotti et al. 2019). In fact, polymeric materials, while being widespread in food packaging and biomedicine for their processability and suitable characteristics, are conversely prone to be colonized by microbial cells, thus being responsible for infections (Scaffaro et al. 2018c). The current demand for novel antimicrobial materials stimulated the research efforts for the formulation of polymer-based systems able to provide microbial growth inhibition and prevention of the subsequent colonization and proliferation of pathogens (Nostro et al. 2010; Nostro et al. 2012; Liu et al. 2016; Scaffaro et al. 2018c).

On the other hand, recent environmental concerns are directing both academic and industrial research toward the use of renewable, biodegradable, compostable plastics. Among the so-called bioplastics, poly(lactic acid) (PLA) plays an important role, owing to its good mechanical performance and low immunogenicity, which make PLA preferable for preparing food packaging films and biomedical devices. PLA can be endowed with antimicrobial activity via the incorporation of additives, including metals, metal oxides, antibiotics, enzymes, and natural compounds (Tawakkal et al. 2014; Scaffaro et al. 2018c). These latter ones are particularly promising in the perspective of retaining high standards in terms of environmental sustainability (Scaffaro et al. 2018c). Among the natural compounds, carvacrol (CAR) appears to be an interesting bioactive component with high potential for food packaging and biomedical applications. CAR can be easily compounded with PLA by adopting the same techniques as those commonly used for PLA manufacturing, whose main advantages and drawbacks will be critically discussed in this review. In fact, the choice of the preparation technique affects morpho-mechanical properties, release behaviour and biocidal activity of PLA-CAR systems. Beyond the current trends, even some possible future perspectives will be proposed in the latter section of this review.

Poly(lactic acid) - properties and application

Polylactic acid is a bioplastic produced by totally renewable sources that belongs to the family of poly(alpha-hydroxy esters) (Inkinen et al. 2011). Although it was first synthesized in 1932 by Carothers (DuPont), its patentation occurred in 1954, as a higher molecular weight was achieved (Scaffaro et al. 2016). Its monomer is the lactic acid molecule, which exists in two different enantiomeric forms owing to its chirality. Therefore, its esterification leads to the existence of stereoisomers, such as poly(L-lactide) (PLLA), poly(D-lactide) (PDLA), and poly(DL-lactide) (PDLLA) (Lopes et al. 2012; Scaffaro et al. 2016). Figure 1a provides the structure of PLLA and PDLA stereoisomers.

PLA can be synthesized via different polymerization routes of lactic acid monomers, involving ring opening polymerization (ROP), polycondensation, azeotropic dehydration and enzymatic polymerization (Inkinen et al. 2011). Among these, however, ROP is the only method allowing a high molecular weight (Inkinen et al. 2011). Basically, ROP can be performed either in solution, in the melt or in bulk, and involving several mechanisms, such as anionic, cationic, and coordination-insertion mechanisms depending on the catalyst (Inkinen et al. 2011). Actually, the most widespread catalyst is stannous octanoate, which provides high conversion and reaction rates, together with the possibility to achieve high molecular weights even under mild conditions (Lopes et al. 2012).

The eco-friendly characteristics of PLA, in terms of renewability, recyclability, non-toxicity, compostability, make it very promising in the perspective of green chemistry applications, whereas its cytocompatibility and the biocompatibility of its degradation products make it attractive as material for biomedical and drug delivery applications (de Velde and Kiekens 2002). Although its biodegradability is extremely slow under acidic and physiological conditions, the possibility to rapidly degrade it in alkaline environments, together with its compostability and recyclability, offer a wide variety of scenarios for its post-consumer disposal, thus meeting the ecological requirements (Scaffaro et al. 2019c).

Apart from its bio/green properties, PLA attracted enormous interest due to its good mechanical performance (Scaffaro et al. 2017a). Indeed, as concerns this latter aspect, it has to be mentioned that properties of PLA depend on several factors, such as the content of stereoisomers, the type of source, the processing techniques, the annealing time and, of course, the molecular weight (Rasal et al. 2010; Nampoothiri et al. 2010).

Depending on these features, PLA may be amorphous or semi-crystalline and its crystallinity may depend on intrinsic chemical-physical properties, such as stereochemistry, or preparation conditions, such as thermal history (de Velde and Kiekens 2002; Nampoothiri et al. 2010; Scaffaro et al. 2018d; Scaffaro and Maio 2019b). PLA tends to be crystalline when the amount of PLLA is higher than 90%, otherwise it tends to be amorphous. The content of PLLA affects even melting temperature (Tm) and glass transition temperature (Tg), as well as mechanical properties (de Velde and Kiekens 2002). Elastic modulus proved to increase from less than 1-3.5 GPa to 2.7-4.1 GPa as a function of L-lactide content, whereas tensile strength was found to vary from 20-50 MPa to 60-80 MPa (de Velde and Kiekens 2002). Notably, elongation at break of PLA seems to be scarcely affected by stereoisomers content and equal to 2-10% (de Velde and Kiekens 2002). Furthermore, the ease of processability of PLA, which can be performed both in the melt or in solution, as well as the possibility to synthesize different grades of PLA with *ad hoc* engineered chemical-physical properties, make such polymer particularly suitable for several applications (Scaffaro et al. 2017b; Scaffaro et al. 2017c; Scaffaro et al. 2018e; Scaffaro et al. 2018f)

Carvacrol - properties and application

Carvacrol (2-Methyl-5-(1-methylethyl) phenol) is a monoterpenoid compound, formed from the bonding of two isoprene molecules with three functional group substituents (Figure 1 b). It is a component of many aromatic plants of the *Labiatae* family that are usually used as spices in culinary and for therapy/prevention purposes in folk medicine. The scientific interest to this molecule is due to its broad-spectrum of biological and pharmacological properties such as anti-inflammatory, antimicrobial, antioxidant, antitumor, anti-hepatotoxic and insecticidal activities (Nostro and Papalia 2012; Suntres et al. 2015; Sharifi-Rad et al. 2018).

With regard to antimicrobial activity, several investigations have demonstrated the efficacy of CAR against pathogens or spoilage microorganisms including drug-resistant and sessile-lifestyle clinically relevant strains (Nostro et al. 2004; Nostro et al. 2007; Nostro et al. 2009; Marchese et al. 2018; Marini et al. 2019; Kachur and Suntres 2019)

The activity of CAR has been attributed to its hydrophobic nature, the presence of a free hydroxyl group and a delocalized electron system. CAR impairs the bacterial cytoplasmic membranes causing leakage of integrity and intracellular material (Ben Arfa et al.; Lambert et al. 2001).

CAR is also effective against various fungi by inducing envelope damage and blocking ergosterol biosynthesis (Ahmad et al. 2011; Chaillot et al. 2015).

CAR is classified as Generally Recognized As Safe (GRAS) by the U.S. Food and Drug Administration and included in the list of additives permitted for direct addition to food for human consumption (http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?FR=172.515). In recent decades, it has been proposed as a natural preservative for the food industry (Burt 2004) and as a disinfectant, fungicide and fragrance ingredient in cosmetic formulations and in health related products (2006).

It is interesting to note that the strong antimicrobial efficacy of its vapors compared to that of the solution suggests further advantageous perspectives (Inouye et al. 2001; Nostro et al. 2009; Mandras et al. 2016)

The recent patents claimed on CAR alone or in combination with other essential oil components are reported in the study of (Nostro and Papalia 2012).

Although the food and pharmaceutical industry has shown great interest in the CAR research, the direct use of CAR still encounters limitations due to its poor solubility and high volatility. The study continues in order to develop new strategies such as polymeric systems able to improve the stability and prolong the biological activity of CAR (Persico et al. 2009; Nostro et al. 2012; Nostro et al. 2015; Krepker et al. 2018).

Incorporation of carvacrol into poly(lactic acid)

The development of PLA/CAR based materials is an innovative antimicrobial approach in the food and biomedical fields. The physicochemical properties of PLA coupled to the biological activities of CAR are likely to lead to next generation of antimicrobial food packaging or innovative delivery systems, wound healing materials, coating for medical devices.

Basically, the most common methods to integrate CAR into a PLA matrix can be divided into two approaches, i.e.: melt processing and solution mixing. The former case can be used since PLA requires temperature of 170–190 °C to be processed and the boiling point of CAR is above 205 °C. Indeed, volatilization of CAR occurs even at room temperature and is promoted at high temperatures, therefore this technique, while bringing several advantages in terms of eco-friendliness, rapidity, eco-sustainability and industrial scalability, presents a low degree of loading efficiency (Yang et al. 2019). By contrast, solvent-aided techniques require mild temperature conditions and hence allow to incorporate higher amounts of CAR without significant loss of this volatile additive during preparation, while displaying drawbacks related to the use of toxic solvents, the difficulty to scale-up the process and the requirement of time-consuming protocols. Regardless of the processing technique used, the assessment of the remaining amount of a volatile compound such as CAR after processing is essential to evaluate the antimicrobial activity of the films. The total content of CAR after processing can be determined by liquid chromatography coupled to ultraviolet spectroscopy detector (HPLC-UV) after a solid–liquid extraction of the films (Armentano et al. 2015). Often, the choice of the preparation technique depends on the type of application. In fact, melt processing is particularly suitable in packaging applications, or more generally, for manufacturing commodities, while solvent-aided preparative techniques are preferable for the realization of high added value materials, such as biomedical devices or advanced drug delivery systems.

To date, there are no standardized methods for evaluating the antimicrobial activity of polymeric materials. The traditional techniques commonly used in microbiology and occasionally adapted *ad hoc* for a specific application field (i.e. food models) involve methods in solid and liquid media including diffusion tests in which the polymeric samples are aseptically cut and placed on the inoculated agar plate, evaluation of minimum inhibitory concentrations (MIC), vapor contact tests, time-kill tests and microscopy investigations

Poly(lactic acid)/carvacrol materials prepared by melt processing techniques

The articles reporting the preparation of PLA/CAR systems in the melt are listed in Table 1, which provide for each work technique, PLA grade, operating parameter adopted, as well as antimicrobial properties including microbial strain and method.

As concerns the melt-based preparation of PLA/CAR films, two crucial factors are represented by the processing temperature and the content of CAR. Both melt viscosity and antimicrobial activity tend to decrease upon increasing temperature, and the use of plasticizers, such as - among others - poly(butylene succinate) or poly(butylene succinate adipate), together with the intrinsic plasticizing effect of CAR itself, allow to reduce processing temperature and to improve the mechanical properties of films, especially in terms of toughness, and stretchability, which are required for packaging applications (Scaffaro et al. 2019c).

Generally, PLA/CAR films in the melt can be prepared continuously or in the batch. The former allows a more rapid manufacturing and an industrial-scalability but poses some limits in terms of formulation. It was generally reported that contents of CAR usually above 10 wt.% determine a huge decrease of viscosity, thus negatively affecting the processability of films (Raouche et al. 2011). By contrast, due to the aforementioned volatility of CAR, films containing less than 5 wt% of active compound might not display any antibacterial activity. Melt blending in a batch mixer or extruder, followed by compression molding, allows preparing films with higher amounts of CAR. Often, nanoparticles are used to modulate release kinetics and/or to protect the active compounds volatilization during processing. In this context, even complexes of CAR with β-cyclodextrins (β-CD) can be conveniently used to prepare PLA-CAR films at higher temperature (T = 190 °C) (Raouche et al. 2011). Despite this, the developed materials were not effective in inhibiting the growth of the widely spread crops pathogen *Botrytis cinerea*, during 10 days at 22 ° C.

Frinè et al. (2019) prepared β-CD/CAR complexes via spray-drying, which were successively added to PLA up to 10 wt.% and melt processed by injection molding. Even in this case, β-CD/CAR complexes proved to act as plasticizers, being particularly able to reduce intermolecular forces of the polymer chains, thus improving the breaking properties. The PLA/β-CD/CAR materials were then analyzed to evaluate the efficacy of CAR vapor released by the PLA matrix against *Alternaria alternata*, a phytopathogen that causes post-harvest diseases but also problems in humans and animals (Frine et al. 2019). The materials containing 2.5% and 5% β-CD/CAR were aseptically cut into 50 mm rectangles and placed on top of the Petri dishes. The results documented that after 10 days of incubation *A. alternata* was completely inhibited (Frine et al. 2019).

PLA/CAR systems plasticized with other additive such as acetyl tributyl citrate (ATBC) can be prepared until a CAR content equal to 20 wt.% (Celebi and Gunes 2018). The materials were processed in a twin-screw compounder at 180 °C and 100 rpm for 4 minutes. When added to PLA, CAR is known to act as a plasticizer, thus leading to a strong decrease of elastic modulus with consequent increase of ductility and toughness. This aspect could be suitable in the perspective of overcoming one of the most limiting issues of PLA, that is, its brittleness. The plasticizer effect of CAR was investigated by analyzing the morphology of specimens subject to tensile failure. Figure 2 a-b reports SEM analysis of fractured cross-sections of neat PLA (a) and PLA containing 20% CAR (b). While neat polymer exhibited the typical morphology of a brittle material, PLA/CAR displayed a fibrous structure and rough fracture surface. The fibrillary morphology and microvoids indicated that the ductile failure was likely achieved owing to the massive shear yielding, which was the energy absorbing mechanism. It is worth mentioning that CAR addition determines a lower thermal stability in PLA-based systems, as pointed out by Thermogravimetric analysis (TGA) measurements. The addition of CAR to PLA showed the potential for food-packaging application. In this context, the samples containing high concentrations of CAR (20 wt.%) demonstrated the best antibacterial activity against *Staphylococcus aureus*, *Salmonella typhimurium* and *Listeria monocytogenes* with inhibition zones from 0.8 mm to 2.5 mm by the agar diffusion method. On the contrary, the same samples were not effective against *E. coli*. The reason for the different results could be due to the morphological differences among these microorganisms.

CAR was added in the melt to a PLA plasticized with poly(3-hydroxybutyrate) (PHB) and oligomeric lactic acid (OLA) (Armentano et al. 2015). The authors reported that systems containing CAR (10 wt.%) retained the high stiffness of the corresponding matrices and practically the same stress-strain curves. The same research group incorporated CAR even in a PLA/PHB/OLA matrix reinforced with nanocellulose (CNC) (Luzi et al. 2019). The authors found that even in this case, 10 wt. % of CAR was able to preserve the stiffness of nanocomposite, while enormously increasing stretchability from 150% to 410%. Even in this case, the increase of ductility was ascribed to a further plasticizing effect exerted by CAR molecules (Luzi et al. 2019). In this context, Burgos et al. (Burgos et al. 2017) demonstrated the importance of the addition of OLA to PLA/PHB blends. Notably, PLA films blended with PHB and incorporating OLA as plasticizers and CAR as active agent were studied for their barrier properties, antioxidant and antibacterial properties and disintegration behavior. The antimicrobial activity against two food-borne bacteria *S. aureus* and *E. coli* was preliminarily assayed by the agar diffusion method and then by evaluation of growth inhibition. The activity was significantly improved by the presence of both 10 wt.% of CAR and 15 or 20 wt.% of OLA, showing a bactericidal effect after 3 h and up to 24 h. Specifically, the order of effectiveness was as follows: PLA/PHB/10 CAR < PLA/PHB/20OLA/10CAR < PLA/PHB/15OLA/10CAR. The addition of OLA to PLA/PHB blends increased mobility of the macromolecular chains resulting in a higher release of CAR from the polymer matrix into the culture medium.

Yang et al. (2019) developed biodegradable films based on PLA blended with poly (butylene succinate adipate) (PBSA) and CAR for food preservation, in particular for aquatic products (Yang et al. 2019). The addition of CAR increased the mobility of PLA/PBSA chains and improved their flexibility and ductility. The shelf life test performed on salmon slices demonstrated that the polymeric films effectively inhibited bacterial growth, extending the shelf life of the salmon slices by 3–4 days during cold storage.

Poly(lactic acid)/carvacrol materials prepared by solution processing techniques

The articles reporting the solvent based techniques adopted to prepare PLA/CAR films, fibers and fibrous membranes are listed in Table 2.

In this case, the choice of solvents and the concentration of each component play a key-role in processing-structure-properties relationship of such materials. The most widespread technique adopted was electrospinning, which enables the preparation of fibrous nanomats providing high specific area and, thus, a quick and relevant release of active CAR. Active membranes containing CAR could find application not only in food packaging and pharmaceutics but even in tissue engineering. In this case, electrospinning parameters, such as flow rate, distance between the needle tip and the collector, and supplied high voltage are crucial. For PLA/CAR system, these parameters were found to be similar in all of the works. In fact, the typical values of flow rate and supplied voltage used were 0.1 mL/h and 15 kV, respectively, whereas distance between the needle tip and the collector was found to vary from 15 to 20 cm. Moreover, in all the cases chloroform was chosen as a solvent, while acetone or DMF were used as a co-solvent.

Differently from melt-based techniques, which displayed strong limitations in terms of formulation, it was demonstrated that an amount of CAR equal to 28-30 wt.% can be easily integrated into a PLA matrix (Scaffaro and Lopresti 2018). As already seen in the case of systems prepared by melt processing, even in this case nanoparticles or other polymers are often used to modulate electrospinnability of the solutions, as well as morpho-mechanical properties of fibrous mats and/or release kinetics of CAR from the matrix. While gelatin, zein, poly(ethylene oxide) and poly(ethylene glycol) proved to provide a faster release, graphene nanoplatelets were found to slow down the delivery kinetics of CAR, presumably due to aromatic interactions and barrier effect (Scaffaro et al. 2018a; Scaffaro et al. 2019b). Notably, when graphene was used, CAR acted as a green exfoliant for these nanoparticles, with the formation of stable suspensions, which promoted a more uniform dispersion of both component throughout the matrix. Consequently, the mechanical properties of membranes were enhanced. Moreover, graphene nanoplatelet (GNP) proved to protect CAR from volatilization and to limit the burst release. Therefore, PLA/CAR/graphene system is promising to realize membranes with mechanical robustness and long-time sustained release activity of active CAR. In particular, the authors found that the best morphomechanical properties of such membranes were achieved at relatively low GNP content, since at higher GNP loading levels (7 wt.%), the fibers morphology of electrospun membranes proved to be less uniform likely due to nanoparticles aggregation, with negative repercussions on the elastic modulus of the mats. In the same study, it was evaluated even the possibility to fabricate PLA/CAR/GNP films via solvent casting and the results put into evidence that, with respect to the analogous systems prepared via electrospinning, the effect of GNP addition in PLA/CAR films was even more remarkable, with relative increments up to +600%, observed in terms of stiffness, tensile strength, ductility and toughness, as showed in Figure 3. As concerns the release behaviour, the presence of GNP led to a progressively more Fickian and sustained release, likely due to the higher hydrophobic character imparted by nanoparticles that hindered the solvent penetration and to the strong π-π* interactions between graphene and carvacrol molecules.

Altan et al. (2018) investigated the effectiveness of PLA/CAR electrospun fibers for the preservation of a fresh food and extend its shelf life (Altan et al. 2018). Notably, slices of bread were placed on the base of Petri dishes and the fibers (25-40 mg) were cut and placed with no direct contact. After 7 days, the microbiological quality of bread samples was evaluated by determining the total number of aerobic bacteria, yeast and mold. The growth inhibition rate was 87.0% and 91.3% for PLA fibers at 20% of CAR.

The fabrication of active fibers is of great concern in biomedicine. Indeed, endowing surgery threads with designable antibacterial properties could represent a key-strategy in the perspective of minimizing the risk of infections after surgery (Scaffaro et al. 2019a). In the scientific literature, only one paper reports on the preparation of PLA/CAR fibers (Martínez-Sanz et al. 2015).The authors prepared a multi-component system containing, beyond PLA and CAR, eventually gelatin and even bacterial nanocellulose nanowhiskers (BCNW). Nanocellulose was incorporated to enhance thermal stability and fibers robustness. This filler, in fact, was found to increase the viscosity of solutions, thus providing a good spinnability. Notably, CAR proved to increase the thermal stability of neat PLA. Gelatin was added to the system as a hydrophilic agent to trigger CAR release, since water sorption experiments performed onto various formulations put into evidence that PLA/CAR showed no water affinity. The addition of gelatin into the system was critical for the antimicrobial effect of CAR since it favored water sorption into the fibers, thus facilitating the release of the compound. In fact, the results of diffusion test documented that fibers of PLA + 30% Gelatin + 12% CAR showed increased activity against *L. monocytogenes*, isolated from cheese associated with an outbreak. Specifically, the blow spun fiber disc was aseptically cut (12-mm diameter) and was placed over the inoculated agar plate. After 24 h of incubation at 37 °C, an inhibition area around the fiber disc was detected. On the contrary, the incorporation of BCNW into PLA fibers was detrimental to the CAR release, showing a reduced antibacterial activity.

The evidence that the most infectious diseases are linked to microorganisms organized in biofilms represents a significant public health problem. From here, the research of innovative antibiofilm systems is crucial. In this context, Zodrow et al. (Zodrow et al. 2012) explored the potential of CAR to prevent biofilm formation when added to PLGA materials.

Notably, PLGA/CAR films with 0.1 and 1.0% CAR, prepared by solvent casting in chloroform, were coated onto the sides and bottom of wells in a glass-coated 96-well plate. Then *E. coli*, *Pseudomonas aeruginosa* and *S. aureus* were inoculated to each well. After 20 h incubation at 25 °C, the biofilm was evaluated by optical density measurements and confocal laser scanning microscopy. The results demonstrated that the PLGA films coated with CAR impaired biofilm formation by both *E. coli* and *S. aureus* whereas no interfered with *P. aeruginosa* biofilm. The surface of PLGA coated with CAR resulted more hydrophilic and therefore more resistant to bacterial adhesion. The authors suggested the use of CAR in low concentrations in polymer coatings to inhibit bacterial adhesion on indwelling devices.

The interaction among microorganisms in mixed culture can influence the biofilm forming capacity thus favoring a stronger biofilm. Polymicrobial biofilms develop aggressive forms of diseases with enhanced antimicrobial resistance. Scaffaro et al. report the potential of PLA/CAR 28 wt.% membranes as new tools for skin and wound polymicrobial infections (Scaffaro et al. 2018b). The efficacy of electrospun PLA-CAR membranes against bacterial–fungal communities of *S. aureus* and *Candida albicans* was studied by optical density measurement, vitality test and fluorescence microscope image. The results showed that PLA/CAR membranes reduced the biofilm production of *S. aureus* and *C. albicans* in single and mixed cultures by 92-96% and 88-95% and were also effective against mature 24-h and 48-h biofilms (Figs. 4-5).

Poly(lactic acid)/carvacrol materials prepared by hybrid technique

Of course, it is possible gathering solvent-based and melt-based protocols into multi-step hybrid techniques, aiming to exploit the advantages of both methods (Maio et al. 2015; Scaffaro and Maio 2017; Scaffaro and Maio 2019a), thus achieving high dispersion levels or graded/hierarchical structures. In this context, the possibility to prepare cast films of PLA/CAR and starch separately with subsequent assembly by compression molding was tested by Requena et al. (2018). More in detail, the authors prepared PLA/CAR films blended with poly(ethylene glycol) and poly(hydroxybutyrate-co-hydroxyvalerate) and starch films by solvent casting that were assembled into mono- and bilayer structures by themocompression at 200 °C (Requena et al. 2018). The authors evaluated the radial distribution of CAR as a function of time for various formulation, aiming to study the CAR retention after thermoforming. The results demonstrated that CAR volatilization was negligible. However, from a mechanical point of view, films prepared by compression molding displayed higher stiffness and tensile strength and lower stretchability than those prepared by solvent casting.

As regards the antibacterial activity, the different kinds of films were evaluated against *L. innocua* and *E. coli* by placing circular samples (55 mm in diameter) on inoculated TSA plates. After incubation of 6 days at 10 °C, the results documented that the incorporation of CAR by spraying between the polyester and starch sheets was not effective, probably due to the insufficient CAR retention. On the contrary, mono and bilayer structure loaded with CAR by solvent casting significantly reduced the growth of *L. innocua* and *E. coli* by 9 and 5 log CFU, respectively. The effect was attributed to the total retention of the CAR during the film processing and its adequate release.

Conclusion and future directions

CAR can be easily compounded with PLA by adopting different melt processing and solution mixing techniques. The review underlines the importance of the method used to develop active materials, in fact the choice of the preparation technique affects morpho-mechanical properties, release behaviour and antimicrobial activity of the polymeric system.

PLA/CAR based materials have the potential to receive further attention due to their remarkable physical-chemical and antimicrobial properties. Despite the results achieved, it is desirable to move forward in research in order to develop antimicrobial PLA/CAR systems with well-defined characteristics for the specific application in the different fields. In this context, the use of nanofillers such as graphene, lignin, chitin and nanocellulose might be useful to modulate mechanical properties and release kinetics of such systems, as well as the possibility to prepare hierarchical or graded structures can be explored by adopting multi-step techniques (Herrera et al. 2015; Maio et al. 2016; Scaffaro et al. 2016; Maio et al. 2018).

PLA/CAR materials could lead to an innovative era in the food packaging industry in order to extend the shelf life of products or in biomedicine to control infectious diseases through new delivery systems, wound healing systems or coating for medical devices. Furthermore, endowing surgery threads with antimicrobial properties could be a key strategy in the perspective of minimizing the risk of infections after surgery. It is also desirable to have antimicrobial standardized methods also adapted for different kinds of samples in order to correctly compare and interpret the results.

Compliance with ethical standards

This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of Interest

The authors declare that they have no conflict of interest

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Figure Captions

Fig. 1. Schematic structure of PLA (a) and CAR (b).

Fig. 2 SEM images (3000×) of (a) neat PLA and (b) PLA containing 20% CAR. Adapted from (Celebi and Gunes 2018).

Fig. 3 Elastic modulus (E) (A), Tensile strength (TS) (B), Elongation at break (EB) (C) and toughness (D) plotted as a function of graphene/CAR (G/C) ratio for materials prepared via electrospinning (ES-series) and solvent casting (SC-series) Reprinted from (Scaffaro et al. 2019b) with permission from Elsevier.

Fig. 4 Effectiveness of PLA/CAR membranes on biofilm formation. Values are expressed as means ± standard deviations. Adapted from (Scaffaro et al. 2018b).

Fig. 5 Effect of PLA/CAR membranes on preformed 24-h biofilms of *S. aureus* and *C. albicans* in single and mixed cultures. Data are presented as the mean ± standard deviations. Adapted from (Scaffaro et al. 2018b)