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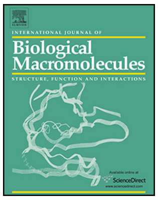

Effect of cellulose and lignin on disintegration, antimicrobial and antioxidant properties of PLA active films

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ABSTRACT

This study reports the effects on antimicrobial, antioxidant, migration and disintegrability activities of ternary nanocomposite films based on poly(lactic acid) incorporating two biobased nanofillers, (cellulose nanocrystals (CNC) and lignin nanoparticles (LNP)), in two different amounts (1 and 3% wt.). Results from antimicrobial tests revealed a capacity to inhibit the Gram negative bacterial growth of *Xanthomonas axonopodis* pv. *vesicatoria* and *Xanthomonas arboricola* pv. *pruni* along the time, offering innovative opportunities against dangerous bacterial plant pathogens. LNP proved to be highly efficient in antioxidation activity, based on the disappearance of the absorption band at 517 nm of the free radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH) upon reduction by an antiradical compound; moreover the combination of LNP and CNC generates a synergistic positive effect in the antioxidation response of PLA ternary films. Furthermore, all the studied formulations showed a disintegrability value up to 90% after 15 days of incubation in composting conditions. Migration results showed that the films can be considered suitable for application in food packaging field.

1. Introduction

The increasing public awareness regarding synthetic antioxidant application not only for food but also for stabilization of polymeric materials moved the interest towards antioxidants of natural origin, in particular polyphenols that, due to their biodegradability and significantly lower toxicity, can represent a good alternative to the synthetic ones [1]. However, most of the naturally originated antioxidants are not efficient enough for the protection of plastics and composites, due to their lower molecular mass and lesser thermal stability in comparison with synthetic systems [2]. On the contrary, biorenewable polymeric polyphenol lignin is a suitable natural antioxidant for use in polymers, due to its lower sensitivity to high temperatures and higher molecular mass in comparison with the low molecular weight natural antioxidants, which are commonly proposed for the stabilization of polymeric materials, e.g. α-tocopherol [3]. Lignin, as the second most abundant renewable and biodegradable natural resource next to cellulose, is a heteropolymeric aromatic compound that has a complex chemical structure, such as carboxyl, carbonyl, phenolic or aliphatic hydroxyls that can be found in different proportions according to lignin origin. Due to the presence of these functional groups, oxidation propagation reaction can be terminated through hydrogen donation. The antioxidant capacity of lignin has been widely studied and its application in fields as medical, pharmaceutical (anti carcinogenic agent) or polymeric applications (thermal behaviour enhancement) has been proved [4]. However, this property depends on the used lignocellulosic material, the extraction methods, and the secondary applied treatments. As a result, studies were carried out with regard to the incorporation of lignin into materials for manufacturing value added antioxidant products in the various fields, aiming to tune the rate of autooxidation [5,6]. For higher antioxidative efficiency, lignin should have higher quantity of phenolic hydroxyl and methoxy groups, lower quantity of aliphatic hydroxyl groups, narrow polydispersity and lower molecular weight. Furthermore, the effects of chemical characterisation, structure of side chain, π-conjugated systems of lignin on its antioxidant activity have been studied [7,8]. It was determined that the main factor that contributes to lignin antioxidant activity was the high content of phenolic moieties, which underwent proton coupled electron transfer mechanism. The remaining factors that could contribute

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to antioxidant activity included relative content of phenylpropane units, phenylpropane units with CH_2 groups in the α-position of side chains, phenylpropane units with oxygen-containing groups in the side chains and size of the π-conjugated systems. This effect has been studied, for example, by Azadfar et al. [9], who revealed that the antioxidant activity of alkali lignin was comparable to the commercial antioxidants (such as butylated hydroxytoluene), as the 2,2-diphenyl-1-picrylhydrazyl (DPPH) inhibition percentages of lignin and butylated hydroxytoluene were 86.9% ± 0.34% and 103.3% ± 1%, respectively. However, despite the well proved antioxidant properties, the absence of rationalized conceptions about lignin structural features and physical–chemical properties (including size dimensions) influencing its antioxidant activity hinders the realization of lignin potential in the area of stabilization of materials and food/feed products against oxidative damage. Domenek et al. [10] investigated the antioxidant activity of microscaled lignin as a natural substitute of synthetic antioxidants for the protection of the food, paving the way to the fabrication of active packaging materials compounded with natural antioxidant substances. After that, Yearla et al. investigated the radical scavenging activity of nanosized lignin and demonstrated that its antioxidant and UV protection properties could be also further exploited in food, pharmaceutical and cosmetic industries [11]. They studied how structure-activity relationship is crucial to identify the most optimal antioxidants and to determine the most promising directions for upgrading of technical lignins, in order to produce effective antioxidants for various application systems. However, no previous studies have been reported on how lignin at the nanoscale dimension could work as antioxidant agent when incorporated in polymeric systems.

Concerning the antibacterial properties of lignin, it seems to be also a promising green principle useful against dangerous microorganisms. Its biocidal activity allows to reduce the environmental problems related to the silver nanoparticles use and could improve, as an example, the release of active principles in agriculture [12,13]. Some studies have shown that lignin has an antimicrobial effect [14], even when it was incorporated into polyethylene film and applied in the finishing processes of textiles [15,16]. However, none of them investigated the mechanism of the antibacterial activity of lignin when included in polymeric matrices. A recent study [17] revealed that the main determining factor of the antimicrobial effect of lignin lies on the phenolic components, specifically the side chain structure and the nature of the functional groups. Typically, the presence of a double bond in α, β positions of the side chain and a methyl group in the γ position grants the phenolic fragments with the most potency against microorganisms.

In addition to lignin based nanoparticles, cellulose nanocrystals (CNC), that can be easily extracted from cellulose based sources, have been proved to be effective in enhancing the barrier to gases when introduced in polymeric systems, due to the synergistic tortuosity, crystal nucleation and chain immobilization effects [18,19]. In the meanwhile, they present the advantage of being renewable when compared to other inorganic fillers, supporting their use in biodegradable materials, such as PLA [5,20,18,1,21,22,23,24,25]. Some previous research works have also highlighted the significant advantages of incorporating CNC in PLA, including important water vapour, oxygen barrier effects, migration level below packaging normative requirements [26] and antimicrobial properties when combined with silver nanoparticles [27] or essential oil [18,28], demonstrating the antimicrobial potential of PLA/CNC films as a promising bioactive packaging to preserve fresh food products against food pathogens.

Composting is nowadays a general treatment method for municipal solid waste. In the European Union, composting is regarded as one recycling method for packages and this will probably favour compostable packages, like films, papers and boards. Tuomela et al. [29] found that the elevated temperatures during the thermophilic phase are essential for rapid degradation of lignocelluloses. Complex organic compounds like lignin are mainly degraded by thermophilic micro fungi and actinomycetes, and then form humus. The optimum temperature for thermophilic fungi is 40 ± 50 °C which is also the optimum temperature for lignin degradation in compost. We have demonstrated [19] that lignin, even at the nanodimension, strongly influences the degradation process in compost of PLA films, and the degradation rate can be modified changing both lignin nanoparticles content and processing methods (solvent casting or extrusion). In that study, PLA filled with 0, 1 and 3 wt.% of lignin nanoparticles (LNPs) was investigated. Disintegrability in composting conditions has been tested and visual observation, chemical, thermal and morphological investigations proved that the incorporation of 1 wt.% of LNP seems to hinder the disintegration of PLA matrix, due to the hydrophobic nature of the filler; when the LNP content rises up to 3 wt.%, some LNP aggregations and rougher film surface structure form, inducing higher degradation rate. For PLA filled with CNC, Bitinis et al. and Fortunati et al. [5,26] studied that the addition of the unmodified CNC did not affect the disintegration rate of the material. Although the addition of hydrophilic filler is expected to accelerate the degradation rate of the PLA/natural rubber material, CNC could also inhibit water diffusion, explaining the obtained results. Actually, the two effects appear to counterbalance each other.

With the aim of evaluating their possible synergic effects, binary and ternary PLA based nanocomposites, prepared by combining two processing procedure (masterbatch and reactive melt grafting) and containing both CNC and LNP without any further modification, were produced. Antibacterial activity, oxidative behaviour, migration and disintegrability activity of all produced films were tested and here reported, taking into account a possible final application of the produced formulation as food packaging materials.

2. Experimental

2.1. Materials

Poly (lactic acid) (PLA 3251D—specific gravity of 1.24 g cm^{-3} and melt flow index of 35 g $(10\,min)^{-1}$ (190 °C, 2.16 kg) was supplied by NatureWorks LLC, USA. Pristine lignin was supplied by CRB (Centro Ricerca Biomasse, University of Perugia) and lignin nanoparticles were synthesized as previously reported [19]. Microcrystalline cellulose (MCC, dimensions of 10–15 μm), used as cellulose nanocrystals precursor during the hydrolysis procedure, was supplied by Sigma-Aldrich®. All the chemical reagents were supplied by Sigma-Aldrich® and used as received.

2.2. Preparation of masterbatches (MBs)

PLA grafting with glycidyl methacrylate was performed in a twin-screw microextruder (DSM Explorer 5&15CC Micro Compounder) in presence of dicumyl peroxide as initiator. Before being used for grafting, GMA was stored in the refrigerator at 5° C. DCP content was set as 1 wt.% of the PLA weight, while GMA was fixed at 10 wt.% of the PLA weight. DCP and GMA (DCP/GMA ratio = 0.1) were mixed and the obtained solution was sprayed onto dried PLA matrix. Screw speed of 100 rpm, mixing time of 8 min and a temperature profile of 165–175–180 °C were selected to realize the grafting reaction. The content of PLA grafted with GMA (g-PLA), to be used as a compatibilizer in the resulting nanocomposites, was fixed at 15 wt.%. The compositions of different masterbatches are MB1 (PLA+1.17 wt.% CNC), MB2 (PLA+3.53 wt.% CNC), MB3 (grafted PLA+6.67 wt.% LNP) and MB4 (grafted PLA+20 wt.% LNP).