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Cellulose nanocrystals from *Actinidia deliciosa* pruning residues combined with carvacrol in PVA_CH films with antioxidant/antimicrobial properties for packaging applications

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ABSTRACT

Kiwi *Actinidia deliciosa* pruning residues were here used for the first time as precursors for the extraction of high performing cellulose nanocrystals (CNC) by applying a bleaching treatment followed by an acidic hydrolysis. The resultant cellulosic nanostructures, obtained by an optimize extraction procedure (0.7% wt/v two times of sodium chlorite $NaClO_2$) followed by an hydrolysis step, were then used as reinforcements phases in poly(vinyl alcohol) (PVA) blended with natural chitosan (CH) based films and also combined, for the first time, with carvacrol used here as active agent. Morphological and optical characteristics, mechanical response, thermal and migration properties, moisture content and antioxidant and antimicrobial assays were conducted. The morphological, optical and colorimetric results underlined that no particular alterations were induced on the transparency and color of PVA and PVA_CH blend by the presence of CNC and carvacrol, while they were able to modulate the mechanical responses, to induce antioxidant activities maintaining the migration levels below the permitted limits and suggesting the possible application in industrial sectors. Finally, inhibitions on bacterial development were detected for multifunctional systems, suggesting their protective function against microorganisms contamination.

1. Introduction

New biodegradable multifunctional, antimicrobial and antioxidant systems were rapidly promoted and investigated in some different application fields and also in food packaging sector as valid eco-friendly possibility to improve the safety and quality of food products. In this scenario, bio-based and/or biodegradable polymers were considered to be a promising solution to reduce and/or limit the environmental impact respect to the traditional plastics [1,2]. The scientific community and the growing interest of humans promoted, in fact, the development of new edible and eco-friendly packaging considered as valid alternatives to reduce wastes and residues [3]. However, bio-based/biodegradable polymers are usually characterized by some restrictions in terms of properties respect to the traditional polymers, that could be addressed by using a nanotechnological approach [4–6].

In the last decade, researchers focused their attention on the use of lignocellulosic by-products as reinforcing fillers in polymeric matrices. The revalorization of lignocellulosic materials was largely investigated as a valid alternative to extract nanoreinforcements completely natural and renewable as cellulose nanofibers, cellulose nanocrystals (CNC) and/or nanolignin [4]. The main constituents of plant structure are: cellulose, hemicelluloses and lignin. Cellulose is the major structural component of plant cell walls, responsible for mechanical strength, hemicellulose macromolecules are often repeated polymers of pentoses and hexoses, while lignin forms a protective seal around the other two components i.e., cellulose and hemicelluloses [7,8]. Cellulosic nanostructures, as i.e., cellulose nanocrystals (CNC), can be recovered from different lignocellulosic wastes (or materials of scarce value) by carrying out applying two step treatments. A first step permits to remove and to clean the cellulosic materials from different components as lignin, hemicelluloses and pectin [9]; the second step consists in the removal of the amorphous components to obtain a high crystalline structure, also at the nanoscaled level, particular useful in a nanocomposite approach.

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Every years, the plants need to be prune to increase their vigorous and splendor while, in the case of fruit plants, the prune is also necessary to improve the quality and the production of fruits. In Kiwi cultivation (*Actinidia deliciosa*) most of crop residues are produced by carrying out the usual pruning practice and the kiwi biomass produced annually by pruning was estimated to be of about 2.51 Mg DM ha^{-1} [10]. Moreover, the *Actinidia* cultivation, showed a growing trend and during the decade 2003–2012, the area under cultivation, excluding China, increased from just over 60,000 to almost 100,000 ha and Italy has become one of the world largest producer of kiwifruit [11].

In this research, the extraction of cellulose nanocrystals (CNC) from kiwi pruning stalks and their application in a nanocomposite approach was proposed for the first time. Nemli et al. previously investigated the suitability of kiwi (*Actinidia chinensis* Planch.) pruning as a raw material to use in the core layer of particleboards [12], while revalorization of pruning residuals from kiwi *Actinidia deliciosa* with and without bark was proposed by Gençer [13].

Here, CNC from kiwi pruning, were used, for the first time, as reinforcement phase in poly(vinyl alcohol) (PVA) and chitosan (CH) based blend. Cellulose nanocrystals are typically characterized by rigid rod monocrystalline domains with diameters ranging from 1 to 100 nm and the length from ten to hundreds of nm [14–18]. CH is a linear polysaccharide of randomly distributed β-(1–4)- linked D-glucosamine and *N*-acetyl-D-glucosamine, classified as Safe (GARS) by the US Food and Drug Administration (FDA) in 2001 [19–21]. CH is obtained from deacetylation of chitin; it is abundant in a variety of crustacean shells, such as crab shells, crawfish shells and shrimp shells [22]. In addition, chitosan presents bacteriostatic and fungistatic properties [23] that perfectly address the active packaging concept and the current trends and opinion regarding the need to inhibit pathogenic microbial activities in food products developing novel active/smart and intelligent packaging solutions [24]. Furthermore, with this objective, some different natural agents, essential oils and/or extracts, were recently involved in the production of biodegradable and eco-friendly packaging solutions [25–27] whereas carvacrol, an already studied antimicrobial additive in thermoplastic biodegradable based blends [28], was here considered as active ingredient and combined, for the first time, with CNC, in PVA_CH based films. Carvacrol is a phenolic compound extracted from oregano and thyme oil that possesses antioxidant and antimicrobial properties, and a particular aroma which makes it an attractive ingredient for certain types of foods [29–31].

In the present research, cellulose nanocrystals were extracted, for the first time of our knowledge, from kiwi pruning stalks by applying a bleaching treatment followed by an acidic hydrolysis and then used as reinforcement phases in water soluble polymers. Poly(vinyl alcohol) and chitosan (10%wt) were selected as polymer matrices while carvacrol was considered as antioxidant agent to improve the radical scavenging activities and antimicrobial response on the final produced packaging formulations. All produced PVA and PVA_10CH based systems were investigated in terms of optical, morphological, thermal and mechanical properties. Finally, functional characteristics of fundamental importance for food packaging sector in terms of antioxidant capacity, antimicrobial activity, overall migration and moisture content, were here deeply investigated to evaluate the effect of chitosan, carvacrol and cellulose nanocrystals on the final properties of PVA based systems.

2. Experimental part

2.1. Materials

Kiwi pruning wastes of *Actinidia deliciosa* were collected during winter pruning time from kiwifruit orchards in Central Italy, Lazio region (Cisterna di Latina, LT), the most important Italian kiwifruit area, where are cultivated around 8,000 ha of *Actinidia* spp.

All chemicals and reagents, including toluene, ethanol, sodium hydroxide (NaOH, reagent grade ≥98%), sodium chlorite ($NaClO_2$, puriss p.a. 80%), acetic acid (CH_3COOH) and sodium bisulfate ($NaHSO_4$, purum, anhydrous, 95.0%) were provided by Sigma-Aldrich® (Milan, Italy).

Polymer matrices, poly(vinyl alcohol) (PVA, average M_w 124–146 kg mol^{-1}, 99% hydrolyzed), and chitosan (CH) (viscosity: 200–800 cPs, degree of deacetylation:75-85%), were provided by Sigma-Aldrich® (Milan, Italy).

Carvacrol (Carv) with 98% purity was purchased from Sigma-Aldrich® (Milan, Italy) and it was selected as antimicrobial and antioxidant additive for the film formulations.

2.2. Chemical pre-treatment of kiwi pruning shoots

Kiwi pruning shoots (Fig. 1: Panel A-a and b) were firstly washed in distilled water several times (5 L of water for 150 g of wastes) in order to remove the powder and environmental contaminations. Subsequently, the pruning residues were treated for 72 h at room temperature (RT) with 1%w/v of NaOH solution (4 L for 150 g of wastes) and then for 2 h at 98 °C with 5%w/v of NaOH solution (2 L for 100 g) to remove the lignin components. The lignocellulosic materials were also mechanically retting and chopped in order to obtain individualized fibres (length around 10–15 mm). After the mechanical retting the fibres were dried at 60 °C for 24 h.

Furthermore, two different procedures were applied for the bleaching treatment in order to optimize this step. In the first case, the fibres were bleached one time with 5% wt/v of sodium chlorite ($NaClO_2$) (liquor ratio 1:50) while in the second case the fibres were treated two times with 0.7% wt/v of $NaClO_2$ (liquor ratio 1:50) [32]. The fibres during both the procedures were boiled for two hours and the solution pH was 4 by means acetic acid. At the end of bleaching treatments the materials were washed several times and treated with sodium bisulphate solution at 5% wt/v for 30 min at RT (liquor ratio 1:40), washed again with deionized water, treated with NaOH solution at 17.5% wt/v for 20 min at RT (liquor ratio 1:50) and then finally dried at 60 °C for 24 h.

2.3. Cellulose nanocrystal extraction

Cellulose nanocrystals (CNC) were prepared from bleached kiwi stalks (treated at both 5% wt/v or 0.7% wt/v of sodium chlorite $NaClO_2$) by means of acid hydrolysis (64% (wt/wt) sulphuric acid at 45 °C for 30 min) [33,34]. Cellulose suspension was centrifuged and dialyzed, prior to its addition for 48 h to a mixed bed ion exchange resin (Dowex Marathon MR-3 hydrogen and hydroxide form). The resultant cellulose nanocrystal aqueous suspension was ultrasonicated by means of a tip sonicator (Vibracell, 750) for 5 min. For both the applied procedures the final yield was calculated as% of initial weight of the used raw material.

2.4. Characterization of raw materials, bleached fibres and extracted cellulose nanocrystals

The morphological structure of kiwi pruning residues, pre-treated stalks and extracted cellulose nanocrystals were investigated by field emission scanning electron microscope (FESEM, Supra 25-Zeiss). The pruning, pre-treated and hydrolyzed materials were prepared following the procedure described by Fortunati and co-authors [35].

X-ray diffraction patterns of cellulose nanocrystals, obtained by means of the acidic hydrolysis of cellulosic materials bleached applying the two different pre-treatments (5% wt/v or 0.7% wt/v of sodium chlorite), were collected with a PANalytical X'PERT PRO