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Green microwave-assisted procedure to generate bio-based pectin materials

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ABSTRACT

New eco-friendly materials have been developed via esterification of several natural fatty acids by pectin alcoholic functions. The quaternization of the obtained pectin-fatty acid conjugates was performed as subsequent synthetic step. By using microwave heating and solvent-free conditions, the conjugation procedure of fatty acid chains and quaternary ammonium groups to the natural polysaccharide was clean, fast and efficient. In other words, it can be considered a low environmental impact process.

1. Introduction

The employment of new materials, endowed with antimicrobial activity in the field of agriculture, medical or food packaging, is always affected by their characteristics of being biodegradable and recyclable, which are considered issues of primary importance (Kenawy et al., 2007; Bordenave et al., 2010; Dutta et al., 2012).

In this regard, we have reported on a chemical modification protocol of the natural pectin, by introducing fatty acid chains on the polysaccharide backbone (Monfregola et al., 2011a, 2011b; Calce et al., 2012). Naturally occurring starting materials were employed and no dangerous waste was generated, since the reaction was performed under solvent-free conditions. The developed bio-based materials (pectin-linoleate, pectin-oleate, pectin-palmitate) were characterized by improved water resistance and barrier properties. Moreover, their bactericidal activity was also investigated, due to the presence of the natural fatty acid alkyl chains (Calce et al., 2014). In fact, the antibacterial activity of long-chain fatty acids are well known, even though the precise mode of action remains unclear. However, the bacterial cell membrane is supposed to be their primary target, and, as a consequence, all the biological processes that occur within and at the membrane. Anyway, within their broad spectrum of biological activity, natural fatty acid are able to inhibit the growth of several pathogenic bacteria.

Therefore, the developed biodegradable pectin derivatives were studied as antimicrobials against several strains, *S. aureus* and *E. coli*. As expected, good results were obtained for the pectin derivatives containing long-chain unsaturated fatty acids, including oleic acid and linoleic acid, while those containing long-chain saturated fatty acids, including palmitic acid, are less active (Calce et al., 2014).

Subsequently, to explore the antimicrobial potential of these new materials in the field of active food packaging, the pectin derivatives were coated on polyethylene films and assessed for their capacity to capture the oxygen molecule, reducing its penetration into the polymer support (Calce et al., 2014).

To further enhance their antimicrobial properties, we decided to introduce a quaternary ammonium (QA) group on these pectin-fatty acids conjugates. In fact, it has been reported that the quaternization of polysaccharides is an important mean to impart an increase of antibacterial activity (Sun et al., 2006; Yu et al., 2007; Song et al., 2008; Sajomsang et al., 2009; Fan et al., 2012; Huang et al., 2013; Mohamed et al., 2013).

The positively charged quaternary ammonium compounds (QAC) are supposed to interact with the negative charge of the cell surface. By this electrostatic interaction process, the long alkylic chains can penetrate through the cell membrane, thus exerting their bactericidal activity. This disruptive penetrating process is enhanced in case the alkyl chains cooperate with the biopolymer backbone, thus tearing the cytoplasmic membrane and causing the leaking of the cell constituents.

Herein, we report on a chemical process for introducing two different chemical modifications, fatty acids and quaternary(alkyl) ammonium moieties, on the pectin. The aim is to develop a green procedure to prepare antimicrobial biopolymers that can readily decompose, thus the conjugation reactions of fatty acids and ammonium groups to the natural polysaccharide can represent an environmental friendly synthetic protocol.

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2. Material and methods

2.1. General

The apple peel Pectin on a dry basis, with high molecular weight (30000–100000 g/mol) and high degree of esterification (70–75%), was purchased from Fluka. Oleic acid, linoleic acid and palmitic acid, N-3-chloro-2-hydroxypropyltrimethylammoniumchloride solution (CHPTAC), all other solvents and reagents were purchased from Sigma-Aldrich.

2.2. Synthesis of pectin-fatty acids conjugates

Fatty acid symmetrical anhydrides were synthesized as follows. A solution of the appropriate fatty acid (10 mmol) in dichloromethane (2 mL) was placed into a 10 mL round bottom flask. The solution was cooled in an ice-water bath and stirred vigorously under argon atmosphere. The dicyclohexylcarbodiimide (5 mmol), previously dissolved in the minimum volume of dichloromethane, was added dropwise and the stirring was continued at ice bath temperature for 2 h. The white solid *N,N'*-dicyclohexylurea was removed by filtration and the solvent was evaporated in vacuum to give the final anhydride (Monfregola et al., 2011a, 2011b).

To obtain the pectin-fatty acids conjugates, 30 mg of pectin were manually milled, in an agate mortar, with 30 mg of the appropriate fatty acid anhydride in the presence of catalytic amount of K_2CO_3. Subsequently, the samples were irradiated in 10 mL tubes in a microwave oven (CEM Discover®) under the following conditions:

–160 °C for 5 min (Power max: 280 W, Pmax: 250 psi).
–180 °C for 3 min (Power max: 280 W, Pmax: 250 psi).
–180 °C for 5 min (Power max: 280 W, Pmax: 250 psi).
–200 °C for 3 min (Power max: 280 W, Pmax: 250 psi).
–220 °C for 2 min (Power max: 280 W, Pmax: 250 psi).

To remove the unreacted fatty acids, the solids obtained were dissolved in water, placed in a 250 mL separatory funnel and extracted with ethyl acetate. The aqueous layers were neutralized by adding 0.5 N HCl aqueous solution, dialyzed (membrane cut off 6000–8000) for 1 day in Milli-Q water and lyophilized to collect the final products. The final yields, after purification steps, was never lower that 40%.

2.3. Synthesis of quaternized pectin-fatty acids conjugates

To a solution of CHPTAC, 60% wt in water, an equimolar amount of NaOH was added and the obtained mixture was kept under stirring for 5–6 h at 50 °C. After lyophilization, the obtained epoxide was manually milled with 10 mg of the pectin-fatty acid conjugates in the presence of catalytic amount of K_2CO_3.

The samples, placed in a 10 mL microwave vial, were irradiated in a microwave oven for 3 min at 160 °C (Power max: 280 W, Pmax: 250 psi). The crude products obtained were dissolved in water, neutralized by adding 0.5 N HCl aqueous solution and then dialyzed (membrane cut off 6000–8000) for 1 day in Milli-Q water. The final products were collected after lyophilization process. The final yields, after purification steps, were around 20–30%.

2.4. NMR characterization

1D [^{1}H] NMR spectra were recorded for pectin-oleate and pectin-linoleate in the temperature range 298–303 K either on a Varian Unity Inova 400 MHz NMR spectrometer equipped with z-axis pulsed-field gradients. For sample preparation, the compounds were dissolved in 600 μL of D_2O (99.9% D, Sigma-Aldrich, Milan, Italy). 1D [^{1}H] spectra were acquired with a relaxation delay of 2 s and 512–2048 scans, without water suppression and processed with Varian software (VNMRJ). The water signal was set at 4.75 ppm for proton chemical shifts referencing.

2.5. FT-IR characterization

The FT-IR spectra of the pectin derivatives were recorded on a Jasco FT/IR 4100spectrometer. For sample preparation, the powder compounds and KBr were very finely ground in an agate mortar and pressed to obtain a disc. The characteristic peaks of IR transmission spectra were recorded at a resolution of 4 cm^{-1}over a wavenumber region of 400–4000 cm^{-1}.

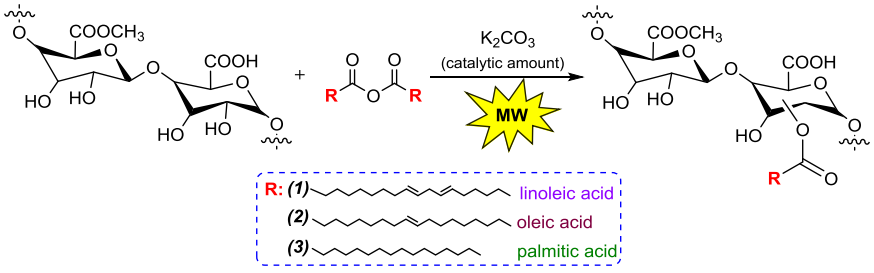
The bands relevant for the structural organization are: pectin-linoleate (a): FT-IR (cm^{-1}): 3421 ν (O–H), 2927 and 2847 ν (C–H), 1744 ν (C=O esters), 1641 ν_{as} (COO^-), 1443 ν_s (COO^-), 1147 ν (C–O); pectin-oleate (b): FTIR (cm^{-1}): 3428 ν (O–H), 2930 and 2851 ν (C–H), 1744 (C=O esters), 1641 ν_{as} (COO^-), 1445 ν_s (COO^-), 1151 ν (C–O); and pectin-palmitate (c): FTIR (cm^{-1}): 3418 ν (O–H), 2923 and 2847 ν (C–H), 1744 ν (C=O esters), 1642 ν_{as} (COO^-), 1444 ν_s (COO^-), 1150 ν (C–O).

3. Results and discussion

The introduction of natural fatty acid chains on the pectin backbone, which consists of primarily 1,4-α-D-galacturonosyl units and their methyl esters, interrupted in places by 1,2-α-L-rhamnopyranosyl units, was obtained via ester bond formation with the polysaccharide alcoholic functions.

In major details, oleic, linoleic and palmitic acids were reacted as anhydride and milled in an agate mortar with the polysaccharide and the inorganic base K_2CO_3, used in catalytic amount. The reaction was performed in neat conditions (Scheme 1) and the mixture was irradiated with microwaves in a CEM Discovery oven for organic synthesis. Concerning with the reaction conditions, a specific temperature value was selected, leaving the microwave irradiation power as unconstrained parameter. To explore the range of temperatures, we took as a reference the oil bath value (T=160 °C, t=15 min), which is characteristic of the conventional heating. In fact, it is worth noting that the reaction was performed under solvent-free conditions, as consequence the solvent temperature could not be taken as a reference for the microwave oven setting (Kappe, 2004). The reaction parameters, that allowed the preparation of the pectin-fatty acid conjugates, were the following: T=180 °C and t=3–5 min. A reduction of the pectin degree of methylation was observed at temperatures higher than 180 °C.

It is proven that MW dielectric heating, by its direct interaction with



Scheme 1. Synthetic strategy of pectin functionalized with fatty acids.