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Antioxidant–polysaccharide conjugates for food application by eco-friendly grafting procedure

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ABSTRACT

In the present study, catechin–alginate and catechin–inulin conjugates were obtained by adopting free radical-induced grafting procedure. In the aim to synthesize the antioxidant–polysaccharide conjugates, ascorbic acid/hydrogen peroxide redox pair was employed as water-soluble and biocompatible initiator system. The insertion of catechin onto the polymeric backbones was verified by performing FT-IR, DSC and fluorescence analyses and molecular weight distributions were analyzed by GPC. Finally, the antioxidant properties of the obtained conjugates were evaluated and compared with that of blank alginate and blank inulin, treated in the same conditions but in absence of catechin. The good antioxidant activity showed by functionalized materials confirmed the efficiency of the adopted reaction method to impart peculiar characteristics to macromolecules of natural origin for specific industrial applications. This kind of systems, indeed, could be very useful in the optimization of food preservation and to help manufacturers in elaboration of new food products and packaging.

1. Introduction

Grafting represents a method of polymeric materials modification (Contreras-García, Burillo, Aliev, & Bucio, 2008) that allows to improve the properties of natural or synthetic polymers. The conventional grafting polymerization technique requires, indeed, the introduction of chemical groups to impart new characteristics, to the modified polymers, for different applications (Dergunov et al., 2008; Joung, Choi, Bae, & Park, 2008; Shin, Lee, & Park, 2008). It is well known that for some specific polymeric products, especially medical equipment and food packaging, sterilization via radiation is needed with a potential risk of degradation, i.e., chain scission and/or cross-linking, resulting in discolouration, cracking of the surface, stiffening, and loss of mechanical properties (Jahan & McKinny, 1999). Natural polysaccharides, such as alginate and inulin are widely employed in industry due to their biocompatibility, biodegradation, non-toxicity and non-immunogenicity. The grafting of antioxidant molecules onto a polysaccharidic structure allows to obtain new functionalized materials characterized by the properties of both grafted molecule and natural polymer (Curcio et al., 2009).

Alginates and their derivatives are widely used in food and beverage industry, as thickening agents, gelling agents and colloidal stabilizers, in cosmetic, drug delivery (Babu, Sairam, Hosamani, & Aminabhavi, 2007; Pongjanyakul & Puttipipatkhachorn, 2007) and agriculture applications (Mishra, Bajpai, & Bajpai, 2004; Puoci et al., 2008a). In literature, many studies report on the use of alginate coatings in packaging technology to improve quality and shelf life of food (Lu, Liu, Ye, Wei, & Liu, 2009; Oms-Oliu, Soliva-Fortuny, & Martín-Belloso, 2008). Alginates represent a class of linear unbranched polysaccharides which contain varying amounts of 1,4′-linked β-D-mannuronic acid and α-L-guluronic acid residues (Fig. 1(a)). The residues may vary widely in composition and sequence and are arranged in a pattern of blocks along the chain. These homopolymeric regions of β-D-mannuronic acid blocks and α-L-guluronic acid blocks are interdispersed with regions of alternating structure (β-D-mannuronic acid–α-L-guluronic acid blocks) (Haug & Larsen, 1962; Haug, Larsen, & Smidsrod, 1967). The physical properties of the alginates are determined by the composition and the extent of the sequences and by the molecular weight. The molecular variability is dependent on the organism and tissue from which the alginates are isolated; generally, these polysaccharides are obtained mainly from brown algas belonging to the *Phaeophyceae*. Alginate is a renewable, biocompatible (Klöck et al., 1997) and biodegradable natural polymer that is used in a variety of commercial applications because of its capacity to form hydrogels. In the presence of multivalent cations, indeed, ionic interactions between the carboxylic acid groups sited on the polymer backbone and the chelating cations are established (Grant, Morris, Rees, Smith, & Thom, 1973).

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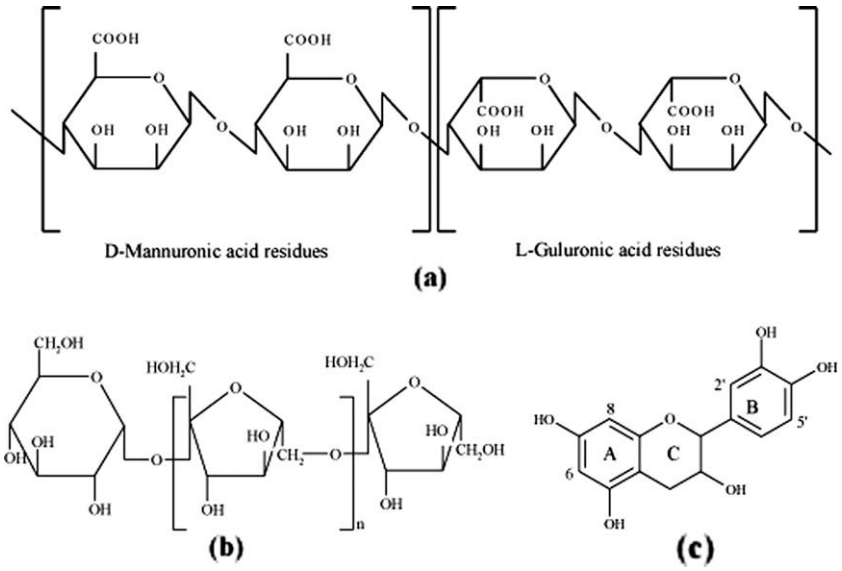

Fig. 1. Chemical structures of alginate (a), inulin (b) and (+)-catechin (c).

Inulin is a dietary fiber composed of a mixture of oligo- and/or polysaccharides consist of fructose unit chains (linked by (2 → 1)-β-D-fructosyl-fructose bonds) of various length, terminated generally by a single glucose unit (linked by an α-D-glucopyranosyl bond) (Fig. 1(b)) (Roberfroid & Delzenne, 1998). This natural polymer is widely distributed in some edible plants including asparagus, garlic, chicory, leek, onion and artichoke as storage carbohydrates (Kaur & Gupta, 2002). Inulin is a prebiotic, a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microbiota, that confers benefits upon host well-being and health (Gibson, Probert, Van Loo, Rastall, & Roberfroid, 2004). It is well known that the colonic microflora exercises a relevant influence on human health (Steer, Carpenter, Tuohy, & Gibson, 2000). Consequently, there is a great interest in the use of prebiotics, such as inulin, as functional food ingredients to influence the composition of colonic microflora in order to improve health (Aryana & McGrew, 2007; Coppa, Zampini, Galeazzi, & Gabrielli, 2006; Losada & Olleros, 2002; Manning & Gibson, 2004; Rao, 2001; Rousseau, Lepargneur, Roques, Remaud-Simeon, & Paul, 2005). The use of inulin in food formulations as fiber ingredient is straightforward and often leads to significantly improved organoleptic characteristics, such as taste and mouthfeel, and texture. Prebiotics show both important technological characteristics and interesting nutritional properties (Chow, 2002; Huebner, Wehling, & Hutkins, 2007) and, to serve as functional food ingredients, they must be chemically stable to food processing treatments such as heat. The insertion of a biocompatible antioxidant agent onto the structure of a prebiotic could be interesting to improve the stability of this kind of food ingredients.

In the aim to improve the antioxidant properties of polysaccharides, our challenge was to covalently bind (+)-catechin (Fig. 1(c)) to alginate and inulin. The antioxidant–polysaccharide conjugates were synthesized by free radical-induced grafting procedure employing ascorbic acid/hydrogen peroxide redox pair as water-soluble and biocompatible initiator system.

Catechins are one of main classes of flavonoids and present in large amounts in green and black tea, in red wine, chocolate, fruits, etc. They are potentially beneficial to human health as they are strong antioxidants, anti-carcinogens, antiinflammatory agents, and inhibitors of platelet aggregation in *in vivo* and *in vitro* studies (Mizugaki, Ishizawa, Yamazaki, & Hishinuma, 2003).

The synthesized antioxidant–polysaccharide conjugates were characterized by FT-IR, DSC and fluorescence analyses, molecular weight distributions were analyzed by GPC. Antioxidant activity of conjugated polymers was tested and both functionalized macromolecules were able to interact with free radical species and to minimize the oxidative damage. The obtained results make the synthesized functionalized polymers useful materials in the optimization of food preservation and to help manufacturers in elaboration of new food products and packaging.

2. Experimental

2.1. Materials

Alginic acid sodium salt (MW ~ 50.000), inulin from Dahlia tubers (MW ~ 5.000), (+)-catechin hydrate (CA), hydrogen peroxide (H_2O_2), ascorbic acid (AA), 2,2′-diphenyl-1-picrylhydrazyl radical (DPPH), Folin–Ciocalteu reagent, sodium carbonate, β-Carotene, linoleic acid, Tween 20, sodium nitrite, aluminium chloride hexahydrate, sodium hydroxide and orthophosphoric acid were obtained from Sigma–Aldrich (Sigma Chemical Co., St Louis, MO, USA).

Ethanol, methanol, water and chloroform were reagent grade or HPLC-grade and provided by Carlo Erba reagents (Milan, Italy).

2.2. Instrumentation

The liquid chromatography consisted of an Jasco BIP-I pump and Jasco UVDEC-100-V detector set at 210 nm. A 250 mm × 4 mm C-18 Hibar® Column, particle size 5 μm (Merck, Darmstadt, Germany) was employed. As reported in literature (Wang, Helliwell, & You, 2000), the adopted mobile phase was methanol/water/orthophosphoric acid (20/79.9/0.1) and run isocratically at a flow rate of 1.0 ml min^{-1}. The column was operated at 30 °C. The sample injection volume was 20 μl.

Freeze drier Micro Modulyo, Edwards was employed. IR-spectra were recorded as films or KBr pellets on a Jasco FT-IR 4200.

The corrected emission spectra, all confirmed by excitation ones, were recorded with a Perkin Elmer LS 50B spectrofluorimeter, equipped with Hamamatsu R928 photomultiplier tube.

Calorimetric analyses were performed using a Netzsch DSC200 PC. In a standard procedure about 6.0 mg of sample was placed inside a hermetic aluminum pan, and then sealed tightly by a hermetic aluminum lid. The thermal analyses were performed from 25 to 400 °C under a dry nitrogen atmosphere with a flow rate of 25 ml min^{-1} and heating rate 5 °C min^{-1}.

Molecular weight distributions of synthesized polymers were analyzed by a GPC system composed of: μBondagel E-125 and E-500 GPC columns (Millipore, Water Associates) connected in series; a Jasco PU-2080 Plus liquid chromatography equipped with a Rheodyne 7725i injector (fitted with a 20 μl loop). The mobile phase employed was phosphate buffer (pH 7.4) at a rate of 0.8 ml min^{-1}. An Agilent ELSD 1200 Light Scattering Detector and Jasco-Borwin integrator were employed: nitrogen flow 3.8 atm; temperature 40 °C.

2.3. Synthesis of grafted antioxidant–polysaccharide conjugates

The synthesis of catechin–alginate **(I)** and catechin–inulin **(II)** conjugates, by employing ascorbic acid/hydrogen peroxide redox pair as initiator system, was carried out as follows: in a 50 ml glass flask, 1.5 g of each polysaccharide were dissolved in 37.5 ml of distilled water, then 12.5 ml of H_2O_2 (120 v) and 0.4 g of ascorbic acid were added. The mixture was maintained at 25 °C under atmospheric air and, after 2 h, 0.1 g of the antioxidant molecule were introduced in the reaction flask. The mixture was maintained at 25 °C for other 24 h under atmospheric air. The obtained polymer solution was introduced into dialysis tubes (MWCO: 12-14000 Dalton; MWCO: 3500 Dalton) and dipped into a glass vessel containing distilled water at 20 °C for 48 h with eight changes of water. The conjugate was checked to be free of unreacted antioxidant and any other compounds by HPLC analysis after purification step.

The resulting solution was frozen and dried with "freezing–drying apparatus" to afford a vaporous solid. Blank alginate and blank inulin, that act as control, were prepared in the same conditions but in the absence of antioxidant agent.

2.4. Evaluation of the antioxidant activity

2.4.1. Determination of scavenging activity on DPPH radicals

In order to evaluate the free radical scavenging properties of **(I)** and **(II)** conjugates, their reactivity towards a stable free radical, 2,2′-diphenyl-1-picrylhydrazyl radical (DPPH), was evaluated according to the literature with some modifications (Spizzirri et al., 2009). For this purpose, 25 mg of each polymer were dissolved in 1 ml of distilled water in a volumetric flask (25 ml) and then 4 ml of ethanol and 5 ml of ethanol solution of DPPH (200 μM) were added, obtaining a solution of DPPH with a final concentration of 100 μM. The sample was incubated in a water bath at 25 °C and, after 24 h, the absorbance of the remaining DPPH was determined colorimetrically at 517 nm. The same reaction conditions were applied on the blank polymers in order to evaluate the interference of polymeric material on DPPH assay. The scavenging activity of the tested conjugates was measured as the decrease in absorbance of the DPPH and it was expressed as percent inhibition of DPPH radicals calculated according the following Eq. (1):

$$\text{inhibition } \% = \frac{A_0 - A_1}{A_0} \times 100 \quad (1)$$

where A_0 is the absorbance of a standard that was prepared in the same conditions, but without any polymers, and A_1 is the absorbance of polymeric samples. Each measurement was carried out in triplicate and data expressed as means (±SEM).

2.4.2. Evaluation of disposable phenolic groups by Folin–Ciocalteu procedure

Amount of total phenolic equivalents was determined using Folin–Ciocalteu reagent procedure, according to the literature with some modifications (Parisi et al., 2009).

Twenty milligrams of **(I)** and **(II)** conjugates were dissolved in distilled water (6 ml) in a volumetric flask. Folin–Ciocalteu reagent (1 ml) was added and the contents of flask were mixed thoroughly. After 3 min, 3 ml of Na_2CO_3 (2%) were added, and then the mixture was allowed to stand for 24 h with intermittent shaking.

The absorbance was measured at 760 nm against a control prepared using the blank polymers under the same reaction conditions. The amount of total phenolic groups in functionalized materials was expressed as catechin equivalent concentrations by using the equation obtained from the calibration curve of the antioxidant molecule. This one was recorded by employing five different catechin standard solutions. 0.5 ml of each solution were added to the Folin–Ciocalteu system to raise the final concentration of 8.0, 16.0, 24.0, 32.0, and 40.0 μM, respectively. After 24 h, the absorbance of the solutions was measured to record the calibration curve and the correlation coefficient (R^2), slope and intercept of the regression equation obtained were calculated by the method of least square.

2.4.3. β-Carotene–linoleic acid assay

The antioxidant properties of synthesized conjugates were also evaluated through measurement of percent inhibition of peroxidation in linoleic acid system by using the β-carotene bleaching test (Conforti, Statti, Uzunov, & Menichini, 2006).

Briefly, 1.0 ml of β-carotene solution (0.2 mg/ml in chloroform) was added to 0.02 ml of linoleic acid and 0.2 ml of Tween 20. The mixture was then evaporated at 40 °C for 10 min in a rotary evaporator to remove chloroform. After evaporation, the mixture was immediately diluted with 100 ml of distilled water. The water was added slowly to the mixture and agitated vigorously to form an emulsion. The emulsion (5.0 ml) was transferred to different test tubes containing 50.0 mg of **(I)** and **(II)** conjugates dispersed in 0.2 ml of 70% ethanol, and 0.2 ml of 70% ethanol in 5.0 ml of the above emulsion was used as a control. The tubes were then gently shaken and placed in a water bath at 45 °C for 60 min.

The absorbance of the filtered samples and control was measured at 470 nm against a blank, consisting of an emulsion without β-carotene. The measurement was carried out at the initial time ($t = 0$) and successively at 60 min. The same reaction conditions were applied for the blank polymers in order to evaluate the interference of the ungrafted polysaccharides on β-carotene bleaching test.

The antioxidant activity ($A_{ox}A$) was measured in terms of successful bleaching of β-carotene using the following Eq. (2):

$$A_{ox}A = 1 - \frac{A_0 - A_{60}}{A_0^o - A_{60}^o} \quad (2)$$

where A_0 and A_0^o are the absorbance values measured at the initial incubation time for samples and control, respectively, while A_{60} and A_{60}^o are the absorbance values measure in the samples and control respectively at $t = 60$ min. All samples were assayed in triplicate and data expressed as means.

2.4.4. Determination of total flavonoid content

A slightly modified version of the spectrophotometric method was used to determine the flavonoid contents of samples (Dewanto, Wu, Adom, & Liu, 2002).

Briefly, in a test tube 20.0 mg of **(I)** and **(II)** conjugates were dissolved in 2.0 ml of distilled water followed by addition of 150 μl of a 5% $NaNO_2$ solution. After 6 min, 300 μl of a 6% $AlCl_3{\cdot}6H_2O$ solution

were added and allowed to stand for another 5 min before 1.0 ml of 1 M NaOH was added. The mixture was brought to 5.0 ml with distilled water and mixed well. The absorbance was measured immediately at 510 nm against a control prepared using the blank polymers under the same reaction conditions.

The amount of total flavonoids in the conjugates was expressed as mean (micrograms of catechin equivalents per gram of polymer) ±SD for five replications, by using the equation obtained from the calibration curve of the antioxidant. This one was recorded by employing five different catechin standard solutions with the same procedure. The final concentrations of catechin in the test tubes were 10, 25, 50, 75, 100 μM, respectively.

3. Results and discussion

3.1. Synthesis of grafted antioxidant–polysaccharide conjugates

Antioxidant–polysaccharide conjugates were synthesized by free radical-induced grafting procedure employing ascorbic acid/hydrogen peroxide redox pair as initiator system.

Alginate and inulin, natural polysaccharides widely used in different fields such as pharmaceutical, cosmetic and food, were chosen as preformed polymeric backbones because of their biocompatibility and biodegradability. In order to obtain conjugates with antioxidant properties, (+)-catechin was covalently bound to the polysaccharides.

For this purpose, ascorbic acid/hydrogen peroxide redox pair was employed as biocompatible and water-soluble initiator system. Comparing to conventional initiator systems (i.e. azo compounds and peroxides), which require relatively high polymerization temperature to ensure their rapid decomposition, redox initiators show several advantages. It is possible, indeed, to perform polymerization processes at lower temperatures reducing the risks of CA degradation; moreover, the generation of any kind of toxic reaction products is avoided.

In Fig. 2 is shown the interaction mechanism of redox reagents involving the oxidation of ascorbic acid by H_2O_2 at room temperature with the formation of hydroxyl radical and ascorbate radical intermediates that initiate the reaction (Kitagawa & Tokiwa, 2006; Puoci et al., 2008b).

The synthetic strategy involved in two steps: the first one allowed to activate polysaccharidic chains towards radical reaction; the second one to covalently bind the antioxidant molecule to preformed macroradical. In Fig. 3 a possible mechanism of reaction is proposed. The hydroxyl radicals, generated by the initiator system, abstract H-atoms from the hydroxyl groups of the polysaccharides (STEP 1) with consequent formation of free-radical sites and the insertion of CA onto the biomacromolecules can occur (STEP 2) (Toti & Aminabhavi, 2004).

The reactivity of CA towards free radical reaction can be explained on the basis of its chemical structure characterized by the presence of phenolic groups. In literature many studies report on the phenolic group compatibility with this kind of polymerization. Monomers with active functional groups (phenolic groups) as side substituents, indeed, were used for the preparation of grafted polymeric systems (Nanjundan, Selvamalar, & Jayakumar, 2004) using free radical initiators. On the other side, phenolic group could be directly involved in polymerization process; it is reported, indeed, that phenolic radical undergoes in dimerization processes by reaction between hydroxyl radical and aromatic ring in the ortho- or para-position relatively to the hydroxyl group (Uyama, Maruichi, Tonami, & Kobayashi, 2002). On the basis of these data, it can be reasonably hypotesized that the insertion of antioxidant agent on the polysaccharidic chains occurs in position 2′, 5′ (B ring) and 6, 8 (A ring) for catechin (Fig. 1(C)).

In the prepolymerization feed, a ratio 1/15 w/w between CA and polysaccharide was chosen because it represents the optimal value to obtain the conjugates with the highest efficiency. A lower amount of CA in the reaction feed is not enough to obtain a material with significant antioxidant properties. On the other hand, an higher amount carries out to a material with no improvement in antioxidant property.

In the aim to remove unreacted CA, physically incorporated in the polysaccharidic structure, the conjugates underwent dialysis process (MWCO: 12-14000 Dalton; MWCO: 3500 Dalton) and washing media were analyzed by HPLC. Finally, the resulting solution of grafted polysaccharide was frozen and dried with freeze-drier to obtain a porous and vaporous solid extensively characterized by FT-IR, fluorescence and calorimetric analyses. Blank alginate and blank inulin were prepared in the same conditions, but in the absence of antioxidant agent, to act as control in the test antioxidant tests.

3.2. Characterization of antioxidant–polysaccharide conjugates

In order to verify the covalent insertion of CA onto the polymeric backbones, the functionalized materials and the respective control polymers were characterized by Fourier Transform IR spectrophotometry, DSC and fluorescence analyses.

IR-spectra of both **(I)** and **(II)** conjugates shown the appearance of new peaks at 1557 and at 1525 cm^{-1}, respectively, awardable to carbon to carbon stretching within the aromatic ring of catechin.

The emission spectra of free antioxidant and conjugates also confirm the covalent functionalization of the polysaccharides. In the spectra of conjugates **(I)** and **(II)**, bathochromic shifts of the emission peaks of CA from 318 to 346 nm and from 318 to 404 nm respectively, are detected (Fig. 4). These spectral red shifts are due to the covalent conjugation, because no emission peak is detected in the same wavelength range for blank alginate and blank inulin.

Thermal characterization of synthesized conjugates was also performed by recording of DSC thermograms of blank sugars (a), dried grafted polysaccharides (b) and pure antioxidant (c), as depicted in Figs. 5 and 6, for **(I)** and **(II)**, respectively.

As far as DSC of pure alginate is concerned, a broad endothermic peak, located around 50–120 °C, was recorded; ΔH_t associated to this transition was −65.93 J per grams of blank polysaccharide

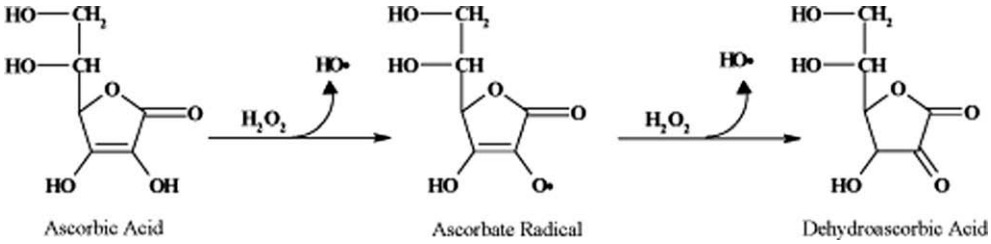

Fig. 2. Interaction between ascorbic acid and hydrogen peroxide.

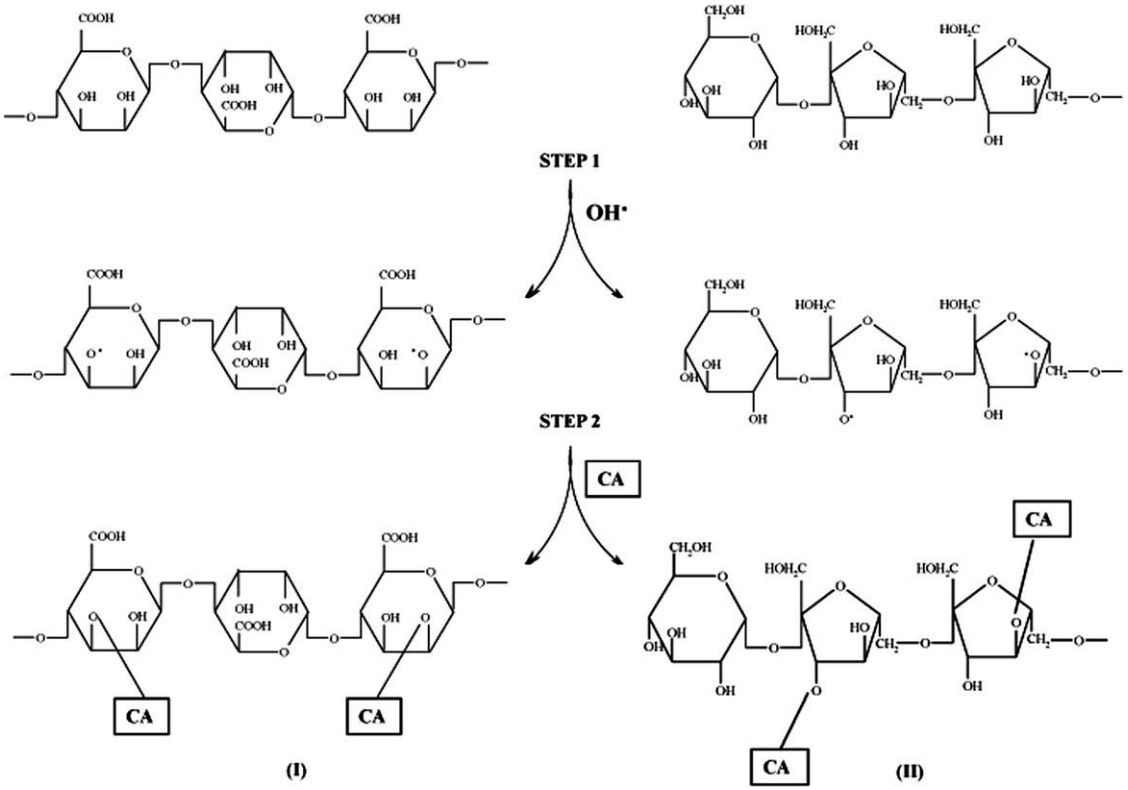

Fig. 3. Insertion of CA onto alginate **(I)** and inulin **(II)** backbones.

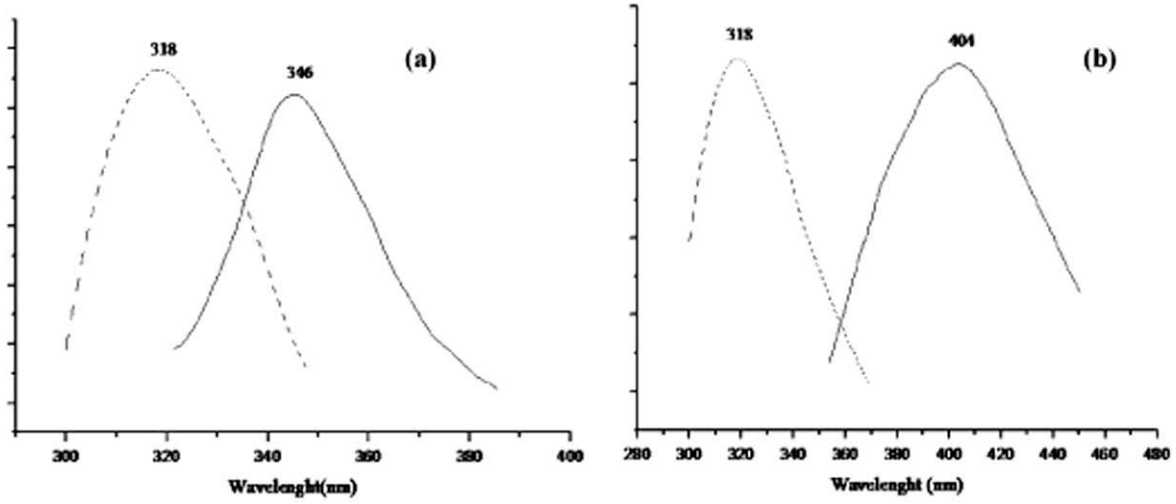

Fig. 4. (a) Emission spectra of CA (—) and conjugate **(I)** (—); (b) emission spectra of CA (—) and conjugate **(II)** (—).

(Fig. 5(a)). The calorimetric analysis of pure CA shows a melting endotherm at 155.8 °C (Fig. 5(c)). Since the grafting of CA produces structural modification onto the polysaccharide chains, in the DSC thermogram of conjugate **(I)** (Fig. 5(b)) marked differences appear. The calorimetric analysis displays the absence of melting endotherm of CA, while, the ΔH_t value associated to the glass transition in the conjugate was −207.2 J per grams of grafted polysaccharide, probably as consequence of more rigidity of polymeric chains. This discrepancy suggests that glass transition conjugate **(I)** needs 214% more heat respect to unmodified polysaccharide.

The same results were observed in the DSC thermogram of catechin–inulin conjugate **(II)** (Fig. 6(b)), where the endotherm due to melting of CA disappears, while the endotherms at 100–125 and 170–250 °C show a ΔH_t equal to −57.52 and −381.13 J per grams of grafted polysaccharides, respectively; the same endothermic peaks in the blank inulin (Fig. 6(a)) were recorded at 110–125 °C (ΔH_t equal to −54.21 per grams of grafted inulin) and 170–225 °C (ΔH_t equal to −289.15 J per grams of blank polysaccharide). In this case to produce glass transition in the conjugate **(II)** about 6% and 32% respectively more heat is necessary respect to unmodified polymer. Thus, the conjugation of polysaccharides with CA causes an increasing in the thermal stability of the native sugar.

Finally, molecular weight distributions of obtained conjugates were analyzed by a GPC instrument equipped with a light scattering

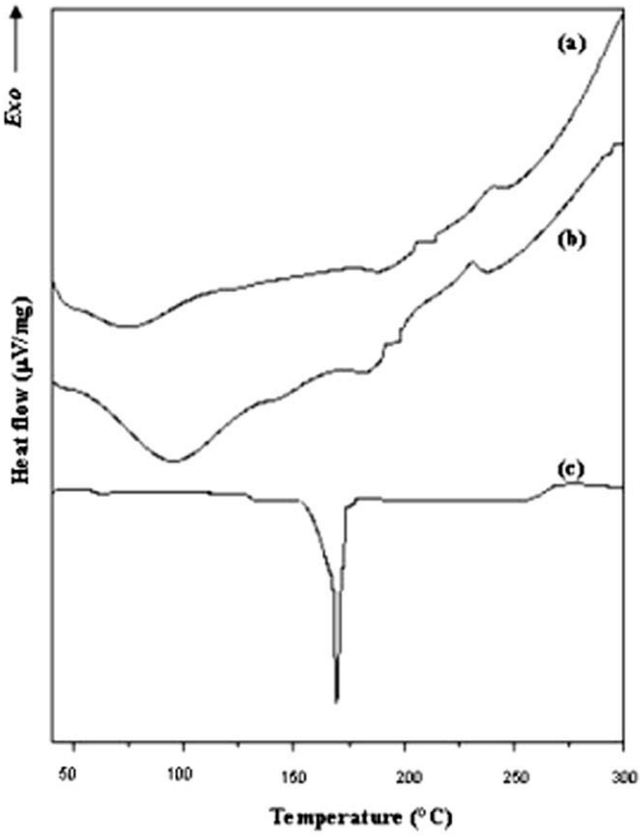

Fig. 5. DSC of blank alginate (a), conjugate **(I)** (b) and CA (c).

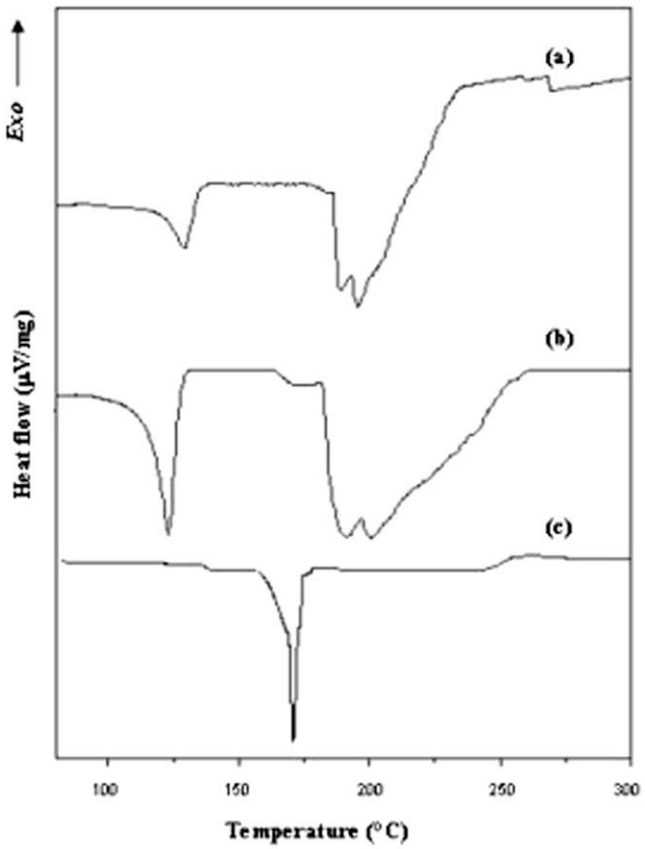

Fig. 6. DSC of blank inulin (a), conjugate **(II)** (b) and CA (c).

detector, and the results show no relevant changes in the molecular weight distribution of commercial, blank and conjugate polysaccharides (Fig. 7).

3.3. Evaluation of the antioxidant activity

3.3.1. Determination of scavenging activity on DPPH radicals

The DPPH radical is a stable organic free radical with an absorption maximum band around 515–528 nm and thus, it is a useful reagent for evaluation of antioxidant properties of compounds.

In the DPPH assay, the antioxidants reduce the DPPH radical to a yellow-colored compound, diphenylpicrylhydrazine, and the extent of the reaction will depend on the hydrogen donating ability of the antioxidants. It has been documented that cysteine, glutathione, ascorbic acid, tocopherol, polyhydroxy aromatic compounds (e.g., ferulic acid, hydroquinone, pyrogallol, gallic acid), reduce and decolorize 1,1-diphenyl-2-picrylhydrazine by their hydrogen donating capabilities (Spizzirri et al., 2009).

Conjugates scavenger ability were evaluated in term of DPPH reduction using catechin as reference compound and data are expressed as inhibition (%). As reported in Table 1, in our operating conditions, antioxidant polymers showed high scavenging activity. In particular, DPPH reductions of 61% and of 74% were recorded for conjugate **(I)** and **(II)** respectively.

3.3.2. Evaluation of disposable phenolic groups by Folin–Ciocalteu procedure

Since the antioxidant activity of conjugates **(I)** and **(II)** is derived from phenolic groups in the polymeric backbone, it is useful express the antioxidant potential in terms of phenolic content. The Folin–Ciocalteu phenol reagent is used to obtain a crude estimate of the amount of disposable phenolic groups present in polymer chain. Phenolic compounds undergo a complex redox reaction with phosphotungstic and phosphomolybdic acids present in the Folin–Ciocalteu reactant. The colour development is due to the transfer of electrons at basic pH to reduce the phosphomolybdic/phosphotungstic acid complexes to form chromogens in which the metals have lower valence (Parisi et al., 2009).

For each grafted polymer, disposable phenolic groups were expressed as mg equivalent of CA by comparing the obtained data with the CA calibration curve. In particular, for conjugates **(I)** and **(II)** these values were 0.53 and 1.27 mg/g of dry polymers, respectively.

3.3.3. β-Carotene–linoleic acid assay

In this model system, β-carotene undergoes rapid discoloration in the absence of an antioxidant, which results in a reduction in absorbance of the test solution with reaction time (Amin, Zamaliah, & Chin, 2004). This is due to the oxidation of linoleic acid that generates free radicals (lipid hydroperoxides, conjugated dienes and volatile byproducts) that attack the highly unsaturated β-carotene molecules in an effort to reacquire a hydrogen atom. When this reaction occurs, the β-carotene molecule looses its conjugation and, as a consequence, the characteristic orange colour disappears. The presence of an antioxidant avoids the destruction of the β-carotene and the orange colour is maintained. Also in this case, good antioxidant activities for conjugates **(I)** and **(II)** were recorded, with inhibition percentages of lipidic peroxidation equal to 50% and 60%, respectively (Table 1).

3.3.4. Determination of total flavonoid content

It has frequently been reported that both phenolic and flavonoids compounds are closely associated with antioxidant activity

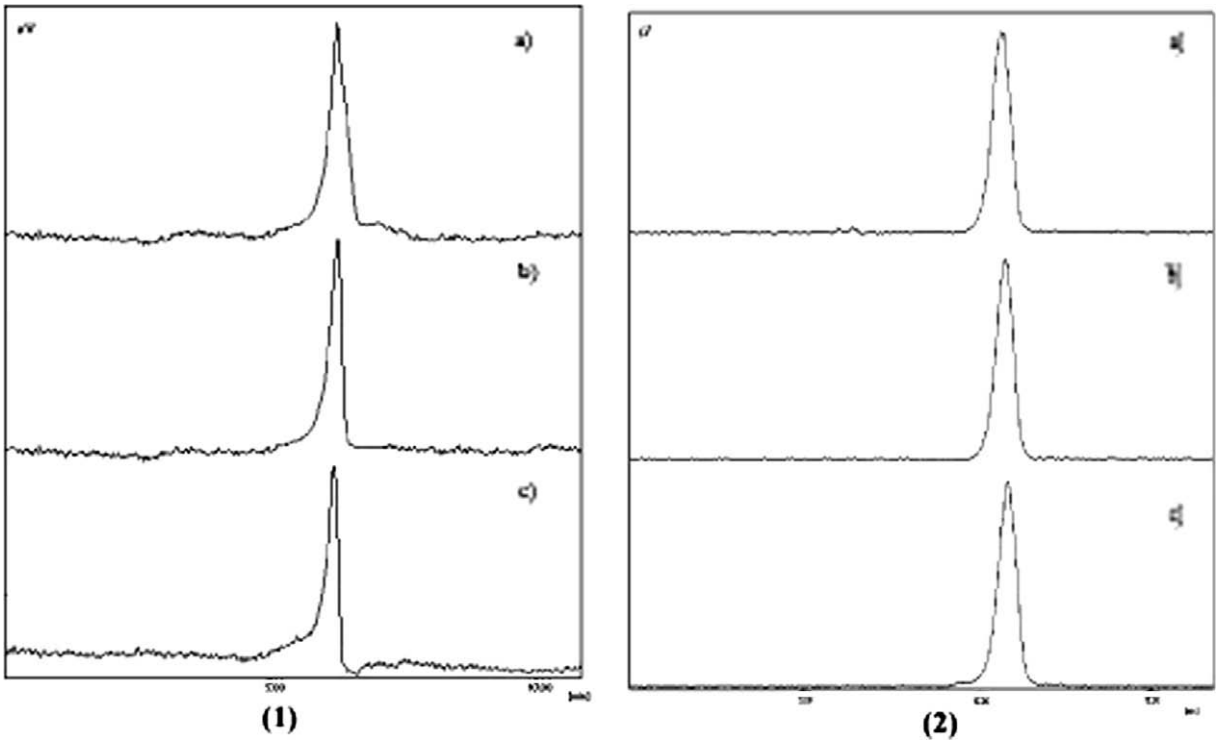

Fig. 7. (1) GPC of native (a), blank (b) and conjugate **(I)** (c); (2) GPC of native (a), blank (b) and conjugate **(II)** (c).

Table 1
Inhibition percentages of DPPH radical and linoleic acid peroxidation by conjugates **(I)** and **(II)** and blank polymers.

Sample	% Inhibition	
	DPPH radical	Linoleic acid peroxidation
Blank alginate	41	22
Conjugate **(I)**	61	50
Blank inulin	20	0
Conjugate **(II)**	74	60

(Dewanto et al., 2002). Thus, $AlCl_3$ assay was employed to have a direct determination of the total flavonoid content, expressed as mg of CA per g of polymer, of conjugates. By comparing the obtained data with the CA calibration curve, the amount of catechin equivalent was determined. In particular, for conjugates **(I)** and **(II)** these values were 1.43 mg/g and 2.47 mg/g of dry polymers, respectively.

4. Conclusion

Catechin–alginate and catechin–inulin conjugates were synthesized by free radical grafting of CA onto polysaccharidic backbones. For this purpose, ascorbic acid/hydrogen peroxide redox pair was employed as water-soluble and biocompatible initiator system.

The insertion of catechin onto the polymeric chains was verified by performing FT-IR, DSC and fluorescence analyses and molecular weight distributions were analyzed by GPC.

Finally, the antioxidant properties of the obtained functionalized materials were evaluated, by performing different assays, and compared with that of blank alginate and blank inulin treated in the same conditions but in absence of CA. In particular, determination of scavenging activity on DPPH radicals, β-carotene–linoleic acid assay, evaluation of disposable phenolic groups and total flavonoid content were performed.

Both functionalized materials showed good antioxidant properties and the best results were obtained with conjugate **(II)**. The data confirmed the efficiency of the adopted reaction method to impart peculiar characteristics to macromolecules of natural origin, that make these materials very interesting from an industrial point of view for different applications. This kind of systems, indeed, could be very useful in the optimization of food preservation and to help manufacturers in elaboration of new food products and packaging.

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