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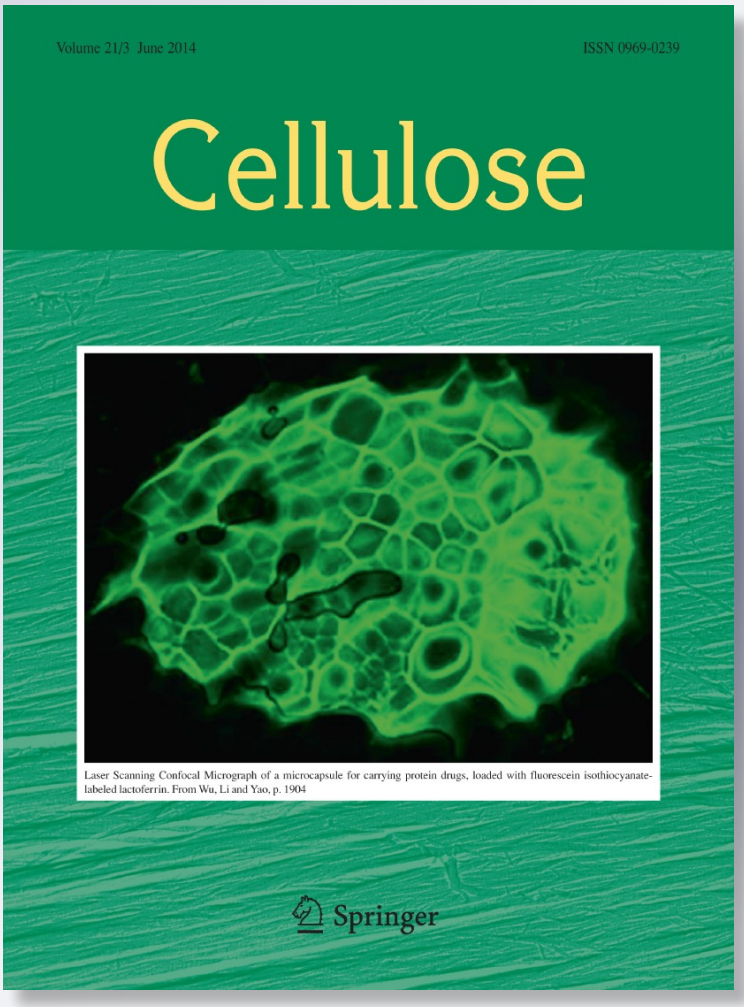

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ORIGINAL PAPER

Antifungal cellulose by capsaicin grafting

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Abstract Cellulose is one of the most abundant materials in nature. Besides its biological function, cellulose can be extracted from the cell wall and used in several industrial applications. Thus, it can be used in papers, pharmaceuticals, food, cosmetics and innovative materials such as nanocomposites, packaging, coatings and dispersion technology. With the aim of extending cellulose applications and producing so-called "smart" materials, new functionality can be introduced by physical or chemical modifications. Taking into account that capsaicin, the active component of chili peppers, is an excellent antifungal agent, a potential new material could be obtained by chemical reaction between this active compound and cellulose. In this work, capsaicin grafting onto cellulose using polycarboxylic acid as linking agent is proposed. The reaction occurrence was corroborated by Fourier transform infrared spectroscopy and UV–Vis spectrophotometry in reflectance mode. Modified cellulose with <2 wt% of capsaicin shows a strong change in antifungal activity with respect to the unmodified one. This activity was evaluated by the fungal growth inhibition test with two different fungi, *Trametes versicolor* and *Gloeophyllum trabeum*. Modified cellulose samples showed a high percentage of fungal growth inhibition, demonstrating the success of the cellulose modification and high antifungal power of the grafting molecule.

Keywords Cellulose · Grafting · Synthetic capsaicin · Antifungal

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Introduction

Cellulose is one of the most abundant organic materials in nature and constitutes the main structural component conferring strength and stability to the plant cell walls. Besides this important biological function, cellulose can be extracted from cell walls by chemical and mechanical processes and applied in several industrial applications. Thus, it can be used in papers, pharmaceuticals, food, cosmetics and paints (Kaith and Kaur 2011). Its exceptionally large specific

surface area implies potential increased interactivity with secondary components/materials. This could create innovative breakthroughs in the use of these materials in areas such as nanocomposites, packagings, coatings and dispersion technology (Stenstad et al. 2008). Specially, the use of cellulose and its derivatives in health care will be particularly promising because of their high strength and stiffness and combined with low weight, biocompatibility and renewability (Kaith and Kaur 2011).

With the aim of extending cellulose applications and produce so-called "smart" materials or products, new functionality can be introduced to cellulose by physical or chemical modifications (Stenstad et al. 2008; Teli and Sheikh 2012; Serrano et al. 2011). Taking advantage of the large number of hydroxyl groups present in cellulose, a surface chemical reaction can be performed to graft bioactive molecules onto it.

In this sense, capsaicin is the active component of chili peppers, which belong to the genus Capsicum. Besides its food applications, this substance is used in medicine as a pain reliever in cream and topical ointments as well as high-dose dermal patches (Jorge et al. 2011; Mason et al. 2004), in marine boats as an anti-fouling agent (Watts 1995) and in agriculture as a repellent of mammalian (Nolte and Barnett 2000; Kimball et al. 2009). Also, capsaicin and analogs show important antimicrobial activity against various microorganisms (Santos et al. 2012; Singh et al. 2011; Soumya and Nair 2012; Xing et al. 2006).

Polycarboxylic acids like 1,2,3,4-butanetetra carboxyic acid (BTCA) have been used as linking agents for cellulose crosslinking with the aim of imparting both wrinkle and shrinkage resistance and smooth drying properties to cotton fabrics (Bertoniere and King 1992; Yang et al. 2010). Polycarboxylic acids can react with hydroxyl groups of cellulose and form stable ester bonds. This esterification can either occur with heat alone or be accelerated by the presence of salts of weak acids, such as sodium hypophosphite (SHPI) (Yang et al. 2011). This reaction was also applied for grafting other molecules containing OH groups onto the cellulose chain. Thus, chitosan and benzophenone are used to provide easy care and antibacterial properties to fabrics (Fouda et al. 2009; Hebeish et al. 2011; Hong and Sun 2008). Also, cyclodextrins are bonded to cellulose in the textile field for many applications: they can absorb unpleasant odors and can retain and release fragrances, "skin-care-active" substances such as vitamins as well as bioactive substances such as biocides and insecticides (Martel et al. 2002; Medronho et al. 2013; Peila et al. 2012; Voncina and Le Marechal 2005).

In this work, the capsaicin grafting onto the cellulose chain using polycarboxylic acid as linking agent is proposed to provide cellulose fibers with antifungal properties. The reaction occurrence was corroborated by Fourier transform infrared spectroscopy (IR-ATR) and UV–Vis in reflectance mode. Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) were applied to evaluate thermal properties and confirm capsaicin grafting. Finally, the antimicrobial activity of final products was evaluated against two different fungi, *Trametes versicolor* and *Gloeophyllum trabeum*. These species belonging to the Basidiomycetes family were selected because they are the most effective microorganisms for degrading cellulose materials (Dhouib et al. 2005).

Experimental

Materials

Cellulose pulp obtained from linen fiber was used as matrix. *N*-Vanillylnonanamide, also called synthetic capsaicin (S-Cap), CAS# 2444-46-4, from Sigma Aldrich (mass fraction $\geq$0.97) was used as active agent. 1,2,3,4-Butanetetracarboxylic acid (BTCA), CAS# 1703-58-8 (mass fraction $\geq$0.98) and sodium hypophosphite monohydrate (SHPI), CAS# 10039-56-2 (mass fraction $\geq$0.99) supplied by Acros Organics were used as ligand and catalyst, respectively.

Cellulose grafting

Synthetic capsaicin and tetracarboxylic acid were dissolved in a beaker with 10 mL ethanol while sodium hypophosphite was dissolved in 7.5 mL water. Both solutions, prepared with minimal amounst of each solvent, were mixed, and immediately 2 g of cellulose pulp was embedded therein. After that, cellulose was dried at room temperature for 12 h in a forced air laboratory hood in order to eliminate the solvents and then cured in an oven at a given temperature and time. The experiments were performed at three different curing temperatures: 120, 140 and 160 °C, and three

Table 1 Names and conditions used in all the reaction experiments performed

Name	S-Cap (wt%)*	BTCA:S-Cap molar ratio	Catalyst:BTCA molar ratio	Temperature (°C)	Time (min)
rA120 (time)	2	4	1	120	15,30, 60
rA140 (time)	2	4	1	140	15,30, 60
rA160 (time)	2	4	1	160	15,30, 60
rB16030	2	4	0	160	30
rC16030	2	4	0.5	160	30
rD16030	2	4	2	160	30

* Relative to cellulose mass

different times: 15, 30 and 60 min. The reaction was also performed in the absence of S-Cap, following the same procedure and with the same acid and catalyst mass used as in the reactions described above. Also, the reaction was performed with different catalyst:BTCA molar ratios, including reaction without catalyst, at optimal conditions (160 °C and 30 min). Table 1 summarizes all the reactions performed with S-Cap and the nomenclature used for each named reaction product. Another reaction was done in the absence of S-Cap with the same BTCA:cellulose mass ratio and catalyst:BTCA molar ratio as in reaction "A" (see Table 1) and using the maximum reaction conditions (160 °C and 60 min). This reaction product was named "rA16060noS-Cap".

After reaction, all cellulose samples were washed with abundant ethanol and water for removing non-reacted synthetic capsaicin, ligand and catalyst. The described washing was repeated up to no S-Cap detection in the residual wash water using UV spectroscopy.

The washed reaction product was divided in two parts; one of them was characterized as such and the other one treated with NaOH solution (0.1 M) at room temperature for 4 min to obtain and characterize carboxylate anions from free carboxylic acid.

In order to contrast and evaluate reaction results, S-Cap/cellulose physical blend was prepared with the relative mass amount used in reaction experiments. This sample was prepared dissolving S-Cap in ethanol, embedding cellulose therein and evaporating the solvent at room temperature for 12 h in a forced air laboratory hood.

Characterization

Fourier transform infrared spectroscopy (IR-ATR)

Identification of pure reagents and reaction products was performed using a Nicolet Nexus 670 FT-IR spectrometer (Madison, WI, USA) equipped with a single horizontal Golden Gate attenuated total reflectance cell (Specac, Kent, UK). The spectra were recorded using a spectral width ranging from 600 to 4,000 cm^{-1}, with 4 cm^{-1} resolution and an accumulation of 20 scans.

Thermogravimetric analysis (TGA)

Thermogravimetric analysis (TGA) was carried out in Discovery TGA equipment from TA instruments. The analysis was performed within a temperature range of 30-800 °C at a heating rate of 10 °C/min. The sample was heated in an aluminum crucible under nitrogen atmosphere. Thermogravimetric (TG) and derivative thermogravimetric (DTG) curves were recorded simultaneously.

Differential scanning calorimetry (DSC)

The thermograms of all samples were obtained in a Perkin-Elmer Pyris I equipment. The samples were heated to 450 °C with a rate of 10 °C/min.

UV-spectrophotometric analysis (UV)

All prepared samples as well as pure materials such as cellulose, S-Cap and BTCA were analyzed as such (solid state) in a UV-3600 Shimadzu spectrophotometer equipped with an ASR absolute specular reflectance attachment (ISR 3100). Spectra were recorded from 200 to 800 nm with a resolution of 0.5 nm.

Antifungal activity analysis

Antifungal activity evaluation of samples against rot fungus was carried out. Each sample (1 mg) was placed in the center of a potato dextrose agar (PDA, Merck) plate. Before each assay, the fungi (*Trametes*

versicolor, Gloeophyllum trabeum) were allowed to grow aerobically for 15 days at 25 °C in potato dextrose broth (PDB). Five replicates of the culture aliquots were aseptically inoculated onto fresh PDA plates containing cellulose, reacted samples and control samples and kept for 90 days at 25 °C. After the time of incubation at 25 °C, the number of colonies was counted in direct microscopic count, and the number of colony-forming units per milliliter (CFU mL^{-1}) was determined. The number of fungal units in a small known volume was directly counted microscopically by a Neubauer counting chamber and the number of fungi in the larger original sample determined by extrapolation. The samples were diluted mixing the sample with deionized water (1:100) in order to avoid interference in observation and after being placed in the counting chamber (this dilution was considered in the final calculations). Once the cover slip had been placed on the slide, a small amount of culture (about 10 µl) was placed in the viewing area. The formula used for the direct microscopic count was (Corry et al. 2003):

$$CFU(\mathrm{mL}^{-1}) = \frac{Nc \times 10^3}{\frac{1}{20} \times \frac{1}{20} \times \frac{1}{50} \times \frac{1}{D}}$$

Here Nc is the average number of cells counted per square, and D is the dilution of the samples placed on the slide.

The fungal growth inhibition (FGI) was calculated as follows (Pinto et al. 2013):

$$FGI(\%) = \frac{C_{cs} - C_{ts}}{C_{cs}} \times 100$$

C_{cs} is the average concentration in the control samples set, and C_{ts} is the average concentration in the treated set (test samples), both expressed in CFU mL^{-1}. The values obtained for the concentration of each sample correspond to the average of independent experiments.

Results

The crosslinking mechanism between cotton cellulose and polycarboxylic acids, such as butane tetracarboxylic acid (BTCA), is well known (El-Tahlawy et al. 2005; Hebeish et al. 2011; Yang et al. 2010). The reaction occurs by dehydration below the curing temperature of the PCAs, which yields an intermediate anhydride that readily reacts with the hydroxyl groups of cellulose by esterification. Then, the remaining carboxylic groups of the reacted BTCA can form a second anhydride that can also react with another cellulosic unit from another macromolecular chain. In the same way, it is expected that the hydroxyl group in the capsaicin analog *n*- vanillylnonanamide can directly react with anhydrides of BTCA, while simultaneously reacting with cellulose. Thus, BTCA can be used as a linking agent to connect the synthetic capsaicin to cellulose fiber through esterification, as shown in Scheme 1 (Hong and Sun 2008).

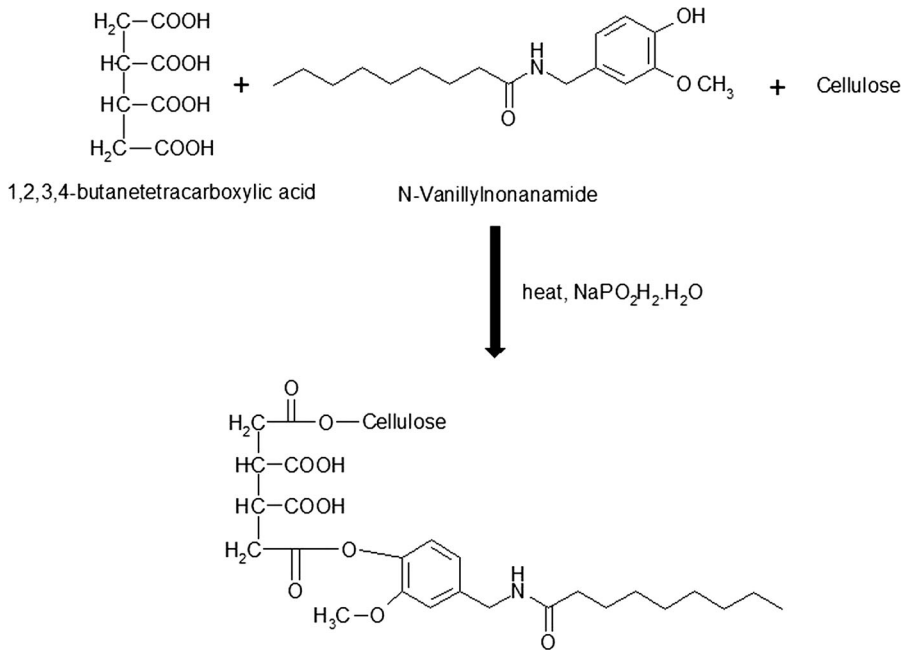
The reaction occurrence was assessed by IR-ATR, following typical synthetic capsaicin and carbonyl (from tetra carboxylic acid) peaks. After cellulose reaction and washing, three carbonyls were found, that is, ester, free carboxylic acid and carboxylate anion (Yang et al. 2010). The carbonyl bands of ester and free acid overlapped at around 1,720 cm^{-1}. For this reason, samples were also treated with 0.1 M NaOH solution to convert the free acid to carboxylate so that the intensity of the ester carbonyl could be measured and used as the basis for quantitative analysis of the ester formed between cotton and the acid. The carboxylate group can be followed by the peak at 1,580 cm^{-1}. Samples were analyzed in order to evaluate the reaction progress after and before NaOH treatment.

For synthetic capsaicin, the IR-ATR spectrum (Fig. 1a) shows two dominant vibration bands around 1,650 cm^{-1} (C = O stretch) and 1,550 cm^{-1} (N–H bend), and a weak band at 1,470 cm^{-1} (C–N stretch).

According to these characteristic peaks, the reacted cellulose samples were analyzed. In all cases, the peaks at 1,720 cm^{-1} corresponding to ester and carboxylic acid were detected in samples washed with ethanol and water, confirming the reaction occurrence. This can be observed in Fig. 1. To ensure this occurrence and quantify the esterification between cellulose and carboxylic acid, also samples treated with NaOH solution were analyzed. In this case, a decrease in the intensity of the peak a 1,720 cm^{-1} and the presence of the carboxylate peak at 1,580 cm^{-1} were observed. The occurrence of the ester peak confirms the reaction between cellulose and carboxylic acid.

The presence of S-Cap is more difficult to determine by this analytical technique. In some samples, a shoulder at 1,570 cm^{-1} was detected (Fig. 2),

Scheme 1 Schematic representation of the pseudocapsaicin grafting onto cellulose using BTCA as linking agent



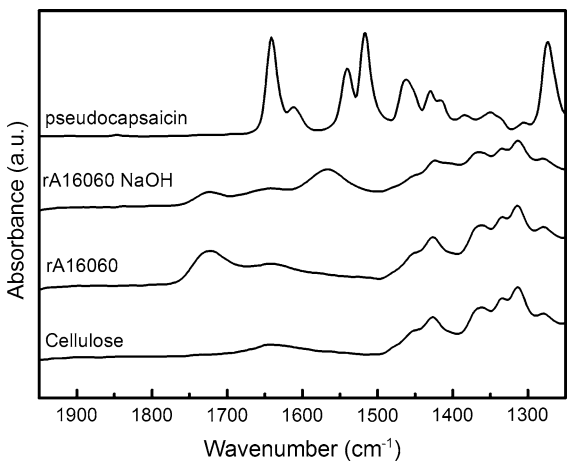


Fig. 1 FTIR spectrum of linen cellulose pulp compared with synthetic capsaicin and reaction performed at 160 °C and 60 min with (rA16060NaOH) and without (rA16060) NaOH treatment

corresponding to a shifting of the characteristic N–H peak. However, this peak was not visible in all of the samples. In order to determine whether it was a problem of limit concentration detection or of capsaicin not reacting, "physical" cellulose/capsaicin samples were prepared. These samples were prepared following a similar procedure as for reaction samples, dissolving S-Cap in ethanol/water, embedding cellulose in this solution and then evaporating the solvent. The spectrum of the physical blend with 0.75 wt% of synthetic capsaicin (PB075) is shown in Fig. 2; no changes were introduced into the spectrum by the presence of capsaicin. This fact demonstrates that the capsaicin concentration is under the detection limit. For this reason, IR-ATR was not a suitable technique for synthetic capsaicin detection and quantification. This problem of detection limit was also observed in NMR and elemental analysis. In the case of elemental analysis, differences in nitrogen content were detected for reacted samples, but the N amount was under the detection limit of the techniques. For this reason, the results were not reported. Additionally, the IR technique was also applied in bulk absorption and using KBr pellets, but S-Cap detection was not possible. In this sense, no differences were observed in the spectral peak position by using both modes of analysis. However, spectra obtained from FTIR-ATR have more peak definition, giving an indication of grafting mainly taking place at the cellulose surface, as is expected because the reaction takes places without cellulose solubilization.

However, the reaction degree can be analyzed by IR-ATR following the intensity of the ester peak. This peak provides information about the esterification

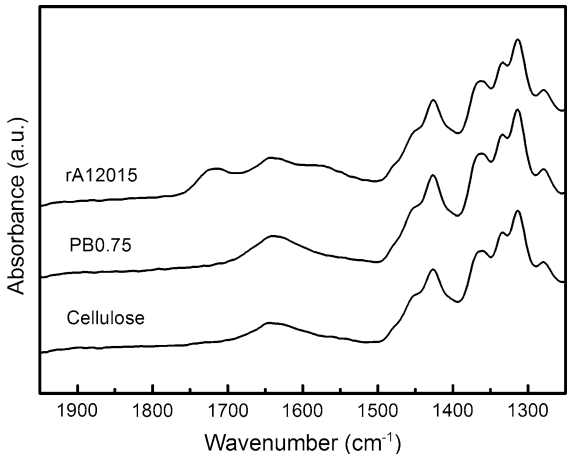


Fig. 2 FTIR spectrum of linen cellulose pulp compared with physical cellulose/capsaicin blends (PB0.75) and reaction performed at 120 °C and 15 min (rA12015)

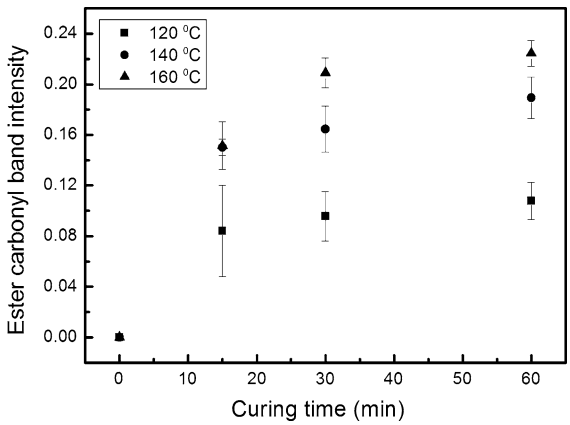


Fig. 3 Ester carbonyl band intensity in the FTIR spectrum as a function of curing time at different temperature reactions

reaction, but it does not indicate capsaicin grafting. Nevertheless, as esterification is the reaction involved in capsaicin grafting, this peak intensity could give some information on the reaction advance, and the effect of time and thus temperature on the reaction degree can be analyzed. For this purpose, the carbonyl band absorbance in the infrared spectra was normalized against the 1,318 cm^{-1} band associated with the C–H bending mode of cellulose. In Fig. 3, it is possible to note an increment of ester peak intensity with both time and temperature. The curing temperature shows an important effect when it is increased from 120 to 140 °C, but a lower increment in cellulose

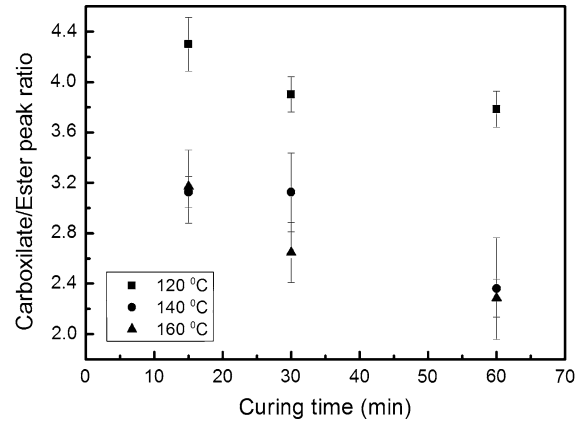


Fig. 4 Carboxylate/ester absorbance ratio as a function of curing time at different temperature reactions

esterification is observed when the temperature is increased to 160 °C.

The reaction time also produces an increment in the reaction advance displaying asymptotic behavior reaching the maximum value at a time between 30 and 60 min.

Another variable analyzed was the carboxylate/ester absorbance ratio, and the results are shown in Fig. 4. As can be observed, this ratio decreases as the time and temperature increase, thus indicating higher polycarboxylic acid substitution due to higher capsaicin grafting and/or cellulose crosslinking.

According to the previous results, a reaction condition with high reaction efficiency was selected in order to analyze the effect of the catalyst:ligand molar ratio. Thus, reaction at 160 °C and 30 min gives a good reaction degree in a short time. At these conditions, reactions with a catalyst:ligand molar ratio of 0.5 and 2 were carried out and compared with the previous results (catalyst:ligand molar ratio = 1). Both a normalized ester peak intensity and carboxylate:ester absorbance ratio does not show significant changes in the catalyst:ligand molar ratio. However, when the reaction was performed in the absence of catalyst (rB16030), the reaction progress was low, obtaining a relative ester band intensity of about 0.13, approximately 60 % of that obtained in the presence of NaH_2PO_2. This difference became higher at lower temperatures (Voncina and Le Marechal 2005; Yang et al. 2010).

Due to the difficulty of capsaicin detection with IR-ATR, UV–Vis spectrophotometric analysis was applied, taking into account the aromatic ring of

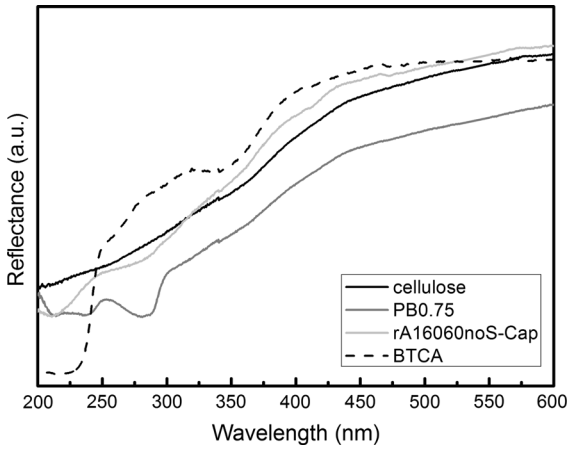


Fig. 5 UV–Vis spectra of pure reagents compared with the synthetic capsaicin/cellulose physical blend and reaction products obtained at 160 °C and 60 without pseudocapsaicin

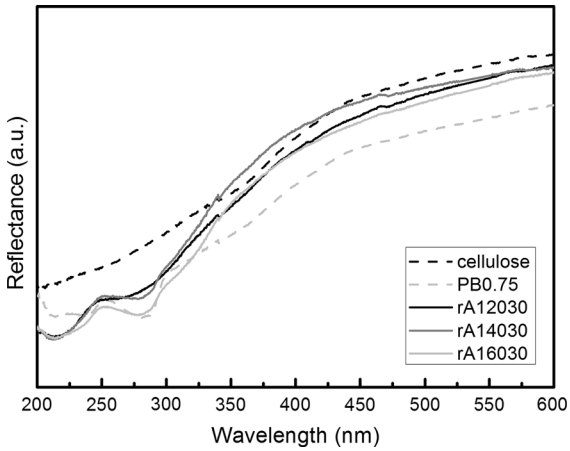


Fig. 6 UV–Vis spectra of reaction products obtained at 30 min and different temperatures compared with pure cellulose and the synthetic capsaicin/cellulose physical blend

S-Cap absorbance at 280 nm. Reflectance was measured rather than absorbance in this case since the reaction product is not transparent to light. In Fig. 5, UV–Vis spectra of pure reagents is compared to a synthetic capsaicin/cellulose physical blend and reaction products obtained at 160 °C and 60 min without pseudocapsaicin. For cellulose a uniform curve is shown, while for BTCA the spectrum presents two shoulders at 250 and 300 nm. Reaction product used as a blank (rA16060noS-Cap) shows a similar but higher shoulder at 250 nm, indicating the presence and reaction of BTCA with cellulose. Finally, the UV spectra of the physical blend prepared at room temperature with *n*-vanillylnonanamide exhibits a decrease in reflectance corresponding to the aromatic ring absorption peak around 280 nm. Based on this evidence, the reaction products were analyzed, and the spectra of samples prepared at 30 min and different temperatures are shown in Fig. 6. Synthetic capsaicin absorbance for the reacted samples at 140 and 160 °C can be observed, being higher as the reaction temperature increases. Thus, the incorporation of the bioactive compound onto cellulose is confirmed. The bioactive compound concentration in the final products is low, and the absorbance intensity for high temperature reacted samples is comparable to that of the physical blend. Also, an increase in capsaicin grafting with increasing time and not changes in this variable with different catalyst:BTCA ratios can be confirmed by this technique. Thus, the UV results are in agreement with the IR-ATR ones. However, the spectrum of the reaction product performed at 120 °C does not show signs of *n*-vanillylnonanamide and is very similar to blank reaction, showing low or null S-Cap grafting at these conditions, although esterification was detected by IR-ATR. The low S-cap concentration in the final products also provides evidence that the incorporation is mainly at the cellulose surface in accordance with the IR study.

Thermal properties of reaction products were evaluated by TGA. In Fig. 7, thermograms of reaction products obtained at 60 min and different temperatures are compared with pure cellulose and S-Cap. For cellulose, two degradation steps can be found up to 700 °C; the first one at a temperature around 100 °C is due to the loss of moisture, while the second one around 360 °C is due to the start of decomposition of the glucose units of cellulose. Synthetic capsaicin shows an initial and slight weight loss around 80 °C and presents the highest degradation degree around 280 °C. For reaction products, the TGA curve is shifted to a lower degradation temperature because of an increase in the disorder of cellulose molecular packing because of the substitution of cellulose hydroxyls (Jandura et al. 2000). The degradation temperature decreases as the temperature increases according to the IR-ATR results in which a higher reaction degree is observed as temperature increases. In Table 2, degradation temperatures of different analyzed samples are listed. In the same way, the degradation residue increases as the reaction temperature increases because of this higher reaction advance

and possible cellulose crosslinking. Also, higher weight loss is detected in reaction products in the low temperature region. This weight decrease can be produced by the evaporation of residual ethanol used in sample preparation and washing.

To evaluate the capsaicin effect, the region between 200 and 300 °C, where the pseudocapsaicin is degraded, was analyzed in detail. For this purpose, thermograms were compared with those obtained for the capsaicin/cellulose physical blend and for a reaction product obtained at 160 °C and 60 min in the absence of synthetic capsaicin (rA16060noS-Cap).

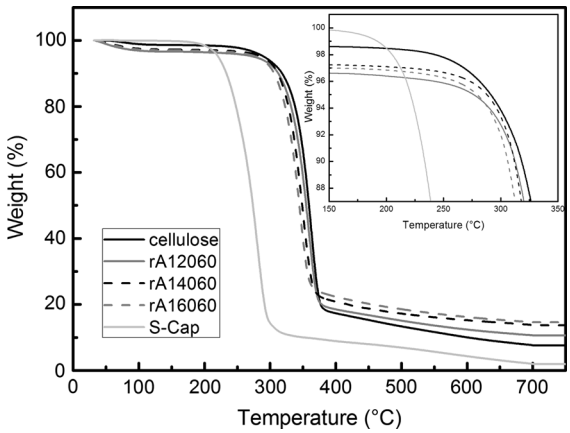


Fig. 7 Thermograms of reaction products obtained at 60 min and different temperatures compared with pure cellulose and synthetic capsaicin

Table 2 Degradation temperature obtained by TGA analysis for pure components and reaction products

Sample	Degradation temperature (°C)
Cellulose	362
N-vanillylnonanamide (synthetic capsaicin)	283
PB 0.75	358
rA16060noS-Cap	351
rA12060	360
rA14060	350
rA16060	341
rA160b15	345
rA160b30	342
rB16030	359
rC16030	341
rD16030	337

This comparison is shown in an inset in Fig. 7. The thermogram of rA16060noS-Cap also shows a lower degradation temperature; see Table 2. The curve presents higher weight loss at low temperature; then it is parallel to the cellulose curve, converging between them at 290 °C where it matches very well up to 310 °C, where they begin to separate. On the other hand, the physical blend shows practically the same degradation temperature as pure cellulose, and its thermogram also expounds a higher weight loss at low temperature as a consequence of evaporation of the residual solvent used in the sample preparation. Additionally, an extra weight loss is observed from about 170 °C, coinciding with pseudocapsaicin degradation. A similar behavior is detected in the thermogram of the reaction product prepared at 160 °C and 60 min, giving some evidence of synthetic capsaicin's presence in the final product.

Samples reacted at different times and the same temperature do not show changes important in their thermograms. They present almost the same degradation temperature and residue amount. Similar behavior is observed with samples prepared at 160 °C, 30 min with a different catalyst:ligand ratio, confirming that the effect of this variable is not important. However, a higher degradation temperature is obtained in cellulose reacted in the absence of catalyst, according to the low reaction progress observed in the IR-ATR analysis.

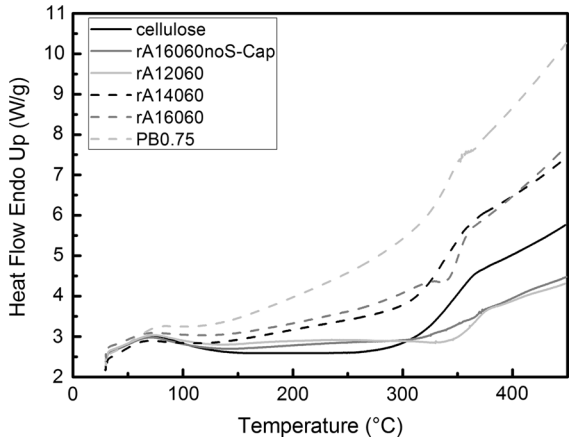


Fig. 8 DSC thermograms of reaction products obtained at 60 min and different temperatures compared with pure cellulose, the synthetic capsaicin/cellulose physical blend and reaction products obtained at 160 °C and 60 min without pseudocapsaicin

Continuing with the thermal study, reaction products were also subjected to DSC analysis. In Fig. 8, the DSC curves of the reaction product obtained at 60 min and different temperatures are shown. As in the TGA analysis, they were compared with those of pure cellulose, the pseudocapsaicin/cellulose physical blend and with the reaction performed without active compound.

For pure cellulose, two endothermic peaks or steps are observed. The first, corresponding to the moisture evaporation, is around 75 °C, and the other endothermic step begins at 300 °C and is mainly due to the cellulose depolymerization with the formation of levoglucosan and its evaporation (Jandura et al. 2000). This result agrees with the TGA study. The DSC curve of the reaction product obtained in the absence of pseudocapsaicin presents almost the same form, with a slight slope before 300 °C. The DSC thermogram of the physical blend suffers a continuous endothermic process from 150 °C, in correspondence with *n*-vanillylnonanamide degradation detected in TGA, and also the step from 300 °C corresponding to cellulose degradation is observed. These differences founded between these DSC curves allow determining the presence of S-Cap. Thus, it is possible to note that the DSC thermogram reaction product obtained at 140 and 160 °C exhibits the pseudocapsaicin degradation slope, being higher at higher temperature. However, in the reaction product obtained at 120 °C, this degradation was very low, in concordance with the results obtained in the UV analysis.

Samples obtained at different times were also analyzed by DSC, and thermograms of the products obtained at 160 °C are shown in Fig. 9. Following the tendency observed in IR-ATR and UV analysis for the ester bond, the endothermic slope increases as the time increases, indicating a higher S-Cap amount in the final product as the reaction time increases.

In both Figs. 8 and 9, an exothermic peak close to 340 °C is detected for all of the samples reacted at 160 °C. This exothermic peak can be attributed to char formation (Jandura et al. 2000) favored in this case by higher cellulose crosslinking.

According to previous analysis, samples obtained with a different catalyst:ligand ratio do not show significant differences in DSC thermograms.

Unfortunately, no typical glass transition was observed in the thermogram, which may be due to the presence of water. It has been demonstrated

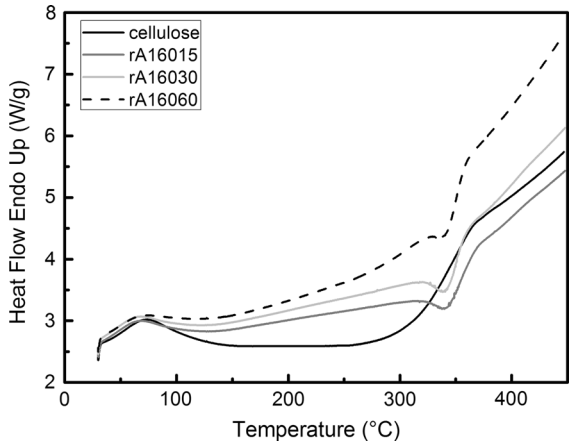


Fig. 9 DSC thermograms of reaction products obtained at 160 °C and different times compared of pure cellulose

(Szcześniak et al. 2008) that it is difficult to obtain absolutely dry cellulose even by heating the cellulose sample up to a higher temperature because of the presence of different types of water in hydrophilic polymers (free water, freezing-bound water and bound water). For the same reason, glass temperature determination is not easy, and several successive heating cycles are required. According these literature data (Szcześniak et al. 2008), the Tg for cellulose with 3 wt% water (value detected in Tga analysis for our samples) is about 100 °C; then this transition could be masked by water peaks. For this reason, no conclusion can be reacheded concerning the effect of capsaicin grafting on the cellulose plasticity.

Another physical property analyzed was the cellulose solubility in different solvents such as acetone, chloroform and dimethyl sulfoxide. In all of the cases, no pure and modified cellulose solubilization was found in these solvents, showing that synthetic capsaicin incorporation does not significantly change the cellulose chemical structure because of its lower content.

Finally, the antifungal activity of prepared samples against rot fungus was evaluated after 90 days of treatment. For this analysis, two species belonging to the Basidiomycetes family were selected because they are the most effective microorganisms for degradation of cellulose materials (Dhouib et al. 2005). Specifically, the fungi of white rot attack the components of the cell wall and degrade carbohydrates producing oxidative enzymes. Within this group the species

Trametes versicolor is highlighted for presenting a high degradation capacity (Dumonceaux et al. 2001). On the other hand, the fungi of brown rot are highlighted for presenting specific degradation of cellulose and hemicelluloses, and the species *Gloeophyllum trabeum* is able to degrade the cellulose very slowly (Jensen et al. 2001).

Previous studies have shown that antimicrobial cellulosic fabrics can be developed with BTCA and CA along with subsequent oxygen bleaching (Orhan et al. 2009), specially for bacteria such as *Staphylococcus aereus* and *Escherichia coli*. This antibacterial activity is produced by the low pH levels given by BTCA. For this reason, the antifungal activity of samples prepared in the absence of S-Cap, namely rA16060noS-Cap, was also analyzed.

Thus, Table 3 shows the total fungal activity of the blank, pure components and modified cellulose with and without S-Cap. Analyzing these results, both the blank and cellulose sample presented high growth with the fungi used. On the other hand, synthetic capsaicin showed high antifungal activity against both rot fungi, completely inhibiting the fungus growth. The sample reacted in the absence of S-Cap presented a slight antifungal activity, perhaps because of the presence of BTCA, as discussed above. However, the antifungal activity was lower with respect to the antibacterial activity (Orhan et al. 2009), perhaps because of the different optimal pH growth conditions for these different kinds of microorganisms and/or the BTCA amount, and then the pH level in the final products. In contrast, rA16030 samples showed a high percentage of fungal growth inhibition even after a long time, especially against *Trametes versicolor*, demonstrating the success of the cellulose modification and the high antifungal power of the inserted molecule. It is important to note that the antifungal characteristics of modified cellulose are achieved with very low synthetic capsaicin grafting, as was demonstrated by UV and IR analysis. Additionally, it is highlighted that S-Cap grafting does not change its antimicrobial activity, showing that the main functional groups involved in this property are not modified during the reaction. Similar behavior was found for other modified cellulose at 140 and 160 °C, but very low antifungal activity was found for samples reacted at 120 °C, in agreement with very low or no S-Cap incorporation.

Table 3 Fungal activity of pure and modified cellulose after 90 days of treatment

Sample	CFU*mL^{-1}	FGI (%)
Blank Tv	126.7×10^8	–
Blank Gt	46.2×10^8	–
Cellulose Tv	16×10^8	–
Cellulose Gt	2.4×10^8	–
Synthetic capsaicin Tv	≤10	100
Synthetic capsaicin Gt	≤10	100
rA16060noS-Cap Tv	15.6×10^8	2.28
rA16060noS-Cap Gt	2.3×10^8	4.2
rA16030 Tv	≤10	100
rA16030 Gt	0.25×10^8	89.5

Conclusions

In this work, synthetic capsaicin grafting onto cellulose is presented, using polycarboxylic acid as linking agent. The reaction occurrence was corroborated by several analytical techniques: FTIR, UV–Vis, TGA and DSC. In all of them, it was demonstrated that the reaction progress is higher as the temperature and time increase, with optimal conditions at 160 °C and 30 min. The presence of catalyst favors the reaction advance, but the catalyst:ligand ratio does not show a significant effect. The modified cellulose showed important antifungal properties against the rot fungus analyzed, confirming the synthetic capsaicin grafting and the preservation of biological activity after the reaction.

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