

Carboxymethylcellulose from bleached organosolv fibers of *Eucalyptus nitens*: synthesis and physicochemical characterization

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Abstract The objective of the present study was to synthesize sodium carboxymethylcellulose from *Eucalyptus nitens* cellulose. The cellulose extraction was carried out in three stages. In the first stage, *E. nitens* wood was fractionated using an organosolv pretreatment (methanol–water 50% v/v, 45 min at 200 °C) followed by an alkaline extraction (NaOH, 1.5 M). Finally, the organosolv pulp was bleached using NaClO₂ (1.3% w/v, pH 3.8). The bleached cellulose obtained was converted to sodium carboxymethylcellulose (NaCMC) by an etherification reaction in isopropanol medium. NaCMC structure, morphology and properties were researched using FTIR, ¹H and ¹³C NMR spectroscopy, scanning electron microscopy, and thermogravimetric analysis. The results indicate substitution mostly occurred mostly at the C-6

position of 1 → 4 β-D-glucopyranosyl residues of cellulose chains.

Keywords *Eucalyptus nitens* · Organosolv · Cellulose · Carboxymethylcellulose

Introduction

Eucalyptus nitens is a species of hardwood angiosperm tree from Southeast Australia, where it is distributed in small isolated populations. Its native range is in the mountainous regions of Central Victoria and East of New South Wales (Roberts et al. 2015).

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Naturally, it develops in temperate climates in mountainous areas (600–1600 m), with not very high temperatures in summer and, cold temperatures, frost and snow in winter. This species is able to withstand temperatures down to $-10\text{ }^{\circ}\text{C}$. It develops well in several types of soils, especially in those with deep clay horizons, in areas with annual rainfall average of 750–1300 mm (Pinkard and Beadle 1998).

In Chile, *E. nitens* adapts and develops well, proving to be the fastest growing species established in our country with a rotation age of 10–12 years. In Chile, the planted area of *E. nitens* reaches 247,207 ha and it is mainly used for the production of bleached hardwood kraft pulp (BEKP) (Gysling et al. 2016). During the last decade, there has been a growing interest in the use of *E. nitens* for sawntimber and for clearwood production (Muñoz et al. 2008).

In organosolv pulping processes, a wide variety of organic solvents have been used for the delignification of lignocellulosic materials (Zhao et al. 2009). Due to their relatively low cost, lower toxicity, flammability and volatility, the solvents of major industrial interest are low molecular weight aliphatic alcohols such as ethanol and methanol (Oliet et al. 2002). Methanol is mostly used as an additional chemical in pulping. The most effective methanol pulping processes are soda pulping method with methanol (Organocell) and alkaline sulfite-anthraquinone-methanol (ASAM) mixture. Both processes have been commercially used and the cellulose pulps obtained have relatively good strength properties (Franco et al. 2012). In addition, it has been demonstrated that, through the autocatalyzed methanol pulping of hardwoods, pulps with low lignin content and acceptable viscosity can be obtained (Muurinen 2000).

Sodium carboxymethylcellulose (NaCMC) is the worldwide most-consumed cellulose ether, with slightly over half of the total consumption volume. Methylcellulose and derivatives represent about 30% by volume, while hydroxyethylcellulose and derivatives account for about 12% by volume. Many companies produce NaCMC around the world, being CP Kelco the world leader with a 32% share, followed by Ashland with a 25% share of the global market. The usual method to obtain sodium carboxymethylcellulose (NaCMC) is the etherification reaction of cellulose hydroxyl groups with sodium monochloroacetate (Heinze and Pfeiffer 1999). The introduction of carboxymethyl groups in anhydroglucose units can

occur in the OH-groups of the C6, C2 and C3 positions reaching a maximum theoretically calculated degree of substitution of 3 (DS = 3). However, the hydroxyl group at the C6 position shows the greatest reactivity due to its lesser spatial impediment and therefore its easier access for reagents (Fengel and Wegener 1989).

In the industrial process, the bleached pulp is pulverized and sprayed with NaOH and subsequently treated with monochloroacetic acid in a kneading reactor and then, in a rotating drum. After dispersion in methanol and neutralization with hydrochloric acid, the product is filtered, washed and dried (Hader et al. 1952).

Due to its high viscosity, hydrophilicity and non-toxicity, NaCMC has a wide range of applications in the fields of Food and Beverages, Oil Field Drilling Fluids, Drugs and Cosmetics, Paper Processing Aids, Detergents, Textile Processing, Ceramic Industry, Paint and Lacquer Industry, Tobacco Industry, Glue and Adhesives Industry, Freezer Pads and Gels Industry, and Other Industrial Applications (Arik Kibar and Us 2017).

In the last decades, NaCMC has been studied for its application in graft copolymers of polyacrylamide via radical polymerization (Biswal and Singh 2004) and NaCMC hydrogels with acrylamide/acrylic acid (AAc) have been obtained using the γ radiation technique (Fekete et al. 2016). Also, NaCMC was used in the synthesis of antibacterial hydrogels and nanocomposites such as NaCMC/CuO nanocomposites (Yadollahi et al. 2015), and NaCMC/silver nanoparticles (Das et al. 2015; Bozaci et al. 2015). Recently, graphene/NaCMC nanocomposites have been employed for Li-ion batteries (Naboka et al. 2016).

On the other hand, many lignocellulosic materials are used as raw material for the synthesis of NaCMC, for instance, sugarcane straw (Candido and Goncalves 2016), sugarcane baggase (Ruzene et al. 2007), cotton gin waste (Haleem et al. 2014), TCF-bleached Milox pulps (Dapía et al. 2005) and others non-wood fibers materials (Barba et al. 2002). Nevertheless, few researches have proposed producing this cellulose derivative from *E. nitens*.

In this context, the first objective of this work was the isolation of cellulose from *E. nitens* using organosolv pulping and the second one was to study the preparation and characterization of its carboxymethylated derivative.

Experimental

Raw material

Eucalyptus nitens chips provided by the Chilean company Empresas CMPC S.A. were screened by particle size of 3.5 cm × 3 cm × 0.5 cm (length, width, thickness). Wood chips were dried in a mechanical convection oven at 50 °C until the content of moisture reached 8% and they were stored in plastic bags at 4 °C.

Extraction of cellulose

Methanol–water organosolv

Methanol–water Organosolv pulping was carried out in a 5 L high pressure Lab. Autoclave-Reactor SS-316 (AMAR EQUIPMENT PVT. LTD., Mumbai, India) equipped with P.I.D. temperature controller Model Neuro 102 ac, thermocouple, and fitted with an internal cooling coil. The reactor was loaded with 200 g of *E. nitens* wood chips (dry basis) and 1.2 L 50% (v/v) methanol–water liquor. The reactor was heated at an average rate of 2.0 °C min⁻¹ until 200 °C. Once the reactor reached 150 °C, the reaction mixture was stirred at 250 rpm and at temperature set point (200 °C), the pulping was continued throughout a residence time of 45 min. Then, the reactor was rapidly water-cooled through the internal cooling coil (serpentine coil). The mixture was vacuum filtered and the solid was washed with 1.2 L warm 50% v/v methanol–water (60 °C) solution followed by 1L of hot water. The pulp was homogenized in 2 L of distilled water for 15 min at 250 rpm in a high speed disperser (IKA® ULTRA-TURRAX, Germany), and the suspension was vacuum filtered using Whatman filter paper, Grade 1. Finally, the wet pulp was centrifuged, weighed, and its moisture content was determined according to the TAPPI method T 210 cm-03. The process severity was calculated using the H-factor parameter (Vroom 1957; Sundquist 2000). H-factor is a single variable model used in the kraft pulping process, combining temperature and time to determine the extent of the cooking. The H-factor is determined by the area under a cooking curve corresponding to the H-factor rate at a given temperature and time (in hours) at each temperature (Eq. 1).

$$H = \int_0^t e^{\left(43.2 - \frac{16117}{T}\right)} dt \quad (1)$$

where T = reaction temperature (K), and t = reaction time (h).

Alkaline pretreatment

In a 2 L beaker, 50 g organosolv pulp (dry basis) were mixed with 1 L 1.5 M NaOH solution in a 20:1 liquor-to-pulp ratio. The mixture was heated up to 70 °C and was mechanically stirred at 150 rpm for 1 h. Pulp and liquor were separated by vacuum filtration on a sintered glass funnel. The extracted organosolv pulp was washed with 6 volumes distilled water at 80 °C and then, with 4 volumes cold water, and air dried, for yield determination.

Bleaching of extracted organosolv pulp

The extracted organosolv pulp (25 g) was transferred to a two neck round-bottom flask (2 L), and 1L NaClO₂ solution (1.3% p/v) was added. The resulting mixture was acidified at pH 3.8 with 6 M CH₃COOH, and refluxed for 3 h. The bleached pulp was cooled to room temperature and vacuum filtered on a sintered glass funnel, washed with four volumes of distilled water and then with 2 L NaHSO₃ solution (2% w/v). Then, it was washed with 1 volume ethanol, and again washed with two volumes of distilled water, filtered and allowed to dry overnight.

Chemical analysis

Klason lignin content in pulps was gravimetrically determined according to TAPPI Standard Method T-222 as the pulp acid insoluble residue after the sample underwent a primary hydrolysis in 72% sulfuric acid followed by a secondary dilute-acid hydrolysis at 121 °C for 1 h. Acid-soluble lignin was determined by absorbance at 205 nm (Lin and Dence 1992). Determination of α , β and γ cellulose in bleached pulp was conducted according to TAPPI Standard Method T 203 cm-99. Bleached pulp was extracted with 17.5 and 9.45% sodium hydroxide solutions at 25 °C, consecutively. The soluble fraction, consisting of β - and γ -cellulose was volumetrically determined by oxidation with potassium

dichromate, and the α -cellulose, as an insoluble fraction, derived by difference.

Synthesis of sodium carboxymethylcellulose (NaCMC)

In a 2 L three-neck round-bottom flask, 30 g of dry bleached pulp were mixed with 430 mL isopropyl alcohol and 100 mL 40% NaOH aqueous solution. The resulting mixture was mechanically stirred for 60 min, then, 18 g monochloroacetic acid (AMA) were added and the reaction mixture was heated at 60 °C for 3.5 h. After this, the mixture was filtered and the fibrous material was suspended in 300 mL methanol–water 70% v/v and neutralized using 6 M acetic acid. The suspension was filtered and the obtained fibers were dispersed in ethanol 95% v/v, stirred for 20 min and filtered. This process was repeated 5 times. Finally, the fibrous material was washed with absolute ethanol and allowed to dry in an oven at 60 °C for 24 h. The NaCMC mass gain (MG) was determined by Eq. 2.

$$\text{MG}(\%) = \frac{\text{NaCMC (g)} - \text{cellulose (g)}}{\text{cellulose (g)}} \times 100 \quad (2)$$

Determination of NaCMC degree of substitution (DS)

The degree of substitution (DS) is defined as the average number of substituent groups per anhydroglucose unit in one polymer cellulose chain on C₂, C₃ and C₆ positions. NaCMC (1 g) was mechanically dispersed in 50 mL 95% (v/v) ethanol. Then, 5 mL 2 M nitric acid were added and the reaction mixture was stirred for 10 min at room temperature. Subsequently, the mixture was heated to its boiling point for 5 min, further stirred for 20 min, cooled to room temperature, and filtered. The resulting solid was washed with 100 mL 95% ethanol until acid and salts were removed; then, it was washed with methanol (100 mL) and dried in oven at 90 °C for 3 h. The dried solid (0.5 g) was mechanically dispersed in water (100 mL) and boiled with 25 mL 0.5 M sodium hydroxide for 20 min under stirring. The resulting solution was titrated with 0.3 M HCl using phenolphthalein as indicator, showing a color change from dark pink to colorless. Determinations were performed in

triplicate. The degree of substitution was calculated using the following Eq. 3 (Togrul and Arslan 2003).

$$A = \frac{BC - DE}{F} \quad (3)$$

$$\text{Degree of substitution} = \frac{0.162 \times A}{1 - (0.058 \times A)}$$

where A = consumed acid per gram of CMC (mEq), B = volume of NaOH added (mL), C = concentration of NaOH, D = volume of HCl consumed, E = concentration of HCl used, and F = sample grams of CMC (g). Used; 162 is the molecular weight of an anhydrous glucose unit and 58 is the net increase in anhydrous glucose weight by monosubstitution with carboxymethyl group.

Characterization methods

Fourier transform infrared (FTIR) spectroscopy

FTIR absorption spectra of polymers in KBr pellets were recorded in the 4000–400 cm⁻¹ region on a BRUKER, VECTOR 22 IR spectrometer.

NMR spectroscopy

NMR spectra were recorded using a Bruker Avance 400 MHz NMR spectrometer (Bruker Instruments Inc. Billerica, MA, USA) equipped with a 5 mm diameter inverse broadband probe with Z gradient, operating at a frequency of 400.13 MHz (¹H) and 100.62 MHz (¹³C). Spectra were recorded in D₂O after isotopic exchange (3 × 0.75 mL) at 70 °C, using 3-(trimethylsilyl)-1-propionic-2,2,3,3-*d*₄ acid sodium salt as internal reference.

Scanning electron microscope (SEM)

Samples of organosolv pulp, bleached pulp cellulose and sodium carboxymethylcellulose (NaCMC) were analyzed by FEI scanning electron microscopy (SEM), Model F50 Inspect. In the analysis, samples were coated with a 10 nm gold layer and a 10 kV electron beam was used.

Thermogravimetric analysis (TGA)

Dynamic thermogravimetric measurements were performed by using TA instruments SDT 2960 Simultaneous DSC-TGA. Temperature programs for dynamic tests were run from 20 to 700 °C at a heating rate of 10 °C/min. Measurements were carried out under a nitrogen atmosphere (20 mL/min) in order to prevent any thermoxidative degradation.

Results and discussion

Cellulose extraction

In this work, *E. nitens* wood was pretreated using methanol–water solution at 50% (v/v), using a liquor-to-wood ratio of 6:1 (v/w), at a temperature of 200 °C and for a residence time of 45 min. The severity factor determined for this process was 10,853. In previous works, these pulping conditions were found to be the most suitable for fractionating *Eucalyptus globulus* using ethanol as solvent, because they are the best conditions for coproducing a high yield cellulose-rich solid fraction and a liquid fraction from which lignin can be recovered (Yañez-S et al. 2013, 2014). In addition, previous works on *E. globulus* autocatalyzed methanol pulping showed that, at 50% (w/w) methanol concentration, up to 73% lignin recovery in the black liquor was obtained (Botello et al. 1999).

Under the pulping conditions set for this work, a pulp with are residual lignin content (22%) higher than the content in organosolv pretreatment ethanol–water was obtained, even with a lower severity factor (Yañez-S et al. 2013). In order to reduce the residual lignin content in the organosolv pulp before bleaching, an alkaline extraction was performed. With the purpose to avoid severe chemical structure alterations, conditions which are milder than the ones of ordinary alkaline pulping (such as kraft and soda pulping processes) were applied (Hussin et al. 2013). These conditions have been effective to remove residual lignin of steam exploded fibers of hardwood, such as *Betula verrucosa* and *Populus tremula*, and softwood such as *Picea abies* and *Pinus sylvestris* (Li et al. 2009). The yield of pulp extracted at this stage was 48.3% and the residual lignin content decreased to 0.72% (see Table 1). This result shows the

effectiveness of the process for removing lignin from *E. nitens* organosolv pulp.

Table 1 shows the yields of cellulose pulps and their lignin content, obtained under organosolv pulping, alkaline pretreatment and chemical bleaching.

During bleaching, sodium chlorite (NaClO_2) was used as reagent. It is a stable compound, highly soluble in water and characterized for being an effective bleaching agent, preserving pulp strength properties. This feature is attributed to the low oxidation potential of NaClO_2 compared to other bleaching agents (Chattopadhyay and Sarkar 1946). In order to bleach the pulp extracted, sodium chlorite was used in acid medium (CH_3COOH , 6 M). In this condition, the metastable chlorous acid (HClO_2) is degraded to chlorite (ClO_2^-), and chlorite is further oxidized to chlorate (ClO_3^-). Subsequently, chlorate is reduced to chlorine dioxide (ClO_2) and chloride (Cl^-). Therefore, the reagent generates an oxidative solution with oxychloro species. After the bleaching process, the pulp was subjected to the action of 2% w/v sodium bisulfite solution, which improved the pulp colour and acted as a good antichlorine agent.

The determination of α , β and γ -cellulose in the *E. nitens* bleached pulp was performed according to the standard TAPPI T method 203 cm-99. This method consists in extracting the pulp from cellulose at 25 °C with 17.5 and 9.45% w/w sodium hydroxide solutions. The process-resistant pulp fraction corresponds to α -cellulose, which is a high molecular weight

Table 1 Mass yield and lignin content of the materials obtained after organosolv pulping (methanol–water), alkaline pretreatment (NaOH) and chemical bleaching (NaClO_2)

Stage	Yield (%)	Klason lignin (%)
Organosolv pulping	62.70	22.00 ± 2.04
Alkaline pretreatment	48.30	0.72 ± 0.18
Chemical bleaching	47.5	0.03 ± 0.01

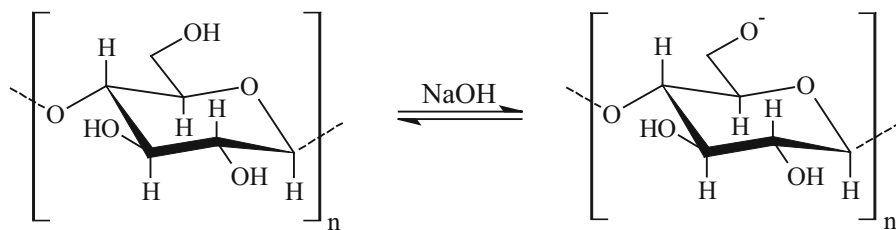
Organosolv pulp yield (%) = [oven-dry organosolv pulp (g)/(oven-dry untreated wood (g)) × 100

Extracted pulp yield (%) = [oven-dry extracted pulp (g)/(oven-dry untreated wood (g)) × 100

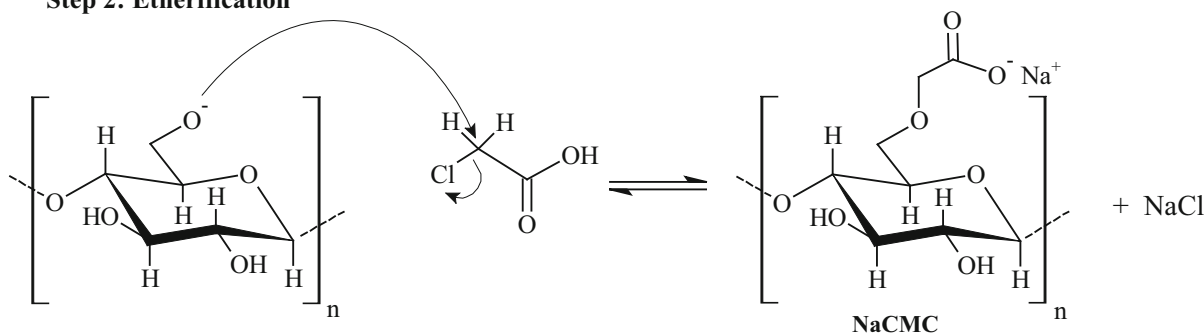
Bleached pulp yield (%) = [Oven-dry bleached pulp (g)/(oven-dry untreated wood (g)) × 100

Klason lignin (includes both acid-soluble and acid-insoluble lignin). Mean of three replicate analyses

Step 1: Alkalization



Step 2: Etherification



Competitive reaction

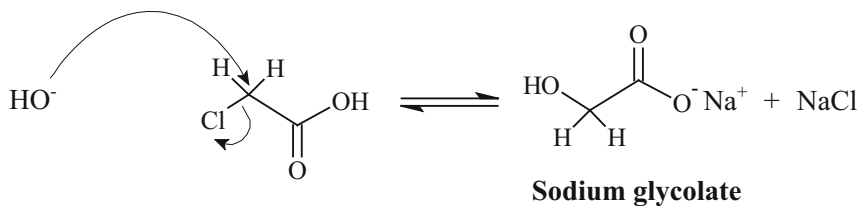


Fig. 1 Synthetic route for the preparation of carboxymethylcellulose, showing a first step with formation of alkoxide groups that act as nucleophile on the monochloroacetic acid aliphatic carbon in a S_N2 substitution reaction

undegraded cellulose, whose chains are made of more than 150 anhydroglucose units (Kuckurek 1989).

This fraction is determined as the difference between the total pulp (100%) and the soluble fraction, in percentage. In contrast, the soluble fraction corresponds to the content of β and γ -cellulose, which are determined together by oxidation with an excess of potassium dichromate ($K_2Cr_2O_7$). β -cellulose is a lower molecular weight degraded cellulose. γ -Cellulose includes chains with 15 units or less and mainly consists of hemicelluloses (Ziaie-Shirkolaei et al. 2007).

Analyses of bleached *E. nitens* organosolv pulp indicated that it consists of about 91.92 wt% α -cellulose, 7.72 wt% β -cellulose and 0.36 wt% γ -cellulose (hemicelluloses). These results are close to

those published by Tonoli et al. (2012), for kraft pulp of other species such as *Eucalyptus urograndis*. In addition, the α -cellulose content is within the range of other bleached pulps obtained from non-wood fibers such as yute, sisal, abacá and *Miscanthus sinensis* (87.8–93.8%) (Barba et al. 2002). The Klason lignin content was < 0.03%, indicating the effectiveness of bleaching. The acid hydrolysis of pulp results in carbohydrate degradation products such as furfural and hydroxymethylfurfural, which, strongly absorb at 280 nm; nevertheless, at this wavelength commonly lignin was monitored in solution. In this work, the acid-soluble lignin absorbance was measured at 205 nm, where the risk of interference from furfural was considerably lower.

Carboxymethylcellulose

Carboxymethylation involves a two-step process under heterogeneous conditions. The first step is an alkalization reaction where hydroxide ion deprotonated hydroxyl groups of glucopyranosyl residues at positions C-2, C-3 and C-6 and produced alkali cellulose (Fengel and Wegener 1989). The second step is an etherification reaction, corresponding to a second-order nucleophilic substitution (S_N2) in which alkoxide ions generated in a basic medium may nucleophilically attack the methylene carbon atom of the monochloroacetic acid. However, the reaction mainly occurs on the OH-groups at C6, which show the highest reactivity followed by the substitution on C2-OH. This is due to the smaller steric hindrance shown by the alkoxy group on C6 carbon (Fox et al. 2011). In a competitive reaction, hydroxide ions can directly attack the acid, generating sodium glycolate as a byproduct. In both reactions sodium chloride is generated as a byproduct (See Fig. 1).

The synthesis of sodium carboxymethylcellulose (NaCMC) from *E. nitens* bleached organosolv pulp was carried out in isopropyl alcohol, given its ability to disperse cellulose fibers (Varshney and Naithani 2011). The cellulose activation step (mercerization) was performed on a cellulose/isopropyl alcohol ratio of 0.069 (w/v) and using a 40% w/v NaOH solution. This concentration has proved to be optimal for the sugarcane straw pulp carboxymethylation, reaching degrees of substitution (DS) within the range of 0.4–0.7 (Candido and Goncalves 2016; Ruzene et al. 2007). In this work, NaCMC mass gain from bleached organosolv pulp was 22%. This yield was lower than that one obtained with other raw materials such as sugarcane bagasse pulp (35%) (Ruzene et al. 2007), cotton gin waste (43.7%) (Haleem et al. 2014) and sugarcane straw (57.6%) (Candido and Goncalves 2016).

On the other hand, under reaction conditions, a DS of 0.32 was obtained. This degree of substitution is close to that obtained in the sugarcane straw carboxymethylation (0.40 ± 0.05) (Candido and Goncalves 2016). More likely, the low degree of substitution is due to the high structural organization of cellulose fibrils in the pulp which implies that glucopyranosyl residue -OH groups are not readily accessible to the reactants (Barba et al. 2002).

FTIR spectroscopy

FTIR spectra of *E. nitens* cellulose pulp and carboxymethylcellulose (NaCMC) are shown in Fig. 2. The infrared spectrum of cellulose pulp shows an intense broad absorption band at 3423 cm^{-1} , which is attributed to the OH group stretching vibration and additionally, it indicates the high proportion of water linked to these groups (Popescu et al. 2007; Hinterstoisser and Salmén 2000). At 1638 cm^{-1} a medium band is seen, resulting from the bending vibration in water plane (adsorbed) related to cellulose (Popescu et al. 2007). The band at 1374 cm^{-1} is assigned to C–H bending vibrations in cellulose II. At 1163 cm^{-1} a medium band, attributed to the asymmetric stretching vibration C–O–C in cellulose II, is observed. Bands between 800 and 500 cm^{-1} result from heavy atom flexing in both C–O and ring vibrational modes, with some minor contributions of ring stretching. In addition, out-of-plane O–H modes may also contribute (Hinterstoisser and Salmén 2000; Michell 1988). The presence of NaCMC was confirmed by two intense absorption bands at 1604 and 1419 cm^{-1} , which are not found in the IR spectrum of bleached pulp. These absorption bands are the result of carboxylate group asymmetric and symmetric stretching vibrations, respectively (Mansouri et al. 2015). The absorption band at 801 cm^{-1} results from the bending vibration of the same group. The band at 1325 cm^{-1} corresponds to the hydroxyl group O–H bending vibration

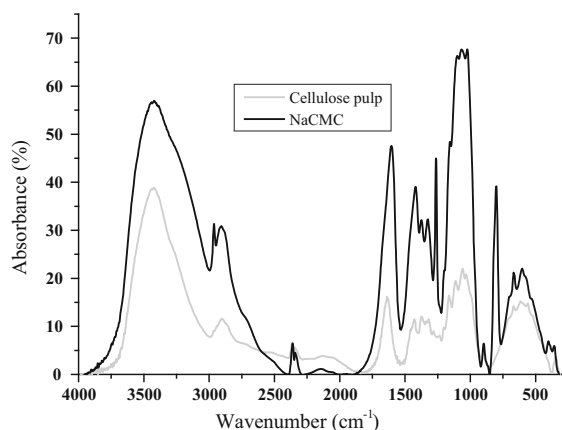


Fig. 2 FTIR spectra of *Eucalyptus nitens* organosolv cellulose pulp and sodium salt carboxymethylcellulose prepared by the reaction of bleached organosolv pulp with 40% sodium hydroxide solution and monochloroacetic acid [cellulose/AMA ratio of 1.67 (w/v)]

Fig. 3 **a** ^1H NMR at 400 MHz and **b** ^{13}C NMR at 100 MHz spectra of NaCMC in D_2O at 27 °C using the 2,2,3,3- d_4 -3-(trimethylsilyl) propionic acid (TSP- d_4) sodium salt as reference

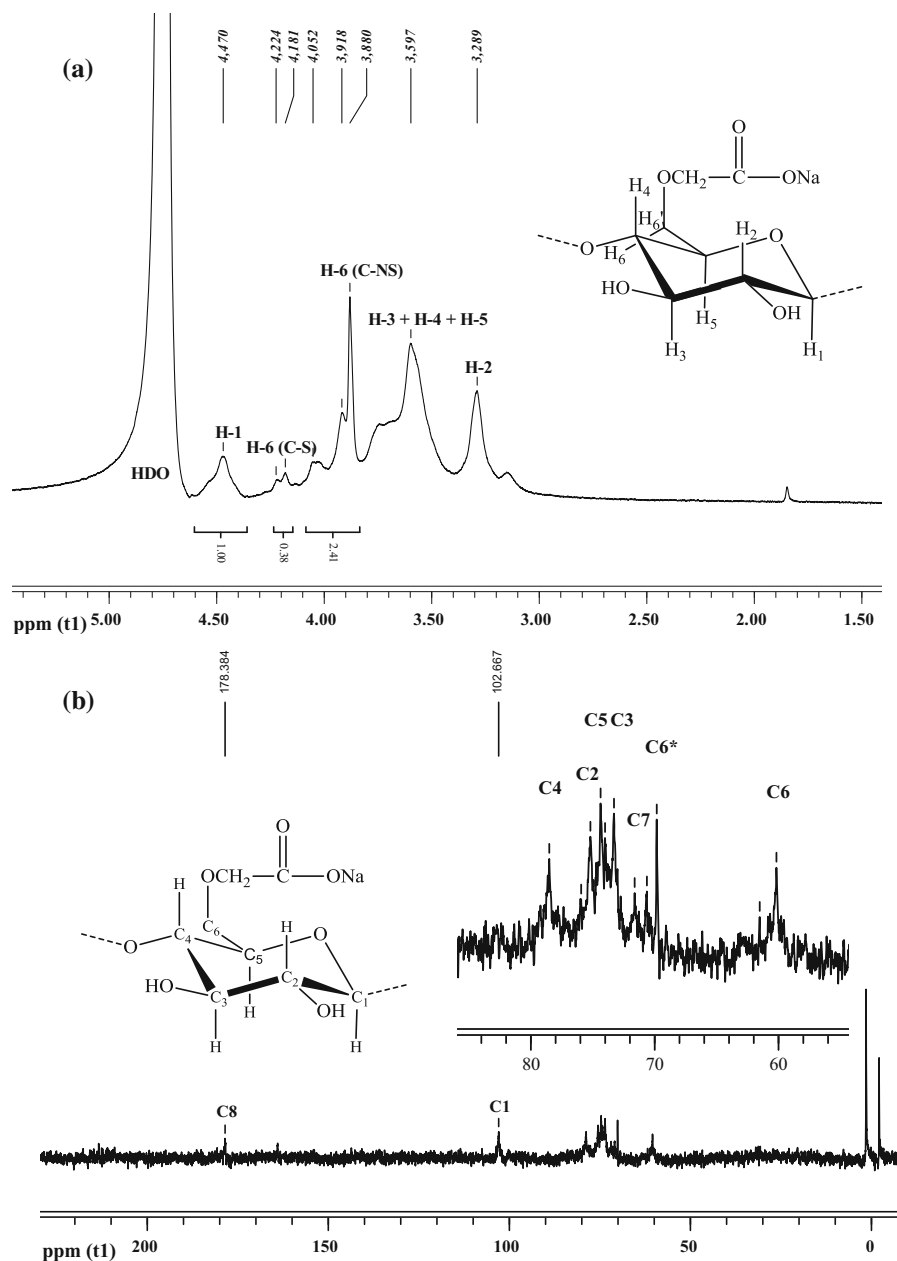


Table 2 Chemical shift assignments (ppm) of ^1H and ^{13}C NMR spectra of NaCMC obtained from *Eucalyptus nitens* bleached organosolv pulp

C_1, H_1	C_2, H_2	C_3, H_3	C_4, H_4	C_5, H_5	$\text{C}_6, \text{H}_{6,6'}$	C_7, H_7
102.667, 4.47	75.140, 3.28	73.229, 3.60	78.469, 3.60	74.315, 3.60	60.087, 3.88	71.632, 4.60
$\text{C}_8 = 178.3$					* $\text{C}_6 = 69.772, 4.18$	

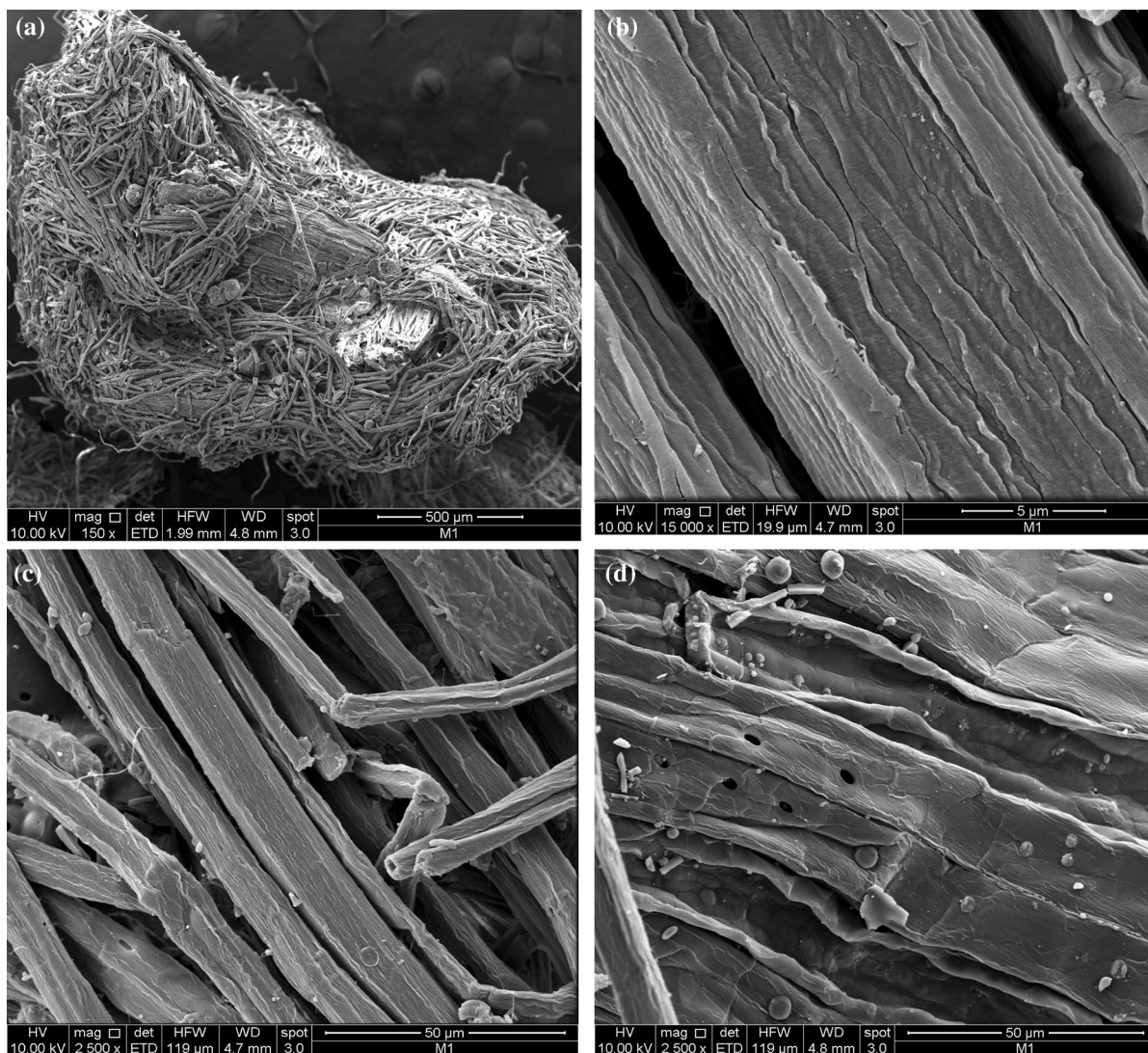


Fig. 4 Scanning electron microscope (SEM) of *Eucalyptus nitens* organosolv pulp cellulose fibers of from. **a** Scale bar is 500 μm , **b** scale bar is 5 μm , **c**, **d** the scale bar is 50 μm .

and the band at 1067.18 cm^{-1} is assigned to $-\text{CH}_2-\text{O}-\text{CH}_2-$ group stretching vibration (Haleem et al. 2014). Finally, the absorption band at 897 cm^{-1} is associated to the $1 \rightarrow 4$ β linked glucopyranosyl residues (Michell 1990).

^1H and ^{13}C NMR spectroscopy

Figure 3a depicts the ^1H NMR spectrum of NaCMC. It shows a signal at 4.47 ppm which was assigned, according to the literature to the anomeric proton (H_1)

Micrographs (c) and (d) show small spherical particles (droplets), attributed to pseudo-lignin

of β -glucopyranosyl residues (Agrawal 1992). The signal at 4.181 ppm was assigned to the H_6 proton when carbon 6 was substituted with the carboxymethyl group, whereas the signal at 3.88 ppm is assigned to the H_6 proton when carbon is unsubstituted. This latter signal is more intense due to the low degree of substitution (DS) in the polymeric structure of the cellulose. Within the 3.40–3.80 ppm range, a wide signal assigned to H_3 , H_4 and H_5 protons is observed. Finally, the signal at 3.29 ppm is assigned to H_2 proton (Heinze and Koschella 2005; Kono 2013).

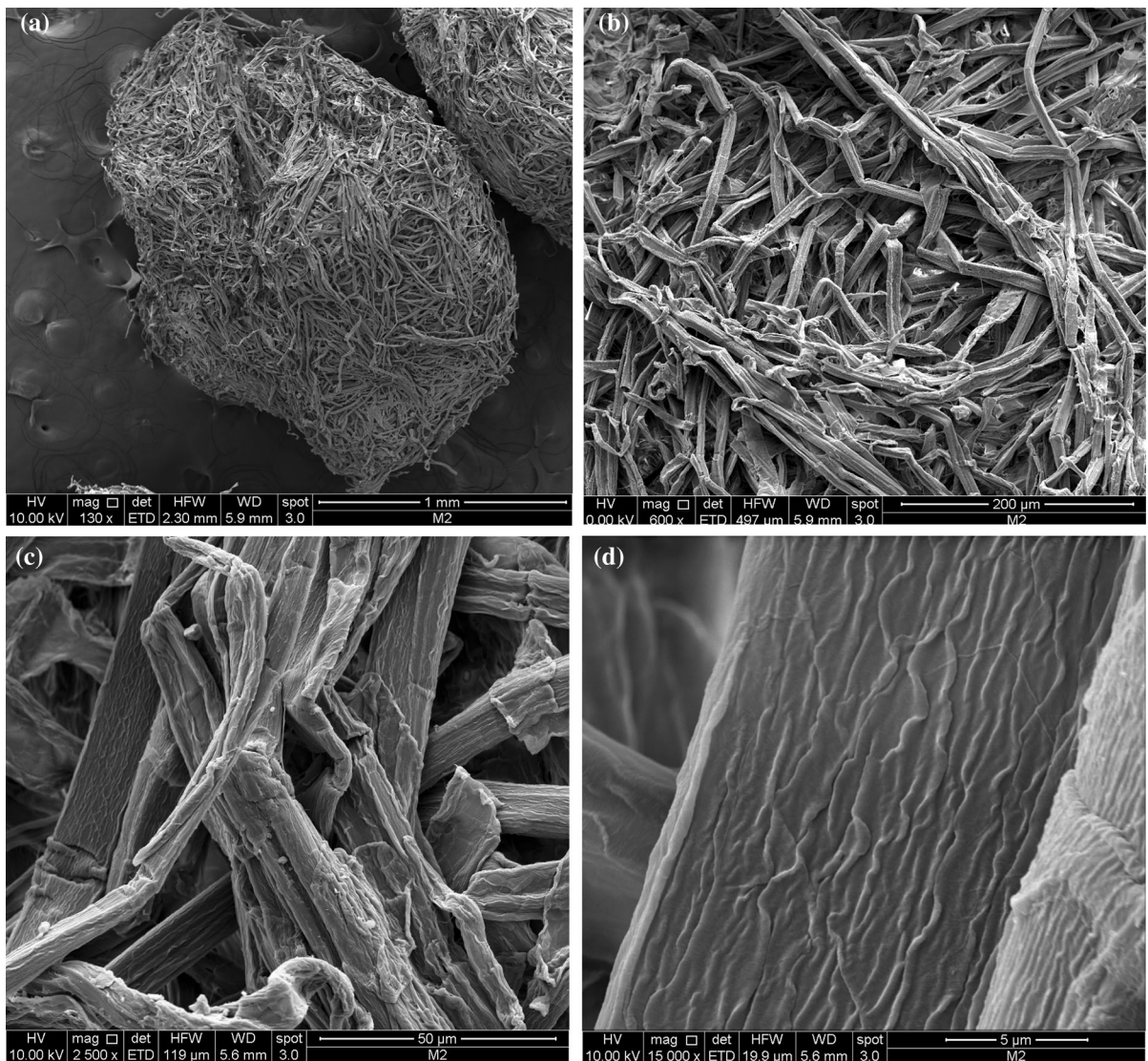


Fig. 5 Scanning electron microscope (SEM) of *Eucalyptus nitens* bleached organosolv pulp cellulose fibers. Scale bars: **a** 1 mm, **b** 200 μm , **c** 50 μm , **d** 5 μm . Micrograph (c) is at higher magnification ($\times 20$) of a. Micrograph (d) is at higher magnification ($\times 40$) of b

Partial carboxymethylation (χ_i) (Heinze et al. 2001) was calculated from ^1H NMR spectrum using the integration of the signals between 3.880 and 4.47 ppm.

The partial degree of substitution determined for C6 position was 0.23. Figure 3b shows the ^{13}C NMR spectrum of NaCMC, with a signal at 180.9 ppm assigned to the carbonyl carbon atom of carboxymethyl group (C8) due to the deshielding effect of the electronegative oxygen atom. At a higher field (105.2 ppm), a signal corresponding to the β anomeric carbon (C1) is shown.

It is worth to note the presence of the signal at 69.7 ppm assigned to carboxymethylated C6 and at 71.55 ppm attributed to methylene carbon (C7) of the carboxymethyl group ($-\text{CH}_2-\text{COO}-$). These results indicate that substitution mostly occurred at the C6 position (Capitani et al. 2000). Chemical shift assignments in the ^1H and ^{13}C NMR spectra of carboxymethylcellulose are shown in Table 2.

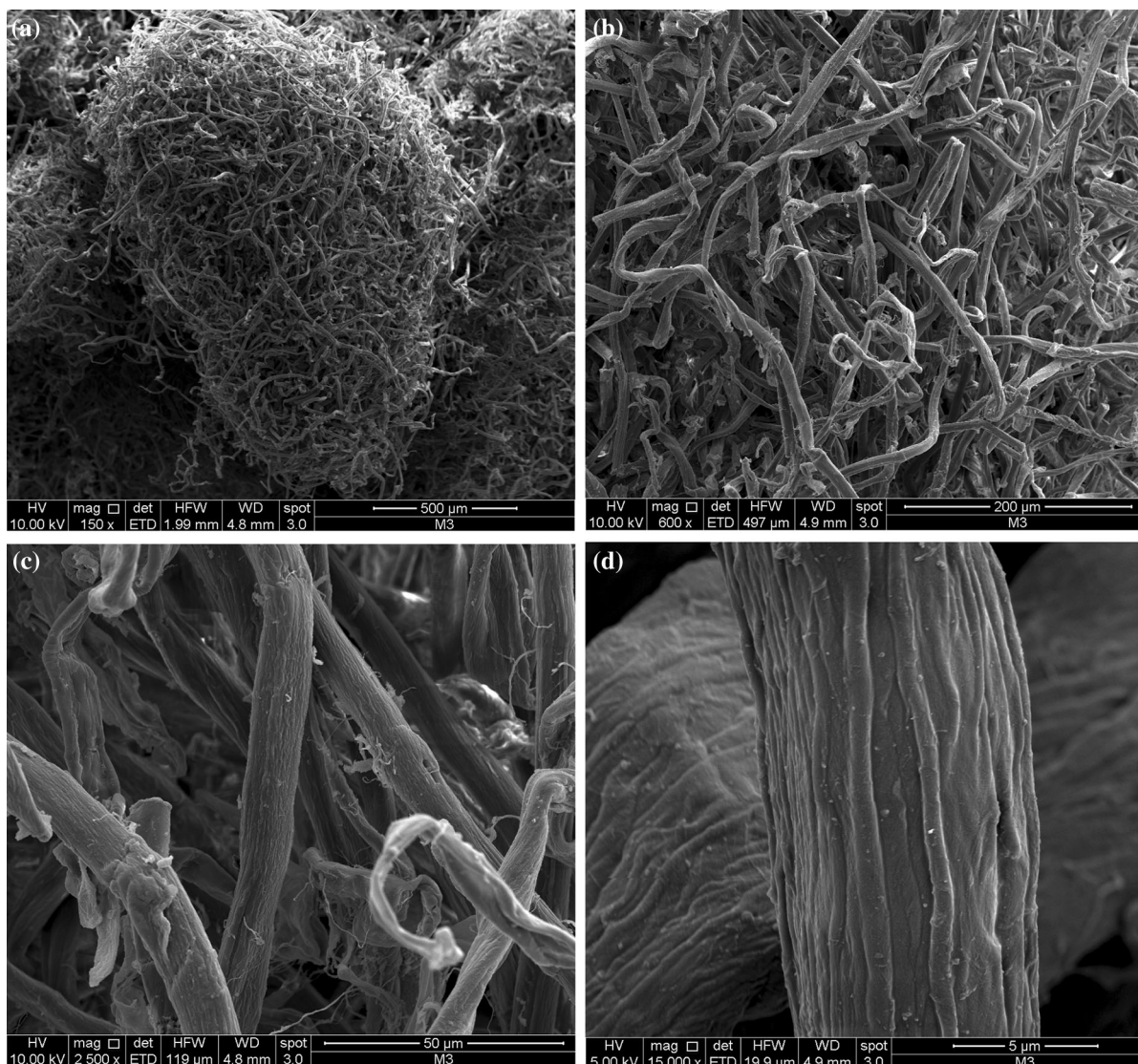


Fig. 6 SEM micrographs of sodium carboxymethylcellulose fibers obtained for the reaction of bleached organosolv pulp with 40% sodium hydroxide solution and monochloroacetic acid (cellulose/AMA ratio of 1.67 (w/v)). Scale bars: **a** 500 µm, **b** 200

µm, **c** 50 µm, **d** bar 5 µm. Micrographs (**b**) and (**c**) show that carboxymethyl group incorporation in cellulose chains produce effects of electrostatic repulsion between fibers

SEM observations

Figure 4 shows the scanning electron micrographs of organosolv pulp macrofibers after pretreatment process and high shear homogenization. The images show the lignocellulosic material defibrillation after the pretreatment process, although internally the fibrous form is retained (Fig. 4a, b). The organosolv pulp cellulose fiber diameters were mainly distributed in

the range of 6.2–13.6 µm. In images 4c, d, small spherical particles (droplets) were observed.

These droplets have been attributed to pseudo-lignin, which usually has a spherical structure (Pu et al. 2013; Hu et al. 2013). Pseudo-lignin is a combination of carbohydrates and lignin degradation products (Kumar et al. 2013; Sannigrahi et al. 2011). Specifically, it consists of carbonyl, carboxylic, aromatic and aliphatic structures (Hu et al. 2012). These pseudo-lignin droplets are formed during pretreatment when

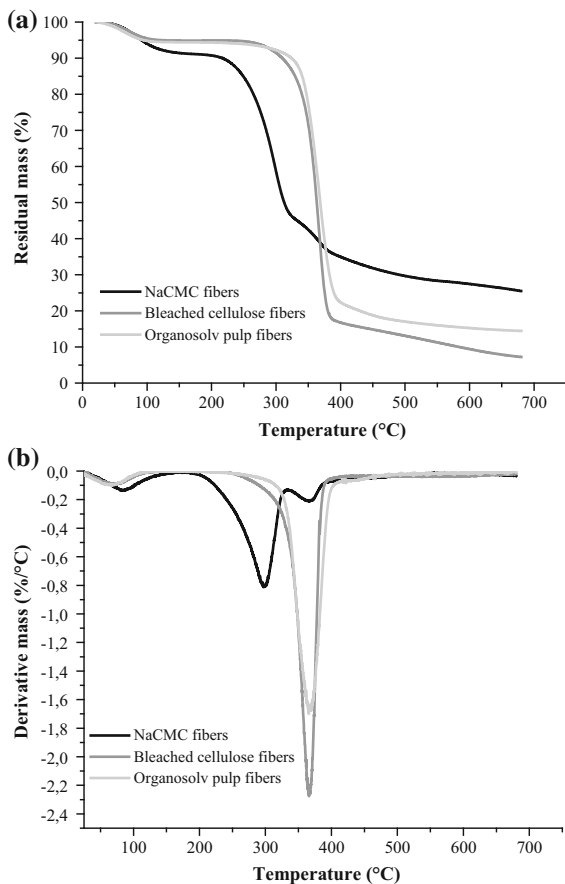


Fig. 7 **a** Thermal analysis of organosolv pulp fibers, bleached cellulose fibers and sodium carboxymethylcellulose (NaCMC). **b** Differential thermogram of sodium carboxymethylcellulose (NaCMC), cellulose fibers, and organosolv pulp

temperatures above the glass transition phase are reached. This causes its coalescence, migration within and out of the cell wall, and re-deposition on the fiber surface (Donohoe et al. 2008; Sannigrahi et al. 2011).

In contrast, bleached cellulose fibers are more randomly distributed than in the organosolv pulp and their diameters are in the range of 10.2–14.1 μm (Fig. 5). This diameter increase indicates that during bleaching, cellulose fibers swell due to water incorporation, breaking the intra and intermolecular hydrogen bonds between cellulose chains. Moreover, in micrographs, no pseudo-lignin is observed, demonstrating the effectiveness of the bleaching process.

NaCMC fiber SEM micrographs are shown in Fig. 6. The average fiber width is 12.5 μm . It can be seen the structure is less compact and ordered than cellulose fiber and organosolv pulp. This is explained

by the incorporation of the carboxymethyl group, which produces both a steric effect between the cellulose chains, modifying the structure crystallinity, and an electrostatic repulsion effect between fibers.

Thermogravimetric analysis (TGA/DTG)

Figure 7a shows the thermal analysis curves of organosolv pulp, bleached pulp cellulose and sodium carboxymethylcellulose (NaCMC).

The thermal analysis shows the residual mass (wt%) percentage of the sample in a nitrogen atmosphere as a function of the temperature. In every material, a small mass loss was observed in the range of 20–150 °C due to humidity evaporation and low molecular weight compounds remaining after the isolation procedures. The cellulose pulp decomposition process started at 230 °C and continued up to 700 °C and at this temperature the cellulose was almost completely pyrolyzed, leaving a solid residue close to 7% w/w. Organosolv pulp showed stability greater than cellulose pulp by about 288 °C, due to its higher residual lignin content (22%). The solid residue at 700 °C is close to 14% w/w. Decomposition of carboxymethylcellulose started at a lower temperature than cellulose (200 °C) and continued up to 700 °C.

In Fig. 7b, the differential mass loss (in wt%/°C) is shown as a function of temperature for the same samples. NaCMC shows a sharp peak at 297 °C, which is attributed to decarboxylation processes of carboxylated glucose units and/or carboxymethyl group decomposition. At 366 °C, another stage of maximum mass loss rate is observed. The solid residue at 700 °C was around 25% w/w.

Conclusions

Organosolv pretreatment employing methanol–water mixture was an effective method to extract cellulose from *E. nitens*. Under these conditions of evaluation, a high yield of cellulose pulp and a low residual lignin content were obtained. Sodium carboxymethylcellulose synthesized from *E. nitens* cellulose showed a low degree of substitution (0.32), this is probably due to the high structural organization of cellulose microfibrils, which makes interaction with reactants difficult. TGA and DTG analysis indicated that thermal stability of NaCMC decreased compared to cellulose fibers.

Further studies, mainly on the improvement of carboxymethylation parameters, must be carried out. The abundance of *E. nitens* and its high content of cellulose makes it a suitable raw material for the production of cellulose derivatives.

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