Food Hydrocolloids 75 (2018) 147–156

Contents lists available at ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

Carboxymethyl cellulose with tailored degree of substitution obtained from bacterial cellulose

Agustina Casaburi [a], Úrsula Montoya Rojo [a, c], Patricia Cerrutti [a, b], Analía Vázquez [a, c], María Laura Foresti [a, c, *]

[a] Grupo de Biotecnología y Biosíntesis, Instituto de Tecnología en Polímeros y Nanotecnología (ITPN-UBA-CONICET), Facultad de Ingeniería, Universidad de Buenos Aires, Las Heras 2214 (CP 1127AAR), Buenos Aires, Argentina
[b] Departamento de Ingeniería Química, Facultad de Ingeniería, Universidad de Buenos Aires, Argentina
[c] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina

ARTICLE INFO

Article history:
Received 4 May 2017
Received in revised form
4 August 2017
Accepted 4 September 2017
Available online 4 September 2017

Keywords:
Bacterial cellulose
Carboxymethylation
Tailored degree of substitution
Characterization

ABSTRACT

Among the multiple industrial applications of carboxymethyl cellulose (CMC), those related to food and beverages industries (e.g. as thickener, stabilizer, emulsifier, binding agent), involve one fourth of the global CMC consumption. In the last years, a number of abundant and underutilized vegetable cellulose sources have been assayed as raw material for CMC production, as alternatives to cotton linters or cellulose feedstocks obtained from bleached pulps derived from wood. Alternatively, cellulose of microbial origin appears as a very promising highly pure raw material for CMC production.

Together with molecular weight and substituents distribution, it is well established that the degree of substitution (DS) of CMC plays a key role in most food and beverages applications. In the current contribution, highly pure cellulose of bacterial origin was used to produce CMC with tailored DS in a two-stage process consisting of alkalinization with sodium hydroxide, followed by etherification with sodium monochloroacetate. Aiming to get insight into how the carboxymethylation extent conferred to BC can be easily tuned within the DS interval allowed for food uses (i.e. 0.2–1.5), the effects of NaOH concentration, molar NaOH/anhydroglucose unit ratio, molar etherifying reagent/anhydroglucose unit ratio, and etherification time, were systematically analyzed. By proper control of those variables, CMC samples with tailored DS within the 0.60–1.52 interval could be successfully obtained. Samples with varying DS were further characterized by means of FTIR, solid state ^{13}C NMR, XRD and TGA. The suitability of using TGA data for estimating the carboxymethylation extent achieved is proposed.

1. Introduction

Carboxymethyl cellulose (CMC) is an anionic water-soluble cellulose ether, produced by reacting alkali cellulose with monochloroacetic acid (MCA) or its sodium salt (NaMCA). CMC finds application in a variety of areas including cosmetics, food, pharmaceutical, adhesives, ceramics, coatings, detergents, paper, textile, oil-drilling and tobacco industries. In those industries, CMC is mainly used as thickener, suspending aid, binder, film-former, gelling agent, stabilizer, water retention agent, protective colloid and/or rheology control agent. Those properties have made CMC the most produced and widely used industrial cellulose ether (Edali, Esmail, & Vatistas, 2001). As CMC is obtained together with a high fraction of by-product sodium salts (i.e. sodium chloride and sodium glycolate), depending on the particular application different purification levels may be required. For example, uses of CMC in detergents, oil drilling and paper industry, require large quantities of crude commercial grade CMC, which requires no refining. On the other hand, uses in food products and pharmaceuticals require high purity CMC grades.

Besides purity, the degree of substitution (DS, average number of hydroxyl groups substituted with carboxymethyl groups per anhydroglucose unit (AGU)) has a major influence on the properties and therefore the potential uses of CMC. This is the case for example of the water solubility of CMC, as well as a number of CMC solutions characteristics such as stability, clarity of the solution, viscosity,

* Corresponding author. Grupo de Biotecnología y Biosíntesis, Instituto de Tecnología en Polímeros y Nanotecnología (ITPN-UBA-CONICET), Facultad de Ingeniería, Universidad de Buenos Aires, Las Heras 2214 (CP 1127AAR), Buenos Aires, Argentina.
E-mail address: mforesti@fi.uba.ar (M.L. Foresti).

http://dx.doi.org/10.1016/j.foodhyd.2017.09.002
0268-005X/

interaction via $-COO^-$ groups/H-bonding, and thixotropy. The DS of CMC has also a direct impact on emulsibility, acid resistance and salt tolerance. The theoretical maximum DS of CMC is 3 (i.e. all OH groups per anhydroglucose unit have been substituted), but the interval of commercially available CMC grades is generally in the range of 0.4–1.5 (Heinze & Pfeiffer, 1999). Particularly, for food uses the DS level of CMC should be within the 0.2–1.5 interval (Joint FAO/WHO Expert Committee on Food Additives, 2011). The impact of the DS of CMC in different food uses is well established (Cai, Wu, Du, & Zhang, 2017; Wüstenberg, 2015; Zecher & Gerrish, 1997).

Commercial CMC is generally produced from cellulose feedstocks obtained from bleached prehydrolyzed kraft and acid sulfite pulps derived from wood, or directly from cotton linters (Barba, Montané, Rinaudo, & Farriol, 2002). However, in the last decade a number of alternative abundant and underutilized cheaper cellulose sources have been assayed as raw material for CMC production, including annual fiber crops, agricultural residues, and wild plants (Adinugraha, Marseno, & Haryadi, 2005; Barai, Singhal, & Kulkarni, 1997; Barba et al., 2002; Bono et al., 2009; Haleem, Arshad, Shahid, & Ashraf Tahir, 2004; Hassan, 2014; Saputra, Qadhayna, & Pitaloka, 2014).

On the other hand, cellulose of bacterial origin has rarely been assayed as raw material for CMC synthesis. Bacterial cellulose (BC) has the same molecular formula as plant cellulose. However, and different from cellulose isolated from wood or plants, microbial cellulose has the advantage of being produced free of lignin, hemicelluloses and pectin, thus avoiding the need for chemical treatments devoted to the removal of these compounds prior to cellulose derivatization. The high intrinsic purity of bacterial cellulose together with the lower environmental impact associated with its isolation, encourages its use in traditional plant derived cellulose applications such as CMC production. However, previous contributions on BC carboxymtehylation are very scarce (Cheng & Mital, 1998; Cheng, Takai, & Ekong, 1999; Geyer et al., 1994; Schlufter & Heinze, 2010) and no methodic study devoted to tailoring the DS of the produced CMC within the range of commercial interest is available. In this context, in the current contribution the effects of selected reaction variables on the DS of BC-derived CMC were systematically assayed. With this aim, the influence of alkali concentration, molar alkali/AGU ratio, molar NaMCA/AGU ratio, and etherification time on the DS conferred to never-dried BC, were evaluated by varying all four parameters one at a time within chosen intervals. Carboxymethylated BC samples with varying DS thus obtained were further characterized by Fourier Transform Infrared Spectroscopy (FTIR) and ^{13}C solid-state nuclear magnetic resonance (^{13}C CP/MAS NMR) to determine their chemical structure, and X-rays diffraction (XRD) and thermogravimetric analysis (TGA) to get insight into the effect of DS on their crystallinity and thermal stability, respectively. The possibility of using TGA data for estimating the DS of carboxymethylated BC samples is also proposed.

2. Materials and methods

2.1. Materials

Carboxymethylation of BC, product recovery and DS determination required the use of sodium hydroxide (Biopack), sodium monochloroacetate (NaMCA, Sigma-Aldrich), isopropanol (Cicarelli), glacial acetic acid (Merck), methanol (Cicarelli), ethanol (Quimicor), hydrochloric acid (Cicarelli) and potassium hydroxide (Biopack). Calibration of TGA for DS estimation was performed with microcrystalline cellulose (MCC, Sigma-Aldrich) and a commercial CMC sample (GELYCEL, 99.5%, DS = 0.65–0.85, Amtex). BC culture medium was formulated using glycerol (Sintorgan) and corn steep liquor (Ingredion). Inocula were cultured in Hestrin and Schramm (HS) medium (Hestrin & Schramm, 1954), formulated with anhydrous dextrose, disodium phosphate.12 H_2O and citric acid (Biopack), and meat peptone and yeast extract (Britania).

2.2. Production of BC

BC was produced in static culture using a *Gluconacetobacter xylinus* strain under optimized conditions previously described (Cerrutti et al., 2016). Briefly, inocula of *G. xylinus* were cultured in 100 mL Erlenmeyers flasks containing 20 mL of Hestrin and Schramm (HS) medium which were incubated under orbital agitation (200 rpm) at 28 °C for 48 h. Inocula (1% v/v) were then transferred to 10 l steel trays with 5.0 L of fermentation medium containing glycerol (4.0% w/v) and corn steep liquor (8.0% w/v), which were statically incubated at 28 °C during 14 days. The produced BC pellicles were then harvested, thoroughly rinsed with distilled water to remove the culture medium, and homogenized in a blender with KOH solution (5% w/v) for 5 min (Castro et al., 2011). The suspension was left in alkali at room temperature for 14 h to eliminate the bacterial cells, and finally rinsed with distilled water till neutralization. This purified bacterial cellulose will be hereafter referred to as “neat BC”, and will be the blank used for characterization assays.

2.3. Carboxymethylation procedure

The neat BC suspension (1 g dry weight, 6.2 mmol AGU (anhydroglucose units)) was solvent-exchanged from water to isopropanol (5 min with stirring, twice) to guarantee water removal without inducing hornification of BC. Carboxymethylation of BC was then carried out heterogeneously following a standard method used for CMC production, which implies first alkalinization to activate cellulose, and then etherification under heterogeneous conditions. In the first step, homogenized solvent exchanged BC was stirred in isopropanol (100 mL) in a 500 mL glass flask during 10 min. After this time and while vigorous stirring, variable volumes of NaOH solution (5–35% w/v) corresponding to NaOH/AGU molar ratios in the 1–5 mol/mol interval were added dropwise. The system was then kept at 30 °C and 300 rpm during 1 h. Etherification was initiated by the addition of sodium monochloroacetate (NaMCA, 0.5–3 mol/AGU). Reaction was carried out at fixed temperature of 55 °C in a thermostatized oil bath and with continuous magnetic agitation (300 rpm) during variable times within the 0.5–5 h interval. A reflux condenser was mounted on the glass flask to prevent isopropanol losses. After the reaction time interval set, the mixture was filtered and the residue was suspended in methanol (75 mL) during 15 min with stirring. The suspension was later neutralized with acetic acid using phenolphthalein as end-point indicator. The suspension was then filtered and washed with 70% v/v ethanol four times. The product (CMC) was dried at 60 °C overnight and grinded to powder.

2.4. Determination of DS

The degree of substitution defined as the average number of sodium carboxymethyl groups substituted per anhydroglucose unit was determined by conductometric titration (Eyler, Klug, & Diephuis, 1947). Briefly, 0.3 g of previously dried (105 °C, 3 h) carboxymethylated BC samples were contacted with 15 mL of methanol solution (70% v/v) in a 600 mL flask, and allowed to soak for 10 min. Then, 200 mL of cold carbon dioxide-free distilled water and 5 mL of 0.3 N NaOH were added and the flask properly stoppered was stirred until the sample dissolved. The solution thus

obtained was titrated with 25 mL of 0.15 N HCl taking the conductivity reading after each 0.5 mL addition of the acid allowing sufficient time for adequate mixing. Conductivity data was then plotted against the HCl volume, and the three linear segments of the curve were extrapolated to determine the volumes V_1 and V_2 corresponding to the intersection points. The degree of substitution (DS) was then calculated as it follows:

$$DS = \frac{A \times 162}{(1000 + 22\,B - 80\,A)} \quad (1)$$

Where 162 accounts for the molecular weight of the anhydroglucose unit of cellulose, 80 is the net increase in the weight of the anhydroglucose unit of cellulose for each sodium carboxymethyl group substituted; and 22 is obtained by substracting the net increase in the weight of the anhydrogluclose unit for each carboxymethyl group substituted (i.e. 58). A and B stand for the milliequivalents of total carboxyl per gram, and the milliequivalents of free carboxyl per gram, respectively and are defined as it follows:

$$A = \frac{(V2 - V1)\,N}{W} \quad (2)$$

$$B = \frac{(VNaOH \times N) - (V1 \times N)}{W} \quad (3)$$

Where V_1 and V_2 (mL) are the volumes of the HCl acid solution corresponding to the intersection points, V_{NaOH} is the total volume of 0.3 N NaOH used (i.e. 5 mL), N is the normality of the HCl solution, and W (g) is the mass of sample used.

2.5. Characterization of carboxymethylated BC samples

Solid-State ^{13}C NMR measurements (^{13}C NMR): High-resolution 13C solid-state spectra of grinded samples were recorded using the ramp {1H} → {13C} CP/MAS pulse sequence (cross-polarization and magic angle spinning) with proton decoupling during acquisition. All experiments were performed at room temperature in a Bruker Avance II-300 spectrometer equipped with a 4-mm MAS probe. The operating frequency for protons and carbons was 300.13 and 75.46 MHz, respectively. Glycine was used as an external reference for the ^{13}C spectra and to set the Hartmann-Hahn matching condition in the cross-polarization experiments. The recycling time varied from 5 to 6 s according to the sample. The contact time during CP was 3 ms for all of them. The SPINAL64 sequence (small phase incremental alternation with 64 steps) was used for heteronuclear decoupling during acquisition with a proton field H1H satisfying $\omega 1H/2\pi = \gamma HH1H = 62$ kHz. The spinning rate for all the samples was 10 kHz.

Fourier Transform Infrared Spectroscopy (FTIR): Fourier transform infrared spectra of carboxymethylated grinded BC samples were acquired on an IR Affinity-1 Shimadzu Fourier Transform Infrared Spectrophotometer in absorbance mode. Carefully dried (5 mg, 110 °C, overnight) samples were mixed with previously dried KBr (110 °C, overnight) in the ratio 1:100 and pressed into discs at 6 kg/cm^2. Discs were further dried at 110 °C overnight, aiming to reduce the band associated with absorbed water which partially overlapped with the COO^- signal. Spectra were then acquired with a resolution of 4 cm^{-1} in the range of 4000 to 650 cm^{-1} and with 40 scans. The derived spectra were baseline corrected and normalized against the intensity of the absorption at 1168 cm^{-1}, corresponding to the (C–O–C) link of cellulose (Ilharco, Garcia, Lopes da Silva, & Vieira Ferreira, 1997; Lee et al., 2011).

X-ray diffraction (XRD): Grinded carboxymethylated BC samples were analyzed in a 2θ angle range of 10–40° at a scan rate of 0.6°/min and with a step size of 0.02° in a Rigaku D/Max-C Wide Angle automated X-ray diffractometer with vertical goniometer (Cu/Kα 0.154 nm, 40 kV, 30 mA).

Thermogravimetric analysis (TGA): Thermogravimetric analysis of preconditioned grinded samples (5 mg, 110 °C, 1 h) was conducted in a TGA-50 Shimadzu instrument. Samples were heated from 25 °C up to 800 °C at a constant heating rate of 10 °C/min under nitrogen atmosphere (30 mL/min, 2 kg/cm^2) in order to provide an inert atmosphere for pyrolysis.

3. Results and discussion

3.1. Carboxymethylation of BC

The range of commercially available CMC grades is generally in the range of 0.4–1.5 (Heinze, 2005). For food uses, the Joint FAO/WHO Expert Committee on Food Additives stated a DS range of 0.2–1.5 (Joint FAO/WHO Expert Committee on Food Additives, 2011). Aiming to analyze how to carboxymethylate BC to the required DS values, the effects of chosen parameters were examined one at a time, i.e.: NaOH/AGU molar ratio (mol NaOH/mol AGU, fixed NaOH solution concentration, variable solution volume), NaOH solution concentration (w/v %, fixed NaOH/AGU mol/mol, variable solution volume), NaMCA/AGU molar ratio (mol NaMCA/mol AGU), and etherification time (h). Table 1 summarizes the assays performed.

In all cases the carboxymethylation of BC was carried out in isopropanol, as this was the organic diluent which, -among others used in the literature (e.g. isobutanol, isopropanol, ethanol, water, DMF, methanol, DMSO)-, has generally led to the highest DS values for other cellulose sources (Barai et al., 1997; Pushpamalar, Langford, Ahmad, & Lim, 2006). NaOH solution is used to induce the formation of alkali cellulose, which modifies the crystalline structure of cellulose and increases its accessibility to chemicals by swelling (Barba et al., 2002). The resulting isopropanol-water-sodium hydroxide medium is a biphasic system with a major (and at rest upper) phase consisting of isopropanol, water, and a very small amount of sodium hydroxide; and a minor (and at rest lower) phase consisting of sodium hydroxide, water, and a very small quantity of isopropanol. The previous is qualitatively illustrated in Fig. 1 upon addition of a few drops of phenolphthalein. In stirred conditions as the ones used herein, bacterial cellulose is dispersed in the isopropanol phase within which fine droplets of the aqueous-NaOH phase supply alkali with high affinity for cellulose. The isopropanol phase promotes an even distribution of sodium hydroxide and water within cellulose, as well as a homogenous distribution of NaMCA in the reaction mass (Yokota, 1985). Besides the target reaction of NaMCA with NaOH-activated BC, NaMCA also reacts with NaOH to form sodium glycolate and sodium chloride by-products.

Besides the diluent used, other factors which were kept at fixed values chosen from the literature in all assays were alkalinization temperature (30 °C), alkalinization time (1 h), and etherification temperature (55 °C). Agitation in both stages was kept at 300 rpm.

In the alkalinization stage the variable that has been studied most in the literature to regulate carboxymethylation extent has been the concentration of sodium hydroxide solution. The biphasic nature of the system previously discussed justifies the reference to the NaOH solution concentration, and not to the total NaOH concentration in the system. Although much less frequently, some contributions have explicitly assayed also the effect of the NaOH/AGU molar ratio in the system (Schlufter & Heinze, 2010; Zhao, Cheng, Li, & Zhang, 2003). Furthermore, most studies dealing with the effect of NaOH solution concentration, have kept the volume of the NaOH solution added constant, so for a fixed

Table 1
Summary of assays performed and resulting DS determined by conductometric titration. Alkalinization temperature: 30 °C, alkalinization time: 1 h, etherification temperature: 55 °C.

Sample No.	mol $_{NaOH}$/mol $_{AGU}$	NaOH conc. (w/v %)	mol $_{NaMCA}$/mol $_{AGU}$	Etherif. Time (h)	DS (conduct.)
1	1	15	3	2	0.61 ± 0.05
2	2	15	3	2	1.20 ± 0.04
3	2.75	15	3	2	1.30 ± 0.02
4	3	15	3	2	1.34 ± 0.06
5	3.25	15	3	2	1.15 ± 0.01
6	4	15	3	2	1.10 ± 0.01
7	5	15	3	2	0.84 ± 0.02
8	2.75	5	3	2	0.80 ± 0.01
9	2.75	25	3	2	1.37 ± 0.01
10	2.75	35	3	2	1.42 ± 0.01
11	2.75	35	0.5	2	0.60 ± 0.04
12	2.75	35	1	2	0.96 ± 0.04
13	2.75	35	2	2	1.52 ± 0.06
14	2.75	35	2	0.5	0.76 ± 0.01
15	2.75	35	2	1	1.16 ± 0.03
16	2.75	35	2	3	1.44 ± 0.01
17	2.75	35	2	5	1.42 ± 0.02

Fig. 1. Photograph of the isopropanol-water-sodium hydroxide at rest upon phenolphthalein addition. The system corresponds to assay No. 7 in Table 1.

cellulose mass the NaOH/AGU molar ratio in the system was actually varied. In the current contribution both factors (i.e. NaOH solution concentration and NaOH/AGU molar ratio) have been assayed separately. Fig. 2a summarizes the results of the effect of NaOH/AGU molar ratio on DS, as obtained by varying the volume of NaOH solution used (15% w/v).

As it is shown in Fig. 2a, the NaOH/AGU molar ratio used in the alkalinization stage was a key factor influencing the DS of the CMC obtained, with DS values in the 0.61–1.34 interval. In the 1–3 mol NaOH/mol AGU range, the DS attained increased when higher NaOH/AGU molar ratios were used. Even if the concentration of the NaOH solution was kept constant at 15% w/v, higher NaOH/AGU molar ratios implied the addition of higher volumes of the alkali solution, resulting in increased probability for fine droplets of the aqueous-NaOH phase dispersed in isopropanol to contact and supply alkali to bacterial cellulose. On the other hand, higher NaOH/AGU molar ratios resulted in lower DS values. The reduction in the DS attained for molar ratios higher than 3 (theoretical NaOH/AGU ratio required to alkalinize all hydroxyls available), may be attributed to the predomination of a competitive/side reaction in which NaOH remaining from the alkalinization stage reacts with NaMCA to produce sodium glycolate. This reaction is known to prevail at high NaOH amounts, thereby lowering the DS. In the following assays to NaOH/AGU molar ratio was kept constant at 2.75 mol NaOH/mol AGU, since the corresponding DS value (DS = 1.30 ± 0.02) was very close to the highest one (DS = 1.34 ± 0.06, 3 mol NaOH/mol AGU) and a 8% reduction in NaOH consumption was achieved.

Fig. 2b illustrates the effect of NaOH solution concentration in the 5–35% w/v interval while keeping a constant amount of NaOH in the system (2.75 mol NaOH/mol AGU). As it is shown, the DS increased with the concentration of NaOH solution in the 5–15% interval, but higher alkali solution concentrations had a minor effect on DS, which showed an almost constant value in the 1.30–1.42 interval for NaOH solutions in the 15–35% w/v range. The distinct influence of NaOH concentration in the alkalinization stage on the subsequent etherification reaction, has been widely highlighted for other cellulose sources in association with the swelling of the polymer and the resulting crystallinity of cellulose (Ambjörnsson, Schenzel, & Germgard, 2013; Heinze & Pfeiffer, 1999). On the other hand, the minor effect of NaOH solution concentration in the 15–35% w/v range shown herein when NaOH/AGU molar ratio was kept at a fixed value of 2.75 mol/mol, further highlights the importance not only of the concentration but also of the volume (as conditioned by a fixed NaOH/AGU molar ratio) of the alkali phase. In the following assays the concentration of the NaOH solution was kept constant at 35% w/v and the NaOH/AGU molar ratio was fixed at 2.75 mol/mol.

Fig. 2c illustrates the effect of NaMCA amount on the DS of the CMC produced. As it is shown, the DS of CMC increased with the NaMCA/AGU molar ratio until a value of 2 mol NaMCA/mol AGU, for which a DS of 1.52 was attained. The increase of DS with NaMCA/AGU molar ratio is attributable to a greater availability of the

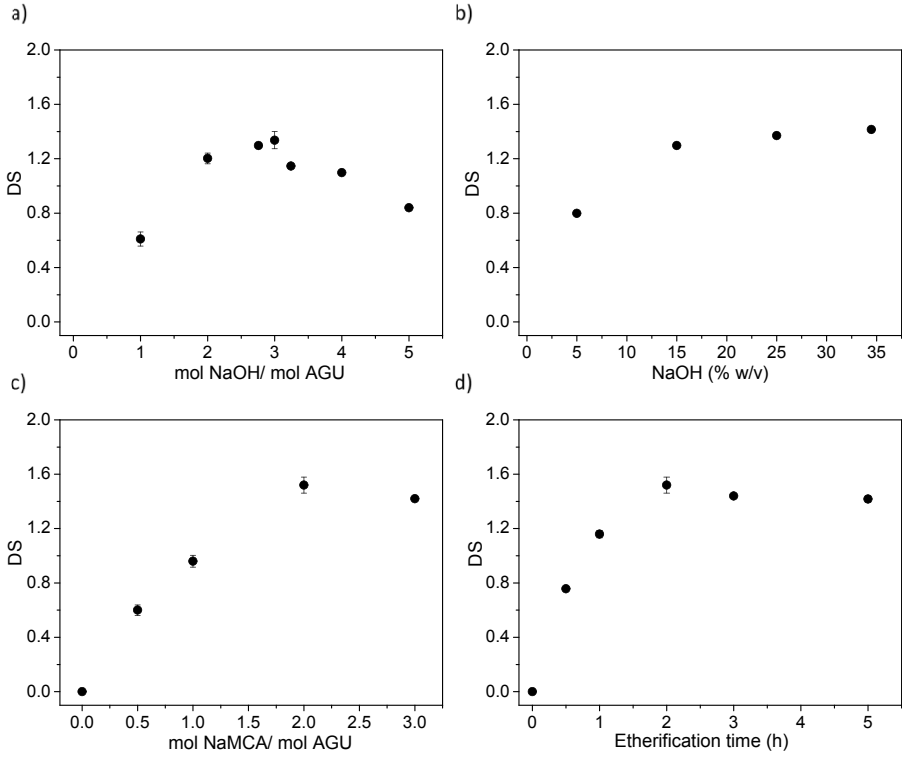

Fig. 2. Effects of reaction conditions on the DS of carboxymethylated BC. See Table 1 for details on constant reaction conditions values.

NaMCA molecules in the proximity of cellulose molecules, as early reported for other cellulosic raw materials (Barai et al., 1997; Joshi et al., 2015). Furthermore, at least up to a NaMCA/AGU molar ratio of one, DS results evidenced an almost linear relationship with NaMCA/mol AGU, with a slope close to *unity*. The previous indicates that all NaMCA added to the reaction was effectively consumed to carboxymethylate alkali activated cellulose to the maximum theoretical value possible. To put it in numbers, results showed that when 0.5 mol of NaMCA per mol of AGU were added to the system, a DS value of 0.5 was attained, which implies that an average number of 0.5 hydroxyl groups per anhydroglucose unit have been substituted with carboxymethyl groups. The same was observed for a NaMCA/AGU molar ratio of 1. In summary, the previous suggests that within the mentioned interval, it was the NaMCA amount which limited the reaction extent, with no apparent reduction in reaction efficiency by side glycolate formation reaction contribution.

On the other hand, the use of NaMCA/AGU molar ratios equal/higher than 2 led to an almost constant DS value (DS ≈ 1.42–1.52). A very similar pattern was observed by Barai et al. (1997) in the carboxymethylation of α-cellulose isolated from a free floating weed; as well as by Zhao et al. (2003) in the carboxymethylation of cotton linters in benzene/ethanol/water mixtures. Lower or constant DS values observed for increasing NaMCA amounts have been previously attributed to a preferential glycolate formation which reduces reaction efficiency (Haleem et al., 2004), or to the non-availability of enough cellulose-alkoxide for reaction with NaMCA (Joshi et al., 2015; Varshney et al., 2006). Actually, Fig. 2c evidences that for NaMCA/AGU molar ratios equal/higher than 2 (and from the pattern shown probably even for NaMCA/AGU molar ratios higher than ≈ 1.5), the DS attained was much lower than the theoretical one considering the NaOH/AGU and NaMCA/AGU molar ratios used (i.e. 2.75). The previous suggests that for NaMCA/AGU molar ratios equal/higher than ≈1.5 the reaction was not any more limited by the etherifying agent amount, but by the alkalinization extent achieved in the first stage, from which unreacted NaOH evidently remained to react with the extra NaMCA to form the glycolate by-product. The maximum DS of ≈1.5 attained up to this point is in accordance with previous contributions which highlighted that, despite the theoretical maximum DS value for CMC is 3.0, applying one-step reaction the maximal DS which can be obtained by the usual heterogeneous reaction is about 1.3–1.5 (Heinze, 2005). In any case, Schlufter and Heinze (2010) have recently attained a DS of 1.84 in the carboxymethylation of freeze dried milled BC with 30% w/v aqueous NaOH and a molar excess of 4 mol NaMCA/mol AGU after a reaction time of 6 h.

Finally, Fig. 2d illustrates the effect of etherification time on the DS of carboxymethylated BC. Under the conditions set, the DS increased within the first two hours of reaction, to later reach a constant DS of ≈1.42–1.52. The increase in DS within the first two hours of reaction is associated with a better and prolonged contact between reagents when more time was given to etherification. On the other hand, the later constant DS close to 1.5 observed herein irrespectively of the time given to etherification, and in excess of NaOH and NaMCA, once again points towards a limiting alkalinization step, in which not all cellulose has been alkalinized.

The results summarized in the current section indicated that under proper conditions carboxymethylation of BC by the well-known slurry process involving NaOH and NaMCA, can successfully lead to CMC with variable DS within the 0.60–1.52 interval. Given that the effect of time has been particularly useful for tuning the DS of the produced CMC within this interval, the resulting samples (Fig. 2d, DS = 0.76–1.52) have been selected for the characterization analysis included in the following section.

3.2. Characterization of carboxymethylated BC

The occurrence of the carboxymethylation reaction was

confirmed by ^{13}C solid state NMR and FTIR spectroscopies. Fig. 3 collects the ^{13}C CP/MAS NMR spectra of pristine and carboxymethylated BC samples with DS in the 0.76–1.52 interval. Native BC spectrum showed carbon resonances at C1: 105 ppm, C4: 89 ppm, C4′: 84 ppm, cluster C2-C3-C5: 80-70 ppm, C6: 65 ppm, and C6′: 63 ppm, which are typical of cellulose I (Earl & VanderHart, 1980). On the other hand, CP/MAS ^{13}C NMR spectra of carboxymethylated BC samples gave evidence of the success of the reaction by appearance of a new resonance centered at 180 ppm assignable to the COO^- groups that have been introduced (Biswas, Kim, Selling, & Cheng, 2014; Eyholzer et al., 2010; Joshi et al., 2015). The intensity of this resonance increased with DS.

NMR spectra of CMC also illustrated the changes in crystallinity that took place during derivatization. Crystalline and disordered components of cellulose are detected in solid state ^{13}C NMR spectra as downfield (C4 and C6) and upfield lines (C4′ and C6') for the C4 or C6 carbons, respectively (Atalla & VanderHart, 1999; Yamamoto, Horii, & Hirai, 2005). Both crystalline and non-crystalline resonances have been identified in the spectra of neat BC in Fig. 3. On the other hand, in carboxymethylated samples spectra, -irrespectively of the DS attained-, resonances assignable to crystalline components disappeared, evidencing the decrystallization of neat BC upon alkalinization + etherification.

FTIR sprectra of carboxymethylated BC samples also provided evidence of derivatization. Fig. 4 collects the spectra of neat BC and CMC with DS = 0.76 and DS = 1.44. Neat BC spectrum showed bands typical of cellulose I, including those of O-H stretching (3360 cm^{-1}); C-H stretching (2895 cm^{-1}); H-*O*-H bending vibration of absorbed water molecules (1647 cm^{-1}); CH_2 symmetrical bending (1427 cm^{-1}); cellulose C-*O*-C bridges (1168 cm^{-1}); C-O bond stretching (1118 cm^{-1}); ether C-*O*-C functionalities (1061 cm^{-1}); and the band at 897 cm^{-1} which is typical of β-linked glucose polymers (Ashori, Babaee, Jonoobi, & Hamzed, 2014; Castro et al., 2011; Morán, Alvarez, Cyras, & Vázquez, 2008). Carboxymethylation of BC resulted in the appearance of an extra absorption band centered at ≈ 1620 cm^{-1} attributed to the stretching vibration of the carboxylate groups introduced (COO^-). Rigorous drying of the samples was extremely important to avoid the contribution and overlapping of absorbed water vibrations.

Fig. 5 shows the X-ray diffractograms of neat and carboxymethylated BC samples with DS in the 0.76–1.52 interval. The effect of the chosen alkali treatment (NaOH 35 w/v %, 2.75 mol NaOH/mol AGU) on the structure of BC is also shown. Hydroxyl groups present in cellulose macromolecules are involved in a number of intra- and intermolecular hydrogen bonds, which result in various ordered crystalline arrangements, i.e. Cellulose I, II, III and IV (Park, Baker, Himmel, Parilla, & Johnson, 2010). The X-ray diffraction pattern as well as the solid-state ^{13}C nuclear magnetic resonance spectra of bacterial cellulose (Fig. 3) showed signals characteristic of Cellulose I allomorph. In the XRD pattern of neat BC this was evidenced by crystalline peaks at diffraction angles at $2\theta = 14.4°$ (101), 16.6° (10–1) and 22.6° (002), all typical of Cellulose I. The crystallinity index for BC determined by the two-phase method was 64%. Alkalinization of BC modified its crystalline structure and increased its accessibility to chemicals by swelling. The mentioned change in cellulose structure is illustrated in the diffractogram of BC treated with NaOH solution (Fig. 5 and 35 w/v %, 2.75 mol NaOH/mol AGU), which correlates with a mixture of crystalline forms of Cellulose I and Cellulose II (i.e. note new peaks at $2\theta = 12.3°$, 20.6°, and 34.8°, characteristic of the 101, 10–1 and 040 planes of Cellulose II, respectively) (Johnson Ford, Mendon, Thames, & Rawlins, 2010). After alkalinization, the resulting crystallinity index was reduced to 39%.

The decrease of crystallinity of BC upon treatment with NaOH solution has been associated with the cleavage of hydrogen bonds by NaOH (Adinugraha et al., 2005) and inclusion of NaOH and water in the crystallites (Heinze, 2005). Further reduction of crystallinity upon carboxymethylation illustrated in Fig. 5 has been attributed to the cleavage of the broadening hydrogen bonds due to carboxymethyl substitution at the hydroxyl groups of cellulose (Adinugraha et al., 2005).

The thermal stability of BC and CMC from BC with increasing DS

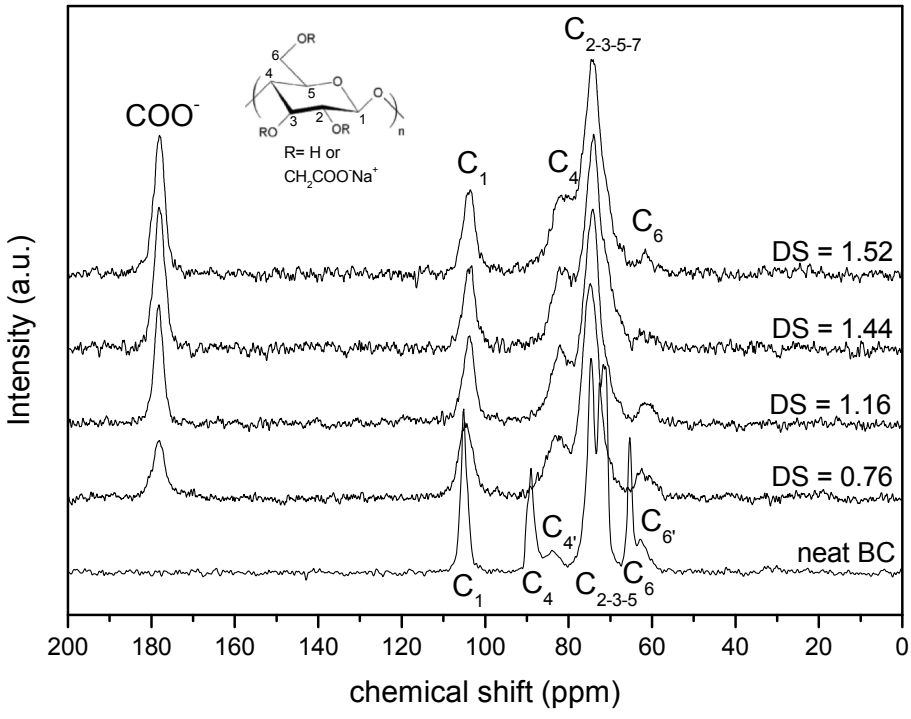

Fig. 3. ^{13}C CP/MAS NMR spectra of neat and carboxymethylated BC with varying DS.

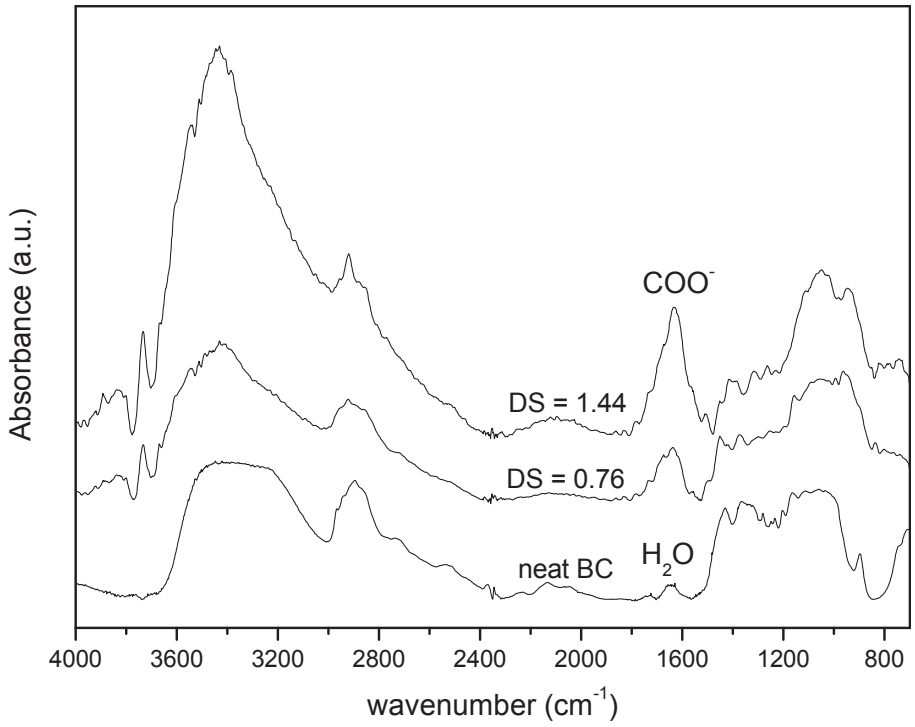

Fig. 4. FTIR spectra of neat and carboxymethylated BC with varying DS.

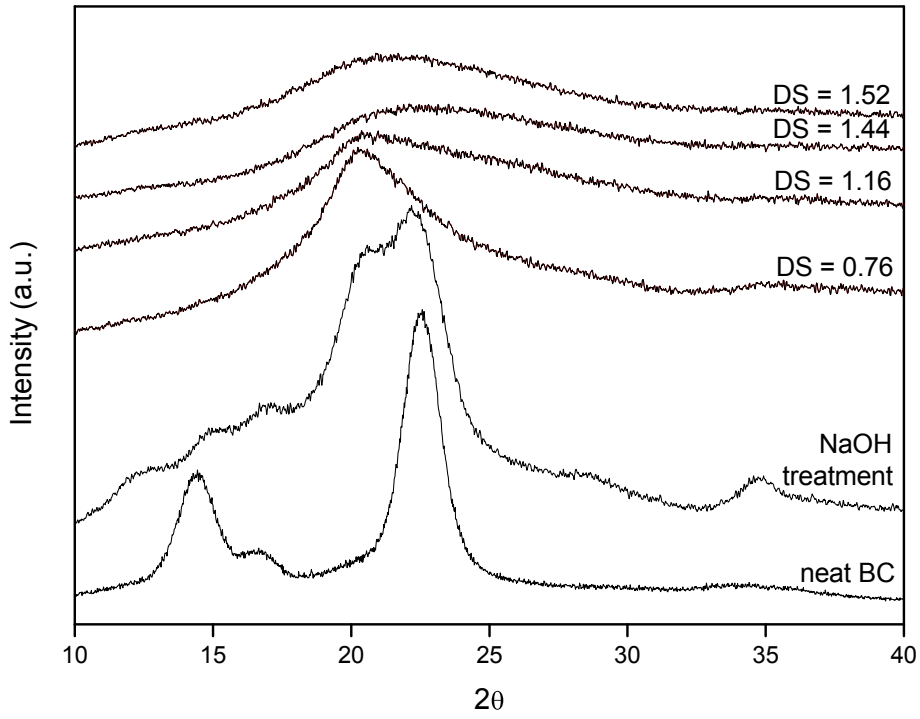

Fig. 5. X-ray diffraction patterns of neat, alkali-treated (35 w/v %, 2.75 mol NaOH/mol AGU) and carboxymethylated BC with varying DS.

was also evaluated. TG and DTG curves obtained have been included in Fig. 6. Heating of BC in nitrogen atmosphere resulted in a first weight loss event between room temperature and ≈130 °C assignable to sample's dehydration. Since all samples were pre-conditioned at 110 °C during 1 h, the remaining moisture removed from BC was below 3%. The second weight loss exhibited in BC thermogram corresponded to BC decomposition and it was characterized by T_{onset} and T_{max} values of 296 °C and 321 °C, respectively. T_{onset} was calculated as the intersection of the extrapolated pre-decomposition ordinate value and a tangential line drawn to the point of steepest slope of the weight loss curve in the decomposition region, and tendencies were confirmed by calculation of

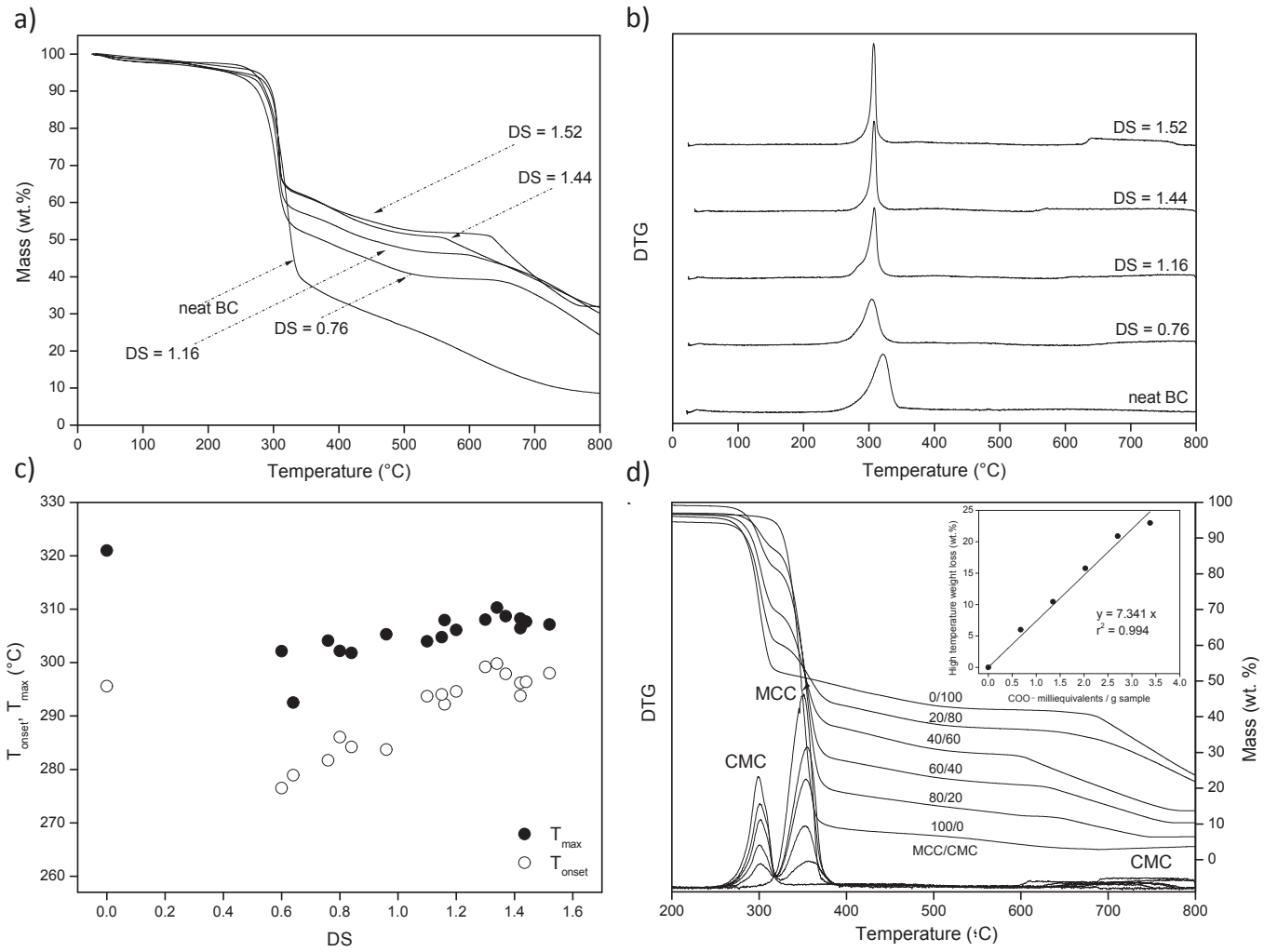

Fig. 6. TG (a) and DTG (b) data of neat and carboxymethylated BC with varying DS. (c) Effect of DS on T_{onset} and T_{max} values. (d) TG and DTG data of commercial MCC/CMC mixtures of varying weight ratios (i.e. 100/0, 80/20, 60/40, 40/60, 20/80, 0/100); inset: linear relationship between the high temperature weight loss and the milliequivalents of COO^- per gram of sample from which DS can be estimated.

T_{onset} as the temperature of 5 wt% weight loss after moisture removal. T_{max} stands for the point of greatest rate of change on the weight loss curve.

Carboxymethylated BC samples also showed a first weight loss associated with samples dehydration, followed by two clearly distinguished decomposition events of different relevance. The first thermal decomposition stage exhibited T_{max} values close to those of BC decomposition, suggesting the contribution of unmodified BC portions (Fig. 6 a–b). The T_{onset} of this weight loss stage was also close to the corresponding BC values. The mass lost within this first decomposition stage decreased with DS, also pointing towards a contribution of the non substituted BC portion. Fig. 6c illustrates the effect of DS on T_{onset} and T_{max} values. Results from all samples listed in Table 1 have been included. As it is shown, carboxymethylation to the lowest DS herein attained (i.e. DS = 0.60) resulted in a 20 °C reduction in both T_{onset} and T_{max} values when compared with neat BC. The reduction in the thermal stability observed could be associated with the reduction in crystallinity upon carboxymethylation. Although thermal stability analysis of CMC samples is not very frequent, reduction of the thermal stability of cellulose and nanocellulose upon carboxymethylation has been previously reported, even for much lower DS values (Eyholzer et al., 2010; Ünlü, 2013). However, samples with increasing carboxymethylation level clearly evidenced a relative increase in their thermal stability. This is illustrated in Fig. 6c for both T_{onset} and T_{max}, which evidence up to a ≈20 °C and 5 °C-recovery, respectively. In fact, the onset decomposition temperature for CMC samples with the highest DS values resembled that of neat BC. The reasons for the increase in the characteristic decomposition temperatures with DS are currently under study. Finally, the fact that all CMC samples produced converged to a single pattern suggests that it was the DS of the samples what determined their thermal stability, and not the conditions used for their production.

As previously introduced, thermal analysis of carboxymethylated samples showed a third weight loss at temperatures higher than 600 °C (Fig. 6a and b). Dodi, Hritcu, and Popa (2011) also observed a high temperature decomposition event in carboxymethylated guar gum samples which was attributed to carboxymethyl groups decomposition (Dodi et al., 2011). In the current contribution, the mass lost within this stage increased with the DS conferred to BC (extended TG analysis up to 900 °C was sometimes necessary to collect the complete weight loss profile), further suggesting that the weight loss corresponded to the groups introduced during derivatization. To further analyze this hypothesis, TG and DTG data from commercial CMC, microcrystalline cellulose (MCC), and their mechanical mixtures at different MCC/CMC mass

ratios were collected. Data is shown in Fig. 6d. The data collected showed that the high temperature weight loss described above for CMC produced from BC was also observed in the thermogram of commercial CMC; and also in TG and DTG data of MCC/CMC mixtures, with its contribution increasing with the fraction of CMC in the sample. Knowledge of the DS of the commercial CMC (i.e. DS = 0.75) allows calculating the milliequivalents of carboxymethyl groups per gram of sample, and from them, the carboxymethyl groups in the target mixture. The inset included in Fig. 6d illustrates the linear correlation found when plotting the contribution of the high temperature weight loss as a function of the milliequivalents of carboxymethyl groups in the sample. Results evidenced the suitability of using TGA data for a rapid estimation of DS in CMC samples upon proper calibration. At the authors best knowledge, it is the first time that the use of TGA for estimating the DS of CMC is reported. On the other hand, and contrarily to weight loss and solid residue left (which both showed a clear positive correlation with the samples' DS); the onset temperature of the last high temperature decomposition event showed no clear correlation with DS (neither for carboxymethylated BC samples nor for MCC/CMC mixtures).

Lastly, given the wide use of CMC in aqueous solution, the water solubility of carboxymethylated BC samples was assayed. Previous contributions on CMC from BC which assayed their water solubility have reported some difficulties for complete dissolution in water, which have been assigned either to highly heterogeneous derivatization of BC (long sequences of unsubstituted anhydroglucose units may aggregate and render part of the sample insoluble) (Cheng et al., 1999); or to BC nanofibrils with highly functionalized chains on the outside and a not/insufficiently functionalized core (Schlufter & Heinze, 2010). However, dried CMC samples herein obtained could be fully dissolved in water, resulting in measured water solubility values in the 26–55 mg/mL range. Since CMC particles have a tendency to lump when first added to water, to promote dissolution CMC samples were slowly added to the vortex of vigorously agitated water aimed at allowing powder dispersion and individual particle wetting. Water solubility values were noticeably higher for samples with DS $\geq$ 1.1 which showed values in the 52–55 mg/mL range. On the other hand, the sample belonging to 0.5 h of carboxymtehylation (DS = 0.76) showed a water solubility of 26 mg/mL. The positive effect of DS on water solubility is associated with the increase in the content of polar carboxyl groups which render the cellulose soluble. Similar findings were also reported for CMC produced from several bleached cellulose pulps obtained from non-wood species, whose solubility improved when DS was raised from $\approx$1 (first carboxymethylation) to $\approx$2 (second etherification of the product) (Barba et al., 2002). Improvements in DS upon second etherification which further resulted in water solubility increases were also reported for CMC derived from rayon grade wood pulps and cotton linters (Latif, Anwar, & Noor, 2007).

4. Conclusions

CMC with tailored DS within the interval allowed for food applications could be successfully obtained using BC as cellulose source. Chosen reaction conditions (i.e. NaOH/AGU molar ratio, NaOH solution concentration. NaMCA/AGU molar ratio and etherification time) were varied within selected intervals to provide a systematic analysis of their effect on the DS of the carboxymethylated BC produced. The analysis performed allowed obtaining water-soluble carboxymethylated BC samples with DS in the 0.60–1.52 interval, in agreement with the DS range commercially required. All variables assayed were significant in manipulating the DS attained. The strong influence of the alkalinization conditions used on the later etherification reaction was particularly illustrated.

The CMC samples obtained were characterized in terms of chemical structure, crystallinity and thermal stability as a function of DS. Accordingly, characteristic signals of the carboxylate groups introduced observed by FTIR spectrometry and ^{13}C solid state NMR spectroscopy confirmed the occurrence of the carboxymethylation reaction. Besides, X-ray diffraction illustrated the reduction in crystallinity of the CMC produced, whereas thermal analysis evidenced that the thermal decomposition pattern was significantly influenced by the carboxymethyl content of the CMC sample (i.e. DS). Actually, results shown herein illustrated the suitability of using TGA data for concomitant estimation of the carboxymethylation level achieved. Water solubility of carboxymethylated BC samples was also confirmed.

Overall, with its high purity which avoids the need for chemical treatments devoted to isolate cellulose prior to derivatization, and the possibility of tuning the DS of the obtained CMC by manipulation of reaction conditions; BC seems an attractive potential feedstock for the production of CMC for food uses with tailored DS. Besides basic characterization results included in the current contribution, studies on functional properties (viscosity, acid resistance) of BC-derived-CMC samples required for particular food applications, are currently in progress. The molecular weight of carboxmethylated samples (as a result of the DS confered, original degree of polymerization of BC, and potential degradation of cellulose during reaction in alkaline conditions) is also of interest, given its positive influence on the viscosity of CMC solutions.

Conflict of interest

The authors confirm that this article content has no conflict of interest.

Acknowledgements

This work was supported by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET- PIP 1122011010 0608) and Agencia Nacional de Promoción Científica y Tecnológica for financial support (PICT 1957 2012 – PRESTAMO BID).

References

Adinugraha, M. P., Marseno, D. W., & Haryadi. (2005). Synthesis and characterization of sodium carboxymethylcellulose from cavendish banana pseudo stem (*Musa cavendishii* LAMBERT). *Carbohydrate Polymers, 62*, 164–169.

Ambjörnsson, H. A., Schenzel, K., & Germgard, U. (2013). Carboxymethyl cellulose produced at different mercerization conditions and characterized by NIR FT Raman spectroscopy in combination with multivariate analytical methods. *BioResources, 8*, 1918–1932.

Ashori, A., Babaee, M., Jonoobi, M., & Hamzed, Y. (2014). Solvent-free acetylation of cellulose nanofibers for improving compatibility and dispersion. *Carbohydrate Polymers, 102*, 369–375.

Atalla, R. H., & VanderHart, D. L. (1999). The role of solid state 13C NMR spectroscopy in studies of the nature of native celluloses. *Solid State Nuclear Magnetic Resonance, 15*, 1–19.

Barai, B. K., Singhal, R. S., & Kulkarni, P. R. (1997). Optimization of a process for preparing carboxymethyl cellulose from water hyacinth (Eichornia crassipes). *Carbohydrate Polymers, 32*, 229–231.

Barba, C., Montané, D., Rinaudo, M., & Farriol, X. (2002). Synthesis and characterization of carboxymethylcelluloses (CMC) from non-wood fibers I. Accessibility of cellulose fibers and CMC synthesis. *Cellulose, 9*, 319–326.

Biswas, A., Kim, S., Selling, G. W., & Cheng, H. N. (2014). Conversion of agricultural residues to carboxymethylcellulose and carboxymethylcellulose acetate. *Industrial Crops and Products, 60*, 259–265.

Bono, A., Ying, P. H., Yan, F. Y., Muei, C. L., Sarbatly, R., & Krishnaiah, D. (2009). Synthesis and characterization of carboxymethyl cellulose from palm kernel cake. *Advances in Natural and Applied Sciences, 3*, 5–11.

Cai, Z., Wu, J., Du, B., & Zhang, H. (2017). Impact of distribution of carboxymethyl substituents in the stabilizer of carboxymethyl cellulose on the stability of acidified milk drinks. *Food Hydrocolloids* (in press).

Castro, C., Zuluaga, R., Putaux, J. L., Caroa, G., Mondragon, I., & Gañan, P. (2011). Structural characterization of bacterial cellulose produced by *Gluconacetobacter*

swingsii sp. from Colombian agroindustrial wastes. *Carbohydrate Polymers, 84*, 96–102.
Cerrutti, P., Roldán, P., Martínez García, R., Galvagno, M. A., Vázquez, A., & Foresti, M. L. (2016). Production of bacterial nanocellulose from wine industry residues: Importance of fermentation time on pellicles characteristics. *Journal of Applied Polymer Science, 133*, 43109–43117.
Cheng, H. N., & Mital, S. (1998). *Polymer Preprints, 39*, 695.
Cheng, H. N., Takai, W., & Ekong, E. A. (1999). Rheology of carboxymethylcellulose made from bacterial cellulose. *Macromolecular Symposia, 140*, 145–153.
Dodi, G., Hritcu, D., & Popa, M. I. (2011). Carboxymethylation of guar gum: Synthesis and characterization. *Cellulose Chemistry and Technology, 45*, 171–176.
Earl, W. L., & VanderHart, D. L. (1980). High resolution, magic angle sample spinning 13C NMR of solid cellulose I. *Journal of the American Chemical Society, 102*, 3251–3252.
Edali, M., Esmail, M. N., & Vatistas, G. H. (2001). Rheological properties of high concentrations of carboxymethyl cellulose solutions. *Journal of Applied Polymer Science, 79*, 1787–1801.
Eyholzer, Ch, Bordeanu, N., Lopez-Suevos, F., Rentsch, D., Zimmermann, T., & Oksman, K. (2010). Preparation and characterization of water-redispersible nanofibrillated cellulose in powder form. *Cellulose, 17*, 19–30.
Eyler, R. W., Klug, E. D., & Diephuis, F. (1947). Determination of degree of substitution of sodium carboxymethylcellulose. *Analytical Chemistry, 19*, 24–27.
Geyer, U., Heinze, Th, Stein, A., Klemm, D., Marsch, S., Schumann, D., et al. (1994). Formation, derivatization and applications of bacterial cellulose. *International Journal of Biological Macromolecules, 16*, 343–347.
Haleem, N., Arshad, M., Shahid, M., & Ashraf Tahir, M. (2004). Synthesis of carboxymethyl cellulose from waste of cotton ginning industry. *Carbohydrate Polymers, 113*, 249–255.
Hassan, M. L. (2014). Cellulose nanocrystals and carboxymethyl cellulose from olive stones and their use to improve paper sheets properties. *International Journal of Nanoparticles, 7*, 261–277.
Heinze, Th (2005). Carboxymethyl ethers of cellulose and starch – a review. *Химия растительного сырья, 3*, 13–29.
Heinze, Th, & Pfeiffer, K. (1999). Studies on the synthesis and characterization of carboxymethylcellulose. *Die Angewandte Makromolekulare Chemie, 266*, 37–45.
Hestrin, S., & Schramm, M. (1954). Synthesis of cellulose by *Acetobacter xylinum*: Preparation of freeze dried cells capable of polymerizing glucose to cellulose. *Biochemical Journal, 58*, 345–352.
Ilharco, L. M., Garcia, R. R., Lopes da Silva, J., & Vieira Ferreira, L. F. (1997). Infrared approach to the study of adsorption on cellulose: Influence of cellulose crystallinity on the adsorption of benzophenone. *Langmuir, 13*, 4126–4132.
Johnson Ford, E. N., Mendon, S. K., Thames, S. F., & Rawlins, J. W. (2010). X-ray diffraction of cotton treated with neutralized vegetable oil-based macromolecular crosslinkers. *Journal of Engineered Fibers and Fabrics, 5*, 10–20.
Joint FAO/WHO Expert Committee on Food Additives. (2011). *Compendium of food additive specifications -FAO JECFA Monographs*. Rome: FAO.
Joshi, G., Naithani, S., Varshney, V. K., Bisht, S. S., Rana, V., & Gupta, P. K. (2015). Synthesis and characterization of carboxymethyl cellulose from office waste paper: A greener approach towards waste management. *Waste Management, 38*, 33–40.
Latif, A., Anwar, T., & Noor, S. (2007). Two-step synthesis and characterization of carboxymethylcellulose from rayon grade wood pulp and cotton linter. *Journal of the Chemical Society of Pakistan, 29*, 143–150.
Lee, K.-Y., Quero, F., Blaker, J. J., Hill, C. A. S., Eichhorn, S. J., & Bismarck, A. (2011). Surface only modification of bacterial cellulose nanofibers with organic acids. *Cellulose, 18*, 595–605.
Morán, J. I., Alvarez, V. A., Cyras, V. P., & Vázquez, A. (2008). Extraction of cellulose and preparation of nanocellulose from sisal fibers. *Cellulose, 15*, 149–159.
Park, S., Baker, J. O., Himmel, M. E., Parilla, P. A., & Johnson, D. K. (2010). Cellulose crystallinity index: Measurement techniques and their impact on interpreting cellulose performance. *Biotechnology for Biofuels, 3*, 1–10.
Pushpamalar, V., Langford, S. J., Ahmad, M., & Lim, Y. Y. (2006). Optimization of reaction conditions for preparing carboxymethyl cellulose from sago waste. *Carbohydrate Polymers, 64*, 312–318.
Saputra, A. H., Qadhayna, l., & Pitaloka, A. B. (2014). Synthesis and characterization of carboxymethyl cellulose (cmc) from water hyacinth using ethanol-isobutyl alcohol mixture as the solvents. *International Journal of Chemical Engineering and Applications, 5*, 36–40.
Schlufter, K., & Heinze, Th (2010). Carboxymethylation of bacterial cellulose. *Macromolecular Symposia, 294*, 117–124.
Ünlü, C. H. (2013). Carboxymethylcellulose from recycled newspaper in aqueous medium. *Carbohydrate Polymers, 54*, 73–82.
Varshney, V. K., Gupta, P. K., Naithani, S., Khullar, R., Bhatt, A., & Soni, P. L. (2006). Carboxymethylation of α-cellulose isolated from *Lantana Camara* with respect to degree of substitution and rheological behavior. *Carbohydrate Polymers, 63*, 40–45.
Wüstenberg, T. (2015). Sodium carboxymethylcellulose. In T. Wüstenberg (Ed.), *Cellulose and cellulose derivatives in the food industry. Fundamentals and applications (pp. 387–478)*. Pinneberg: Wiley-VCH.
Yamamoto, H., Horii, F., & Hirai, A. (2005). Structural studies of bacterial cellulose trough the solid-phase nitration and acetylation by CP/MAS ^{13}C NMR spectroscopy. *Cellulose, 13*, 327–342.
Yokota, H. (1985). The mechanism of cellulose alkalization in the isopropyl alcohol-water-sodium hydroxide-cellulose system. *Journal of Applied Polymer Science, 30*, 263–277.
Zecher, D., & Gerrish, T. (1997). Cellulose derivatives. In A. P. Imeson (Ed.), *Thickening and gelling agents for food* (pp. 60–85). Hong Kong: Springer Science.
Zhao, H., Cheng, F., Li, G., & Zhang, J. (2003). Optimization of a process for carboxymethyl cellulose (CMC) preparation in mixed solvents. *International Journal of Polymeric Materials, 52*, 749–759.