



# Microwave-assisted carboxymethylation of cellulose extracted from brewer's spent grain

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## ABSTRACT

Cellulose was extracted from brewer's spent grain (BSG) by alkaline and bleaching treatments. The extracted cellulose was used in the preparation of carboxymethyl cellulose (CMC) by reaction with monochloroacetic acid in alkaline medium with the use of a microwave reactor. A full-factorial  $2^3$  central composite design was applied in order to evaluate how parameters of carboxymethylation process such as reaction time, amount of monochloroacetic acid and reaction temperature affect the average degree of substitution ( $\overline{DS}$ ) of the cellulose derivative. An optimization strategy based on response surface methodology has been used for this process. The optimized conditions to yield CMC with the highest  $\overline{DS}$  of 1.46 follow: 5 g of monochloroacetic acid per gram of cellulose, reaction time of 7.5 min and temperature of 70 °C. This work demonstrated the feasibility of a fast and efficient microwave-assisted method to synthesize carboxymethyl cellulose from cellulose isolated of brewer's spent grain.

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## 1. Introduction

Carboxymethylcellulose is the most widely used cellulose ether today, with applications in paper, textile, pharmaceutical, food exploration and paint industries (Singh & Khatri, 2012). Production of carboxymethyl cellulose is carried out commercially on a large scale, by the slurry process and includes two steps; first, cellulose fibers are swollen in concentrated NaOH solution then, still under conditions, the hydroxyl groups of cellulose react with the monochloroacetic acid (MCA) (Bhandari, Jones, & Hanna, 2012; Heinze & Koschella, 2005).

The etherification processes commonly employed for the production of carboxymethyl cellulose require temperature between 40 and 80 °C and long reaction time (1–6 h) (Cheng & Biswas, 2011; Heinze & Koschella, 2005; Singh & Khatri, 2012). Under these conditions, the occurrence of side reactions, such as depolymerization of cellulose and the formation of sodium glycolate, negatively affect both the yield of the process and the characteristics of the derivatives obtained, and the development of conversion processes

that minimize the occurrence of side reactions, the reaction time and amount of reagents employed and energy is very important.

In recent years, microwave chemistry has become increasingly popular within the organic synthesis (Gawande, Shelke, Zboril, & Varma, 2014; Moseley & Kappe, 2011). Compared with conventional heating, microwave-assisted heating, under controlled conditions, has been shown to be an advanced technology in reducing reaction time besides increasing product yield and purity (Caddick & Fitzmaurice, 2009; Nuchter, Ondruschka, Bonrath, & Gum, 2004; Zhu & Chen, 2014). Microwave irradiation-assisted synthesis and modification has been widely used in chemical functionalization of polymer materials. It has been developed for cellulose modification processes including acetylation (Li et al., 2009) and carboxymethylation (Biswas, Kim, Selling, & Cheng, 2014). Therefore, microwave irradiation is a promising method to modify the physical–chemical properties of cellulose.

Most of the sources of cellulose that were modified to carboxymethyl cellulose are wood and cotton (Singh & Singh, 2013). However, many others resources could be used such as corn cobs (Singh & Singh, 2013), rice straw (Ragheb, Nassar, Abd El-Thalouth, Ibrahim, & Shahin, 2012), cotton by-products (Cheng & Biswas, 2011), cavendish banana pseudo stem (Adinugraha, Marseno, & H, 2005), however none of them uses the brewer's spent grain (BSG) as a cellulose source.

Brewer's spent grain is the main solid by-product generated in the brewing process. Approximately 15–20 kg of BSG is produced

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per every hectolitre of beer, which corresponds to an annual production of more than 34 million tons of wet BSG (8.5 million tons of dry BSG) (Mussatto, Dragone, & Roberto, 2006; Xiros & Christakopoulos, 2012). This material is composed of the barley malt residual constituents and includes the barley grain husk in the greatest proportion, and also minor fractions of pericarp and fragments of endosperm (Forsell et al., 2008).

BSG is rich in cellulose (16–21%), hemicellulose (15–29%), lignin (19–28%) and proteins (24–39%) (Meneses, Martins, Teixeira, & Mussatto, 2013; Mussatto, Rocha, & Roberto, 2008; Pires, Ruiz, Teixeira, & Vicente, 2012). Some recent studies suggest the possibility of reusing this material for industrial applications such as the production of activated carbon (Poerschmann et al., 2014), ethanol (Forsell et al., 2008) and xylitol (Mussatto & Roberto, 2008), but BSG is still traditionally used as a relatively low value cattle feed (Niemi, Martins, Buchert, & Faulds, 2013). So, there is a need to find new value-added end-uses for this by-product.

The aim of this work was to increase the economic value of brewer's spent grain by the extraction of the cellulosic component from this by-product through chemical methods. The obtained cellulose was used in the preparation of carboxymethyl cellulose, which has many important industrial applications. Carboxymethyl cellulose was obtained through reaction of the extracted cellulose with monochloroacetic acid in alkaline medium employing microwave reactor.

## 2. Materials and methods

### 2.1. Materials

Brewer's spent grains (BSG) were supplied by Ambev, S.A. (Anápolis, Goiás, Brazil). As soon as obtained, the material (approx. 80% moisture content) was dried at 105 °C to 94% dry matter. Then the dried BSG was milled using a knife mill fitted with a 1 mm-sized grating and used in all experiments. Sodium chlorite ( $\text{NaClO}_2$ ), monochloroacetic acid (MCA), sodium hydroxide and isopropanol were obtained from Sigma-Aldrich. All of the other reagents were analytical grade and were used without further purification.

### 2.2. Cellulose extraction from Brewer's spent grains (BSG)

#### 2.2.1. Alkaline treatment

Initially, the dried BSG was treated with a 2% (w/w) sodium hydroxide aqueous solution in a solid:liquid ratio of 1:20 (w:v) for 2 h at 90 °C, as reported by Mussatto et al. (2006b) with some modifications. The obtained black slurry was filtered and the solid material was washed several times with distilled water until the alkali was completely removed, and dried at 50 °C for 12 h in an air-circulating oven. The yield was calculated based on the dried solid product weight and the starting weight.

#### 2.2.2. Bleaching process

After the alkali treatment, the fibers were treated with a solution made up of equal parts (v:v) of acetate buffer and aqueous sodium chlorite (2 wt%  $\text{NaClO}_2$  in water) in a solid:liquid ratio of 1:50 (w:v) for 4 h at 80 °C. Then the mixture was allowed to cool and was filtered using excess distilled water until the pH of the fibers became neutral. The bleached fibers were dried at 50 °C for 12 h in an air-circulating oven. The bleaching process was carried out in a single step. The yield was calculated based on the dried solid product weight and the starting weight.

### 2.3. Preparation of carboxymethyl cellulose (CMC)

Synthesis of CMC was carried out in two steps i.e., alkalization and etherification under heterogeneous conditions. Alkalization

**Table 1**

Uncoded and coded levels of the independent variables of the carboxymethylation process.

Independent variables	Symbol	Levels		
		-1	0	1
Time (min.)	$X_1$	2.5	5	7.5
MCA/cellulose (g/g)	$X_2$	2	3.5	5
Temperature (°C)	$X_3$	70	80	90

was conducted into glass reaction vessels with an internal volume 35 mL as follows: bleached cellulose (0.5 g) was suspended in isopropanol (2 mL) and 2 mL of 40% (w/v) aqueous NaOH was added dropwise under magnetic stirring at room temperature over a period of 15 min.

The etherification was carried out in a monomode microwave reactor (Discover-SP DC-7196, CEM, USA). The desired amount of monochloroacetic acid (MCA) was dissolved in 2 mL of isopropanol. The mixture was subjected to microwave irradiation at 200 W up to the desired temperature (70–90 °C) and stirred at that temperature for the desired duration (2.5–7.5 min). When the irradiation was complete, the slurry was neutralized with glacial acetic acid and then filtrated. The solid obtained as CMC was washed with 70% ethanol for four times to remove undesirable by-products. The obtained cellulose derivative (CMC) was dried at 60 °C in an oven.

For the purification of these derivatives, 1.5 g of the sample was dissolved in 750 mL of aqueous  $0.4 \text{ mol L}^{-1}$  NaCl. The resulting solution was submitted to positive filtration through  $0.45 \mu\text{m}$  membrane (Millipore®) and the carboxymethyl cellulose was precipitated upon addition of ethanol. Subsequently, the carboxymethyl cellulose was sequentially washed with ethanol/water mixtures of increasing ethanol content (70%, 80%, and 90%), with absolute ethanol and then it was dried at room temperature. This procedure resulted in purified sodium carboxymethyl cellulose samples.

### 2.4. Experimental design

A full-factorial  $2^3$  central composite design was employed to analyze the main effects and interactions of the following variables: reaction time, amount of monochloroacetic acid (MCA) per gram of cellulose and reaction temperature on the average degree of substitution ( $\overline{DS}$ ). The independent variables and their levels are shown in Table 1. Maximum and minimum treatment levels were chosen by carrying out preliminary screening tests. Each experiment was performed in triplicate.

### 2.5. Characterization of brewer's spent grain fibers and carboxymethyl cellulose

#### 2.5.1. Chemical composition of brewer's spent grain fibers

The chemical composition of the BSG at each stage of treatment was measured as follows: the holocellulose ( $\alpha$ -cellulose + hemicellulose) and cellulose content were estimated according to standard methods (Browning, 1967). The  $\alpha$ -cellulose content was determined treating the holocellulose with potassium hydroxide solutions (Browning, 1967). The hemicellulose content was found by subtracting the  $\alpha$ -cellulose part from the holocellulose content. The lignin content was determined according to a standard method of Technical Association of Pulp and Paper Industry TAPPI T222 om-88. The viscosity of pulp (cP) dissolved in a cupriethylene-diamine solution was determined according to Tappi standard (T230 om-99). An average of three measurements was calculated for each sample.

### 2.5.2. Determination of average degree of substitution ( $\overline{DS}$ )

The average degree of substitution of CMC was determined according to the methodology proposed by Ho and Klosiewicz (1980), with modifications. For this purpose, the CMC samples were hydrolyzed in a mixture of 25% (v/v) D<sub>2</sub>SO<sub>4</sub>/D<sub>2</sub>O (75 mg mL<sup>-1</sup>) 1.5 h at 90 °C. The <sup>1</sup>H NMR spectra were obtained at room temperature on a Bruker AVANCE III 500–11.75 Tesla spectrometer operating at 500.13 MHz. The chemical shifts were expressed in dimensionless values (ppm) relative to an internal standard of acetic acid.

### 2.5.3. Field emission scanning electron microscopy (FE-SEM)

A field emission scanning electron microscope (LEO-440) was used to evaluate the surface morphology of the untreated, alkali-treated, bleached brewer's spent grain fibers and carboxymethyl cellulose. The acceleration voltage was 15 kV. Prior to analysis, the samples were coated with an ultrathin gold layer in a sputter coating system.

### 2.5.4. Fourier transform infrared (FTIR) spectroscopy

Fourier transform infrared spectra were recorded using a Perkin-Elmer FTIR spectrophotometer. Samples were finely ground and mixed with potassium bromide, KBr. The mixture was then compressed into pellet form. FTIR spectral analysis was performed within the wave number range of 400–4000 cm<sup>-1</sup>.

### 2.5.5. X-ray diffraction (XRD)

XRD patterns of samples were obtained using an X-ray diffractometer (D8 Advance, Bruker AXS) equipped with CuK $\alpha$  radiation ( $\lambda = 1.5406 \text{ \AA}$ ) in the  $2\theta$  range 5–50° with a scan rate of 5° per min at room temperature. The operating voltage was 40 kV, and the current was 40 mA.

### 2.5.6. Thermogravimetric analysis (TGA)

The thermal stability of the different samples was determined by TGA measurements carried out using a Shimadzu TGA 50 equipment. The amount of sample used for each measurement was approximately 7 mg. All measurements were carried out under a nitrogen atmosphere with a gas flow of 50 mL min<sup>-1</sup> by heating the material from room temperature to 600 °C at a heating rate of 10 °C min<sup>-1</sup>.

## 2.6. Statistical analysis

The statistical treatment of the experimental data consisted in fitting a polynomial function to the set of experimental data collected from full-factorial 2<sup>3</sup> central composite design. Multiple regression analysis was used to fit Eq. (1) to the experimental data by means of the least squares method.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{123} X_1 X_2 X_3 + \varepsilon \quad (1)$$

where,  $Y$  represents the predicted response,  $\beta_0$ , is the model intercept,  $\beta_1$ ,  $\beta_2$ ,  $\beta_3$ , and  $\beta_{12}$ ,  $\beta_{13}$ ,  $\beta_{23}$ ,  $\beta_{123}$ , are linear and interaction coefficients respectively, and  $X_1$ ,  $X_2$  and  $X_3$  are the independent variables.  $\varepsilon$  corresponds to the model residue.

The statistical significance of the model was determined by evaluating the  $p$ -value,  $F$ -value and lack of fit with 95% confidence level obtained from the analysis of variance (ANOVA). The extent of fitting of the experimental results to the polynomial model equation was expressed by the adjusted coefficient of determination ( $R^2_{\text{adj}}$ ). Response surface was obtained by using the fitted model and keeping one independent variable constant and varying the other two variables. All calculations and graphs were obtained by the Statistica software (Statsoft version 7.0, USA).

## 3. Results and discussion

### 3.1. Chemical composition of fibers

The chemical composition of the BSG was determined at each stage of treatment and the data are summarized in Table 2. The original brewer's spent grain contained 45.87 wt% holocellulose, 22.36 wt% cellulose, 20.09 wt%  $\alpha$ -cellulose, 25.78 wt% hemicellulose and 30.48 wt% lignin. The values found for the main constituents of brewer's spent grain are in agreement with literature data (Meneses et al., 2013; Mussatto et al., 2008; Pires et al., 2012).

It was found that at the end of the chemical treatment the cellulose and  $\alpha$ -cellulose content increased from 22.36% to 90.12% and from 20.09% to 89.71%, respectively. The alkali and bleaching treatments were efficient in removing hemicellulose and lignin, which decreased from 25.78% to 5.97% and from 30.48% to 3.23%, respectively, following treatments. The sum of the percentage of cellulose, hemicelluloses and lignin to the brewer's spent grain after bleaching stage corresponds to 99.32% of the total dry matter, therefore the treatment not only removed hemicelluloses and lignin but also other components such as proteins, extractives and ash.

Viscosity was strongly decreased after bleaching (from 16.80 to 9.18 cp), indicating that the average cellulose chain length (polymerization degree) was reduced, since viscosity loss occurs due to the cleavage of glycosidic bonds in this polysaccharide chain. The yield after alkaline treatment was 22.96% while after the bleaching treatment was 66.96% (Table 2). Therefore, starting from 100 g of brewer's spent grain was possible to obtain 15.37 g of pulp at the end of the chemical treatments. The composition and viscosity of the fibers after bleaching is quite suitable for the production of carboxymethyl cellulose (Heinze & Koschella, 2005).

### 3.2. Preparation of CMC from cellulose

The manufacturing of carboxymethyl cellulose involved two reaction stages. In the first stage, cellulose was treated with NaOH, in the presence of isopropanol, which acts both as a swelling agent and as a dilutant and facilitates penetration of NaOH into the cellulose structure. In the second stage, carboxymethyl cellulose was synthesized by reacting monochloroacetic acid (MCA) and alkali cellulose under microwave irradiation (Gu, He, Huang, & Guo, 2012; Singh & Khatri, 2012).

The various properties of sodium carboxymethylcellulose depend upon three factors: molecular weight of the polymer, average number of carboxyl content per anhydroglucose unit [degree of substitution ( $\overline{DS}$ )] and the distribution of carboxyl substituents along the polymer chains (Ruzene, Gonçalves, Teixeira, & Pessoa de Amorim, 2007). The  $\overline{DS}$  is most important factor because the industrial utility of the CMC exclusively depends on the  $\overline{DS}$  (Singh & Singh, 2013) so all the optimization experiments were done with respect to the  $\overline{DS}$  and not the yield.

**Table 2**  
Chemical composition of brewer's spent grain at different stages of treatment.

Parameter	BSG <sup>a</sup>	After alkali treatment <sup>a</sup>	After bleaching <sup>a</sup>
Holocellulose (%) <sup>b</sup>	45.87 ± 1.24	81.80 ± 1.16	95.68 ± 0.29
Cellulose (%)	22.36 ± 0.41	66.43 ± 0.87	90.12 ± 0.71
$\alpha$ -cellulose (%)	20.09 ± 0.86	64.71 ± 1.23	89.71 ± 1.05
Hemicellulose (%)	25.78 ± 0.97	17.09 ± 1.10	5.97 ± 0.92
Lignin (%)	30.48 ± 0.54	11.85 ± 0.27	3.23 ± 0.03
Others (%) <sup>c</sup>	21.38 ± 1.06	4.63 ± 0.34	0.62 ± 0.04
Viscosity (cP)	–	16.80 ± 0.19	9.18 ± 0.06
Yield (%)	–	22.96 ± 0.34	66.96 ± 0.99

<sup>a</sup> Values are mean ± SD of three replicates.

<sup>b</sup> Holocellulose (%) =  $\alpha$ -cellulose (%) + Hemicellulose (%).

<sup>c</sup> Other components include ashes, protein and extractive.

**Table 3**  
Independent variables of the full-factorial  $2^3$  central composite design and the experimental results.

Assay	Time (min.)	MCA <sup>a</sup> /cellulose (g/g)	Temperature (°C)	$\overline{DS}$	Distribution of substituent		
					C <sub>2</sub>	C <sub>3</sub>	C <sub>6</sub>
1	2.50 (-1)	2.00 (-1)	70.00 (-1)	0.62	0.32	0.10	0.20
2	2.50 (-1)	2.00 (-1)	90.00 (+1)	0.58	0.28	0.12	0.18
3	2.50 (-1)	5.00 (+1)	70.00 (-1)	1.23	0.51	0.34	0.38
4	2.50 (-1)	5.00 (+1)	90.00 (+1)	1.22	0.50	0.33	0.39
5	7.50 (+1)	2.00 (-1)	70.00 (-1)	0.54	0.26	0.10	0.18
6	7.50 (+1)	2.00 (-1)	90.00 (+1)	0.64	0.25	0.19	0.20
7	7.50 (+1)	5.00 (+1)	70.00 (-1)	1.46	0.61	0.35	0.5
8	7.50 (+1)	5.00 (+1)	90.00 (+1)	1.42	0.65	0.33	0.44
9	5.00 (0)	3.50 (0)	80.00 (0)	0.93	0.48	0.17	0.28
10	5.00 (0)	3.50 (0)	80.00 (0)	0.84	0.45	0.15	0.24
11	5.00 (0)	3.50 (0)	80.00 (0)	0.91	0.46	0.18	0.27
12	5.00 (0)	3.50 (0)	80.00 (0)	0.91	0.48	0.16	0.27

<sup>a</sup> MCA = monochloroacetic acid.

The previous work on the optimization of the carboxymethylation reaction has shown that  $\overline{DS}$  depends on various reaction parameters such as reaction temperature, reaction time and monochloroacetic acid concentration (Adinugraha et al., 2005; Barai, Singhal, & Kulkarni, 1997; Pushpamalar, Langford, Ahmad, & Lim, 2006; Singh & Singh, 2013). Hence, to optimize the CMC preparation conditions and the relationship between these factors, a full-factorial  $2^3$  central composite design and response surface methodology were applied to determine the optimal levels of the selected three variables (Table 3).

The  $^1\text{H}$  NMR spectroscopy was used to study the completely depolymerized samples. The representative spectra are shown in Fig. 1. The set of doublets in the spectral region between 4.5 and 5.5 ppm at the lower field can be assigned to the proton at C<sub>1</sub> of the  $\alpha$ -anomer, while the set of doublets at the higher field was attributed to the proton at C<sub>1</sub> of the  $\beta$ -anomer. The S and U identifications of the doublets in Fig. 1 refer to substituted and unsubstituted hydroxyl group at C<sub>2</sub>, respectively. The signals between 3 and 4 ppm correspond to the protons of the glucose unit. Protons from the carboxymethyl ( $-\text{OCH}_2\text{COO}-$ ) group were observed in a spectral region between 4.0 and 4.5 ppm (Ho & Klosiewicz, 1980).

The  $^1\text{H}$  NMR spectroscopy used to evaluate the distribution of the carboxymethyl substituents at the C<sub>2</sub>, C<sub>3</sub>, and C<sub>6</sub> hydroxyl groups of the cellulose polymer chains and determine the degree of substitution ( $\overline{DS}$ ). The degree of substitution of the samples was calculated using the Eq. (2).

$$\overline{DS} = \frac{(a/2)}{[(b/6) + c]/2} \quad (2)$$

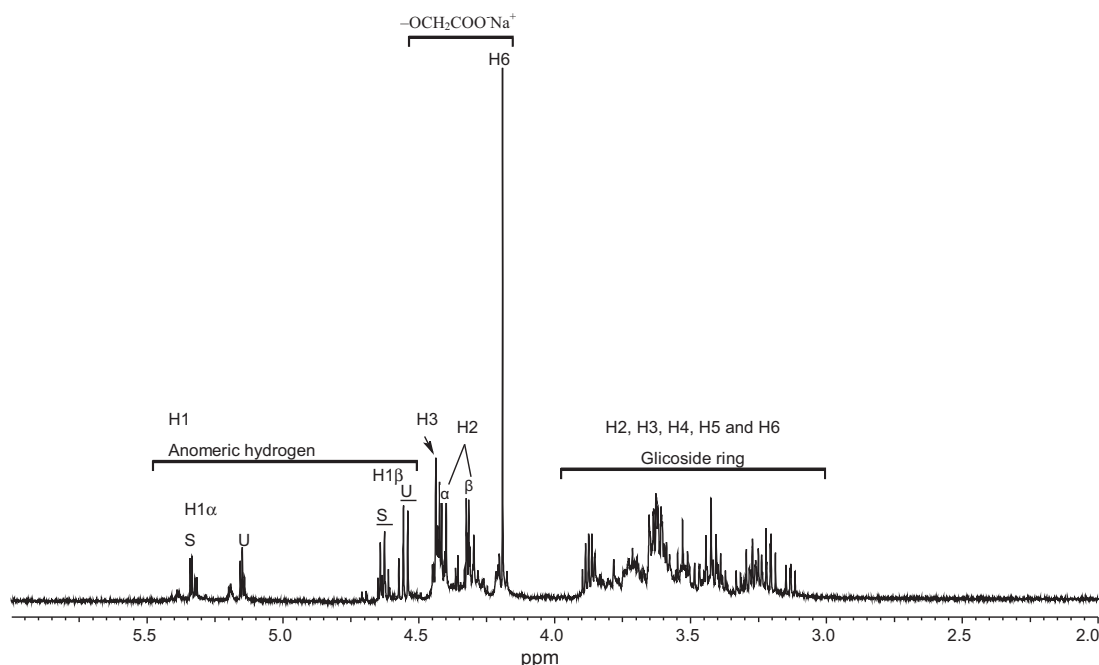
where,  $a$  = Integral which refers to methylene protons of carboxymethyl ( $4 \text{ ppm} < \delta < 4.5 \text{ ppm}$ );  $b$  = Integral which refers to the C–H protons of the glucose unit ( $3 < \delta < 4 \text{ ppm}$ );  $c$  = Integral which refers to the set of doublets in the spectral region between 4.5 and 5.5 ppm.

The distribution of the carboxymethyl groups at C<sub>2</sub>, C<sub>3</sub>, C<sub>6</sub> were determined based on the Eqs. (3)–(5).

$$c_2 = \overline{DS} (I_{C(2)\alpha} + I_{C(2)\beta} / I_{C(2)\alpha} + I_{C(2)\beta} + I_{C(3)} + I_{C(6)}) \quad (3)$$

$$c_3 = \overline{DS} (I_{C(3)} / I_{C(2)\alpha} + I_{C(2)\beta} + I_{C(3)} + I_{C(6)}) \quad (4)$$

$$c_6 = \overline{DS} (I_{C(6)} / I_{C(2)\alpha} + I_{C(2)\beta} + I_{C(3)} + I_{C(6)}) \quad (5)$$



**Fig. 1.**  $^1\text{H}$  NMR spectrum of a CMC sample with  $\overline{DS} = 1.46$  obtained under the conditions of assay 7 (7.5 min.; 5 g of MCA/g of cellulose; 70 °C).

**Table 4**  
Analysis of variance (ANOVA) results.

Source of variation <sup>a</sup>	Sum of square	df	Mean square	F-value	p-value
X <sub>1</sub>	0.021013	1	0.021013	13.4840	0.034950 <sup>b</sup>
X <sub>2</sub>	1.087813	1	1.087813	698.0615	0.000119 <sup>c</sup>
X <sub>3</sub>	0.000013	1	0.000013	0.0080	0.934279 <sup>d</sup>
X <sub>1</sub> X <sub>2</sub>	0.025313	1	0.025313	16.2433	0.027460 <sup>c</sup>
X <sub>1</sub> X <sub>3</sub>	0.001513	1	0.001513	0.9706	0.397174 <sup>d</sup>
X <sub>2</sub> X <sub>3</sub>	0.001513	1	0.001513	0.9706	0.397174 <sup>d</sup>
Lack of fit	0.015317	2	0.007658	4.9144	0.113083 <sup>d</sup>
Pure error	0.004675	3	0.001558		
Total SS	1.157167	11			
R <sup>2</sup> <sub>adj.</sub> = 0.9620					

<sup>a</sup> X<sub>1</sub> = Time (min.); X<sub>2</sub> = MCA/cellulose (g/g); X<sub>3</sub> = Temperature.

<sup>b</sup> = Significant at 5% probability ( $p < 0.05$ ).

<sup>c</sup> = Significant at 1% probability ( $p < 0.01$ ).

<sup>d</sup> = Non-significant.

where,  $I$  represents the integral value of the peaks of C<sub>3</sub>, C<sub>2</sub>α, C<sub>2</sub>β, and C<sub>6</sub>, respectively (Gu et al., 2012). A summary of these data is presented in Table 3.

It can be noted that the  $\overline{DS}$  varied to each condition employed (from 0.54, assay 5, to 1.46, assay 7). It is generally observed that there is slightly more substitution at C<sub>2</sub> than at C<sub>6</sub>, with the least amount at C<sub>3</sub>, indicating that the relative reactivity of the hydroxyl groups is in the following order: OH(2) > OH(6) > OH(3).

### 3.3. Statistical analysis and the model fitting

Table 4 shows the coefficients of the variables in the models that were calculated using the least square technique, and their statistical significances were analysed by ANOVA. The F-value and p-value was used as a tool to check the significance of each parameter. It can be seen from Table 4, the predominant effect on the developed model for the degree of substitution corresponded to the linear term amount of monochloroacetic acid (MCA) (X<sub>2</sub>), followed by the interaction term between time and amount of monochloroacetic acid (X<sub>1</sub>X<sub>2</sub>) and the linear term of time (X<sub>1</sub>). Other terms, such as the linear terms of temperature (X<sub>3</sub>) and the interaction terms (X<sub>1</sub>X<sub>3</sub> and X<sub>2</sub>X<sub>3</sub>) are not significant ( $p > 0.05$ ).

The fitted model for  $\overline{DS}$  without the non-significant terms and in uncoded form is given in Eq. (6).

$$\overline{DS} = 0.20 - 0.076X_1 + 0.24X_2 - 0.015X_1X_2 \quad (6)$$

The model for degree of substitution showed a good fit with the experimental data, as the value of adjusted determination coefficient ( $R^2_{adj.}$ ) was 0.9620. This confirms that the fitted model could explain above 96.00% of the total variability within the range of values studied. The lack of fit is an indication of the failure of a model representing the experimental data at which points are not included in the regression or variations in the models cannot be accounted for random error. The non-significant value of lack of fit ( $p$ -value > 0.05) revealed that the model is statistically significant for the response.

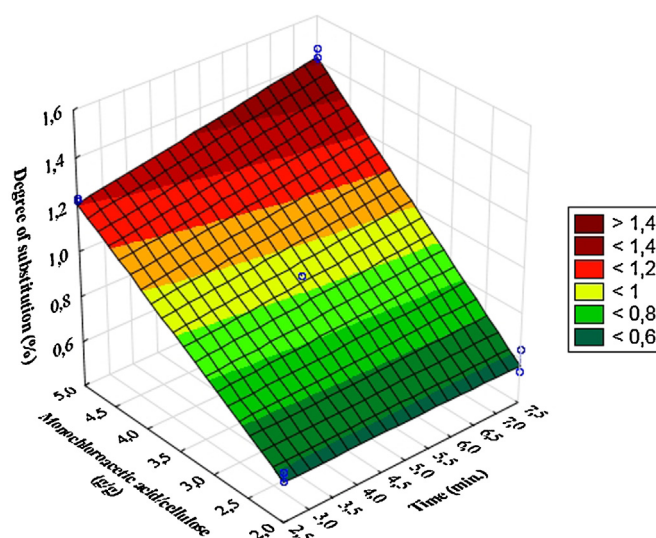
Three-dimensional surface was generated based on Eq. (6) and is shown in Fig. 2 which present the relationship between time and amount of monochloroacetic acid on the degree of substitution. It was found that increasing the reaction time and the amount of monochloroacetic acid results in larger values  $\overline{DS}$ . The increase in  $\overline{DS}$  with increasing amounts of monochloroacetic acid and reaction time is due to the fact that there is a better reaction environment created (i.e., greater availability of the acid molecules at higher concentrations) and a prolonged time available for carboxymethylation. Similar findings have been reported by Barai et al. (1997) and Singh and Singh (2013).

Based on Table 3 and in Fig. 2, the optimum conditions for carboxymethylation were achieved using 5 g de monochloroacetic

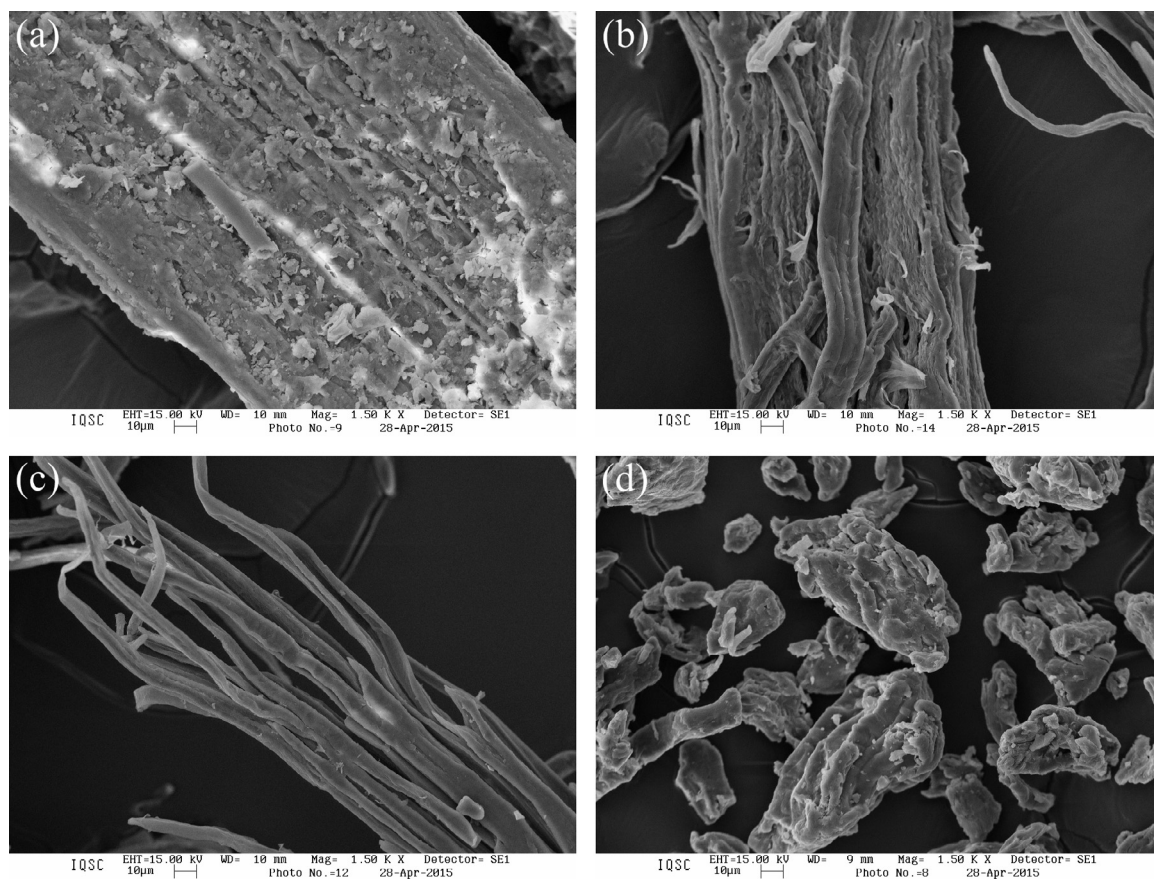
acid per gram of cellulose and carrying out the reaction at 70 °C for 7.5 min. Under these conditions, it was possible to obtain carboxymethyl cellulose with  $\overline{DS}$  of 1.46. In addition, microwave heat allowed obtaining CMC samples with similar  $\overline{DS}$  values compared to the obtained by conventional heat. However, the average reaction time when using conventional heat (1–6 h) for carboximethylation is much greater than microwave heat (Biswas et al., 2014; Heinze & Koschella, 2005; Pushpamalar et al., 2006; Singh & Khatri, 2012). Microwave technology thus appears to be promising in CMC synthesis as a means to reduce reactions times, thereby potentially increasing throughput and reducing energy cost.

### 3.4. Morphological analysis

The morphological changes of the brewer's spent grain fibers after each stage of treatment were evaluated by field emission scanning electron microscopy (Fig. 3). The untreated BSG presents smooth surface (Fig. 3a) and diameter in the range 150–350 μm. After alkali treatment (Fig. 3b), the fiber surface becomes rougher and the diameter decrease to 80–200 μm. The effect of bleaching treatment was evident from the comparison of micrographs in Fig. 3b and c. It can be seen that the BSG fiber bundles separated into individual fibers whose diameters were in the range 5–30 μm. These morphological changes are due to the progressive removal of the outer non-cellulosic layer composed of materials such as



**Fig. 2.** Response surface plot show the effect of reaction time and amount of monochloroacetic acid on the response variable degree of substitution  $\overline{DS}$ .



**Fig. 3.** Scanning electron micrographs (magnification: 1500 $\times$ ) of (a) brewer's spent grain (BSG), (b) alkali-treated BSG, (c) bleached BSG, and (d) carboxymethyl cellulose obtained under the conditions of assay 7 (7.5 min.; 5 g of MCA/g of cellulose; 70  $^{\circ}$ C).

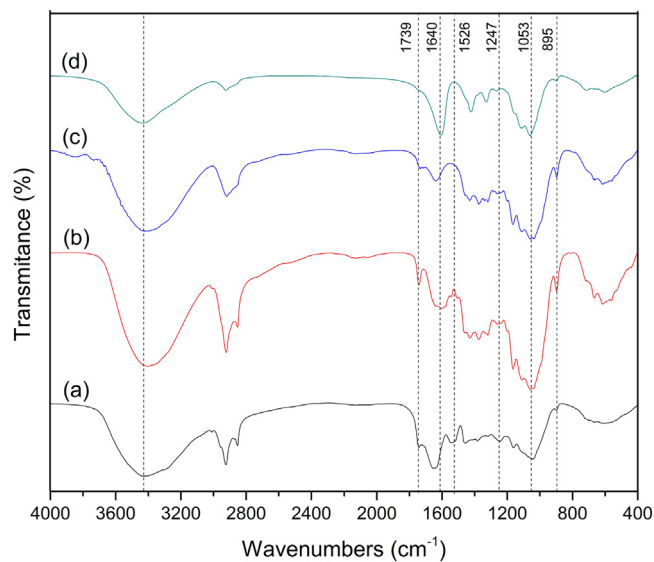
hemicelluloses, lignin, wax, and other impurities contained in the brewer's spent grain upon chemical treatments.

The SEM micrograph of carboxymethyl cellulose, synthesized via chemical modification cellulosic fibers is given in Fig. 3d. It was observed that the carboxymethyl cellulose appear to be irregular and relatively smooth. This morphological change as compared to bleached fiber was ascribed to the heterogeneous introduction of the bulky carboxymethyl groups into the fiber surface after carboxymethylation.

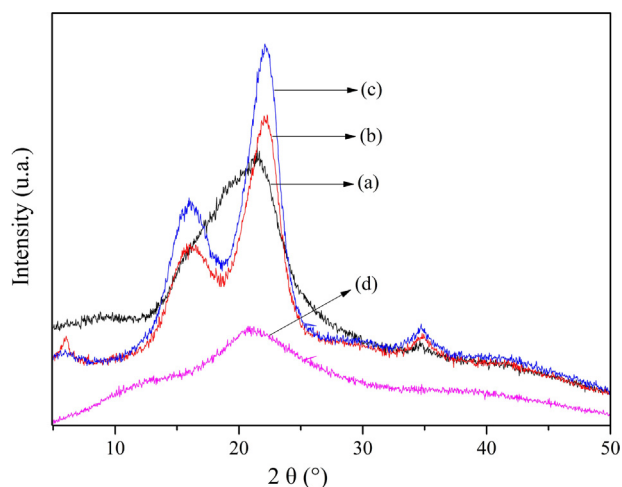
### 3.5. FTIR spectroscopy analysis

Fourier transform infrared spectroscopy (FTIR) allows the characterization of a chemical structure by identifying the functional groups present in each sample. Fig. 4 shows the FTIR spectra recorded for (a) untreated BSG, (b) alkali-treated BSG, (c) bleached BSG and (d) carboxymethyl cellulose. The peak located at 1739  $\text{cm}^{-1}$  that is seen in the spectrum of the untreated BSG can be attributed either to the acetyl and uronic ester groups of the hemicelluloses or to the ester linkage of carboxylic group of the ferulic and p-coumeric acids of lignin and/or hemicelluloses (Sun et al., 2005). This peak almost disappeared after the bleaching treatment, indicating the removal of most of the hemicelluloses and lignin from the BSG. The peak at 1526  $\text{cm}^{-1}$  in the spectrum of the untreated brewer's spent grain represent the aromatic C=C of the aromatic rings of lignin. The peak observed at 1247  $\text{cm}^{-1}$  corresponds to C–O–C (aryl-alkyl ether) (Sheltami, Abdullah, Ahmad, Dufresne, & Kargarzadeh, 2012). These two peaks disappeared after the bleaching treatment, which suggests the removal of lignin.

The peaks at 1053 and 895  $\text{cm}^{-1}$  are associated with the C–O stretching and C–H rock vibrations of cellulose (Alemdar & Sain, 2008), which appeared in all of the spectra. The increment of these peaks is directly related to the increase in the cellulosic components percentages.



**Fig. 4.** FTIR spectra of (a) brewer's spent grain (BSG), (b) alkali-treated BSG, (c) bleached BSG, and (d) carboxymethyl cellulose obtained under the conditions of assay 7 (7.5 min.; 5 g of MCA/g of cellulose; 70  $^{\circ}$ C).



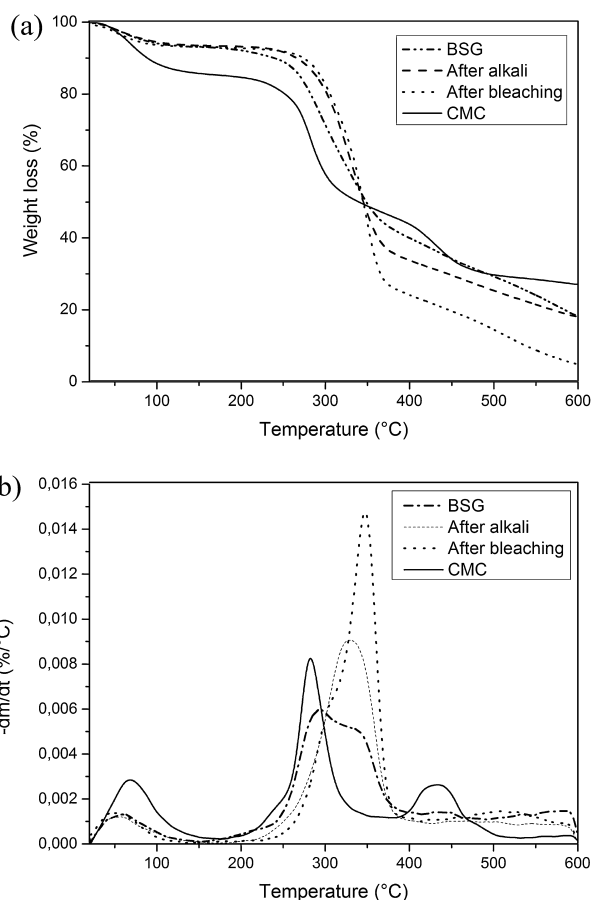
**Fig. 5.** X-ray diffractograms for (a) brewer's spent grain (BSG), (b) alkali-treated BSG, (c) bleached BSG, and (d) carboxymethyl cellulose obtained under the conditions of assay 7 (7.5 min.; 5 g of MCA/g of cellulose; 70 °C).

The occurrence of carboxymethylation was confirmed by infrared spectroscopy. The IR spectra of the prepared CMC were recorded, as displayed in Fig. 4 which shows that the appearance of the typical band at  $1610\text{ cm}^{-1}$  associated with the presence of the carboxylate anion  $\text{COO}^-$  and confirms the formation of CMCNa (Heinze & Koschella, 2005).

### 3.6. X-ray diffraction (XRD)

The X-ray diffraction patterns obtained for untreated, alkali-treated, bleached brewer's spent grain as well as for the carboxymethyl cellulose are shown in Fig. 5. It is observed that the diffractogram of brewer's spent grain has a main peak at  $2\theta = 21.5^\circ$  and a discrete peak at  $2\theta = 34.7^\circ$ . Three peaks can be observed for alkali-treated BSG and bleached BSG at  $2\theta = 16.0^\circ$ ,  $22.2^\circ$  and  $34.7^\circ$ . They are typical of cellulose I and become more defined upon chemical treatments (Azubuike, Rodríguez, Okhamafe, & Rogers, 2012; Chen et al., 2011; Flauzino Neto, Silvério, Dantas, & Pasquini, 2013; Kumar, Negi, Bhardwaj, & Choudhary, 2012). The crystallinity index (CrI) of all samples was calculated following the amorphous subtraction method proposed by Park, Baker, Himmel, Parilla, & Johnson (2010). By this method, the CrI was found to be 27.3%, 46.7% and 56.5% for untreated BSG, alkali-treated BSG and bleached BSG, respectively. The higher CrI value of bleached BSG compared to alkali-treated BSG and untreated BSG is due to the removal of amorphous non-cellulosic compounds such as lignin and hemicellulose by the alkali and bleaching treatments carried out in the purification process.

The comparison of the X-ray patterns of bleached fibers and carboxymethyl cellulose revealed that the carboxymethylation of fibers provoked important changes in the arrangement of the polymer chains in the solid state. It can be seen that after carboxymethylation all characteristic peaks for bleached fibers have almost disappeared and transformed into an amorphous phase. Although the diffractogram of carboxymethyl cellulose has not been quantitatively treated to determine the CrI, it is assumed that the carboxymethyl cellulose adopt a less ordered arrangement as compared to bleached fibers. This is attributed to the presence of the carboxymethyl moieties which substitute the hydrogen atoms of the hydroxyl groups of cellulose affecting the establishment of hydrogen bonds involving these groups that are responsible for the adoption of a more ordered arrangement by the cellulose (Johar, Ahmad, & Dufresne, 2012).



**Fig. 6.** (a) TG and (b) DTG curves for brewer's spent grain (BSG), alkali-treated BSG, bleached BSG, and carboxymethyl cellulose obtained under the conditions of assay 7 (7.5 min.; 5 g of MCA/g of cellulose; 70 °C).

### 3.7. Thermogravimetric analysis

Fig. 6 shows the TGA and derivative thermogravimetry DTG curves obtained for raw brewer's spent grain, as well as those obtained after alkali treatment and bleaching and for carboxymethyl cellulose. In all cases, an initial weight loss of the fibers occurs in the range of 35–150 °C, due to the evaporation of adsorbed and bound water (Flauzino Neto et al., 2013).

Degradation of hemicellulose and cellulose starts at temperatures 220 and 250 °C, respectively, while lignin degrades at a lower temperature, 200 °C (Morán, Alvarez, Cyras, & Vázquez, 2008). At higher temperatures lignin is more heat resistant than hemicelluloses and cellulose, due to its low degradation rate. The weight loss between 200 and 300 °C is mainly due to hemicelluloses decomposition and the parallel slow decomposition of lignin, while the weight loss between 250 and 500 °C is attributed to cellulose (250–350 °C) and lignin (200–500 °C) decomposition (Nanda et al., 2013). At temperatures higher than 400 °C, there is oxidation and breakdown of the charred residue to lower molecular weight gaseous products (Morán et al., 2008).

By the comparison of the untreated and alkali treated fibers, it was observed that the bleaching treatment induces an increase in the materials thermal stability. This is due to the presence of hemicellulose and lignin in the chemical composition of the untreated and alkali treated BSG fibers. These components have a lower decomposition temperature compared to cellulose and their progressive removal improves the thermal stability of the fibers. The lower thermal stability of alkali treated compared to the bleached fibers is attributed to the partial elimination of

non-cellulosic material. However, after 400 °C, the thermal stability order of the materials is reversed, because lignin at higher temperature is more heat resistant than hemicelluloses and cellulose.

In the DTG curve for BSG, a shoulder is observed at around 300 °C, but is no longer present after the chemical treatments, which very likely reflects the removal of a portion of the hemicellulose. The weight fraction of material that still exists after heating between 400 °C and 600 °C is representative of the carbon content in the fibers. The increased residue amount at high temperature, or the char fraction, for BSG compared to chemically treated fibers is due to the presence of ash as well as lignin. As it can be seen in Fig. 6, the amount of residue at temperature around 600 °C in bleached fibers was only 5%.

Fig. 6 shows that the introduction of carboxymethyl groups in the cellulose chain decreased the thermal stability of the polymer. The decrease in the thermal stability of the carboxymethyl cellulose as compared to the parent cellulose is justified by the presence of the negatively charged carboxymethyl groups and is supported by the X-ray diffraction analyses which evidenced the less ordered arrangement of CMC.

#### 4. Conclusions

The present work shows that cellulose can be isolated from brewer's spent grain. Chemical treatment with alkali and sodium chlorite removed the non-cellulosic constituents resulting in fibers with high content of cellulose and  $\alpha$ -cellulose, hence suitable for production of carboxymethyl cellulose. The results obtained suggest that BSG is an industrial by-product with great potential to be a feedstock for producing this derivative. The carboxymethylation of cellulose was successfully achieved and samples of different average degrees of substitution ( $0.58 < \overline{DS} < 1.46$ ) were prepared according to the reaction conditions employed. The use of a higher amount of monochloroacetic acid and reaction time resulted in the more substituted samples but the reaction temperature did not strongly affect the average degree of substitution. Using optimized set of conditions: 5 g of monochloroacetic acid per gram of cellulose; reaction time of 7.5 min and temperature of 70 °C, CMC having a  $\overline{DS}$  of 1.46 can be prepared.

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#### 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(2), 164–169. <http://dx.doi.org/10.1016/j.carbpol.2005.07.019>
- Alemdar, A., & Sain, M. (2008). Isolation and characterization of nanofibers from agricultural residues—Wheat straw and soy hulls. *Bioresource Technology*, 99(6), 1664–1671. DOI <http://dx.doi.org/10.1016/j.biortech.2007.04.029>.
- Azubuikwe, C., Rodríguez, H., Okhamafe, A., & Rogers, R. (2012). Physicochemical properties of maize cob cellulose powders reconstituted from ionic liquid solution. *Cellulose*, 19(2), 425–433. DOI 10.1007/s10570-011-9631-y.
- 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(3–4), 229–231. DOI [http://dx.doi.org/10.1016/S0144-8617\(96\)00166-X](http://dx.doi.org/10.1016/S0144-8617(96)00166-X).
- Bhandari, P. N., Jones, D. D., & Hanna, M. A. (2012). Carboxymethylation of cellulose using reactive extrusion. *Carbohydrate Polymers*, 87(3), 2246–2254. DOI <http://dx.doi.org/10.1016/j.carbpol.2011.10.056>.
- 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(0), 259–265. <http://dx.doi.org/10.1016/j.indcrop.2014.06.004>
- Browning, B. L. (1967). *Methods of wood chemistry*. New York: John Wiley & Sons.
- Caddick, S., & Fitzmaurice, R. (2009). Microwave enhanced synthesis. *Tetrahedron*, 65(17), 3325–3355. DOI <http://dx.doi.org/10.1016/j.tet.2009.01.105>.
- Chen, W., Yu, H., Liu, Y., Chen, P., Zhang, M., & Hai, Y. (2011). Individualization of cellulose nanofibers from wood using high-intensity ultrasonication combined with chemical pretreatments. *Carbohydrate Polymers*, 83(4), 1804–1811. DOI <http://dx.doi.org/10.1016/j.carbpol.2010.10.040>.
- Cheng, H. N., & Biswas, A. (2011). Chemical modification of cotton-based natural materials: Products from carboxymethylation. *Carbohydrate Polymers*, 84(3), 1004–1010. DOI <http://dx.doi.org/10.1016/j.carbpol.2010.12.059>.
- Flauzino Neto, W. P., Silvério, H. A., Dantas, N. O., & Pasquini, D. (2013). Extraction and characterization of cellulose nanocrystals from agro-industrial residue—Soy hulls. *Industrial Crops and Products*, 42(0), 480–488. DOI <http://dx.doi.org/10.1016/j.indcrop.2012.06.041>.
- Forssell, P., Kontkanen, H., Schols, H. A., Hinz, S., Eijssink, V. G. H., Treimo, J., et al. (2008). Hydrolysis of brewers' spent grain by carbohydrate degrading enzymes. *Journal of the Institute of Brewing*, 114(4), 306–314. DOI 10.1002/j.2050-0416.2008.tb00774.x.
- Gawande, M. B., Shelke, S. N., Zboril, R., & Varma, R. S. (2014). Microwave-Assisted chemistry: synthetic applications for rapid assembly of nanomaterials and organics. *Accounts of Chemical Research*, 47(4), 1338–1348. DOI 10.1021/ar400309b.
- Gu, H., He, J., Huang, Y., & Guo, Z. (2012). Water soluble carboxymethylcellulose fibers derived from alkalization-etherification of viscose fibers. *Fibers and Polymers*, 13(6), 748–753. DOI 10.1007/s12221-012-0748-x.
- Heinze, T., & Koschella, A. (2005). Carboxymethyl ethers of cellulose and starch—A review. *Macromolecular Symposia*, 223(1), 13–40. DOI 10.1002/masy.200550502.
- Ho, F. F. L., & Klosiewicz, D. W. (1980). Proton nuclear magnetic resonance spectrometry for determination of substituents and their distribution in carboxymethylcellulose. *Analytical Chemistry*, 52(6), 913–916. DOI 10.1021/ac50056a032.
- Johar, N., Ahmad, I., & Dufresne, A. (2012). Extraction, preparation and characterization of cellulose fibres and nanocrystals from rice husk. *Industrial Crops and Products*, 37(1), 93–99. DOI <http://dx.doi.org/10.1016/j.indcrop.2011.12.016>.
- Kumar, A., Negi, Y. S., Bhardwaj, N. K., & Choudhary, V. (2012). Synthesis and characterization of methylcellulose/PVA based porous composite. *Carbohydrate Polymers*, 88(4), 1364–1372. DOI <http://dx.doi.org/10.1016/j.carbpol.2012.02.019>.
- Li, J., Zhang, L.-P., Peng, F., Bian, J., Yuan, T.-Q., Xu, F., et al. (2009). Microwave-assisted solvent-free acetylation of cellulose with acetic anhydride in the presence of iodine as a catalyst. *Molecules*, 14(9), 3551–3566. <http://www.scopus.com/inward/record.url?eid=2-s2.0-70349556803&partnerID=40&md5=e02cbf146d55cf2124e4c3e16d1149e3>.
- Meneses, N. G. T., Martins, S., Teixeira, J. A., & Mussatto, S. I. (2013). Influence of extraction solvents on the recovery of antioxidant phenolic compounds from brewer's spent grains. *Separation and Purification Technology*, 108, 152–158. <http://www.scopus.com/inward/record.url?eid=2-s2.0-84875197003&partnerID=40&md5=780402e79a46f25790f3e137b8b6c10e>.
- Morán, J., Alvarez, V., Cyras, V., & Vázquez, A. (2008). Extraction of cellulose and preparation of nanocellulose from sisal fibers. *Cellulose*, 15(1), 149–159. DOI 10.1007/s10570-007-9145-9.
- Moseley, J. D., & Kappe, C. O. (2011). A critical assessment of the greenness and energy efficiency of microwave-assisted organic synthesis. *Green Chem.*, 13(4), 794–806. DOI 10.1039/C0GC00823K.
- Mussatto, S. I., Dragone, G., & Roberto, I. C. (2006). Brewers' spent grain generation characteristics potential applications. *Journal of Cereal Science*, 43(1), 1–14. DOI <http://dx.doi.org/10.1016/j.jcs.2005.06.001>.
- Mussatto, S. I., Dragone, G., Rocha, G. J. M., & Roberto, I. C. (2006). Optimum operating conditions for brewer's spent grain soda pulping. *Carbohydrate Polymers*, 64(1), 22–28. <http://www.scopus.com/inward/record.url?eid=2-s2.0-33745041751&partnerID=40&md5=4f844fc40fcdab87c203d70b419fd0bb>.
- Mussatto, S. I., & Roberto, I. C. (2008). Establishment of the optimum initial xylose concentration and nutritional supplementation of brewer's spent grain hydrolysate for xylitol production by *Candida guilliermondii*. *Process Biochemistry*, 43(5), 540–546. DOI <http://dx.doi.org/10.1016/j.procbio.2008.01.013>.
- Mussatto, S. I., Rocha, G. J. M., & Roberto, I. C. (2008). Hydrogen peroxide bleaching of cellulose pulps obtained from brewer's spent grain. *Cellulose*, 15(4), 641–649. <http://www.scopus.com/inward/record.url?eid=2-s2.0-45949090863&partnerID=40&md5=0e7d84be260a9f56417dfc3e5442a686>.
- Nanda, S., Mohanty, P., Pant, K. K., Naik, S., Kozinski, J. A., & Dalai, A. K. (2013). Characterization of North American lignocellulosic biomass and biochars in terms of their candidacy for alternate renewable fuels. *Bioenergy Research*, 6(2), 663–677. <http://www.scopus.com/inward/record.url?eid=2-s2.0-84876969305&partnerID=40&md5=c72e7b3a6a0d56ae56311755983c8fd9>.
- Niemi, P., Martins, D., Buchert, J., & Faulds, C. B. (2013). Pre-hydrolysis with carbohydrases facilitates the release of protein from brewer's spent grain. *Bioresource Technology*, 136(0), 529–534. DOI <http://dx.doi.org/10.1016/j.biortech.2013.03.076>.
- Nuchter, M., Ondruschka, B., Bonrath, W., & Gum, A. (2004). Microwave assisted synthesis—A critical technology overview. *Green Chemistry*, 6(3), 128–141. DOI 10.1039/B310502D.
- 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 cellulase performance. *Biotechnology for Biofuels*, 3. DOI 10.1186/1754-6834-3-10.
- Pires, E. J., Ruiz, H. A., Teixeira, J. A., & Vicente, A. A. (2012). A new approach on brewer's spent grains treatment and potential use as



- lignocellulosic yeast cells carriers. *Journal of Agricultural and Food Chemistry*, 60(23), 5994–5999. <http://www.scopus.com/inward/record.url?eid=2-s2.0-84862208224&partnerID=40&md5=81e1fa71dd5784d096021d78bf42dabd>.
- Poerschmann, J., Weiner, B., Wedwitschka, H., Baskyr, I., Koehler, R., & Kopinke, F.-D. (2014). Characterization of biocoals and dissolved organic matter phases obtained upon hydrothermal carbonization of brewer's spent grain. *Bioresource Technology*, 164(0), 162–169. DOI <http://dx.doi.org/10.1016/j.biortech.2014.04.052>.
- 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(2), 312–318. DOI <http://dx.doi.org/10.1016/j.carbpol.2005.12.003>.
- Ragheb, a. a., Nassar, S. H., Abd El-Thalouth, I., Ibrahim, M. a., & Shahin, a. a. (2012). Preparation, characterization and technological evaluation of CMC derived from rice-straw as thickening agents in discharge, discharge-resist and burn-out printing. *Carbohydrate Polymers*, 89(4), 1044–1049. DOI [10.1016/j.carbpol.2012.03.054](http://dx.doi.org/10.1016/j.carbpol.2012.03.054).
- Ruzene, D., Gonçalves, A., Teixeira, J., & Pessoa de Amorim, M. (2007). Carboxymethylcellulose obtained by ethanol/water organosolv process under acid conditions. *Applied Biochemistry and Biotechnology*, 137–140(1–12), 573–582. <http://dx.doi.org/10.1007/s12010-007-9080-0>
- Sheltami, R. M., Abdullah, I., Ahmad, I., Dufresne, A., & Kargarzadeh, H. (2012). Extraction of cellulose nanocrystals from mengkuang leaves (*Pandanus tectorius*). *Carbohydrate Polymers*, 88(2), 772–779. DOI <http://dx.doi.org/10.1016/j.carbpol.2012.01.062>.
- Singh, R. K., & Khatri, O. P. (2012). A scanning electron microscope based new method for determining degree of substitution of sodium carboxymethyl cellulose. *Journal of Microscopy*, 246(1), 43–52. <http://www.scopus.com/inward/record.url?eid=2-s2.0-84857996843&partnerID=40&md5=4d62bc77c70e9695a02235776957e2ae>.
- Singh, R., & Singh, A. (2013). Optimization of reaction conditions for preparing carboxymethyl cellulose from corn cobs agricultural waste. *Waste and Biomass Valorization*, 4(1), 129–137. DOI [10.1007/s12649-012-9123-9](http://dx.doi.org/10.1007/s12649-012-9123-9).
- Sun, X. F., Xu, F., Sun, R. C., Geng, Z. C., Fowler, P., & Baird, M. S. (2005). Characteristics of degraded hemicellulosic polymers obtained from steam exploded wheat straw. *Carbohydrate Polymers*, 60(1), 15–26. DOI <http://dx.doi.org/10.1016/j.carbpol.2004.11.012>.
- Xiros, C., & Christakopoulos, P. (2012). Biotechnological potential of brewers spent grain and its recent applications. *Waste and Biomass Valorization*, 3(2), 213–232. DOI [10.1007/s12649-012-9108-8](http://dx.doi.org/10.1007/s12649-012-9108-8).
- Zhu, Y.-J., & Chen, F. (2014). Microwave-assisted preparation of inorganic nanostructures in liquid phase. *Chemical Reviews*, 114(12), 6462–6555. DOI [10.1021/cr400366s](http://dx.doi.org/10.1021/cr400366s).