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| 1 2 | Non thermal plasma in liquid media: effect on inulin depolymerization and functionalization | | | |
|--------|---|--|--|--|
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| 6 | Corresponding author: elodie.fourre@univ-poitiers.fr | | | |
| 7 | Keywords | | | |
| 8 | Gas-liquid plasma reactor; non thermal plasma; inulin; depolymerization; infrared | | | |
| 9 | spectroscopy; ssNMR spectroscopy | | | |
| 10 | Highlights | | | |
| 11 | • A novel double dielectric barrier discharge plasma reactor with a liquid interface has | | | |
| 12 | been designed | | | |
| 13 | • It is possible to totally convert inulin into 100 % fructose and glucose | | | |
| 14 | • No degradation products are generated | | | |
| 15 | • Combined analytical results evidenced the acidic attack of the glycosidic bond leading | | | |
| 16 | to depolymerization | | | |
| 17 | Abstract | | | |
| 18 | We report the complete conversion of inulin in gas/liquid media by a dielectric barrier discharge | | | |
| 19 | plasma at atmospheric pressure. Depending on the plasma treatment time (from 1 to 30 min) | | | |
| 20 | and the chemical nature of the gases (air, oxygen, nitrogen), it was possible to depolymerize | | | |

21 inulin into fructo-oligosaccharides with a degree of polymerization inferior to 5 or to achieve a

total conversion of inulin into its two monomeric constituents, fructose and glucose in 20 min,

23 without any degradation products. Combined results from liquid chromatography (HPLC),

solid state Nuclear Magnetic Resonance (ssNMR) and mass spectroscopy revealed that the

breakage of the β 1-4-bridged oxygen occurs by an acidic attack, following the oxidation of the

polymer. Infrared spectroscopy revealed the oxidation and breakage of the polymer and alsoadsorption of nitrate species.

28 Introduction

Since the industrial revolution, chemistry has developed processes, catalysts and technologies 29 for the conversion of fossil carbon, aiming to create complex and diverse molecules. However, 30 increasing environmental awareness is prompting scientists and manufacturers to develop 31 32 strategies for environmental sustainability by using processes and materials with low cost, low energy consumption and low toxicity. For several years now, a new approach has been focusing 33 on the use of new raw material from the biomass or waste (Ong et al., 2019; Sheldon, 2018). 34 This change of strategy was revolutionary in the world of chemistry and it has dramatically 35 changed the way a process is designed (Jérôme, Chatel & De Oliveira Vigier, 2016; Farmer & 36 Mascal, 2015; Sylla-Iyarreta Veitía & Ferroud, 2015; Horváth & Anastas, 2007; Baig & Varma, 37 2012; Benoit et al., 2012). For this reason, the use of advanced technologies, such as non-38 39 thermal plasma, ultrasounds or ball-milling have been extensively investigated (Farmer & 40 Mascal, 2015). Current and future society needs scientists and manufacturers to focus on new strategies and develop low cost processes for sustainable materials, easy to produce and widely 41 available (Jérôme, 2016) 42

The recent extensive use of non-thermal plasma is the result of a range of reaction parameters 43 that cannot be accessible otherwise, or to a lesser extent. No other media can provide gas 44 temperatures or energy densities as high as those of plasmas; no other media can excite atomic 45 and molecular species to radiate as efficiently; no other media can be arranged to provide 46 comparable transient and non-equilibrium conditions (National Research Council, 1991). This 47 technology is safe, versatile, easy to carry out and allows the generation of highly reactive 48 chemical species with low energy consumption, low toxicity and the possibility of continuous 49 processing (Kan, Lam, Chan, & Ng, 2014). 50

The development of atmospheric pressure plasma technologies has dramatically increased in 51 recent years due to their potential impact in a very wide range of applications that include 52 surface treatments (cleaning, etching), surface activation, surface coating (air plasma spray, 53 plasma enhanced chemical vapor deposition) (Tendero, Tixier, Tristant, Desmaison & 54 Leprince, 2006), but also food (decontamination, toxin degradation, packaging), medicine 55 (sterilization, wound healing, skin treatments) and water (pesticide and dyes degradation, 56 decontamination) (Pankaj & Keener, 2017). The technological progress has encouraged the 57 interest and advancement in the understanding of plasmas. The possibility of performing 58 reactions at atmospheric pressure is becoming increasingly attractive and the fundamental and 59 essential role of technological plasmas is set to expand significantly in the coming years 60 0(Mariotti, Patel, Švrček & Maguire, 2012). 61

In this context, production of fructose from fructans such as inulin using plasma, is an 62 alternative to the current approaches, such as acid or enzymatic hydrolysis (Raccuia et al., 63 2016). Inulin is constituted of fructose units connected by β (1-2) linkages with a glucose at its 64 65 extremity linked in α (1-2) form. In the inulin chain, the fructose is blocked in the furanose form and glucose is in glucopyranose form. Inulin, its derivatives and its two constitutive monomers 66 have received considerable interest as food ingredients (Blecker, Fougnies, Van Herck, 67 68 Chevalier & Paquot, 2002). Inulin has also been chemically modified in several ways (neutral, anionic, and cationic modification as well as cross-linking and slow release applications) to 69 obtain highly biodegradable compounds at the industrial scale (Stevens, Meriggi & Booten, 70 2001). 71

The effect of non-thermal atmospheric plasma on inulin was already investigated on solid material in gas phase but the reaction mechanism was not completely elucidated [Nastase, Tatibouët & Fourré, 2018; Benoit & al., 2012). The authors reported the depolymerization of inulin with a yield of 16 wt % of fructose (other products being fructo-oligosaccharides (FOS) with a degree of polymerization (DP) lower than 6) via the surrounding water initially contained
in polysaccharides that promotes the cleavage of the glycosidic bonds (Benoit &al.). In a recent
study (Nastase, Tatibouët & Fourré, 2018), it was suggested that reactive oxidizing species
generated by oxygen (ROS) and nitrogen (RNS) played a key role in the depolymerization
process, via OH radicals or nitric acid attack.

Nowadays, within the field of plasma science and technology, the attention is increasing over 81 82 the plasma-liquid interactions, and particularly on the physical and chemical mechanisms leading to complex reaction at the plasma-liquid interface (Bruggeman et al, 2016). It is well 83 admitted in the literature that discharges generated at the gas-liquid interface provide gaseous 84 85 reactive species that can dissolve in the liquid media, inducing the formation of species presenting high reactivity, such as H_2O_2 , NO_2^-/NO_3^- , OH^+ , $HOO^+/O_2^{\bullet-}$ to name a few. The 86 fundamental properties of liquid-phase plasma (like generation, state or reactive species) have 87 not been fully described, but the presence of liquid in the system leads to higher reaction rate 88 since the molecular density in the liquid phase is much higher than in the gas phase (Takai, 89 90 2008). However, plasmas in liquids are more difficult to control and stabilize: the liquid is often 91 an electrode, therefore evaporation and chemical modification occurs, which adds significant complexity compared to the gas phase plasmas (Bruggeman & Leys, 2009). 92

93 In this respect, a reactor with a double dielectric (DD) barrier, combining a liquid and gas phase DD-LG plasma has been developed. The configuration of the reactor used along this study 94 allows the initiation of various types of reactions: plasma active species formed in the gas and 95 at the gas-liquid interface are further transferred into the liquid giving rise to more chemical 96 reactions. The novelty of this configuration resides in the isolation of the liquid phase between 97 98 the 2 electrodes, which can avoid problems like electrode evaporation (when using liquid electrode) or contamination from the metal electrode in contact with the liquid. This plasma 99 configuration has been used to follow depolymerization of inulin into FOS, fructose and glucose 100

investigated in controlled conditions in power, time, chemical nature of the gas phase andsample concentration.

- 103 1. Experimental
- 104 1.1. Materials
- 105

106 Throughout this study, commercial inulin (from chicory, Sigma Aldrich) was used as a 107 substrate. Inulin was solubilized in ultra-pure water under stirring at room temperature, to 108 obtain a concentration of 8 g.L⁻¹ without further treatment.

- 109
- 110 1.2.Gas-liquid plasma device
- 111

Plasma treatment was carried out in a dielectric barrier discharge reactor consisting in a double 112 wall glass cylinder separated in two parts by a fritted glass (figure 1). This reactor can be 113 classified as multiphase discharge (Bruggeman et al., 2016) except that both electrodes are 114 isolated from the liquid phase by two glass walls. The first electrode (inox tube) stands in the 115 center of the cylinder and was protected from the liquid by a first dielectric wall. The second 116 electrode (copper adhesive tape, from Advance tape) was wrapped around the second dielectric 117 wall, on the outside wall of the cylinder. The gap between both dielectrics was 3 mm. A high 118 119 voltage supply (A2E Technologies Enertronic), was connected to the electrodes and providing bipolar voltage pulses, allows the variation of the voltage from 0 to 40 kV. The gas (flow of 30 120 121 mL.min⁻¹) was introduced in the reactor from the bottom entry and was flown through a fritted glass which not only created bubbles, but also prevented the liquid from draining. The volume 122 of the solution to be treated was set at 5 mL, allowing the generation of plasma in the gas phase, 123 right above the surface of the liquid and, in a lesser extent, in the gas bubbles through the liquid. 124 125 The solution was injected in the system kept at atmospheric pressure, room temperature and with the gas flow set at 30 mL min⁻¹. In order to determine the optimal parameters for inulin 126 conversion, the electrical parameters (voltage, 16-21 kV) and the treatment time (1-40 min) 127

were modified. The discharge was generated under nitrogen, oxygen, helium and airatmosphere



130

131 Figure 1: Drawing of the experimental setup

132

| 133 1.3.Characterizatio |
|-------------------------|
|-------------------------|

- 134 *Hydrogen peroxide content*
- 135

Hydrogen peroxide produced during the plasma treatment was calculated from colorimetric titration with potassium permanganate, in acidic conditions. 10 mL of plasma treated sample was placed in a beaker with 20 mL distilled water and 10 mL of H₂SO₄ solution (3 M). The solution was magnetically stirred and a solution of KMnO₄ (0.025 M) was added dropwise through a graduated burette. The concentration was calculated from the equivalent point volume.

142 *HPLC*

High Pressure Liquid Chromatography was used to determine the presence of oligosaccharides
after plasma processing. Samples were analyzed on a Shodex Sugar KS-802 column
(maintained at 40°C) that allows the separation of oligosaccharides by steric exclusion and

ligand exchange. The stationary phase consists of polystyrene-divinylbenzene coated with a
cation exchange resin and the mobile phase consists of ultra-pure water at a flow rate of 1
mL.min⁻¹.

Molecules were eluted depending on their hydrodynamic volume. The concentrations of fructose and glucose were defined for each compound by a calibration curve established with different concentrations (supplementary information), giving the concentration in $g.L^{-1}$ of the analyzed compound as a function of the area of the chromatographic peaks.

153 Solid state Nuclear Magnetic Resonance

Solid state ¹³C NMR experiments were carried out using a Bruker Avance III 400 MHz 154 spectrometer operating at a ¹³C frequency of 100.62MHz and equipped with a CP/MAS 4 mm 155 1 H/ 13 C probe. Prior to the analysis, the samples were neutralized with a solution of NaOH (0.1 156 157 M) and lyophilized. The pH was measured with a pHmeter (Eutech instrument pH510). The solid samples were packed in a 4 mm NMR rotor without any other preparation. The sample 158 were spun at a rate of 9 kHz at room temperature. The cross polarization pulse sequence 159 parameters were as follow : 3.95 µs for proton 90° pulse, a contact time between 0.8 and 2.0 160 ms and 10 s recycle time. Usually, the accumulation of 5120 scans was used. The carbonyl 161 signal of glycine (176.03 ppm) was used to calibrate the chemical shift of the ¹³C NMR spectra. 162 The chemical shift, peak half-width and peak area of the different peaks were determined with 163 a least squares fitting method using Peakfit® software 164

165 *Mass spectrometry*

166

167 The samples analyzed by ssNMR were also analyzed by matrix-assisted laser 168 desorption/ionization (MALDI)-time-of-flight (TOF) MS. For the measurements, an ionic 169 preparation of 2,5-dihydroxybenzoic acid (DHB) and N,N-dimethylaniline (DMA) was used as 170 the MALDI matrix, as described in (Ropartz, 2011). Briefly, the matrix consists of a mixture of DHB and DMA (DHB 100 mg.ml⁻¹, in H₂O/acetonitrile/ DMA (1:1:0.02)) and was mixed with the samples in a 1:1 ratio (v/v). The mixture (1 μ L) was then deposited on a polished steel MALDI target plate. MALDI measurements were then performed on an Autoflex Speed MALDI-TOF/TOF spectrometer (Bruker Daltonics, Bremen, Germany) equipped with a Smartbeam laser (355 nm, 1000 Hz) and controlled using the Flex Control 3.0 software package. The mass spectrometer was operated with positive polarity in reflectron mode. Spectra were acquired in the range of 180–3500 *m/z*.

178 The evolution of the percentage of DP 1 was then monitored using the following formula:

179
$$\% = \frac{I_{DP1}}{I_{DP1} + I_{DP2} + I_{DP3} + I_{DP4}}$$
 equation 1

180 *FT-IR*

Fourier transform infrared spectroscopy was performed before and after plasma treatment in order to observe the eventual functionalization and/or stabilization of species on the surface of inulin. Prior to analysis, the sample was lyophilized. The powder sample (5 mg) was mixed with KBr (100 mg) and pressed in a hydraulic press and then recovered in the form of a pellet. The analysis was carried out in a Nicolet IS50 spectrometer in transmission mode and the resulting spectra were an average of 200 scans at a resolution of 16 cm⁻¹. All spectra were baseline corrected and normalized to be compared to each other.

- 188 2. Results and discussion
- 189 2.1. Reactor optimization
- 190

In order to identify the reactivity sites (in gas bubbles, in the upper gas phase or at liquid-gas interface) and to evaluate the discharge propagation, the effect of the volume of liquid submitted to plasma was studied, introducing 5 mL, 10 mL and 15 mL of an aqueous solution of inulin at 8 g.L⁻¹ (figure 2). The experiments were carried out at constant voltage (19 kV), frequency (2 kHz) and time (20 min). The efficiency of the plasma discharge was evaluated by its impact on inulin depolymerization via the following of fructose yields, pH, and NO_2^- / NO_3^- and H_2O_2 concentrations.

When 15 mL were introduced, the inulin solution filled the reactor with 5 mL of the volume standing above the external electrode limit. No depolymerization occurred in this configuration. Additionally, no pH change was observed and no other species were detected (Table 1). It appeared that the observed discharge was occurring in the tube holding the central electrode, as a very small air gap was present between the electrode and the inner wall of the dielectric barrier (visible on figure 1). When the inner tube was sealed with glue, no plasma discharge was observed, neither depolymerization.

When 10 mL of solution were inserted in the reactor, it allowed the development of the plasma at the gas-liquid interface but without (or very little) plasma in the gas phase. A concentration of only 0.14 g.L⁻¹ of fructose was detected and, in the same time, pH dropped from 6.5 (untreated solution) to 5.0. Using semi quantitative test strips (Quantofix), a small fraction of NO_x⁻ species were measured indicating the dissolution of nitrous oxides into nitrites (1 mg.mL⁻ 1) and nitrates (≤ 10 mg.mL⁻¹).

Finally, 5 mL of solution introduced resulted in a system with two phases of plasma, at interface and in the gas phase. In this case, inulin was totally depolymerized into fructose (7 g.L⁻¹), glucose (0.25 g.L⁻¹) and a compound of DP 2. It clearly showed that the most effective reaction occurred when the gas-liquid interface and even more importantly, the plasma in gas phase are present. From these results, the configuration using 5 mL of solution was kept throughout the study.

The fact that the depolymerization was enhanced in presence of a gas phase highlighted the fact that the long lived plasma species from the gas phase may be responsible of the reactivity. Their dissolution in the liquid media would lead to active species capable to dissociate the polymer.

In order to verify this hypothesis the reactor was turned upside down, allowing only the contact 220 of the plasma long lived species with the liquid. It resulted in no modification of the inulin 221 chain. The pH of the solution decreased to 3.4, indicating the dissolution of acidic species from 222 223 the gas phase in the liquid, but no depolymerization occurred. It has been established in the literature (Bruggeman et al; 2016; Takai, 2008) that the active species generated in the gas 224 phase are transferred from the gas to the liquid phase, creating more reactive species such as 225 H₂O₂, peroxonitrites (ONOO⁻) and nitric acid (HNO₃). Among the species commonly produced 226 in the liquid phase, H_2O_2 , NO_2^- and NO_3^- were detected in the samples after plasma treatment 227 (Table 1). In the case of air treatment, NO_3^- was detected up to 500 mg.mL⁻¹. 228

Table 1: pH, NO₂⁻ / NO₃⁻ and H₂O₂ concentrations measured after 20 min of air plasma treatment for different liquid volumes. Parameters: P = 28 W, gas flow = 30 mL.min⁻¹, f = 2 kHz; [inulin] = 8 g.L⁻¹

| Volume | 15 ml | 10 ml | 5 ml | Upside down (5 |
|--|-------|-------|------|----------------|
| | | | | mL) |
| pH | 6.5 | 5 | 1.5 | 3.4 |
| H_2O_2 mg.mL ⁻¹ | 0 | 10 | 11.2 | 1.5 |
| NO ₂ ⁻ mg.mL ⁻¹ | 0 | 1 | 15 | 0 |
| NO ₃ ⁻ mg.mL ⁻¹ | 0 | 15 | 500 | 350 |

232

233 2.2. Varying the chemical nature of the plasma gas

234

In order to further identify and evaluate the reactivity of plasma chemical active species in such a system, the depolymerization reaction of inulin was performed using different gases. The results are summarized in table 2. The highest concentration of H_2O_2 was measured under oxygen and O_2 /He plasma. Under nitrogen and air plasma the H_2O_2 concentrations were lower, due to the presence of NO_2^- , which in acidic conditions can lead to the H_2O_2 degradation

- 240 (Gorbanev, O'Connell & Chechik, 2016; Lukes, Dolezalova, Sisrova & Clupek, 2014). The
- 241 lowest concentration was measured under pure helium plasma.

Table 2: fructose concentration, pH and oxidized species concentrations measured after 20 min of plasma treatment for different gas phases. Parameters: gas flow = 30mL.min-1, f = 2 kHz; [inulin] = 8 g.L⁻¹

| | Gas nature | 100 % O ₂ | 100 % He | 50% He / 50% O ₂ | 100 % N ₂ | Synthetic air |
|--|------------------------------------|----------------------|-------------|--------------------------------|------------------------------------|---------------|
| | Power (W) | 12 | 12 | 28 | 14 | 28 |
| | Fructose conc. $(g.L^{-1})$ | 0.3 | 0.02 | 0.3 | 0.9 | 7 |
| | pН | 3-4 | 3-4 | 3-4 | 1.5 | 1.5 |
| | $H_2O_2 (mmol.L^{-1})$ | 15.5 | 3.6 | 13.7 | 10 | 11.2 |
| | NO_{3}^{-} (mg.L ⁻¹) | none | none | none | 250≥ x ≥500 | ≥500 |
| | NO_2^{-} (mg.L ⁻¹) | none | none | none | 5 <x<10< td=""><td>15</td></x<10<> | 15 |

245

The fructose concentration measured after plasma treatment under helium was the lowest (0.02 246 g.L⁻¹). The actives species generated under helium plasma such as He^{*}, He₂^{*}, He⁺ and He₂⁺, but 247 also dissolved oxygenated species generated from electron and helium impact on water 248 249 molecules, are not participating in the reaction in the first 30 min. Oxygen gas appeared also quite inert since the addition of oxygen to helium gas and higher injected power in the reactor 250 did not affect the fructose yield as compared with pure oxygen (0.3 g.L⁻¹). When pure nitrogen 251 was flown through the system, a slight increase of fructose concentration was observed but to 252 a lesser extent compared to air treatment where inulin was totally converted. It is worth 253 mentioning that an air plasma treatment at 12W resulted in no conversion. 254

The HPLC results of the plasma treatment under synthetic air showed the progressive depolymerization of inulin into smaller fractions and its two constituents, glucose and fructose. Air plasma is the most effective treatment for the inulin depolymerization. A treatment time of approximatively 20 min allowed the total conversion of inulin (figure 2). The concentration of fructose was 7 g.L⁻¹ reaching a plateau at 20 and 30 min. Interestingly, the glucose concentration

(figure 2b) increased until 30 min of treatment (0.4 g.L⁻¹). It appears that the reactive species 260 derived from the reactions between nitrogen and oxygen in the air plasma are responsible for 261 the depolymerization. Nitrous oxides solvation in water would lead to nitric and nitrous acids, 262 providing H⁺ ions and consequently acid hydrolysis of the polymer. 263

264



Figure 2: plot of a) fructose and b) glucose yields as a function of treatment time. Conditions: 2 kHz, 30 270 mL.min⁻¹; P = 28 W and P (He) = 12 W; [inulin] = 8 g.L⁻¹ 271

272

A series of tests with nitric acid were performed in order to confirm this hypothesis. When 5ml 273 of inulin solution were mixed with nitric acid (1 mol.L⁻¹) till a pH = 1.5, followed by 80°C 274 heating from 20 to 100 min, a total conversion into fructose (8mg.ml⁻¹) was achieved. The same 275 result was obtained when inulin powder was added to plasma treated water (in air; P = 28 W) 276 and heated up at 80°C. The pH of the water treated with air plasma equaled 1.5. These 277 experiments confirmed the participation of H⁺ in the depolymerization process. 278

279

280

2.3. Elucidating the depolymerization process

¹³C CP/MAS NMR spectroscopy was used for anomer analyses of reducing terminal units of 281 the treated chains in order to follow depolymerization under air plasma treatment, which proved 282 to be the most efficient treatment. A freeze dried reference (no plasma) was prepared and 283 compared to samples treated in air up to 20 min. The structure of inulin (a), along with the β-284

D-fructopyranose form (b) detected in some samples is represented in figure 6. When glucopyranose is present at the reducing end, the fructose molecules are in the furanose form. Without glucose at the end, the fructose molecules are in the pyranose form (Levy & Fügedi, 2005). The labelling on the NMR spectra are also referenced in figure 3.



289

290 Figure 3: Structure of a) inulin and b) β -D-fructopyranose

291

292 After spectral deconvolution, the average degrees of polymerization have been calculated using

the next formula:

For untreated inulin, the lyophilized untreated inulin and inulin treated by plasma for 3 min:

295
$$DP average = \frac{\left[\frac{\sum area from 83 to 57 ppm}{5}\right] - area at 93 ppm}{area at 93 ppm}$$
 Equation 2

The numerator corresponds to the signal of fructose monomers subtracted from glucose contribution (figure 4). The denominator corresponds to the signal of anomeric carbons of ending glucose.

Inulin treated by plasma from 7 to 40 min:

300
$$DP average = \frac{[(\sum area from 83 to 57 ppm)/5] - (area at 93 ppm+area at 98 ppm)}{(area at 93 ppm+area at 98 ppm)}$$
 Equation 3

301 The peak at 93 ppm corresponds to the glucose reducing end in the α conformation and the 302 peak at 98 ppm in the β conformation or C₂ from fructopyranose (Colombo, Aupic, Lewis & 303 Mario Pinto, 2015).

The NMR analysis of the freeze dried reference (figure 4) highlighted the presence of fructopyranose form of inulin and showed that the freeze-drying step was also affecting the supramolecular organization of inulin. The widening of the bands at 103 ppm and in both regions 86-70 ppm and 68-54 ppm reflected a less ordered structure after lyophilization, most probably arising from a loss of the crystallinity. In addition, the chemical shift of C₁ (F1) of fructose, between 80 and 74 ppm, was modified suggesting a significant change of the magnetic environment.





Figure 4: ¹³C CP/MAS inulin spectra, (a) initial state, (b) after solubilization and lyophilization.
(Annotations are referring to figure 3).

314



315

Figure 5: ¹³C CP/MAS spectra zones of non-anomeric carbons (left panel) and anomeric carbon (right panel) of (a) lyophilized inulin (reference) and after plasma treatment of (b) 3min, (c) 7 min, (d) 13 min, (e) 20 min. (Annotations are referring to figure 6)

319

Figure 5 shows the ¹³C CP/MAS spectra of inulin before and after up to 20 min of plasma 320 treatment. As the time of plasma treatment increased, peaks in the 110 - 90 ppm and 90 - 55321 ppm regions evolved differently. The anomeric and non-anomeric carbon peaks of furanose 322 ring, labelled F1 to F6, are decreasing as the treatment time increases. At 7 min of treatment, 323 324 an anomeric carbon (FP2) presenting a chemical shift at 98.4 ppm is emerging, identified as C₂ carbons of free fructose in the β -D-fructopyranose form (Colombo, Aupic, Lewis & Mario 325 Pinto, 2015; Shiomi & Onodera, 1990). The proportion of this signal increases with treatment 326 time. The presence of free β -D-fructopyranose is confirmed by peaks in the non-anomeric 327 carbon region at 70 ppm and 84 ppm, which increases as a function of treatment time. It is 328 worth mentioning that the pyranose form of fructose was predominant during the solubilization 329 step of monomeric fructose. This result indicates that the conversion from the furanose to 330

331 pyranose form is most likely to occur during the neutralization step and is not due to the plasma

332 treatment.

From equations (2) and (3), average values of the degrees of polymerization were calculated (table 3). It shows that after 3 min, a clear depolymerization occurs as already shown by the HPLC. A progressive decrease of the DP was observed as a function of the plasma treatment time up to monomers, without further degradation.

Table 3: Calculated average degree of polymerization (DP) of air plasma treated inulin: P = 28 W; f = 2kHz; flow rate: 30 mL.min⁻¹

| | DP |
|--------------------|----|
| Commercial inulin | 30 |
| Lyophilized inulin | 29 |
| 3min | 27 |
| 7min | 13 |
| 13min | 3 |
| 20min | 1 |

339

To investigate the molecular polydispersity of the released products and more specifically the proportion of DP 1 throughout the treatment of inulin by the plasma, MALDI-TOF MS analyses were performed (figure 6). The evolution of the proportion of DP 1 compared to DP 2, 3 and 4 was monitored by mass spectrometry, using the equation 1. These experiments showed that above 20 min, it remains almost only DP 1 species (glucose and/or fructose). This result is according to NMR measurements.



346

Figure 6: Evolution of the signal of DP 1, 3 and 4 by mass spectrometry as a function of plasma treatment
time. Oligosaccharides are detected as sodium adducts. The percentage of DP 1 was determined using
equation 1. The DP2 is not represented for esthetic reason, due to the presence of numerous matrix peaks
in the same region.

351

While fructose and glucose both crystallize as cyclic forms, in solution the free sugars show an 352 equilibrium with an acyclic form in small amount, the formation of which creates a carbonyl 353 354 group (Levy & Fügedi, 2005). This carbonyl group can react with hydroxyl groups restructuring the hemiacetal cyclic form. For six carbon carbohydrates, like glucose and fructose, the ring 355 closing reaction can occur with more than one hydroxyl group, leading to isomerization and 356 multiple cyclic forms. Inulin is relatively chemically inert, although cleavage of the polymer 357 chain at any of the glycosidic bonds will produce a reactive reducing end, prone to further 358 359 reaction (Stevens, Meriggi & Booten, 2001; Wack & Blaschek, 2006).

360 2.4. Infrared bands attribution

361

Infrared analysis was used routinely for the assessment of chemical structure of treated inulin. 362 The full IR description of raw inulin has been described elsewhere (Grube, Bekers, Upite & 363 Kaminska, 2002; Ibrahim, Alaam, El-Haes, Jalbout & de Leon, 2006; Wack & Blaschek, 2006), 364 and can be used as reference. In the first region, between 4000-2000 cm⁻¹, the large broad band 365 at 3300 cm⁻¹, corresponding to the stretching of OH groups, did not change during the plasma 366 treatment (Figure 7). A sharp band of middle intensity was noticeable at 2930 cm⁻¹, assigned to 367 the valence vibration of C-H asymmetric stretching of CH₂ and a shoulder at 2890 cm⁻¹ 368 attributed to C-H symmetric stretching of CH₂. A deviation of the baseline with treatment time, 369 giving rise to a broad absorption between 2400 - 3000 cm⁻¹ was attributed to the formation of 370 carboxylic acids, under their dimeric form (Bellamy, 1962). 371



372

Figure 7: Infrared spectra of inulin as a function of plasma treatment time In the following region, between 2000 and 1100 cm⁻¹, multiple modifications were observed. Plasma treated samples showed the formation of a new band at 1740 cm⁻¹, corresponding to stretching vibrations of C=O group. The band at 1640 cm⁻¹, based on the existing literature

(Higgins, Stewart& Harrington, 1961), was assigned to adsorbed water. The light shift of the 377 band to 1660 cm⁻¹ was attributed to a change of the electronic environment due to intermediate 378 states generated during the oxidation steps, i.e. C=O formation. The bands at 1430 and 1334 379 cm⁻¹ of the raw inulin are no longer visible after plasma treatment, hidden by the intensive broad 380 band at 1385 cm⁻¹, corresponding to adsorbed nitrate ion (Elmelouky, Mortadi, Chahid & 381 Elmoznine, 2018). From T10 to T30, the presence of two bands at 1280 cm⁻¹ and at 1205 cm⁻¹ 382 383 indicate a structure modification from the polymer to the monomer as these bands are related to OCH and CCH bending vibrations bands of fructose and glucose. A complete disappearance 384 of the 1130 cm⁻¹ band indicates the loss of C-O-C bridge character. 385

In the third region 1100-500 cm⁻¹, also known as the fingerprint region, modifications are also 386 visible. Firstly, the band at 1030 cm⁻¹ is reduced and hidden by a band at 1080 cm⁻¹ (D-fructose). 387 All the bands (1035, 990, 935, 872 and 820 cm⁻¹) related to the fructose/glucose ring structure 388 (C-O-C ring group and ring vibrations) shift slightly towards lower values but are still present, 389 indicating that the integrality of the monomer ring was not affected during the 390 depolymerization. Finally, a new band is formed at 778 cm⁻¹ corresponding to CCO and CCH 391 bending of D-fructose and D-glucose. From these data, inulin is depolymerized without 392 degradation of the fructose and glucose rings. First, an oxidation of C-OH groups into 393 394 carboxylic acids takes place. As the plasma treatment time increases, breakage of the C-O-C bridge of the polymer is observed. 395

It has been established in the literature (Bruggeman & Leys, 2009; Gorbanev, O'Connell & Chechik, 2016; Nastase, Tatibouët & Fourré, 2018; Shainsky, 2012), that various types of plasma-chemical species and reactions are initiated in air plasma in and in contact with liquids. Among the chemical species produced by plasma at the gas-liquid interface, OH• radical, atomic oxygen, ozone and hydrogen peroxide are the main reactive oxygen species generally accepted (Lukes, Dolezalova, Sisrova & Clupek, 2014; Sunka, 1999) to play the dominant role

in the reactivity. As for the nitrogen based species, nitric oxide and its derivatives formed with 402 403 water (nitrites, nitrates and peroxynitrites) are to be considered. For example, the dissolution of the nitrous oxide gas generated in air plasma (Machala, 2013) leads to the formation of nitric 404 405 and nitrous acids. The most likely reaction pathway for the depolymerization would be via the hydrolysis of the C-O-C bridge from H⁺ ions of HNO₃ arising from the NO_x dissolution in the 406 liquid. The slight depolymerization observed in nitrogen free plasmas could be attributed to OH 407 radicals attack of the glycosidic bond. However, this does not explain the decrease of pH after 408 a plasma treatment without nitrogen. Hydrogen peroxide concentration is too low to induce 409 such pH decrease. Literature reports the formation of acids (Machala, 2013; Shainsky, 2012), 410 arising from superoxide ion, O_2^{-} , that participate in the decrease of pH under nitrogen free 411 plasma discharge and could explain our observation. 412

413 <u>Conclusions</u>

414 The use of renewable polysaccharide feedstocks to produce chemicals is stimulating a revival 415 in carbohydrate chemistry employing green and sustainable processes. In this study, a new 416 reactor has been successfully designed for the treatment of solutions or suspensions in a double dielectric barrier discharge plasma reactor. This specific reactor configuration was used in the 417 depolymerization reaction of inulin. The conversion was strongly dependent on the gas 418 419 chemical nature and reactor configuration. Pure gases of helium, nitrogen and oxygen had little effect on the depolymerization. However, plasma treatment under air led to a complete 420 depolymerization into fructose, glucose and a DP2 compound. It appears that reactivity is at 421 play at the gas-liquid interface, where electrons and gas species can be solvated and either attack 422 glycosidic bonds of inulin or recombine into more reactive species. Hydrogen peroxide, nitrous 423 424 and nitric oxides were identified. It appeared that the breakage of the glycosidic bond is achieved by nitric acid hydrolysis under an air plasma discharge, while OH radicals attack 425 seems to be responsible of the small depolymerization under nitrogen free plasma. There is no 426

| 427 | doubt with these results that the modification of biomass by non-thermal plasma in liquid media |
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| 428 | represents a new and non-toxic approach that would reconsider the traditional ways. |

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