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# Ozonized 2-hydroxypropyl- $\beta$ -cyclodextrins as novel materials with oxidative and bactericidal properties

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## Abstract

Ozonized (2-Hydroxypropyl)- $\beta$ -cyclodextrins (Oz-HPbCDs) were produced by direct gas/solid reaction between gaseous ozone (O<sub>3</sub>) and solid HPbCD. The solid materials obtained were first characterized using physical and chemical methods and compared to the initial HPbCD. The main process parameters of the synthesis were studied independently to assess their effect on the oxidizing power of Oz-HPbCDs. The ability of the Oz-HPbCDs to retain their oxidative properties over time was evaluated, at different storage temperatures, for a period of at least two months. Lastly, aqueous solutions of HPbCD and Oz-HPbCD at different concentrations were contacted with bacterial strains of *Escherichia coli* and *Streptococcus uberis* to see whether these materials might have bactericidal properties. Since normal bacterial growth was noted with HPbCD, the antimicrobial efficiency of Oz-HPbCDs was clearly demonstrated on these two types of bacteria.

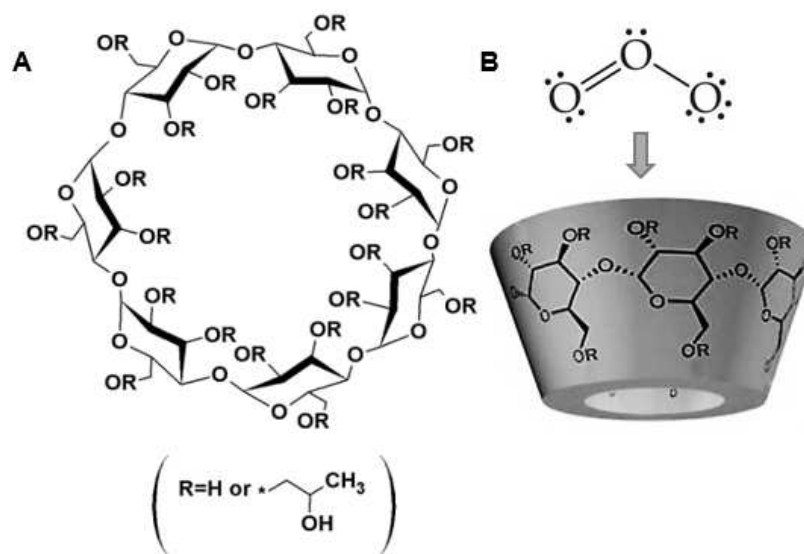
**Keywords:** Cyclodextrin; ozone; oxidant; material; antimicrobial activity; bactericide.

## 1. Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides composed of bridged glucopyranose subunits, shaped like a truncated cone or a lampshade (see **Figures 1A** and **1B**) and forming a cavity at the center (Morin-Crini et al., 2021; Szejtli, 1998; Szejtli & Osa, 1996). CDs are used for their versatile inclusion properties in the food, pharmaceutical and biological industries among others (Harada, Takashima, & Yamaguchi, 2009; Li et al., 2014; McCray, Boving, & Brusseau, 2000). Despite the fact that these materials have been used and studied for decades (Szejtli, 1997; Szente, Szemán, & Sohajda, 2016), understanding the mechanisms at play is still a scientific challenge in many respects.

There are different types of CDs, the most common being composed of 6, 7 and 8 glucopyranose units, named  $\alpha$ -CD,  $\beta$ -CD and  $\gamma$ -CD, respectively (Ibrahim & El-Zairy, 2009; Szejtli, 1998). These native CDs are obtained from the enzymatic degradation of starch under the action of the enzyme glucosyl-transferase (also called CGT-ase), meaning they have the same advantageous qualities as bio-based products. The hydrophobic nature of the cavity is due to the non-polar carbon skeleton. Conversely, the hydroxyl groups are what give the CD its hydrophilic exterior, meaning that these compounds are particularly soluble in aqueous media (Amiri & Amiri, 2017; Crini, 2014). The water solubility of  $\beta$ -CD can be greatly improved by modifying certain hydroxyl groups to break this hydrogen bond network. For example, the modified CD (2-hydroxypropyl)- $\beta$ -cyclodextrin (HP- $\beta$ -CD, written HPbCD for convenience in the following) is obtained industrially by treating the  $\beta$ -CD with propylene oxide in an alkaline medium (Nasongkla et al., 2003). This process makes it possible to substitute part of the hydroxyl functions for hydroxypropyl (HP) ones on the CD faces, as shown in **Figure 1**. HPbCDs can be characterized by their degree of substitution (*DS*), corresponding to the average number of HP functions present on the CD (the *DS* of HPbCDs usually ranges from 2.8 to 10.5), or their degree of molar substitution (*MS*), which gives the number of substituents per glucose unit (ranging from 0.4 to 1.5 for HPbCDs). HPbCDs have a high solubility in water ( $> 600 \text{ g.L}^{-1}$ ) (Loftsson & Duchêne, 2007), which is much greater than that of native  $\beta$ -CD ( $18.5 \text{ g.L}^{-1}$ ). As a high product solubility in water is advantageous for the biological applications targeted in this study, we therefore selected HPbCD as a relevant raw material for our experiments. Further information on the history, synthesis, properties and detailed characterizations of HPbCDs can be found in Malanga et al. (Malanga et al., 2016).

65  
66



67  
68

69 **Figure 1.** Example of the A) Molecular structure of (2-hydroxypropyl)-β-cyclodextrin, B) schematic view of the  
70 truncated cone-shape of the CD in contact with the ozone molecule.

71 The resistance of bacterial pathogens is increasing dramatically, resulting in multidrug  
72 resistance (MDR) to antibiotics. MDR bacteria are giving rise to serious health issues  
73 identified by several organizations such as the World Health Organization (WHO) and the  
74 European Centre for Disease Prevention and Control (ECDC). Studies are conducted by the  
75 European Food Safety Authority (EFSA) on the growing hazards of MDR bacteria in the food  
76 industry (Roca et al., 2015). The unreasonable use of antimicrobial agents has likewise caused  
77 this problem to spread to the natural environment (Wellington et al., 2013). This buildup  
78 should not be underestimated. The medical, food and agricultural sectors are faced with  
79 microbial issues, which can be dealt with by using chemicals such as pesticides. The French  
80 and European public authorities have introduced many regulations to control their use, but it  
81 appears necessary to propose more healthy and environmentally friendly alternatives (e.g.  
82 safer chemicals or less hazardous material) to better control microbial development.

83

84 Because of its oxidizing power and high effectiveness in killing bacteria, fungi, molds, and  
85 viruses (Cristiano, 2020), ozone is currently used for many applications such as wastewater  
86 treatment (Vittenet et al., 2015), air depollution (Vitola Pasetto et al., 2020) and is regarded as  
87 a very promising option for disinfection and sanitation measures (Tizaoui, 2020). Ozone is

88 also being looked at for potential applications such as crop protection, including treating  
89 plants and protecting fruit and vegetables like green peppers, raspberries and melons (Özen,  
90 Koyuncu, & Erbaş, 2021; Piechowiak, Grzelak-Błaszczak, Sójka, & Balawejder, 2020; Zhang  
91 et al., 2021). In practice however, spraying ozone-enriched water on outdoor crops is a very  
92 difficult task due to the gas desorption phenomenon that occurs when the ozone-enriched  
93 water droplets come in contact with the air. The efficiency of this treatment is limited because  
94 ozone is so easily desorped from the water droplets (Canado et al., 2020). Moreover, ozone is  
95 well-known for its high instability due to its specificity of decomposing into oxygen very  
96 quickly (Muromachi, Ohmura, & Mori, 2012). Its half-life is about 20 minutes at ambient  
97 temperature when it is dissolved in water. Although this property is interesting as it confers to  
98 ozone a very low remanence (Pagès, Kleiber, Pierron, & Violleau, 2016), the gas' instability  
99 is a severe disadvantage as it makes storage and transportation difficult. Ozone must be  
100 produced continuously, as close as possible to the place where it is used. Ozone gas is  
101 generally produced by a high-voltage electrical discharge (3 - 20 kV) through an oxygen flow  
102 ( $O_2$ ) or air in a continuous gas flow. Concentrations of up to 13 wt% of  $O_3$  in the gas phase  
103 can be obtained by means of ozone generators designed specifically for medium industrial  
104 applications. Despite the fact that this type of ozone production system is very popular for  
105 stationary applications — such as water treatment — these electrical devices are relatively  
106 difficult to use in mobile applications, such as the in-field treatment of leaves, vegetables and  
107 fruit.

108  
109 The contacting of ozone with CDs is scarcely documented in the literature. Just a few authors  
110 have proposed different concepts and protocols with CDs, the aim being to try and stabilize  
111 the ozone molecule — due to its very short half-life — in the CD cavity. Wang et al. (Wang,  
112 Peng, Li, Bai, & Huang, 2016) studied the complexation of HPbCD with  $O_3$  in solution and  
113 observed its oxidative properties on potassium indigotrisulfonate (indigo) and 2,2'-azino-bis  
114 (3-ethylbenzothiazoline-6-sulfonate) (ABTS). They showed that only the solution containing  
115 HPbCD/ $O_3$  decreased the indigo absorbance after 72 hrs. at 600 nm, and that the  
116 decomposition of ABTS increased by increasing the doses of ozone/CD solution. Dettmer et  
117 al. (Dettmer et al., 2017) concluded that  $O_3$  can be stabilized with cyclodextrin in an aqueous  
118 solution and they showed that the half-life of  $O_3$  increases proportionally with the HPbCD/ $O_3$   
119 molar ratio. The formation of an inclusion complex of  $O_3$ , trichloroethene (TCE), 1,1,1-  
120 trichloroethane (TCA) and 1,4-dioxane (1,4-D) with HPbCD was investigated by Khan et al.  
121 (Khan, Johnson, & Carroll, 2018) to remove the guest contaminants (TCE, TCA and 1,4-D) or

122 apply reagents during water treatment. Recently, Fan et al. (Fan et al., 2021) used nanobubble  
123 technology in an HPbCD inclusion to remove organic micropollutants from contaminated  
124 water. Nanobubbles increased the solubilization of O<sub>3</sub> and, according to the authors, formed  
125 an inclusion complex with HPbCD unlike macrobubbles. Using this technique, the removal  
126 proficiency of the main micropollutant 4-chlorophenol was found to be 6.9 higher compared  
127 to the standard macrobubble ozonation method. In all these studies, the CD-ozone contacting  
128 was always done in an aqueous solution with a prior step of CD solubilization in the water.  
129 Interestingly enough, to the best of the authors' knowledge, it is the first time that synthesis  
130 and characterization experiments with gaseous ozone contacting solid HPbCDs followed by  
131 tests of the ozonized CDs for biological applications, are presented.

132

133 The main target of this paper is therefore to report results on the synthesis and  
134 characterization of novel materials obtained by contacting gaseous ozone and solid  
135 cyclodextrins, and to evaluate their antimicrobial activity for potential biological applications.  
136 The key hypothesis suggests that CDs are oxidative materials, that physical and chemical  
137 changes are visible compared to native CDs, and that they have bactericidal properties. The  
138 results of physical and chemical characterizations of the HPbCD and ozonized HPbCD  
139 (denoted Oz-HPbCD) are discussed first. Then, the effect of the main process parameters on  
140 the oxidative power of the Oz-HPbCD obtained by direct gas/solid reaction are presented. The  
141 stability of the oxidative properties of the ozonized CDs stored at different temperatures for  
142 several months is also evaluated. Finally, to illustrate potential applications of these types of  
143 oxidative materials in microbiological treatments, the antimicrobial efficiency of ozonized  
144 CDs is studied.

145

146

## 147 **2. Materials and methods**

148

### 149 **2.1. Materials**

150

151 (2-Hydroxypropyl)- $\beta$ -cyclodextrin (HP $\beta$ CD) (purity  $\geq$  94%,  $MS = 0.9$ , produced by Wacker  
152 Chemie AG, Burghausen, Germany) was purchased from Sigma-Aldrich, and used without  
153 further purification. The chemicals used for the iodometric method were potassium iodide  
154 (KI) (reagent grade), sulfuric acid at 1 mol.L<sup>-1</sup> (purity  $\geq$  99.9%) purchased from Fisher  
155 Scientific, and a sodium thiosulfate solution at 1 mol.L<sup>-1</sup> provided by Merck. The potassium  
156 bromide (KBr) for IR spectroscopy Uvasol® (purity  $\geq$  99%) was purchased from Merck  
157 KBaA, Darmstadt, Germany. Ultra-pure water was used in all preparations with an ELGA  
158 Purelab Option-Q 7 model from VWS (for chemical characterizations and oxidative power  
159 determination) and with a Millipore Milli-Q Integral 15 water purification system from Merck  
160 (for microbiological tests). Oxygen (purity  $>$  99.999%, purchased from Air Liquide, France)  
161 was used to produce ozone in the experimental rig.

162

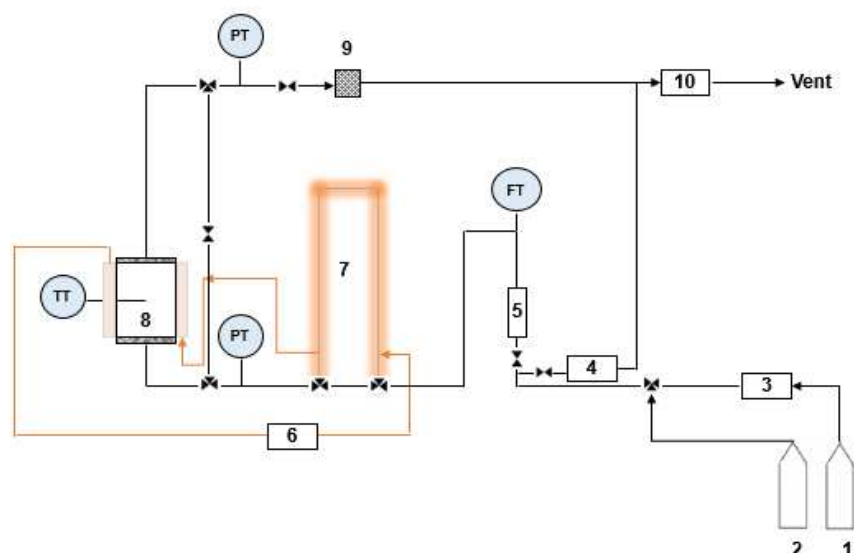
### 163 **2.2. Methods**

164

#### 165 **2.2.1. Synthesis protocol**

166

167 The ozonized CDs are synthesized using an experimental rig at bench scale. A diagram of  
168 the different elements of the experimental rig used in this study is shown in **Figure 2**, and  
169 detailed technical information is given in **Appendix A (section A1)**.



170

171 **Figure 2.** Process and instrumentation diagram of the experimental rig used for the synthesis: 1. oxygen bottle; 2.  
 172 nitrogen bottle; 3. ozonator; 4. ozone BMT analyzer; 5. float flowmeter; 6. thermostatic bath; 7. heat exchanger;  
 173 8. reactor; 9. filter; 10. ozone destructor; FT. mass flowmeter, TT. temperature probe; PT. pressure sensor

174

175 The reactor is first loaded with the HPbCD, directly on a balance with a precision of  $\pm 0,005$   
 176 g. A mass of  $\sim 5$  g is used for the process parameter study and  $\sim 7$  g are used for all the  
 177 characterization studies. The reactor is then closed, the temperature probe connected to the  
 178 supervision system, the reactor mounted on the rig, and the coolant hoses connected to the  
 179 reactor jacket. Before starting the synthesis, a 5-minute leak test is always performed with  
 180 nitrogen at 2 bar to ensure perfect sealing of all the pieces composing the rig. All the process  
 181 lines are flushed with oxygen and the reactor, regulated at temperature ( $T_r$ ), is first bypassed  
 182 to start the ozone production. The pressure drop is adjusted in the by-pass line to equalize the  
 183 pressure drop in the reactor to avoid any change in the gas flow when this line is closed. The  
 184 gas flow ( $Q_f$ ) and ozone concentration ( $C_{O_3}$ ) are then set to the correct values and stabilized  
 185 using the reactor bypass line before the start of the synthesis. When all the process parameters  
 186 are perfectly stable, the  $O_2/O_3$  gas coming from the ozonator is sent to the reactor by turning  
 187 the valves to shut the bypass line. The time  $t = 0$  of the synthesis is defined at this moment.  
 188 Contact between the ozone and the CD powder is then maintained for a given reaction time  
 189 (noted  $t_r$ ). At the end of the synthesis, the ozone concentration is gradually decreased to zero,  
 190 and the ozonator is then switched off. After this, the reactor is vented with nitrogen,  
 191 disassembled from the rig and transferred to a glove box (under air) to be opened safely. The  
 192 ozonized CDs are finally discharged from the reactor into a capped glass vessel using a  
 193 funnel. The product is immediately sampled for analysis and stored.



### 194 2.2.2. Oxidative power (*OP*)

195

196 The total oxidation capacity of the solid materials obtained from the synthesis is determined  
197 by iodometric titration carried out according to the following procedure: 20 mL of an aqueous  
198 solution of potassium iodide (KI) at 0.1 mol.L<sup>-1</sup> is stirred in a 125-mL Erlenmeyer flask; then,  
199 the pH of the solution is adjusted to ~ 2 with few drops of sulfuric acid (1 mol.L<sup>-1</sup>); finally, a  
200 precise amount of ozonized CD ( $m_{oz-CD} \sim 0.05-0.1$  g) measured at  $\pm 10^{-4}$  g, is added to the  
201 acidified KI solution. The reactants are kept in contact under stirring for 60 minutes before  
202 titration.

203 The oxidative power of the material, noted *OP*, is therefore directly proportional to the  
204 quantity of iodine generated. The *OP*, expressed here as the ratio of the mass of iodine  
205 produced to the mass of *Oz-HPbCD*, is calculated by **Eq. (1)** as follows:

$$206 \quad OP\left[\frac{mg_{I_2}}{g_{Oz-HPbCD}}\right] = \frac{C_{thio} \cdot V_{thio} \cdot M_{I_2} \cdot 10^3}{2 m_{Oz-HPbCD}} \quad (1)$$

207 with  $M_{I_2}$  the molar mass of diiodine (253.81 g.mol<sup>-1</sup>),  $C_{thio}$  the concentration of the thiosulfate  
208 solution,  $V_{thio}$  the volume of the thiosulfate solution poured at equivalence, and  $m_{Oz-HPbCD}$  the  
209 mass of ozonized CDs.

210 The decrease in the oxidative properties of the material during the storage time ( $t_s$ ) is  
211 quantified by the rate at which oxidative power is lost (noted  $OP_{loss}$ ). This indicator quantifies  
212 the stability of the *OP* versus time: the lower  $OP_{loss}$ , the more stable the oxidative properties  
213 of the product over time.  $OP_{loss}$  (in %) is defined as the difference in *OP* between the initial  
214 *OP* (measured at  $t_s = 0$ ) and the *OP* measured at the storage time  $t_s$ , normalized by the initial  
215 *OP* obtained at  $t_s = 0$ . The  $OP_{loss}$  is expressed by **Eq. (2)** as follows:

$$216 \quad OP_{loss} [\%] = \left(1 - \frac{OP_{t_s}}{OP_{t_s=0}}\right) \times 100$$

217 (2)

218 With  $OP_{t_s=0}$ , *OP* at  $t_s = 0$  and  $OP_{t_s}$  the *OP* at  $t_s$ .

219 To ensure a robust determination of the *OP*, the iodometric titration is triplicated for each  
220 sample, and the given *OP* is the arithmetic mean of these 3 values.

221

### 222 2.2.3. Physico-chemical characterizations

223

224 The morphology of the particle before and after the synthesis is observed by scanning electron  
225 microscopy (SEM), and the size distribution of the particles by granulometric analysis based  
226 on light diffraction. Helium pycnometry is used to measure the real density ( $\rho$ ) of the  
227 particles, and the specific area ( $a_{s,BET}$ ) of the samples is obtained from N<sub>2</sub> adsorption-  
228 desorption isotherms at 77 K. The CDs before and after synthesis are characterized using FT-  
229 IR and Raman vibrational spectroscopies. Details of the techniques and methods are given in  
230 **Appendix A (section A2).**

231

#### 232 **2.2.4. Antimicrobial activity assessment**

233

234 **Preculture of bacteria.** The different bacterial strains (*Escherichia coli* L.1112 and  
235 *Streptococcus uberis* L.1111) are stored in glycerol milk at  $-80^{\circ}\text{C}$ . After thawing, 50  $\mu\text{L}$  are  
236 cultured in 5 mL of brain-heart broth (BHB) (Biokar diagnostics BK015HA). These  
237 suspensions are incubated at  $37^{\circ}\text{C}$  under agitation ( $\approx 150$  rpm) until bacterial growth is visible  
238 (cloudy culture medium). On the day of the experiment, 100  $\mu\text{L}$  of the bacterial preculture are  
239 placed in 10 mL of BHB and incubated at  $37^{\circ}\text{C}$  under agitation at 150 rpm (in triplicate for  
240 each strain). A tube of pure broth serves as a blank. The optical density at 600 nm (UV  
241 Jenway ThermoFisher Scientific) is then measured for each suspension. The values obtained  
242 are used to dilute bacterial suspensions in order to obtain bacterial inocula standardized at  
243  $5.10^5$  CFU/mL.

244

245 **Minimum Inhibitory Concentrations (MIC)** are used to assess the antibacterial activity of the  
246 ozonized powder and are determined by means of the microdilution method principle (CLSI,  
247 2012). Solutions of Oz-HPbCD and HPbCD at seven different concentrations (between 30  
248 and  $0.003\text{ g}\cdot\text{L}^{-1}$ ) are prepared in brain-heart broth. The solutions are distributed over 48 wells  
249 on 96-well microplates, as shown in **Appendix A (section A3, Figure A1)**. The highest  
250 concentration of the powder (50  $\mu\text{L}$ /well) is deposited in three wells in the first row (line 1).  
251 Following the same configuration, decreasing concentrations are placed in wells from rows 2  
252 to 7. 50  $\mu\text{L}$  of bacterial suspension are added to the first two wells for each different dilution  
253 (total volume 100  $\mu\text{L}$  in each well) whereas 50  $\mu\text{L}$  of BHB are added to the last well of each  
254 row. The last row (no. 8) holds 50  $\mu\text{L}$  of the bacterial suspension and 50  $\mu\text{L}$  of the BHB  
255 (positive control). The final concentration of the bacterial suspensions is therefore  $2.5.10^5$   
256 CFU/mL and those of the ozonized and non-ozonized HPbCD are between 15 and  $0.0015\text{ g}\cdot\text{L}^{-1}$

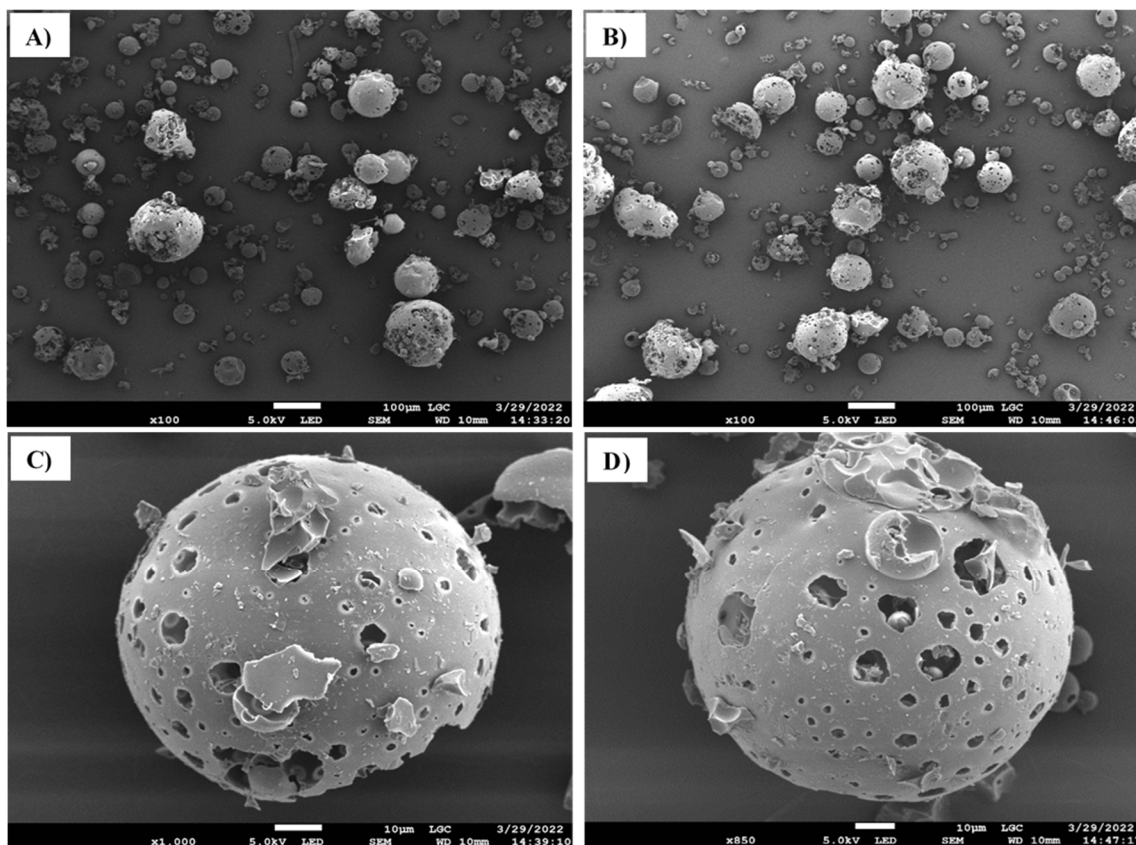
257 <sup>1</sup>. The microplates are placed in an incubator shaker (TECAN infinite M200 PRO) at 37°C  
258 and 125 rpm for 24 hrs. After this period, the measured absorbance is 600 nm in each well.  
259 This experiment is triplicated. Using this method, it is possible to simultaneously test the  
260 effects of HPbCD and Oz-HPbCD on bacteria. Antimicrobial activity is assessed on two  
261 bacterial strains: *Escherichia coli* and *Streptococcus uberis*. MICs are determined as the  
262 minimum concentrations of HPbCD and/or Oz-HPbCD stopping the proliferation of bacteria.  
263

## 264 **3. Results and Discussion**

265

### 266 **3.1 Physical and chemical characterization**

267 In order to compare the particles before and after ozonation (i.e. HPbCD and Oz-HPbCD,  
268 respectively), the results of the physical characterizations of the powders are presented first.  
269 Concerning the morphology of the powders, the SEM images of both samples presented in  
270 **Figures 3A** and **3B**, show spheroidal faceted particles of various sizes, presenting (in most  
271 cases) a smooth surface perforated by numerous holes, with the smallest particles visible  
272 inside the largest spheres. No salient differences can therefore be noted between the particles  
273 before (**Figure 3C**) and after ozonation (**Figure 3D**).



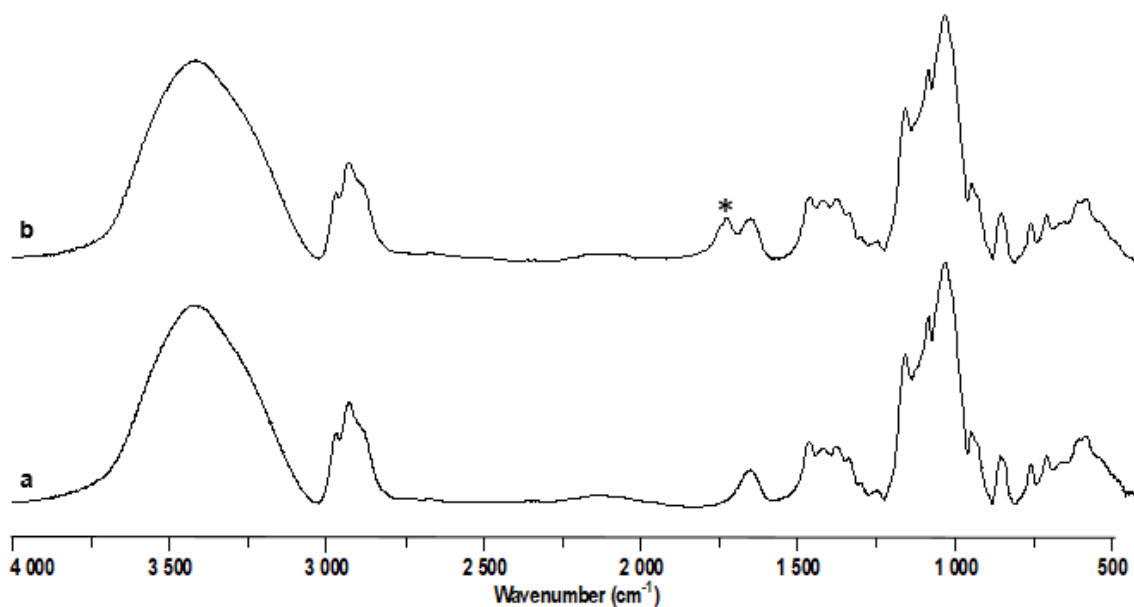
274  
 275 **Figure 3.** SEM images of the materials. A) and C) : HPbCD; B) and D): Oz-HPbCD; the scale (horizontal white  
 276 bar at the bottom of the picture) is 100  $\mu\text{m}$  for A) and B), and 10  $\mu\text{m}$  for C) and D).

277 **Table 1.** Densities, specific surface areas, and median particle diameters of *HPbCD* and *Oz-HPbCD*

Physical characterization	$\rho$ ( $\text{g}\cdot\text{cm}^{-3}$ )	$a_{s,BET}$ ( $\text{m}^2\cdot\text{g}^{-1}$ )	$Dv(50)$ ( $\mu\text{m}$ )
<b>HPbCD</b>	$1.3199 \pm 0.0071$	$0.39 \pm 0.01$	$42.3 \pm 0.2$
<b>Oz-HPbCD</b>	$1.3062 \pm 0.0073$	$0.43 \pm 0.01$	$42.3 \pm 0.2$

278  
 279 The real particle densities ( $\rho$ ), specific surface areas ( $a_{s,BET}$ ) and median particle diameters  
 280 ( $D_{v,50}$ , i.e. the point in size distribution below which 50% of the material is contained) of the  
 281 HPbCD and Oz-HPbCD, are presented in **Table 1**. Given the very small differences between  
 282 the values and considering their absolute uncertainties, it can be concluded that ozonation has  
 283 no effect on the physical parameters in these conditions. No variation in the specific surface  
 284 area proves that the contact of HPbCD with ozone creates no meso/microporosity inside the  
 285 particles. In addition, the fact that the  $D_{v,50}$  was exactly the same between the initial and final  
 286 products demonstrates that the phenomena of fragmentation, attrition and dislocation of the  
 287 initial particles during the synthesis are negligible in our case.

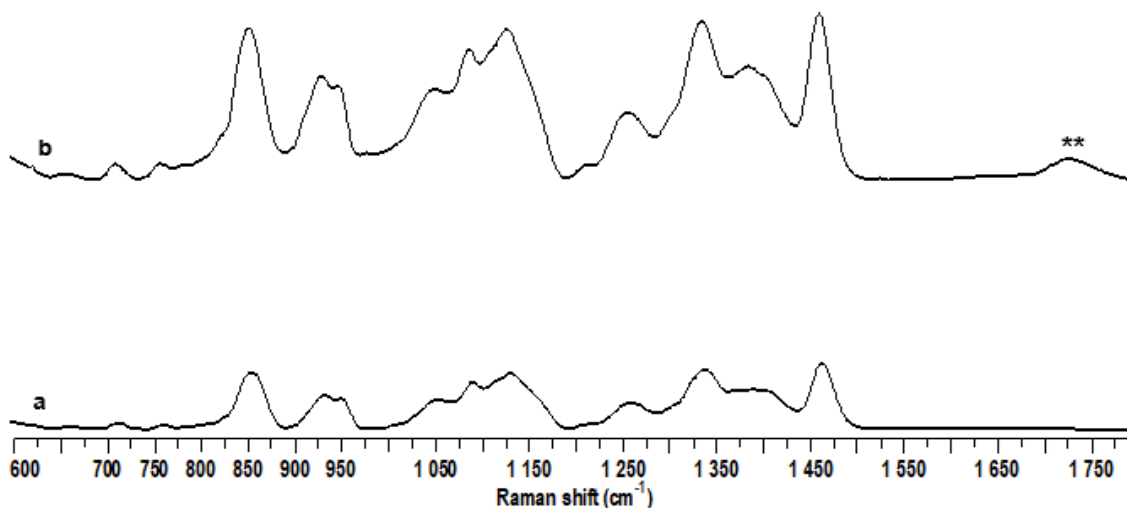
288 To characterize the possible chemical transformation after contact of the CD with O<sub>3</sub>, HPbCD  
289 and Oz-HPbCD were analyzed using FT-IR and Raman spectroscopy. Note that these two  
290 techniques are complementary, particularly when the molecules are not symmetrical (Farber  
291 et al., 2019). FT-IR and Raman spectra are shown in **Figure 4** and **Figure 5**, respectively.



292

293 **Figure 4.** FTIR spectra of a) HPbCD, b) Oz-HPbCD . (\*) new peak identified on the spectrum.

294



295

296 **Figure 5.** Raman spectra of a) HPbCD, b) Oz-HPbCD. (\*\*) new peak identified on the spectrum.

297

298 As shown in **Figure 4**, both the HPbCD and Oz-HPbCD spectra reveal strong bands at 3,300  
299 cm<sup>-1</sup> (O—H stretching vibrations), 2,930 cm<sup>-1</sup> (C—H stretching vibrations), 1,160 cm<sup>-1</sup>, 1,090

300  $\text{cm}^{-1}$  and  $1,031 \text{ cm}^{-1}$  corresponding to C—H, C—O stretching vibrations (Stancanelli et al.,  
301 2008), and  $1,650 \text{ cm}^{-1}$  corresponding to O—H bending vibrations of the water molecules  
302 physisorbed or stabilized in the CDs (Yuan, Liu, & Liu, 2015). A new peak is clearly  
303 noticeable at  $1,730 \text{ cm}^{-1}$  in the spectrum of Oz-HPbCD (identified by the \* in **Figure 4**),  
304 which could be attributed to the —C=O stretching of carbonyl groups.

305 The Raman spectra of HPbCD and Oz-HPbCD shown in **Figure 5** exhibit strong peaks at  
306  $1,460 \text{ cm}^{-1}$  and  $1,330 \text{ cm}^{-1}$  corresponding to C—H bending and  $1,135 \text{ cm}^{-1}$ ,  $1,083 \text{ cm}^{-1}$  and  
307  $1,048 \text{ cm}^{-1}$  which can be correlated with C—O stretching (Egyed, 1990; Martins et al., 2017).  
308 Again, a clear difference exists between the two spectra with the presence of a new peak at  
309  $1,728 \text{ cm}^{-1}$  in the Oz-HPbCD spectrum (identified by the \*\* in **Figure 5**). This contribution  
310 may be attributed to the creation of novel carbonyl groups by ozonation of the native CD, in  
311 agreement with the FT-IR results.

312

313 It can therefore be inferred from these characterization results that, in these conditions, the  
314 ozonation of HPbCD did not induce noticeable changes in the physical parameters considered  
315 for this study, i.e. particle morphology, density, specific surface area and median particle  
316 diameter. However, as new vibrational contributions were detected both by FT-IR and Raman  
317 spectroscopy on the spectra of the ozonized CD, this means that chemical changes did occur  
318 in the product during ozonation. And it seems logical that, in the presence of ozone, some of  
319 the primary and secondary alcohols initially present in the HPbCD could be oxidized to form  
320 different types of new organic functions such as aldehydes, ketones, carboxylic acids,  
321 peracids and so on. These functions could either be linked (i.e. chemically attached) to the  
322 CDs, or found in organic by-products if some parts of the CDs were broken by ozonation.  
323 However, we are aware that a detailed quantitative analysis will be necessary, using NMR for  
324 example, to identify and precisely determine the possible chemical modifications of the CDs  
325 and any by-product(s) that may form in these conditions.

326

### 327 **3.2 Influence of the process parameters on *OP* values**

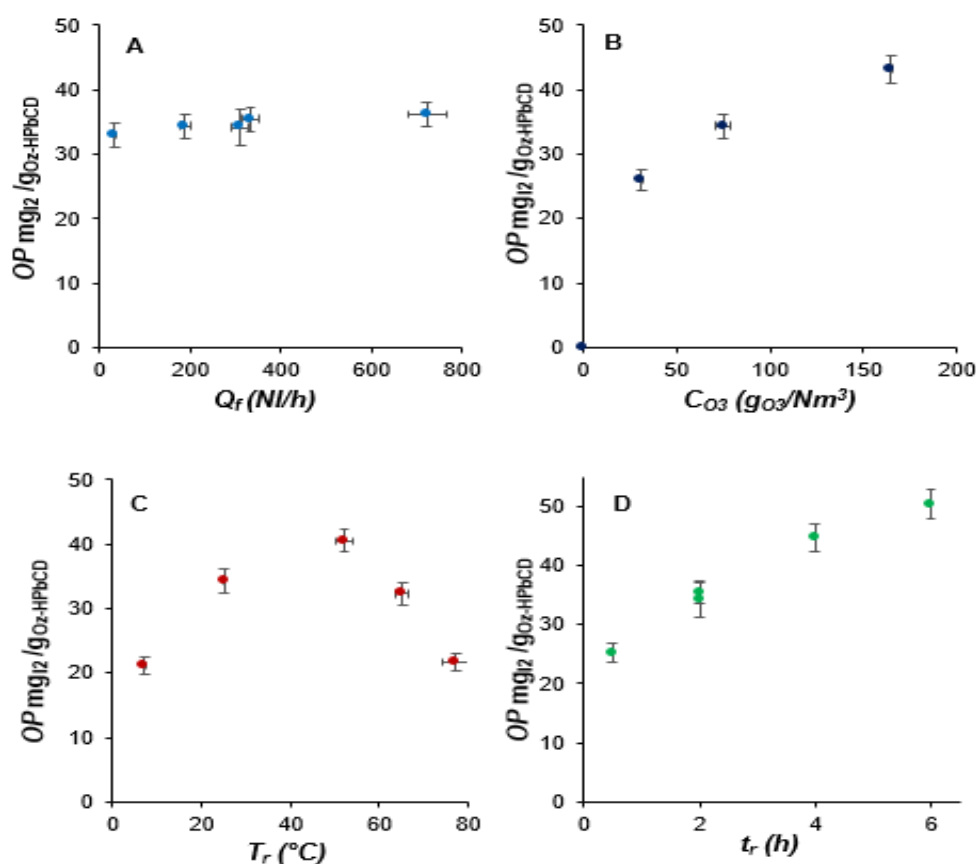
328 In order to provide robust information as regards the reproducibility of the experiments, 5  
329 syntheses were carried out in the same process conditions. The mean average values of the  
330 synthesis parameters were:  $Q_f = 49 \text{ NI/h}$ ,  $C_{O_3} = 96 \text{ gO}_3/\text{Nm}^3$ ,  $T_r = 25.8^\circ\text{C}$ , and  $t_r = 2 \text{ hrs}$ . The  
331 measurement uncertainties of the process parameters  $Q_f$ ,  $C_{O_3}$ , and  $T_r$ , calculated as the average

332 of the standard deviations of each parameter obtained for the 5 experiments, were estimated to  
333 be  $\Delta Q_f = \pm 3 \text{ NL/h}$ ,  $\Delta C_{O_3} = \pm 3 \text{ g}_{O_3}/\text{Nm}^3$ , and  $\Delta T_r = \pm 0.8^\circ\text{C}$ .

334  
335 Based on all the experiments carried out, we noted that when the solid HPbCD was in contact  
336 with gaseous ozone, an exothermic phenomenon occurred inside the reactor, leading to an  
337 increase in temperature of about  $5.0 \pm 1.5^\circ\text{C}$ . Immediately after  $t_0$ , the reactor temperature  
338 increased rapidly, reached a maximum, and then progressively decreased to stabilize close to  
339 the temperature set for the synthesis. In these conditions, the maximum peak temperature was  
340 reached in a few minutes, and this exothermicity could be measured for a few dozen minutes  
341 from  $t_0$ . At the end of each synthesis, the oxidative power of the ozonized CDs was measured  
342 by iodometry, and the mean value and standard deviation of  $OP$  were equal to  $45 \text{ mg}_{I_2} / \text{g}_{Oz-}$   
343  $\text{HPbCD}$  and  $\sigma = 3 \text{ mg}_{I_2} / \text{g}_{Oz-HPbCD}$ , respectively. These observations and results highlight that: (i)  
344 the contact between HPbCD and ozone leads to exothermic reaction(s); (ii) the synthesis  
345 process and the  $OP$  determination method, both evaluated using a set of 5 independent and  
346 identical runs, give good reproducible results, with a  $\pm 7\%$  variation of the  $OP$  values ; and  
347 (iii) as the  $OP > 0$ , it is clear that the process of contacting gaseous ozone with solid HPbCD  
348 generates materials with oxidative properties.

349  
350 The effect of the process parameters (i.e. the gas flow rate  $Q_f$ , the ozone concentration in the  
351 gas  $C_{O_3}$ , the reactor temperature  $T_r$ ) on the  $OP$  of the Oz-HPbCD was first studied more  
352 precisely by maintaining the reaction time at  $t_r = 2 \text{ hrs.}$ : when one parameter varied (i.e.  
353 ranging from 33 to  $723 \text{ NL.h}^{-1}$  for  $Q_f$ , from 31 to  $165 \text{ g}_{O_3}/\text{Nm}^3$  for  $C_{O_3}$ , and from 7 to  $77^\circ\text{C}$  for  
354  $T_r$ ), the others were maintained at constant values at  $Q_f = 186 \text{ NL.h}^{-1}$ ,  $C_{O_3} = 76 \text{ g}_{O_3}.\text{Nm}^3$ , and  
355  $T_r = 25.5^\circ\text{C}$ . Then, to study the effect of the reaction time  $t_r$ , the same methodology was used,  
356 using a higher gas flow rate value ( $Q_f = 330 \text{ NL.h}^{-1}$ ). The results obtained are presented  
357 **Figure 6.**

358



360  
 361 **Figure 6.** Effects of the different process parameters on the oxidative power of the Oz-HPbCD: A. gas flow  
 362 rate; B. ozone concentration in the gas; C. mean reactor temperature; D. reaction time.

363 As shown in **Figure 6A**, the gas flow rate has a negligible effect on the *OP*, as a variation in  
 364 the *OP* of less than 10% (from 33 to 36 mg<sub>12</sub> /g<sub>Oz-HPbCD</sub>) was measured across a very wide  
 365 range of gas flow rates (from 33 to 723 NI/h). As expected however, the *OP* was found to be  
 366 strongly dependent of the ozone concentration in the gas as shown in **Figure 6B**: stepping up  
 367 the *C*<sub>O<sub>3</sub></sub> from 31 to 165 g<sub>O<sub>3</sub></sub>/Nm<sup>3</sup> enhanced the *OP* of the Oz-HPbCD from 26 to 43 mg<sub>12</sub> /g<sub>Oz-</sub>  
 368 *HPbCD*. In an additional experiment performed using a gas containing only pure oxygen (*C*<sub>O<sub>3</sub></sub> =  
 369 0 g<sub>O<sub>3</sub></sub>/Nm<sup>3</sup>), we found that the powder obtained at the end of the synthesis was not oxidant  
 370 (the KI solution remained uncolored during the iodometric titration). As oxygen could also be  
 371 a potential oxidizing agent, this latter point confirms with any doubt that the oxidative  
 372 properties of the solid powder obtained at the end of the synthesis are due only to the presence  
 373 of ozone in the O<sub>2</sub>/O<sub>3</sub> gaseous mixture which contacts the CD. Interestingly enough, the mean  
 374 reactor temperature had a singular non monotonic effect on the *OP* compared to the other  
 375 parameters studied, as shown in **Figure 6C**: the *OP* variation curve first increases with *T*<sub>r</sub> (*OP*  
 376 = 21 to 40 mg<sub>12</sub> /g<sub>Oz-HPbCD</sub> when the temperature *T*<sub>r</sub> goes from 7 to 52°C), reaches a maximum  
 377 (extremum obviously located between ~ 30 and ~ 50°C), and finally drops from 40 to 21 mg<sub>12</sub>



378  $/g_{O_3-HPbCD}$  for temperatures between 52 and 77°C. Finally, it is clear from **Figure 6D** that  
379 increasing the reaction time of the synthesis significantly enhances the *OP* of the final  
380 ozonized product: the *OP* was increased from 25 to 50  $mg_{I_2} / g_{O_3-HPbCD}$  when the reaction time  
381 was increased from 0,5 hrs. to 6 hrs., respectively.

382 As reaction kinetics are generally enhanced by a temperature increase, we initially expected  
383 that the *OP* would be higher at high synthesis temperatures. The fact that we obtained the  
384 exact opposite (i.e. we measured a drastic decrease in the *OP* for  $T_r > \sim 50^\circ C$ ) reveals a  
385 certain instability of the oxidative properties of the ozonized product at high temperature. This  
386 can be correlated with the creation of oxidative chemical functions on the ozonized CDs,  
387 and/or the encapsulation of oxidative species in the CD cavity, and/or the creation of by-  
388 products, which are unstable in these temperature conditions. For example, it is a well-known  
389 fact that the ozone molecule, as well as many peroxides (Batakliiev, Georgiev, Anachkov,  
390 Rakovsky, & Zaikov, 2014), are naturally unstable from moderate to high temperatures.  
391 Because the oxidative power of the ozonized CD increased with the reaction time, with the  
392 ozone concentration in the gas and with the reactor temperature (for  $T_r < \sim 50^\circ C$ ) while being  
393 quasi-independent of the gas flow rate (i.e. the gas velocity inside the reactor), it is likely to  
394 be directly correlated with kinetics. The reaction and/or encapsulation did not appear to be  
395 limited by the external mass transfer (as the *OP* is independent of the velocity of the gas  
396 through the particles in the reactor), but it is likely it was limited by the reaction/encapsulation  
397 step and/or the diffusion of the gaseous ozone inside the solid CD particles. We therefore  
398 believe that it is possible that CDs did not react completely when in contact with ozone in  
399 these conditions. Unfortunately, it has been impossible to verify this assumption to date as the  
400 conversion (i.e. the fraction of the initial ozonized CDs) could not be determined in this study  
401 and requires additional specific characterizations with complementary analytical techniques,  
402 such as NMR. Work is in progress in this respect.

403

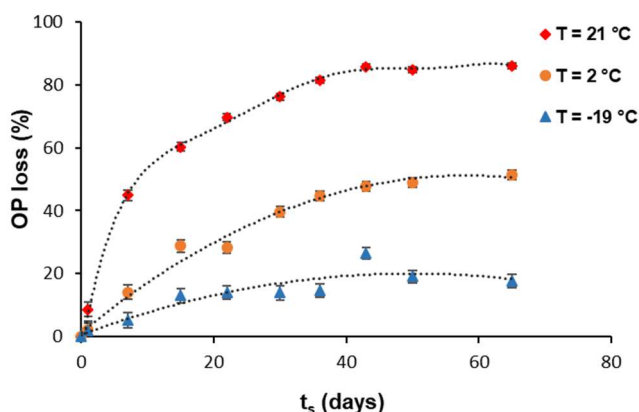
### 404 **3.3. Stability study**

405 In order to test the stability of the oxidizing power of the material over time, a synthesis was  
406 carried out in the following conditions:  $t_r = 6$  hrs.,  $Q_f = 335$   $Nl/hr.$ ,  $C_{O_3} = 69$   $g_{O_3}/Nm$  and  $T_r =$   
407  $27.1^\circ C$ . The stability test was performed using the same ozone treated with HPbCD. A mass  
408 of  $\sim 5$  g of the powder obtained from the synthesis was separated into three equal parts (i.e.  $\sim$   
409  $1.7$  g), and each sample was stored in the same model of glass flask (sealed with a septum)  
410 under different temperatures: at ambient temperature at  $21 \pm 2^\circ C$ , in a refrigerator at  $2 \pm 2^\circ C$ ,

411 and in a freezer at  $-19 \pm 2^\circ\text{C}$ . A sample was taken periodically in each flask to measure the  
412 *OP* by iodometric titration. The analyses were performed over a period of 65 days. The loss of  
413 the *OP* of the ozonized CD over time ( $t_s$ ) for the three different storage temperatures is  
414 presented in **Figure 7**.

415

416



417

418 **Figure 7.** Evolution of the rate of the loss of OP (%) of the oxidative power over storage time ( $t_s$ ) for Oz-  
419 HPbCD stored at different temperatures

420

421 After 65 days, the ozone-treated powder lost 86% of its *OP* when stored at  $21^\circ\text{C}$ , 51% at  $2^\circ\text{C}$   
422 and 18% at  $-19^\circ\text{C}$ . Looking at these results, it is obvious that the stability of the ozonized CDs  
423 depends strongly on the storage temperature of the materials: the lower the temperature, the  
424 more stable the oxidative material. As the chemical characterization of the material revealed  
425 that ozonation creates new chemical functions on the CDs and/or forms by-products, it might  
426 be possible that the ozonized CDs contain oxidative products or functions, such as ozone, an  
427 ozone derivative, or oxidative organic species such as peroxides. Such species might be  
428 unstable during long storage periods under moderate or high temperatures (Clark, 2001). This  
429 assumption is in agreement with the previous conclusions made in the section relative to the  
430 effect of the process parameters, where it was shown that the *OP* significantly decreased when  
431 the synthesis temperature was increased to values greater than  $50^\circ\text{C}$ . To improve our  
432 understanding of the product stability over time, it might be interesting to perform additional  
433 analytical experiments on the cyclodextrin samples, using NMR. Accordingly, it can be  
434 concluded that these ozonized CDs are unstable in certain conditions, but are nevertheless  
435 able to hold on to the most of their oxidative properties for a relatively long period of time

436 (more than two months) if the product is stored in a freezer at  $\sim -19 \pm 2^\circ\text{C}$ . In the light of  
437 these results, we might expect to achieve an even better long-term stability over time if the  
438 product is stored in hermetically closed vessels (instead of semi-hermetic flasks opened  
439 regularly for sampling purposes).

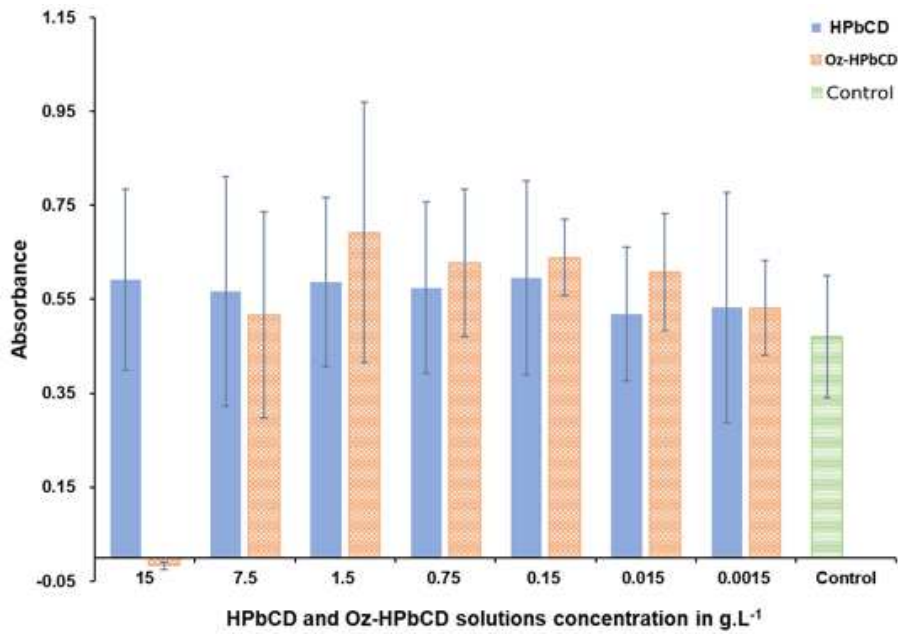
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441

### 442 **3.4. Minimum inhibitory concentrations (MIC)**

443

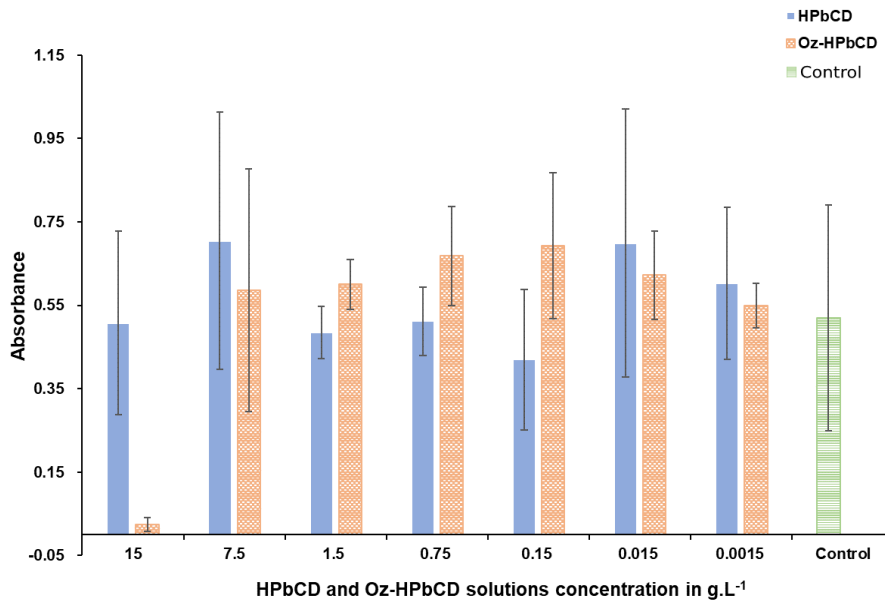
444 The results obtained for the MIC values are presented in **Figure 8** and **Figure 9** for the  
445 bacterial strains *Streptococcus uberis* and *Escherichia coli* respectively. It is important to note  
446 that these mean and standard deviation values result from 6 measurements, as each test  
447 condition was replicated within the plate, and each plate was triplicated. The absorbance with  
448 the different concentrations of powder for both strains was between 0.4 and 0.6. The positive  
449 control, the bacterial suspension with the BHB, showed a normal bacterial growth. The well  
450 with  $15 \text{ g.L}^{-1}$  of the Oz-HPbCD solution exhibited 0 absorbance for both strains *Streptococcus*  
451 *uberis* and *Escherichia coli*. However, at the same concentration, the wells with HPbCD  
452 presented a normal growth. Antibacterial activity was therefore clearly visible with the  
453 ozonized powder, although no difference was recorded between the two kinds of powder at  
454 any of the lower concentrations (between  $7.5$  and  $0.0015 \text{ g.L}^{-1}$ ). As no concentrations were  
455 tested in the range between  $7.5$  and  $15 \text{ g.L}^{-1}$ , the MIC value for Oz-HPbCD is considered to  
456 be  $15 \text{ g.L}^{-1}$  and above.



457

458 **Figure 8.** Antimicrobial activity assessment of HPbCD and Oz-HPbCD against the *Streptococcus uberis* strain.

459



460

461 **Figure 9.** Antimicrobial activity assessment of HPbCD and Oz-HPbCD against the *Escherichia coli* strain.

462

## 463 **4. Conclusion**

464

465 The results obtained in this study demonstrate the possibility of producing solid oxidative  
466 materials by contacting gaseous ozone with HPbCD powder. Comparing the physical  
467 characterizations of the HPbCD used as raw material for the synthesis and that of the  
468 ozonized HPbCD, there was no apparent modification of the morphology of the solid  
469 particles, density, and specific area, leading to the conclusion that the contact with  $O_3$  in these  
470 conditions does not induce fragmentation or the creation of meso/micro porosity in the initial  
471 particles. However, the characterization of Oz-HPbCD by FTIR and Raman spectroscopy  
472 revealed a new band which could be attributed to the  $-C=O$  stretching of carbonyl groups,  
473 demonstrating that the contact with  $O_3$  leads to chemical modifications of the CDs, which  
474 may be directly related to new functions created on the CD and/or to the formation of by-  
475 products. Further analytical work, in particular NMR studies, will be required to more  
476 precisely identify and quantify both the by-products formed as a result of the synthesis and the  
477 chemical changes to the solid CDs by reaction with the ozone. Looking at the influence of the  
478 process parameters, the oxidative power ( $OP$ ) of Oz-HPbCD was found to increase with the  
479 ozone concentration in the gas, the reactor temperature, and the duration of the synthesis,  
480 while the gas flow rate in the reactor had a low impact. These results seem to highlight the  
481 fact that the  $OP$  values are directly correlated with the reaction kinetics between CDs and  $O_3$ .  
482 The global reactivity is likely to be more limited by the chemical reactions and diffusivity of  
483  $O_3$  inside the solid particles than by the external mass transfer. It can therefore be  
484 hypothesized that, in the conditions of this study, the HPbCD particles may have not reacted  
485 completely. The Oz-HPbCDs, stored at low temperature were found to be relatively stable  
486 products, as only 18% of their oxidative properties were lost after 65 days in a freezer at -  
487  $19^\circ\text{C}$ . The microbiological results obtained by contacting Oz-HPbCDs and bacteria  
488 demonstrated that the ozonized products have bactericidal properties, with a minimum  
489 inhibitory concentration determined at  $15\text{g}\cdot\text{L}^{-1}$  for both *Escherichia Coli* and *Streptococcus*.  
490 We believe that these results may open up new avenues for developing novel solid materials  
491 with tunable oxidative and antimicrobial properties, usable in biological applications.

492

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498

## 499 **Author Contributions**

500 The manuscript was written based on contributions from all the authors. All the authors have  
501 given their approval to the final version of the manuscript.

502

## 503 **Declaration of competing interest**

504 The authors declare that they have no known competing financial interests or personal  
505 relationships that could have appeared to influence the work reported in this paper.

506

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516

## 517 **References**

518 Amiri, S., & Amiri, S. (2017). *Cyclodextrins: Properties and Industrial Applications* (1<sup>st</sup>  
519 ed). John Wiley & Sons Ltd, Hoboken, NJ, (Chapter 2).  
520 <https://doi.org/10.1002/9781119247609>.

521 Batakliiev, T., Georgiev, V., Anachkov, M., Rakovsky, S., & Zaikov, G. E. (2014). Ozone  
522 decomposition. *Interdisciplinary toxicology*, 7(2), 47–59. [https://doi.org/10.2478/intox-](https://doi.org/10.2478/intox-2014-0008)  
523 2014-0008.

524 Canado, A., Tournois, M., Pages, M., Roustan, M., Remus-Borel, W., Dietrich, N.,  
525 Violleau, F., & Hébrard, G. (2020). The sudden decrease of the dissolved ozone  
526 concentration in sprays: a mass transfer phenomenon? *Industrial & Engineering Chemistry*  
527 *Research*, 59(33), 14914–14924. <https://doi.org/10.1021/acs.iecr.0c03216>.

528 Clark, D. E. (2001). Peroxides and peroxide-forming compounds. *Chemical Health &*  
529 *Safety*, 8(5), 12–22. [https://doi.org/10.1016/S1074-9098\(01\)00247-7](https://doi.org/10.1016/S1074-9098(01)00247-7).

530 CLSI (2012). *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That*  
531 *Grow Aerobically*; Approved Standard—Ninth Edition. CLSI document M07-A9. Wayne,  
532 PA: Clinical and Laboratory Standards Institute.

533 Crini, G. (2014). Review: a history of cyclodextrins. *Chemical Reviews*, 114(21), 10940-  
534 10975. <https://doi.org/10.1021/cr500081p>.

535 Cristiano, L. (2020). Could ozone be an effective disinfection measure against the novel  
536 coronavirus (SARS-CoV-2)? *Journal of Preventive Medicine and Hygiene*, 61(3), E301-  
537 E303. <https://doi.org/10.15167/2421-4248/jpmh2020.61.3.1596>.

538 Dettmer, A., Ball, R., Boving, T.B., Khan, N.A., Schaub, T., Sudasinghe, N., Fernandez,  
539 C.A. & Carroll, K.C. (2017). Stabilization and prolonged reactivity of aqueous-phase ozone  
540 with cyclodextrin. *Journal of Contaminant Hydrology*, 196, 1-9,  
541 <https://doi.org/10.1016/j.jconhyd.2016.11.003>.

542 Egyed, O. (1990). Spectroscopic studies on  $\beta$ -cyclodextrin. *Vibrational Spectroscopy*, 1(2),  
543 225-227. [https://doi.org/10.1016/0924-2031\(90\)80041-2](https://doi.org/10.1016/0924-2031(90)80041-2).

544 Fan, W., An, W., Huo, M., Xiao, D., Lyu, T. & Cui, J. (2021). An integrated approach  
545 using ozone nanobubble and cyclodextrin inclusion complexation to enhance the removal of  
546 micropollutants. *Water Research*, 196, 117039.  
547 <https://doi.org/10.1016/j.watres.2021.117039>.

548 Farber, C., Li, J., Hager, E., Chemelewski, R., Mullet, J., Rogachev, Yu. A., & Kurouski,  
549 D. (2019). Complementarity of Raman and Infrared spectroscopy for structural  
550 characterization of plant epicuticular waxes. *ACS Omega*, 4(2), 3700-3707.  
551 <https://doi.org/10.1021/acsomega.8b03675>.

552 Harada, A., Takashima, Y., & Yamaguchi, H. (2009). Cyclodextrin-based supramolecular  
553 polymers. *Chemical Society Reviews*, 38, 875-885. <https://doi.org/10.1039/b705458k>.

554 Ibrahim, N.A., & El-Zairy, E.M.R. (2009). Union disperse printing and UV-protecting of  
555 wool/polyester blend using a reactive  $\beta$ -cyclodextrin, *Carbohydrate Polymers*, 76(2), 244-  
556 249. <https://doi.org/10.1016/j.carbpol.2008.10.020>.

557 Khan, N. A., Johnson, M.D., & Carroll, K.C. (2018). Spectroscopic methods for aqueous  
558 cyclodextrin inclusion complex binding measurement for 1,4-dioxane, chlorinated co-  
559 contaminants, and ozone. *Journal of Contaminant Hydrology*, 210, 31-41.  
560 <https://doi.org/10.1016/j.jconhyd.2018.02.002>.

561 Li, N., Chen, Y., Zhang, Y.M., Yang, Y., Su, Y., Chen, J.-T., & Liu, Y. (2014).  
562 Polysaccharide-gold nanocluster supramolecular conjugates as a versatile platform for the  
563 targeted delivery of anticancer drugs. *Scientific Reports*, 4, 4164.  
564 <https://doi.org/10.1038/srep04164>.

565 Loftsson, T., & Duchêne, D. (2007). Cyclodextrins and their pharmaceutical applications.  
566 *International Journal of Pharmaceutics*, 329(1-2), 1-11.  
567 <https://doi.org/10.1016/j.ijpharm.2006.10.044>.

568 Malanga, M., Szemán, J., Fenyvesi, E., Puskás, E., Csabai, K., Gyémánt, G., Fenyvesi, F.,  
569 & Szente, L. (2016). “Back to the future”: a new look at hydroxypropyl beta-cyclodextrins.  
570 *Journal of Pharmaceutical Sciences*, 105(9), 2921-2931.  
571 <https://doi.org/10.1016/j.xphs.2016.04.034>.

572 Martins, M.L., Eckert, J., Jacobsen, H., Dos Santos, E.C., Ignazzi, R., Ribeiro de Araujo,  
573 D., Bellissent-Funel, M.-C., Natali, F., Marek Koza, M., Matic, A., De Paula, E., &  
574 Bordallo, H.N. (2017). Raman and Infrared spectroscopies and X-ray diffraction data on  
575 bupivacaine and ropivacaine complexed with 2-hydroxypropyl- $\beta$ -cyclodextrin. *Data in*  
576 *Brief*, 15, 25-29. <https://doi.org/10.1016/j.dib.2017.08.053>.



577 McCray, J., Boving, T., & Brusseau, M. (2000). Cyclodextrin-enhanced solubilization of  
578 organic contaminants with implications for aquifer remediation. *Ground Water Monitoring  
579 & Remediation*, 20, 94-103. <https://doi.org/10.1111/j.1745-6592.2000.tb00256.x>.

580 Morin-Crini, N., Fourmentin, S., Fenyvesi, É., Lichtfouse, E., Torri, G., Fourmentin, M., &  
581 Crini, G. (2021). 130 years of cyclodextrin discovery for health, food, agriculture, and the  
582 industry: a review. *Environmental Chemistry Letters*, 19, 2581–2617.  
583 <https://doi.org/10.1007/s10311-020-01156-w>.

584 Muromachi, S., Ohmura, R., & Mori, Y. H. (2012). Phase equilibrium for ozone-  
585 containing hydrates formed from an (ozone + oxygen) gas mixture coexisting with gaseous  
586 carbon dioxide and liquid water. *The Journal of Chemical Thermodynamics*, 49, 1-6.  
587 <https://doi.org/10.1016/j.jct.2012.01.009>.

588 Nasongkla, N., Wiedmann, A. F., Bruening, A., Beman, M., Ray, D., Bornmann, W. G.,  
589 Boothman, D. A., & Gao, J. (2003). Enhancement of solubility and bioavailability of  $\beta$ -  
590 lapachone using cyclodextrin inclusion complexes. *Pharmaceutical Research*, 20, 1626-  
591 1636. <https://doi.org/10.1023/a:1026143519395>.

592 Özen, T., Koyuncu, M.A. & Erbaş, D. (2021). Effect of ozone treatments on the removal of  
593 pesticide residues and postharvest quality in green pepper. *Journal of Food Science and  
594 Technology*, 58, 2186–2196, <https://doi.org/10.1007/s13197-020-04729-3>.

595 Pagès, M., Kleiber, D., Pierron, R. J. G., & Violleau, F. (2016). Ozone effects on *Botrytis*  
596 *cinerea* Conidia using a bubble column: germination inactivation and membrane  
597 phospholipids oxidation. *Ozone: Science & Engineering*, 38(1), 62-69.  
598 <https://doi.org/10.1080/01919512.2015.1074856>.

599 Piechowiak, T., Grzelak-Błaszczak, K., Sójka, M., & Balawejder, M. (2020). Changes in  
600 phenolic compounds profile and glutathione status in raspberry fruit during storage in ozone-  
601 enriched atmosphere. *Postharvest Biology and Technology*, 168, 111277.  
602 <https://doi.org/10.1016/j.postharvbio.2020.111277>.

603 Roca, I., Akova, M., Baquero, F., Carlet, J., Cavaleri, M., Coenen, S., Cohen, J., Findlay,  
604 D., Gyssens, I., Heur, O.E., Kahlmeter, G., Kruse, H., Laxminarayan, R., Liébana, E.,  
605 López-Cerero, L., MacGowan, A., Martins, M., Rodríguez-Baño, J., Rolain, J.-M., Segovia,  
606 C., Sigauque, B., Tacconelli, E., Wellington, E., & Vila, J. (2015). The global threat of

607 antimicrobial resistance: science for intervention. *New Microbes and New Infections*, 6, 22-  
608 29. <https://doi.org/10.1016/j.nmni.2015.02.007>.

609 Stancanelli, R., Ficarra, R., Cannavà, C., Guardo, M., Calabrò, M.L., Ficarra, P., Ottanà,  
610 R., Maccari, R., Crupi, V., Majolino, D., & Venuti, V. (2008). UV-vis and FTIR-ATR  
611 characterization of 9-fluorenon-2-carboxyester/(2-hydroxypropyl)- $\beta$ -cyclodextrin inclusion  
612 complex. *Journal of Pharmaceutical and Biomedical Analysis*, 47(4-5), 704-709.  
613 <https://doi.org/10.1016/j.jpba.2008.02.018>.

614 Szejtli, J. (1998). Introduction and general overview of cyclodextrin chemistry. *Chemical*  
615 *Reviews*, 98(5), 1743-1754. <https://doi.org/10.1021/cr970022c>.

616 Szejtli, J. (1997). Utilization of cyclodextrins in industrial products and processes. *Journal*  
617 *of Materials Chemistry*, 7(4), 575-585. <https://doi.org/10.1039/A605235E>.

618 Szejtli, J., & Osa, T. (1996). *Comprehensive Supramolecular Chemistry, Volume 3:*  
619 *Cyclodextrins*. (1st ed.). Oxford: Pergamon.

620 Szente, L., Szemán, J., Sohajda, T. (2016). Analytical characterization of cyclodextrins:  
621 *History, official methods and recommended new techniques. Journal of Pharmaceutical and*  
622 *Biomedical Analysis*, 130, 347-365. <https://doi.org/10.1016/j.jpba.2016.05.009>.

623 Tizaoui, C. (2020). Ozone: a potential oxidant for COVID-19 Virus (SARS-CoV-2).  
624 *Ozone: Science & Engineering*, 42(5), 378-385.  
625 <https://doi.org/10.1080/01919512.2020.1795614>.

626 Vitola Pasetto, L., Simon, V., Richard, R., Pic, J.-S., Violleau, F., & Manero, M.-H.  
627 (2020). A catalyst-free process for gas ozonation of reduced sulfur compounds. *Chemical*  
628 *Engineering Journal*, 387, 123416. <https://doi.org/10.1016/j.cej.2019.123416>.

629 Vittenet, J., Aboussaoud, W., Mendret, J., Pic, J.-S., Debellefontaine, H., Lesage, N.,  
630 Faucher, K., Manero, M.-H., Thibault-Starzyk, F., Leclerc, H., Galarneau, A., & Brosillon, S.  
631 (2015). Catalytic ozonation with  $\gamma$ -Al<sub>2</sub>O<sub>3</sub> to enhance the degradation of refractory organics  
632 in water. *Applied Catalysis A: General*, 504, 519-532.  
633 <https://doi.org/10.1016/j.apcata.2014.10.037>.

- 634 Wang, T., Peng, D., Li, Y., Bai, Y. & Huang, T. (2016). HP- $\beta$ -Cyclodextrin assisted  
635 ozonation. *Proceedings of the 2016 International Conference on Civil, Transportation and*  
636 *Environment*, 1362-1365. Aug 28, 2016 - Aug 29, 2016, Phuket, Thailand.  
637 <https://doi.org/10.2991/iccte-16.2016.239>.
- 638 Wellington, E. M. H., Boxall, A. B. A., Cross, P., Feil, E. J., Gaze, W. H., Hawkey, P.  
639 M., Johnson-Rollings, A. S., Jones, D. L., Lee, N. M., Otten, W., Thomas, C. M., &  
640 Williams, A. P. (2013). The role of the natural environment in the emergence of antibiotic  
641 resistance in Gram-negative bacteria. *The Lancet Infectious Diseases*, 13(2), 155-165.  
642 [https://doi.org/10.1016/S1473-3099\(12\)70317-1](https://doi.org/10.1016/S1473-3099(12)70317-1).
- 643 Yuan, C., Liu, B., & Liu, H. (2015). Characterization of hydroxypropyl- $\beta$ -cyclodextrins  
644 with different substitution patterns via FTIR, GC-MS, and TG-DTA. *Carbohydrate*  
645 *Polymers*, 118, 36-40. <https://doi.org/10.1016/j.carbpol.2014.10.070>.
- 646 Zhang, X., Tang, N., Zhang, H., Chen, C., Li, L., Dong, C., & Cheng, Y. (2021).  
647 Comparative transcriptomic analysis of cantaloupe melon under cold storage with ozone  
648 treatment. *Food Research International*, 140, 109993.  
649 <https://doi.org/10.1016/j.foodres.2020.109993>.

## GRAPHICAL ABSTRACT

