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► **To cite this version:**

Fangxin Zou, Tatiana Budtova. Tailoring the morphology and properties of starch aerogels and cryogels via starch source and process parameter. *Carbohydrate Polymers*, 2021, 255, pp.117344. 10.1016/j.carbpol.2020.117344 . hal-03525459

HAL Id: hal-03525459

<https://hal.science/hal-03525459>

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1 *Submitted to Carbohydrate Polymers 28 August 2020*

2 *Revised 12 October 2020*

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6 **Tailoring the morphology and properties of starch aerogels and**
7 **cryogels via starch source and process parameter**

8

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10

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21

22 **Abstract**

23 Porous starch materials with various morphology and properties were made via starch dissolution,
24 retrogradation and drying either with supercritical CO₂ (“aerogels”) or lyophilisation (“cryogels”). Their
25 properties were correlated with the rheological response of retrograded starch gels and crystallinity of
26 aerogels and cryogels. All starch cryogels possess very low density (0.07 – 0.16 g/cm³), very large
27 macropores and low specific surface area (around 3 – 13 m²/g). Their morphology is mainly the replica
28 of sublimated ice crystals. The properties of starch aerogels strongly depend on starch source: the
29 lowest density (around 0.1 g/cm³) and highest specific surface area (170 – 250 m²/g) was recorded for
30 pea starch aerogels and the highest density (0.3 – 0.6 g/cm³) and lowest specific surface area (7 – 90
31 m²/g) for waxy maize starch aerogels. The morphology and properties of starch aerogels are
32 interpreted by amylose and amylopectin evolution during retrogradation.

33

34 Keywords: supercritical drying, freeze-drying, morphology; density; specific surface area

35

36

37 1. Introduction

38 Starch is one of the most abundant polysaccharides used in multiple applications: in food and non-
39 food (biodegradable films and foams for packaging, as additive in paper and textile) and also as a
40 source of low molecular weight chemicals. Porous starch makes a special family of lightweight
41 materials in which pore sizes, porosity and cellular morphology depend on the processing method: for
42 example, voids are formed during extrusion of starch/water at high temperatures, baking a starch
43 paste followed by water evaporation, microwave heating which generates steam bubbles, freeze-
44 drying/sublimation of ice crystals and supercritical fluid extrusion. Rather recently, supercritical fluid
45 extraction technique, used to make aerogels, has also been applied to starch.

46 Aerogels are nanostructured lightweight mesoporous/small macropores materials with high specific
47 surface area (of several hundreds of m^2/g); the latter distinguishes them from foams which usually are
48 lightweight but with very large macropores and thus low surface area. The very first aerogels were
49 reported by Kistler in 1931 (Kistler, 1931); inorganic aerogels were then developed in the 70s-80s of
50 the past century followed by aerogels based on synthetic polymers (Pierre, 2011). Aerogels are
51 versatile materials: they can be used for water-oil separation (Sai et al., 2015; Zou, Peng, Fu, Zhang,
52 & Li, 2015), thermal insulation (Groult & Budtova, 2018a; Zou et al., 2016), drug delivery (Mehling,
53 Smirnova, Guenther, & Neubert, 2009; Ulker & Erkey, 2014) and electro-chemical/energy storage
54 (Hamedi et al., 2013; Rooke et al., 2011) applications. In the past two decades, a variety of so-called
55 bio-aerogels made from polysaccharides such as cellulose (Lavoine & Bergström, 2017; Budtova,
56 2019), pectin (Zhao, Chen, & Chen, 2017; Groult & Budtova, 2018b), starch (García-González, Uy,
57 Alnaief, & Smirnova, 2012; Zhu, 2019), and alginate (Mallepally, Bernard, Marin, Ward, & McHugh,
58 2013; Robitzer, David, Rochas, Renzo, & Quignard, 2008) gained widespread attention in the view of
59 the need of sustainable and biodegradable materials on the one hand, and their potential use in
60 biomedical applications on the other hand.

61 As most of bio-aerogels (except those based on nanocellulose), starch aerogels are made via
62 dissolution-gelation (retrogradation for starch)-solvent exchange-drying with supercritical carbon
63 dioxide. Starch aerogels were shown to be promising matrices for drug release (García-González et
64 al., 2012; Mehling et al., 2009) and also for thermal insulation (Druel, Bardl, Vorweg, & Budtova,
65 2017). It was reported that higher starch concentration, higher aerogel density (García-González &
66 Smirnova, 2013; Ubeyitogullari & Ciftci, 2016), as expected. It seems that higher amylose content

67 leads to higher specific surface area, for example, 200 – 250 m²/g for pea and high amylose corn
68 (Druel et al., 2017; García-González et al., 2013) vs 20 – 80 m²/g for potato (Druel et al., 2017) and
69 wheat starches (Ubeyitogullari et al., 2016). However, low specific surface area, 90 m²/g, was reported
70 for high amylose starch Eurylon 7 (Mehling et al., 2009), most probably because of incomplete starch
71 dissolution. There are still several open questions such as the influence of the starch type and
72 retrogradation time on aerogel structure and properties.

73 It should be noted that the first mention of using drying with supercritical CO₂ for making starch
74 aerogels was made by Glenn *et al*, and the materials were called “microcellular foams” (Glenn & Irving,
75 1995). This seminal work compared the structure and properties of freeze-dried and supercritically
76 dried unmodified wheat starch, regular corn and high amylose corn starches. However, starch granule
77 remnants were present in the dried samples and samples’ specific surface area was not measured;
78 low-resolution scanning electron microscope (SEM) did not allow the analysis of microstructure below
79 one micron.

80 As mentioned above, another way of making porous starch materials avoiding pores’ collapse
81 during drying is freeze-drying. Freeze-dried starch can be used in multiple applications, mainly in food
82 and also in biomedical area (for example, in tissue engineering). Numerous studies have been
83 performed on freeze-dried starches, either on starch granules (B. Zhang et al., 2014), or on starch
84 solutions alone or mixed with other natural components (Nakamatsu, Torres, Troncoso, Min-Lin, &
85 Boccaccini, 2006; Svagan, Samir, & Berglund, 2008), or on starch emulsions (Silva, Azevedo, Cunha,
86 Hubinger, & Meireles, 2016; Spada, Norena, Marczak, & Tessaro, 2012). In food applications, freeze-
87 drying is considered as a way of granules’ preservation, and the influence of drying method (oven
88 drying from water or ethanol, freeze-drying, microwave drying, etc) on starch gelatinization and
89 digestibility was investigated (Glenn et al., 2008; B. Zhang et al., 2014). In materials science freeze-
90 drying is used to shape the pores of starch solution (usually strong retrogradation is avoided) upon
91 removing water (Nakamatsu et al., 2006; Svagan et al., 2008). When using starch for encapsulation,
92 freeze-drying allows obtaining powders and is often compared with spray drying (Silva et al., 2016;
93 Spada et al., 2012).

94 To the best of our knowledge, there is no study, except the one of Glenn *et al* (Glenn et al, 1995),
95 on the comparison of the structure and properties of freeze-dried and supercritical dried starches
96 made from the same starting gels. How does the way of drying and starch type (i.e.

97 amylose/amylopectin ratio) influence starch morphology and properties? It is well documented that
98 retrogradation kinetics depends on amylose/amylopectin ratio: gelation is faster in high-amylose
99 starches as amylose undergoes rapid recrystallization forming semi-crystalline clusters. What is the
100 impact, if any, of retrogradation time on freeze-dried and supercritically dried starch gels? The
101 answers to these questions will help tuning starch morphology and properties for the desired
102 applications.

103 The goal of this work is to understand the influence of starch type (amylose content) and
104 processing conditions (way of drying, starch concentration, retrogradation time) on the morphology
105 and properties of porous starches starting from the same precursor. For simplicity, we will use
106 “aerogels” for samples dried with supercritical CO₂, and “cryogels” for freeze-dried samples. We
107 correlate the morphology and properties of porous starches with their crystallinity and gel strength, and
108 we suggest using “aerogel approach” as a way to follow starch gel structure evolution upon
109 retrogradation and as a function of amylose content.

110

111 **2. Experimental**

112 2.1 Materials

113 Three types of starches were kindly provided by Roquette, France: waxy maize, potato and pea
114 with amylose content around 1%, 18-21%, and 33-36%, respectively. Absolute ethanol (>99%) was
115 purchased from Fisher Scientific. Distillated water was used. All chemicals were used without any
116 further purification.

117 2.2 Preparation of starch solutions and gels

118 Starch needs to be dissolved as well as possible in order to avoid the presence of non-dissolved
119 granules and their remnants. The residual granules and their remnants are non-porous and may
120 decrease the specific surface areas of aerogels in a similar manner as non-dissolved cellulose fibers
121 decreased specific surface area in cellulose aerogels (Korhonen & Budtova, 2020).

122 Different methods were adapted to dissolve the three types of starches because of their different
123 solubility in water. Optical microscopy (LEICA DM4500P, Leica Microsystems) was used for the
124 screening of the influence of the dissolution conditions on the presence of granules' remnants (Table
125 S1 in the Supporting Information). The final conditions selected for each starch type were when no
126 non-dissolved fragments were seen in optical micrographs. For example, no non-dissolved granules

127 were detected when stirring potato starch dispersed in water at 1000 rpm at 95 °C for 2 h, but 3 h
128 were needed to dissolve waxy maize starch in the same conditions (Table S1). To dissolve pea starch
129 two steps method (Druel et al., 2017) was used: first, pea starch-water was heated to 95 °C under
130 stirring at 1000 rpm for 1 h; then this mixture was placed in the autoclave (452HC, Parr instrument),
131 heated during 50 min up to 130 °C and kept at 130-140 °C for another 20 min under stirring at 250
132 rpm.

133 Starch solutions of various concentrations, from 5 to 11 wt% (in dry weight), were prepared as
134 described above for each type of starch. Hot starch solutions were centrifuged, if needed, for 3 min
135 under 8000 rpm to remove bubbles and poured into plastic molds. After cooling down to room
136 temperature, all samples were stored at 4 °C for retrogradation. The retrogradation time for potato and
137 pea starch was from 1 to 4 days and for waxy maize from 15 to 45 days. Long retrogradation times of
138 waxy maize starch solutions were needed as in non-gelled state solution viscosity was too low not
139 allowing making homogeneous aerogels and cryogels. In the following, starch gels are named as
140 "starch type-Xwt%-Nday-gel", where X is starch concentration in solution and N is retrogradation time.

141 2.3 Preparation of starch aerogels and cryogels

142 Starch aerogels were prepared according to the following steps: dissolution-retrogradation-solvent
143 exchange-supercritical CO₂ drying. Before drying, water was replaced by ethanol through solvent
144 exchange as water is not miscible with supercritical CO₂. The solvent exchange was performed by
145 gradual increase of ethanol in ethanol/water mixtures: first, the gels were soaked in ethanol/water
146 50/50 (v/v) for 0.5 day, then in ethanol/water 75/25 (v/v) for another 0.5 day, and finally in pure ethanol
147 for 3 days during which fresh ethanol was exchanged twice a day.

148 Supercritical CO₂ drying was performed as follows. First, ethanol in the gel was slowly drained by
149 gaseous CO₂ while the system was pressurized at 50 bar and 37 °C. Then, the temperature was set at
150 37 °C, and the pressure was increased to 80 bar above CO₂ critical point. After that, a dynamic
151 washing step was performed at 80 bar and 37 °C with an output of 5 kg/h of CO₂ for 1 h to remove the
152 residual ethanol from the gel. It was followed by a static mode for 1-2 h under the same temperature
153 and pressure and dynamic washing step again for 2 h. Finally, the system was kept at 37 °C and
154 slowly depressurized to ambient conditions with pressure decrease speed 5 bar/h. Slow
155 depressurization was used to prevent sample strong shrinkage. The autoclave was cooled down to

156 room temperature before being opened. Starch aerogels are named as “starch type-Xwt%-Nday-
157 aerogel” where X and N are the same as for starch gels.

158 Starch cryogels were prepared from gels using freeze drying. The gels were unidirectionally frozen
159 by placing starch gel on a metal plate which was immersed in liquid nitrogen for 20 min, and then
160 freeze-dried for 72 h at -82 °C and pressure below 2 mTorr by using the Lyophilisateur Cryotec
161 COSMOS-80 (Cryotec). In the following, the starch cryogels are named as “starch type-Xwt%-Nday-
162 cryogel” where X and N are the same as for starch gels.

163 2.4 Characterization

164 2.4.1 Rheology

165 The rheological properties of starch gels were probed using Gemini 150 rheometer (Bohlin
166 Instruments) with parallel plate geometry (40 mm diameter) and gap 1000 μm. A hot starch solution
167 was put on the lower plate, kept for 1.5 min, gap closed and the whole kept for another 3 min.
168 Temperature was then set at 4 °C for different retrogradation times varying from 1 to 4 days before
169 starting the oscillation. Strain response of elastic (G') and viscous (G'') moduli was tested at 4 °C to
170 determine the mechanical response of starch gels to shear strain in the range 0.1% - 1000% at
171 frequency 1 Hz. The diameter of plates was varied to test the potential slippage; no influence of plates'
172 geometry on G' and G'' values was recorded, within the experimental errors, indicating the absence of
173 slippage.

174 2.4.2 Linear shrinkage

175 The total linear shrinkage of the samples due to the solvent exchange and drying was calculated
176 according to the following equation with at least three measurements per formulation (Himmel, Gerber,
177 Biiirger, Holzfhfiter, & Olbertz, 1995; Saliger, Heinrich, Gleissner, & Fricke, 1995):

$$178 \text{ Linear shrinkage} = \frac{D_b - D_a}{D_b} \times 100\% \quad (1)$$

179 where D_b and D_a are the diameters of sample before solvent exchange and after drying, respectively.

180 2.4.3 Bulk density and porosity

181 Bulk density of starch aerogels and cryogels was determined by Geopyc 1360 (Micromeritics)
182 powder densitometer with Dryflo as the powder, with at least five measurements per formulation. The
183 diameter of the test chamber was 19.1 mm, and the force was 25 N.

184 The porosity (%) of each sample was calculated as follows:

$$185 \text{ Porosity} = \frac{\rho_s - \rho_b}{\rho_s} \times 100\% \quad (2)$$

186 where ρ_b and ρ_s refer to the bulk and skeletal densities, respectively, with $\rho_s = 1.45 \text{ g/cm}^3$ (García-
187 González et al., 2013).

188 2.4.4 Specific surface area

189 Specific surface area (S_{BET}) of starch aerogels and cryogels was calculated using nitrogen
190 adsorption method with ASAP 2020 specific surface area and porosity analyzer (Micromeritics
191 Instrument Corporation). Prior to the measurement, all samples were degassed at 70 °C for 10 h. The
192 maximal standard deviation was 12 m²/g.

193 2.4.5 Scanning electron microscope

194 The morphology of starch samples was characterized by Scanning Electron Microscope (SEM)
195 Supra40 Zeiss SEM FEG (Fields Emission Gun) (ZEISS) with an accelerating voltage of 3 kV and
196 diaphragm 10 μm. Before the measurements, a roughly 7 nm thin layer of platinum was sputtered onto
197 the sample's surface with Q150T coater (Quorum).

198 2.4.6 X-ray diffraction analysis

199 X-ray diffraction (XRD) analysis (in reflection mode) was performed on diffractometer “XPRT-PRO”
200 (PANalytical) with Cu K α radiation at wavelength of 1.5406 Å. The samples were grinded into powder
201 and scanned in 2 θ range from 5° to 50°. The relative crystallinity was calculated by the following
202 equation:

$$203 \text{ Relative crystallinity} = \frac{A_c}{A_c + A_a} \times 100\% \quad (3)$$

204 where A_c and A_a are the integrated areas of crystalline and amorphous regions, respectively, over the
205 2 θ range from 5° to 28°. A_c and A_a were calculated using the method of Komiya and Nara (Komiya &
206 Nara, 1986; Cheetham & Tao, 1998). Komiya & Nara showed that XRD in symmetrical reflection and
207 symmetrical transmission modes provide almost the same values of the relative crystallinity (Komiya &
208 Nara, 1986).

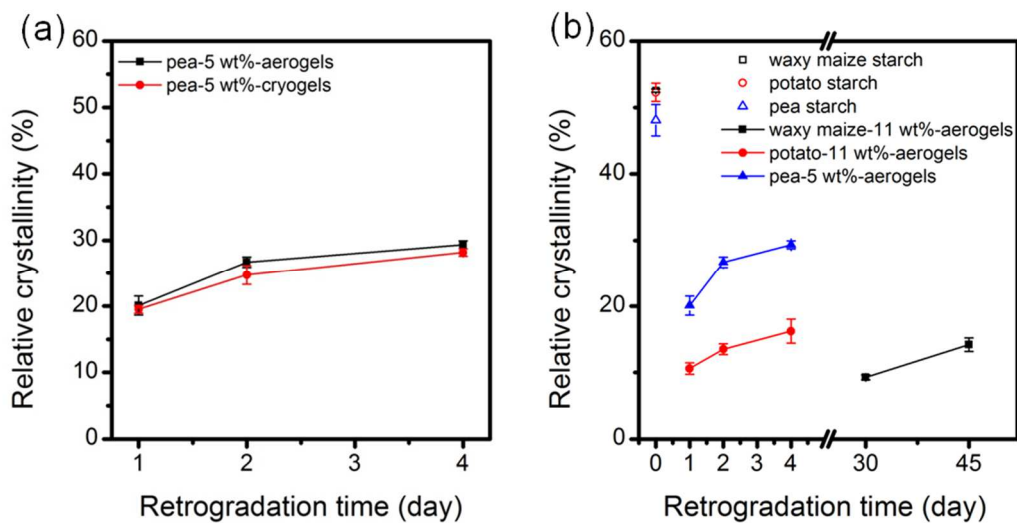
209

210 3. Results

211 3.1 X-ray diffraction

212 An example of the relative crystallinity of starch aerogels and cryogels as a function of
213 retrogradation time is shown in Figure 1a for pea starch aerogels and cryogels, and in Figures S1b
214 and S1c (Supporting Information) for other starches. The examples of the diffraction profiles are
215 shown in Figure S1a of the Supporting Information; sharp peaks on XRD profiles of the initial starches

216 become much weaker and broader after dissolution-retrogradation, as expected (Shi & Gao, 2016;
 217 Soest, Tournois, Wit, & Vliegenthart, 1995; Cheetham & Tao, 1998; Zhang, Hou, Liu, Wang, & Dong,
 218 2019). As a consequence, the crystallinity of the initial starch significantly decreases after the
 219 dissolution-retrogradation-drying for all starches studied (Figure 1b). The decrease of the crystallinity
 220 of starch in wheat starch aerogels as compared to the initial starch have been reported previously
 221 (Ubeyitogullari et al., 2016). Figure 1a shows that drying method does not influence the crystallinity of
 222 pea starch; the same result was observed for potato and waxy maize starch (Figure S1b and S1c,
 223 respectively), and also for cellulose aerogels and cryogels (Buchtova, Pradille, Bouvard, & Budtova,
 224 2019). The crystallinity of aerogels slightly increases with the increase of retrogradation time for all
 225 starches (Figure 1b), and it is the highest for pea starch which contains the highest amount of amylose.
 226 The latter is known for much faster crystallization than amylopectin (Ma, Ma, Zhou, Li, & Hu, 2019; Shi
 227 et al., 2016).
 228

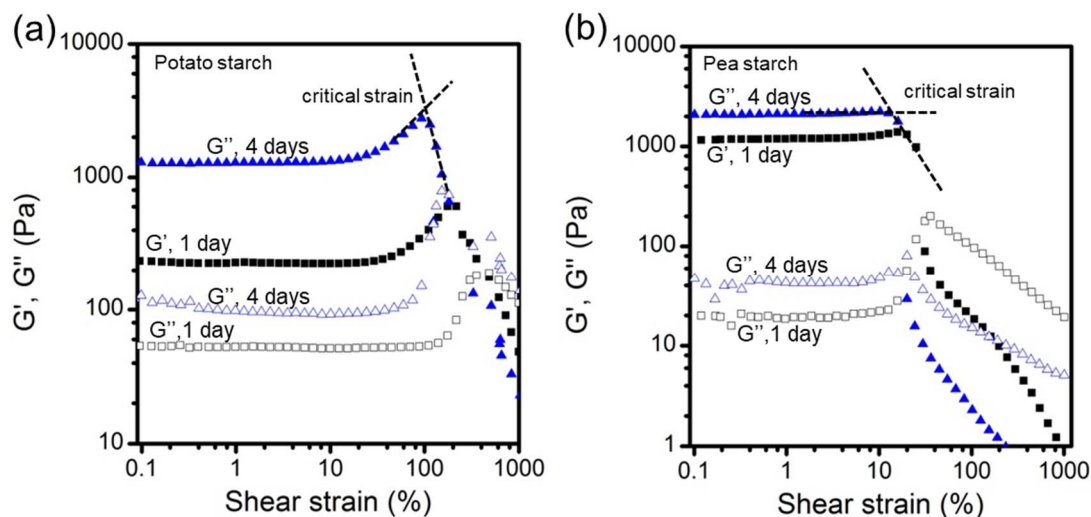


229
 230 **Figure 1.** Relative crystallinity as a function of retrogradation time for (a) pea starch aerogel and
 231 cryogel and (b) aerogels from all three types and initial starches (retrogradation time 0). Lines are
 232 given to guide the eye. When standard deviation is not visible, it is smaller than the size of the symbol.
 233

234 3.2 Rheological properties of starch gels

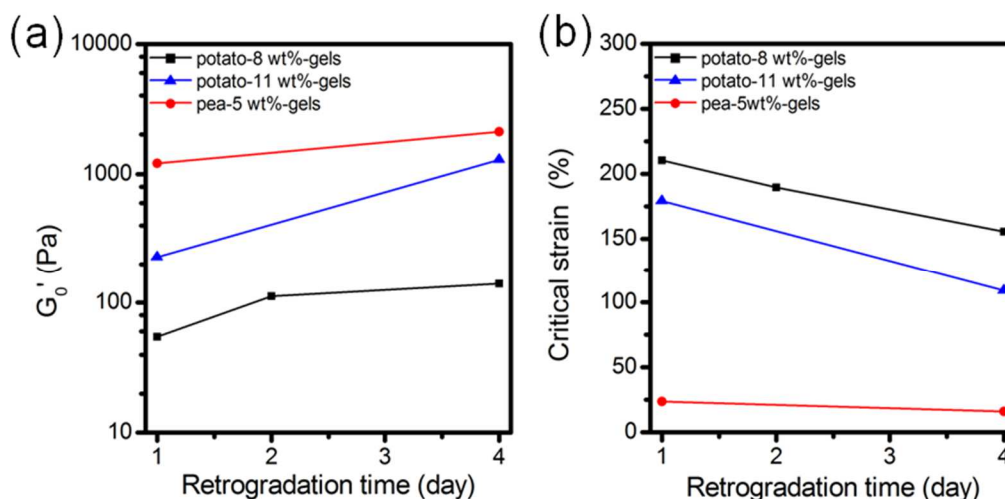
235 The examples of the dependences of storage and loss moduli on shear strain for potato and pea
 236 starch gels are presented in Figure 2. For all gels the elastic behavior dominates the viscous one, as
 237 expected, as potato and pea starch solutions are retrograded. For potato gels both moduli remain

238 constant within the wide strain interval: at high strains, around 100%, these gels first show strain
 239 hardening and then break (Figure 2a). No strain hardening was observed for pea starch gels (Figure
 240 2b).
 241



242
 243 **Figure 2.** Representative dependences of storage modulus (G' , filled points) and loss modulus (G'' ,
 244 open points) as a function of shear strain at different retrogradation times for (a) potato-11wt%-gels
 245 and (b) pea-5wt%-gels. Dashed lines are showing the determination of the critical strain.

246
 247 The storage modulus in the linear region, G_0' , is plotted vs. retrogradation time in Figure 3a for all
 248 starch gels. Higher starch concentration, denser the network and thus higher G_0' , as expected. For
 249 example, for potato starch gels retrograded for 4 days the G_0' value increases from 141 Pa to 1290 Pa
 250 for starch concentration varying from 8 wt% to 11 wt% (Figure 3a). The increase of retrogradation time
 251 also leads to G_0' increase for both types of starches. Interestingly, G_0' of 5 wt% pea starch gels is
 252 higher than that of potato starch gels of even higher concentrations, 8 wt% and 11 wt% (Figure 3a).
 253 The reason is that pea starch contains higher amount of amylose which is known to retrograde quicker
 254 than amylopectin and forms more crystalline regions (Putaux, Bule, & Chanzy, 2000) as reflected by
 255 higher relative crystallinity (Figure 1b).



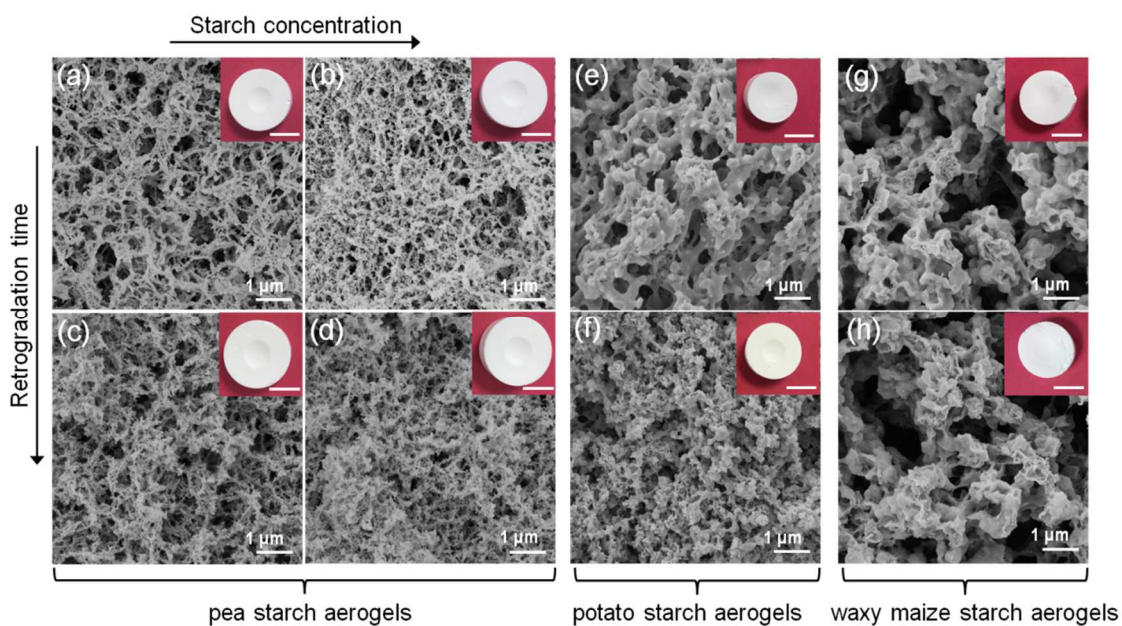
256
 257 **Figure 3.** Storage moduli G_0' (a) and critical strain (b) of pea and potato starch gels as a function of
 258 retrogradation time. Lines are given to guide the eye
 259

260 Critical strain is determined as the value at which gel starts to break, see details in Figure 2a and b.
 261 The critical strain is plotted vs. retrogradation time in Figure 3b for the studied starch gels. Opposite to
 262 the storage modulus G_0' , critical strain decreases with increasing retrogradation time and starch
 263 concentration indicating that gels become more brittle. The most brittle are pea starch gels. Overall,
 264 the mechanical properties of starch gels correlate well with relative crystallinity data: the higher
 265 amylose content and longer retrogradation time, the higher is relative crystallinity and the stronger and
 266 more brittle are starch gels.

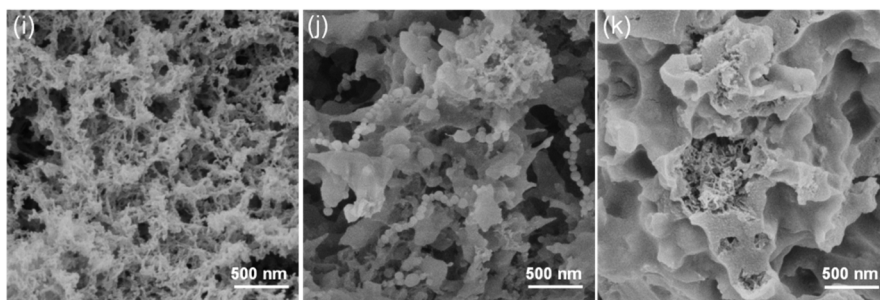
267
 268 **3.3 Starch aerogels: morphology and properties**

269 The examples of the inner morphology of starch aerogels is shown in Figure 4 (a-h for lower
 270 magnification and i-k for higher magnification) for starches of different concentrations and retrograded
 271 at different times. More examples are presented in Figure S2 and S3 of the Supporting Information.
 272 Starch type has a significant influence on aerogel morphology: higher amylose content
 273 (pea>potato>waxy maize), finer and more ramified is aerogel structure. Waxy maize starch aerogels
 274 consist of rather smooth beads assembled together. Waxy maize starch solutions are retrograding
 275 very slowly as they are based on amylopectin and even at long retrogradation times the gels are very
 276 weak. We hypothesize that when these weak gels are placed in a non-solvent (ethanol), phase
 277 separation occurs according to spinodal decomposition mechanism, which is also the case when

278 cellulose is coagulated in a non-solvent directly from solutions resulting in aerogels with morphology
 279 represented by assembled together “hairy” beads (Buchtová & Budtova, 2016; Pircher et al., 2016;
 280 Sescousse, Gavillon, & Budtova, 2011). Interestingly, the surface of the beads in the internal structure
 281 of waxy maize aerogels is smooth but it seems that inside they are porous (Figure 4k). However, this
 282 porosity is observable in the fracture zones only where aerogel was broken for SEM observations.
 283 Potato starch aerogels contain a “mixture” of smooth beads and fine network while pea starch
 284 aerogels’ morphology is represented by a fine network. The strength of a starch gel before the sample
 285 is placed in a non-solvent plays an important role in structure formation: stronger is the gel (case of
 286 pea starch), better it is keeping the ramified network structure. In weak gels (case of waxy maize
 287 starch) amylopectin chains have more freedom to move, they form polymer rich phases upon the
 288 addition of non-solvent and undergo phase separation driven by spinodal decomposition.
 289



290 pea starch aerogels potato starch aerogels waxy maize starch aerogels

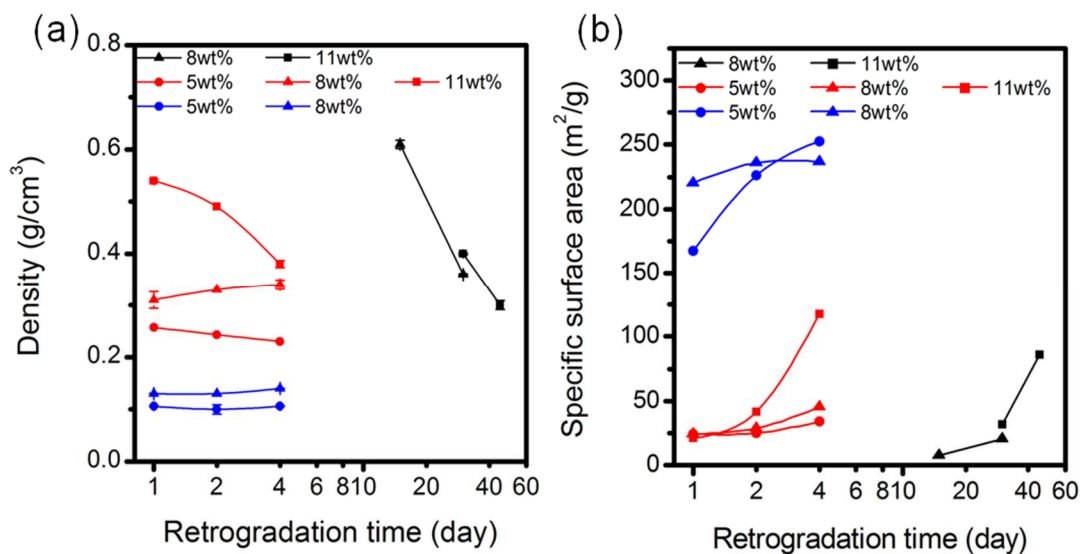


291
 292

293 **Figure 4.** SEM images of aerogels internal morphology, insets are the digital photos of aerogel
294 samples: (a) pea-5wt%-1day-aerogel, (b) pea-8wt%-1day-aerogel, (c) pea-5wt%-4day-aerogel, (d)
295 pea-8wt%-4day-aerogel, (e) potato-11wt%-1day-aerogel, (f) potato-11wt%-4day-aerogel, (g) waxy
296 maize-11wt%-30day-aerogel, (h) waxy maize-11wt%-45day-aerogel, (i) pea-8wt%-4day-aerogel, (j)
297 potato-8wt%-4day-aerogel and (k) waxy maize-8wt%-15day-aerogel. The scale bars for photos of
298 aerogels are 1 cm.

299
300 Higher starch concentration leads to denser network morphology (see Figure 4 for pea starch
301 aerogels), the same was observed for other bio-aerogels (Buchtová et al, 2016; Groult et al, 2018b).
302 Longer retrogradation times induce more ramified structure for amylose-containing starches (Figure 4).

303 Aerogel density and specific surface area are summarized in Fig. 5, and linear shrinkage (eq.1)
304 and porosity (eq. 2) in Figure S4. Pea starch aerogels possess the lowest shrinkage, lowest density
305 and highest porosity at all concentrations and retrogradation times: higher amylose content in starch
306 leads to quicker retrogradation, highest relative crystallinity (Figure 1) and strongest gels (Figure 3a),
307 helping to “resist” solvent exchange and drying. Higher starch concentration, lower shrinkage, as
308 higher polymer concentration also leads to stronger gels. The same phenomenon was observed for
309 cellulose and pectin aerogels (Buchtová et al, 2016; Groult et al, 2018b). Despite lower shrinkage with
310 the increase of starch concentration, density increases (García-González et al., 2013; Ubeyitogullari et
311 al., 2016) and porosity decreases, which shows that added matter “dominates” small gain in volume
312 (Figure 5a and S4b). A strong influence of retrogradation time on shrinkage (Figure S4a), density
313 (Figure 5a) and porosity (Figure S4b) is recorded for waxy maize aerogels as even retrograded for a
314 long time (15 days) the gels are very weak and contract under solvent exchange and drying, as in the
315 case of non-gelled pectin solutions (Groult et al., 2018b).



316
 317 **Figure 5.** Density (a) and specific surface area (b) of starch aerogels from different starch sources and
 318 concentrations as a function of retrogradation time. Black, red, and blue data points correspond to
 319 waxy maize, potato and pea starch aerogels, respectively. Lines are given to guide the eye. If
 320 standard deviations for density values are not visible, they are within the dimension of the symbol.

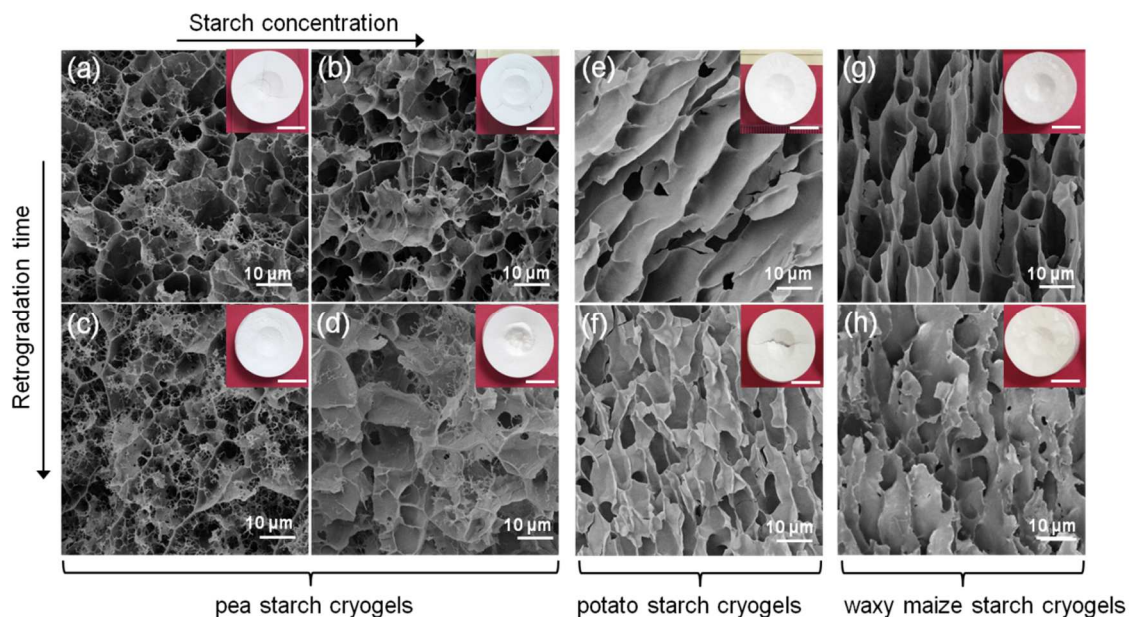
321
 322 Pea starch aerogels show the highest specific surface area among all aerogels studied, from
 323 around 160 - 250 m²/g vs 8 - 120 m²/g for potato and waxy maize aerogels (Figure 5b). Fine and
 324 ramified morphology of pea starch aerogels is the reason of higher specific surface area, see Figure 4.
 325 Specific surface area increases with the increase of starch concentration and of retrogradation time
 326 (Figure 5b). For cellulose aerogels it was suggested that higher polymer concentration leads to the
 327 “division” of pores into smaller ones thus increasing specific surface area (Buchtová et al., 2016); the
 328 same can also be assumed for starch aerogels. Network ramification with retrogradation time shown
 329 by SEM (Figure 4) confirms the increase of specific surface area.

330
 331 **3.4 Starch cryogels: morphology and properties**

332 The morphology of starch cryogels is shown in Figure 6, more SEM images for potato and waxy
 333 maize starch cryogels can be found in Figures S5 and S6, respectively. All cryogels have very large
 334 macropores which are the replica of ice crystals that grew under freezing and “pushed aside” pore
 335 walls of starch gel. The pore sizes, as seen from SEM images, are smaller (especially for pea cryogels)
 336 than those reported in literature for the cases of freeze-dried amylopectin (7 days retrogradation) or

337 non-retrograded potato and corn starch solutions (Nakamatsu et al., 2006; Svagan et al., 2008). As all
 338 our samples were prepared in the same freezing and lyophilization conditions, it is the intrinsic
 339 properties of the starch that influence cryogel morphology. Stronger are the gels, better they “resist” to
 340 the growth of ice crystals. The pores in the retrograded pea starch aerogels do not show any
 341 preferential orientation despite unidirectional freezing; potato starch gels are weaker than pea starch
 342 and pores become oriented, and the weakest gels are those based on waxy maize starch and pores
 343 are strongly oriented in the direction of the growth of ice crystals as in the case of nanocellulose (Chau
 344 et al., 2016). Waxy maize starch is thus suitable for ice templating method. Potato and waxy maize
 345 starch cryogels have smooth pore walls, as in the case of amylopectin freeze-dried starch (Svagan et
 346 al., 2008), while those of pea starch are thinner and the network is more ramified. Pores with smooth
 347 walls and no preferential orientation were observed for cellulose cryogels made via unidirectional or
 348 isotropic freeze drying (Buchtová et al., 2016). In that case the structure of the network of cellulose
 349 coagulated in water resisted the unidirectional growth of ice crystals but resulted in smooth and thick
 350 pore walls and pore’s size smaller than in starch cryogels.

351



352

353 **Figure 6.** SEM images of cryogels' internal morphology, the insets show the digital photos of cryogels:

354 (a) pea-5wt%-1day-cryogel, (b) pea-8wt%-1day-cryogel, (c) pea-5wt%-4day-cryogel, (d) pea-8wt%-
 355 4day-cryogel, (e) potato-8wt%-1day-cryogel, (f) potato-8wt%-4day-cryogel, (g) waxy mazie-8wt%-
 356 30day-cryogel and (h) waxy mazie-8wt%-45day-cryogel. The scale bars for photos of cryogels are 1

357

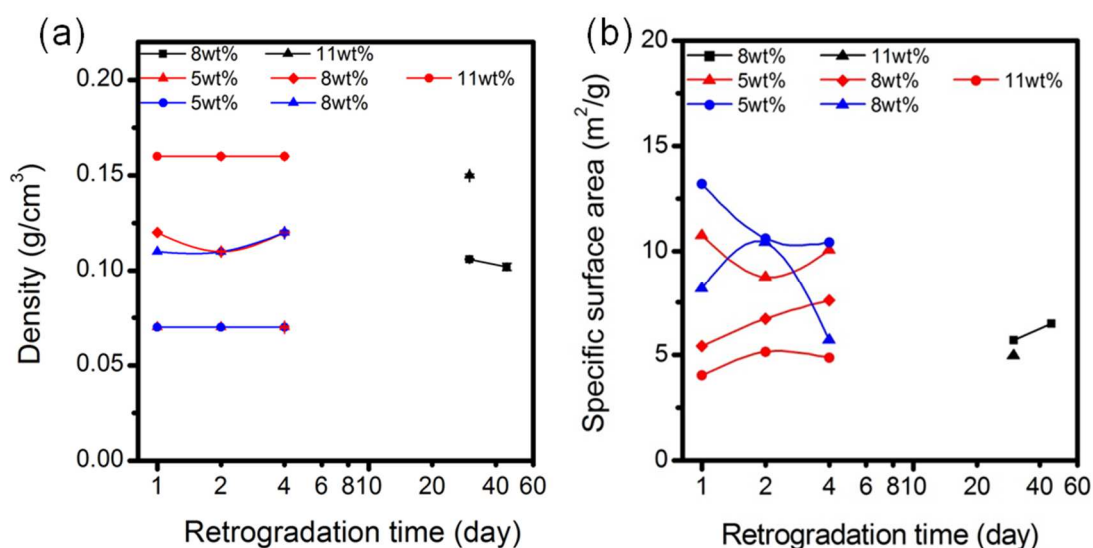
cm.

358

359 The increase of starch concentration seems to slightly decrease pore sizes of cryogels (Figures 6,
360 S5 and S6), however, it is retrogradation time which impacts starch cryogel morphology: the longer
361 retrogradation, the smaller the pores for all types of starches used in this work. The same was
362 observed for all starch aerogels and is the sign of starch network ramification during retrogradation.

363 The density, porosity and specific surface area of all starch cryogels are summarized in Figure 7
364 and S4c as a function of retrogradation time. Practically no shrinkage was observed as no
365 solvent/non-solvent exchange was performed for the preparation of cryogels. All cryogels have very
366 low density, lower than that of aerogels, 0.07 - 0.16 vs 0.1 - 0.6 g/cm³; a similar trend was reported for
367 cellulose aerogels and cryogels (Buchtová et al., 2016). Higher starch concentration led to higher
368 cryogel density and lower porosity, as expected, and no influence of retrogradation time was observed
369 (Figure 7a).

370



371

372 **Figure 7.** (a) Density and (b) specific surface area of cryogels from starch different concentrations and

373 sources as a function of retrogradation time. Black, red, and blue data points correspond to waxy

374 maize, potato, and pea starch cryogels, respectively. The lines are given to guide the eye. When

375 standard deviations for density values are not seen, they are within the dimensions of the symbol.

376

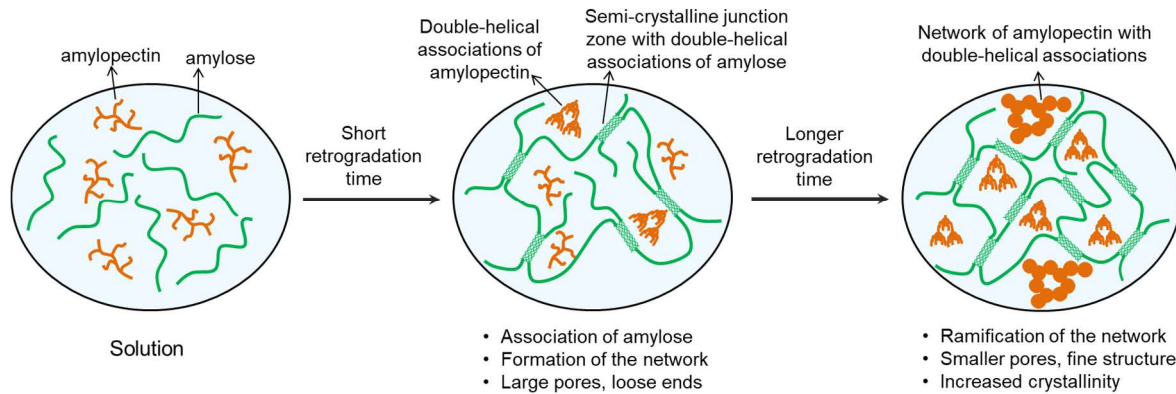
377 The specific surface area of all cryogels (Figure 7b) is very low, within 3 - 13 m²/g, which is at least
378 20-30 times lower than that of aerogels (see Figure 5b). We consider all values obtained for cryogels
379 being similar within experimental errors which is around 10 m²/g. Thus, no trend can be deduced.

380

381 **4. Discussion**

382 Based on all results obtained, we propose the following structure evolution in starch gels resulting
383 in different aerogel morphology and properties. It is known that during retrogradation amylose
384 associates into parallel double helices connected by amorphous regions, the whole forms semi-
385 crystalline clusters (Putaux et al., 2000). We assume that at the early stages of retrogradation,
386 amylose network with large pores, loose ends and junctions based of semi-crystalline clusters is
387 formed (Figure 8); these gels are weak. At this stage, amylopectin starts to slowly associate to form
388 necklace-like nano-structures (Putaux et al., 2000). With the increase of retrogradation time the
389 crystallinity increases and gel becomes stronger: amylose chains, if present, form more and more
390 junction zones leading to network reinforcement and ramification, the latter inducing the increase of
391 specific surface area of aerogels. For the neat amylopectin solutions, it was shown that amylopectin
392 forms a fractal network built of necklace-like structures (Putaux et al., 2000). We suppose that these
393 nano-structures are too weak to resist solvent/non-solvent exchange: they collapse into much larger
394 entities according to spinodal phase separation mechanism. We also hypothesize that with the
395 increase of retrogradation time, amylose does not form longer and/or thicker "zip"-type structures
396 which would lead to the decrease of specific surface area of aerogels. We assume that it is larger
397 number of crystalline junctions that are formed with longer retrogradation resulting in stronger gels with
398 higher crystallinity and more ramified network, the latter reflected by the increase of aerogels specific
399 surface area (Figure 8). Higher amylose content in pea starch amplifies this phenomenon. It may also
400 be possible that the presence of amylopectin in pea and potato starches prevents amylose
401 condensing into thick aggregates, as was found for amylose alone in (Putaux et al., 2000), thus
402 helping network ramification. As at higher amylose content gels are stronger, the shrinkage during
403 aerogels' preparation is lower and the density of aerogels is also lower as compared to that of low-
404 amylose content starch aerogels. The influence of amylose content of aerogel and cryogel density and
405 on aerogel specific surface area is summarized in Figure 9.

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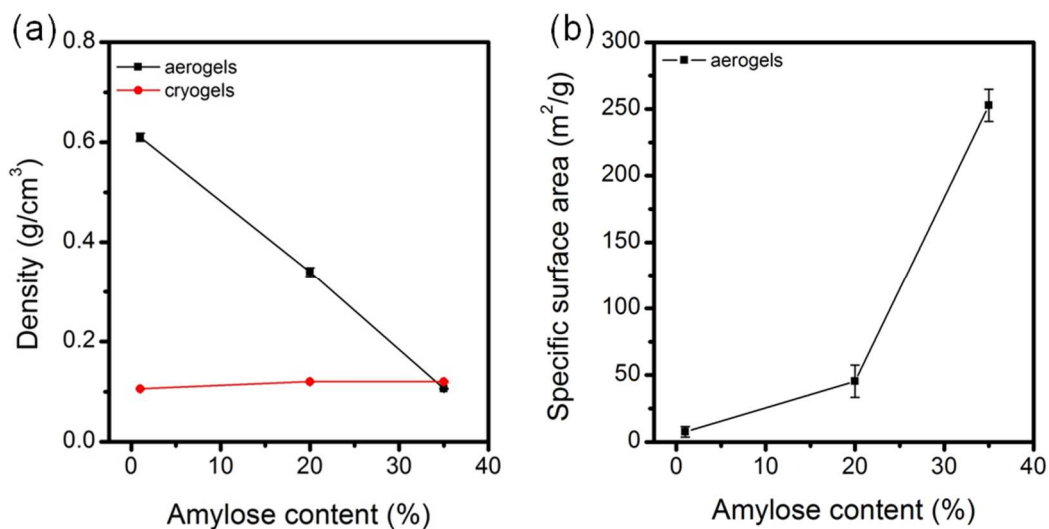
Figure 8. A schematic presentation of structure evolution in starch gels with the increase of

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retrogradation time.

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413

Figure 9. Density (a) and specific surface area (b) of starch aerogels and cryogels from 8 wt%

414

solutions as a function of amylose content. The retrogradation time is 4 days for pea and potato

415

starches and 15 days for waxy maize. The lines are given to guide the eye. When standard deviations

416

values are not seen, they are within the dimensions of the symbol.

417

418

In the view of the said above, a schematic presentation summarizing structure evolution in all

419

samples studied as a function of amylose content, starch concentration and retrogradation time is

420

presented in Figure 10. In all the cases, with the increase of starch concentration and retrogradation

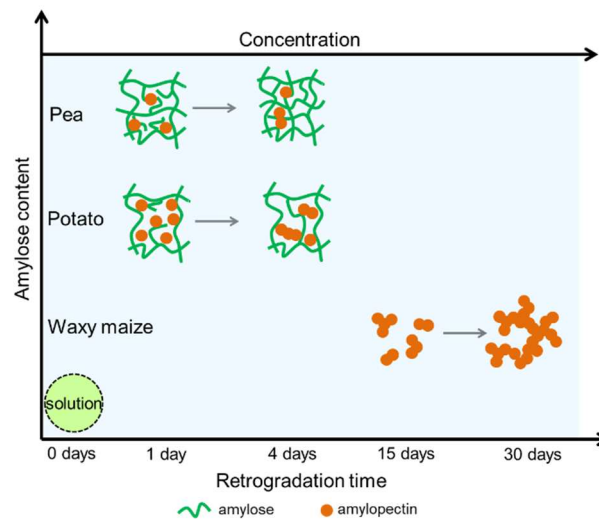
421

time, the specific surface area of aerogels increases as the network becomes more ramified. Pea

422

starch aerogels with the highest amylose content (33~36%) possess the highest specific surface area

423 due to the finest morphology amylose network (see Figure 9b). As pea starch gels are the strongest,
 424 their density is the lowest (Figure 9a). Potato aerogels with 18-21% of amylose present a mixed
 425 morphology of amylopectin beads and amylose network. Their specific surface area is lower than that
 426 of pea starch aerogels, and as the gels are weaker, the density is higher due to stronger shrinkage
 427 (Figure 9a). Waxy maize starch aerogels with only 1 % of amylose have the largest pores with the
 428 lowest specific surface area and the highest density (Figure 9).



429
 430 **Figure 10.** A schematic presentation summarizing structure evolutions in all starch samples, as
 431 deduced from aerogel morphology and properties, as a function of amylose content, starch
 432 concentration and retrogradation time. “0 days” correspond to the initial solution.

433
 434 Various morphologies obtained in porous starches are interesting for different biomedical
 435 applications, in particular as no toxic compounds were used. For example, larger pores are suitable for
 436 making scaffolds for cells growth, and smaller pores are attractive for control release applications.

437
 438 **5. Conclusions**

439 Highly porous starch materials were made from starch gels using two ways of drying: with
 440 supercritical CO₂ and freeze-drying. The influence of starch type (amylose content) and process
 441 parameters (starch concentration and retrogradation time) on the morphology, density and specific
 442 surface area were systematically studied and correlated with relative crystallinity and gel strength.

443 Drying mode had a significant impact on the properties of the dry material: freeze-dried starch gels
 444 had lower density and much lower specific surface area as compared to those of aerogels. This was

445 an expected result as the morphology and properties of materials freeze-dried from water are
446 dominated by the growth of ice crystals. Relative crystallinity dropped significantly after starch
447 dissolution-retrogradation-drying but was not influenced by the drying mode.

448 As drying with supercritical CO₂ is well preserving the morphology of starch gels, amylose content
449 and retrogradation time were playing an important role in the understanding of aerogel properties.
450 Higher amylose content, higher relative crystallinity and gel strength, lower sample shrinkage and
451 lower aerogel density. Different ways of amylose and amylopectin chains reorganization during
452 retrogradation and their different gelation kinetics is evidenced by the evolution of aerogel specific
453 surface area: pea starch aerogels possess the highest surface area, followed by potato and then by
454 waxy maize starch. With retrogradation, amylose forms more and more fine network leading to the
455 increase of aerogel surface area. At very long retrogradation times amylopectin (waxy maize starch) is
456 also gelling, but the gels are weak and shrinking during processing, resulting in higher density and
457 lower specific surface area.

458 The results obtained offer a new way of investigation of starch gels properties using drying with
459 supercritical CO₂. Opposite to freeze-dried starch, aerogels reflect the structure and its evolution in
460 starch gels; this mode of drying can thus be used as a tool for a deeper understanding of the structure
461 and properties of starch gels.

462

463 **Acknowledgments**

464 Authors are grateful to Roquette for sponsoring the work and providing starches, to Pierre Ilbizian
465 (PERSEE, MINES ParisTech) for the supercritical CO₂ drying, to Suzanne Jacomet (CEMEF, MINES
466 ParisTech) for the help with SEM and to Gabriel Monge (CEMEF, MINES ParisTech) for performing
467 XRD.

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