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(Article begins on next page)

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## **Freeze-drying and cultural heritage: An overview**

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1 **Abstract**

2

3 This paper aims to provide an overview of the application of freeze-drying in the field of  
4 cultural heritage. The possibility of removing water from an object at low temperature, thus  
5 preserving most of its specific features that would be lost at high temperature, makes the process  
6 the elective choice when restoring water damaged materials. Flooded archives and libraries,  
7 due to natural events or to the intervention of fire extinguishing systems, are a typical case  
8 where freeze-drying can be applied since the material, after flooding, is brought to a low  
9 temperature as soon as possible to cause the water to freeze and, therefore, to block the  
10 degradation of the material. Besides, freeze-drying may be used to preserve archaeological  
11 finds, *e.g.* waterlogged woods or wet textiles, by means of water removal. Anyway, in all these  
12 cases specific issues must be faced, aiming to preserve the mechanical properties of the object,  
13 and this may require ad hoc pre-treatments. It must be highlighted that in some cases the  
14 occurrence of a freeze-drying process, in particular of the atmospheric freeze-drying process,  
15 is undesired for the preservation of finds, and this must be carefully managed. Finally, the  
16 possibility of using freeze-drying to create works of art will also be addressed.

17

18

19 **Keywords**

20

21 Freeze-drying, paper drying, microbial contamination, waterlogged wood, historical textiles,  
22 atmospheric freeze-drying.

## 23 1. Introduction

24

25 Freeze-drying, also known as lyophilization, is considered a gentle drying process as water is  
26 removed as vapor phase from a product previously frozen. Therefore, the first step of the freeze-  
27 drying process involves lowering the temperature of the object to be dried, in such a way the  
28 liquid water inside turns into ice. Actually, not all the water freezes, but some of it remains  
29 bounded to products molecules (and, thus, it is called “bound water”). Usually, in this step the  
30 product has to reach a temperature of -40/-50°C, and this may be accomplished either in the  
31 freeze-dryer or in a separate equipment. After freezing, the pressure in the environment  
32 surrounding the object to be dried is lowered to a value that may reach up to 5 Pa (or even less)  
33 to promote ice sublimation. At the same time, energy must be supplied to the object as ice  
34 sublimation is a process that needs energy. This is usually accomplished by increasing the  
35 temperature of the shelf on which the object to be dried is placed, thus transferring heat through  
36 conduction, in the points of contact, by radiation, due to the temperature difference between the  
37 shelf and the object, and by conduction in the layer of gas between the object and the shelf.  
38 Also, radiation from chamber walls may play a role in this phenomenon. This step is known as  
39 primary drying, as it is followed by what is called secondary drying, in which the desorption of  
40 the bound water is accomplished, till the target value of final water content is reached. This  
41 result is generally obtained by further increasing the temperature of the heating surface.

42 The books by Mellor<sup>[1]</sup>, Jennings<sup>[2]</sup>, Oetjen and Haseley<sup>[3]</sup>, Costantino and Pikal<sup>[4]</sup> and  
43 Franks<sup>[5]</sup> may be considered the cornerstones in the field, addressing several issues related to  
44 the process, *e.g.* the features of the equipment and the effect of the operating conditions on the  
45 final product characteristics and on the time needed to get the product dried.

46 The role of the freezing stage is particularly important as the temperature decrease velocity  
47 may affect the size of the ice crystals and this has an impact on both the drying rate and on the

48 final product features. Small ice crystals may be helpful in preserving the original material  
49 shape, but the porous structure arising from the ice removal will present low diameter holes (as  
50 the holes correspond to the ice crystals), thus decreasing the permeability to vapor flux and  
51 resulting in a longer drying time.

52 Also, primary and secondary drying stages affect in a non-negligible way process and  
53 product features. The temperature of the heating shelf and the pressure in the drying chamber  
54 may, in fact, result in different values of temperature of the object, which may affect its  
55 properties and the duration of the overall process. In fact, higher temperatures result in faster  
56 drying, due to the higher water vapor pressure, but they may be responsible for the loss of  
57 mechanical properties (in case of soft dried materials, with low mechanical properties, the  
58 collapse of the porous structure may occur) or of other properties.

59 Monitoring the primary drying, that is the most critical step of the overall process in this  
60 framework, is a must to check if desired features are obtained in the final product. When  
61 referring to pharmaceutical products, usually formulated as aqueous solutions either poured  
62 into vials or processed as bulky product filling trays, the goal is usually to maintain product  
63 temperature below a value corresponding to the collapse temperature of the dried product, in  
64 order to maintain its elegance and the possibility of a fast reconstitution.<sup>[6]</sup> This requires the use  
65 of temperature probes as well as of *ad hoc* designed process analytical technologies.<sup>[7],[8]</sup> The  
66 other key issue is the detection of the ending point, thus avoiding any extra time in the process  
67 or extracting the product from the dryer before the completion of ice sublimation<sup>[9]</sup> In case of  
68 food products, also nutritional properties have to be accounted for, as product temperature in  
69 the process may affect, *e.g.*, the total antioxidant capacity, the phenolic content, the amount of  
70 ascorbic acid and others.<sup>[10]</sup> Less attention was paid to the secondary drying stage, due to the  
71 very low value of humidity, although the role of the operating conditions on the duration of this  
72 stage and on final humidity was also assessed.<sup>[11],[12]</sup>

73 The operating conditions resulting in the desired final product features may be found either  
74 by experimental investigation, possibly using a suitable design of experiments, or by  
75 mathematical modeling and *in silico* simulation of the process. In the first case, several  
76 experiments are needed to assess the effect of shelf temperature and chamber pressure on  
77 product temperature, drying duration and on some product features, *e.g.* residual moisture. This  
78 approach is certainly time consuming, and needs the availability of non-negligible amounts of  
79 materials to be dried, that may be an issue in the case of pharmaceutical products. As an  
80 alternative, it is possible to minimize the experimental effort by carrying out *ad hoc* experiments  
81 to get the parameters through which it is possible to model the heat transfer from the equipment  
82 to the product inside the drying chamber and the mass transfer from the product to the drying  
83 chamber. The following *in silico* simulation of the evolution of the temperature and of the  
84 residual humidity in the object during the freeze-drying process allows getting the best  
85 operating conditions that minimize the drying time beside preserving final product quality. Both  
86 simplified models<sup>[13],[14]</sup>, where a specific geometry of the moving interface between the frozen  
87 and the dried layers is assumed, and detailed multi-dimensional models<sup>[15]</sup> were proposed to  
88 this purpose, showing that they could be used to calculate off line the design space of the  
89 primary<sup>[16],[17]</sup> and of the secondary drying<sup>[12],[18]</sup> stages, *i.e.* the set of operating conditions that  
90 allows obtaining the target features in the final product.

91 Besides the operating conditions, in the case of pharmaceutical products the possibility of  
92 modifying the composition of the product to be freeze-dried was also investigated, considering,  
93 among the others, the addition of excipients like bulking agents, surfactants and others. By this  
94 way it is possible to modify the limit temperature of the product in the drying stages, as well as  
95 the structure of the dried product, and this may result in a different drying rate and final  
96 properties.<sup>[19]</sup>

97 Great attention was paid in the past to freeze-drying of pharmaceuticals and of food

98 products, where drying at low temperature, through ice sublimation, makes the process the  
99 elective choice to preserve the quality features of the product. For similar reasons this technique  
100 was successfully applied also in the cultural heritage field, mainly for the purpose of recovering  
101 water damaged materials, *e.g.* books and archival materials as well as archaeological finds like  
102 waterlogged woods or wet textiles. In all these cases the material to be treated must be frozen  
103 as soon as possible, to block the degradation due to contact with liquid water, before being dried  
104 through ice sublimation. While in the case of books and archival materials this process may be  
105 carried out directly on the original objects, in case of waterlogged wood or wet textiles some  
106 pre-treatments are needed in order to prevent further damage due to the drying process and  
107 preserve their mechanical properties. The aim of this paper is thus to provide an overview of  
108 the literature in this field, not as broad as that for the pharma and food sectors, to point out the  
109 common features and the specific issues of each case study, and the open questions for research.  
110 Finally, two peculiar case studies will be discussed, namely the use of freeze-drying to create  
111 works of art and the freeze-drying of frozen stored materials, *e.g.* iceman mummies, where the  
112 occurrence of (atmospheric) freeze-drying is undesired.

113

114

## 115 **2. Freeze-drying of flooded papers and archival materials**

116

117 Flooding of archives and libraries is, unfortunately, a common event. This may be due to natural  
118 events as well as to the intervention of fire extinguishing systems or to breakdowns of fire-  
119 fighting systems or of plumbing systems in the building where the archive or the library is  
120 located. Water seriously damages the books and the archival materials: it causes mechanical  
121 distortions, due to swelling and adhesion of leaves, it may seriously corrupt the content of the  
122 archival material, through inks and dyes migration and solubilization of water-soluble

123 components, and it may promote microbiological infections. As a consequence, it is necessary  
124 to stop this sequence of events as soon as possible, and freezing of water is the elective choice  
125 as an emergency intervention.<sup>[20]</sup> After that, the traditional approach consists of defrosting the  
126 material and drying it manually, with the action of air at a moderate temperature<sup>[21]</sup> and, in some  
127 cases, by interleaving it with absorbent paper. This way of working, although it allows us to  
128 obtain a high-quality product, requires a lot of time and, therefore, is expensive. Furthermore,  
129 the time that elapses between the accident and the moment in which the material is again  
130 available to the user can be particularly high. This motivated the study of alternative drying  
131 methods, *e.g.* vacuum drying<sup>[22]</sup>, microwave drying<sup>[23]</sup>, freeze-drying<sup>[24]-[26]</sup>, allowing for a  
132 faster ice removal. In all cases it must be highlighted that no drying techniques may repair the  
133 damage caused by water in the time interval between the accident and the moment in which  
134 water is turned into ice. The goal of the drying process is “just” to remove water as soon as  
135 possible without causing additional damages.<sup>[27]</sup> In this framework a pivotal role may be played  
136 by freeze-drying mainly due to the low operating temperatures. One of the first studies in the  
137 field are those by Flink and Hoyer<sup>[28]</sup> and Flink<sup>[29]</sup> who used freeze-drying to dry the  
138 Kleinschmidt collection, damaged after a fire extinguished in the Greenland Regional Library  
139 in Godthab in 1968. They evidenced the excellent results obtained for paper documents: each  
140 page separated easily and in no case did the ink run, while in case of a photograph album the  
141 prints tended to stick together after drying, mainly due to their chemical composition. Ref. [30]  
142 presents the Records and Archives Management Programme (RAMP) study, presenting a broad  
143 summary of data about freeze-drying of water-soaked paper, comparing the vacuum freeze-  
144 drying (a dry process) and the vacuum drying by evaporation (a wet process), and providing  
145 useful guidelines for practitioners in the field. More recently Capolongo and Barresi<sup>[31]</sup>  
146 investigated the use of freeze-drying to recover water-damaged library materials comparing the  
147 results obtained in case of standard freezing and in case of evaporative freezing. In the first

148 case water freezing was carried out in a conventional freezer, at  $-20^{\circ}\text{C}$ , while in the second case  
149 water freezing was the result of the sudden pressure decrease: it caused the evaporation of part  
150 of the liquid water, resulting, at the same time, in the decrease of product temperature and,  
151 finally, in water freezing. At first, they set up a method to produce reproducible water-soaked  
152 samples, moving from the method of Fisher<sup>[32]</sup>, and then pointed out that no excessive damages  
153 occurred in samples for both freezing methods. Unfortunately, over-drying could not be avoided,  
154 and re-conditioning of brittle and rigid paper was recommended to avoid potential mechanical  
155 damage.

156 Model-based *in silico* simulation of the process was proposed by Carapelle et al.<sup>[33]</sup>: the  
157 main hypothesis at the basis of this simple phenomenological model are the followings:

- 158 - the ice core has the same shape of the object and retains the same proportions as drying  
159 goes on, always remaining in the centre of the object;
- 160 - the heat flux transferred from the equipment to the product is directly proportional to  
161 the area of the ice core facing the heating shelf, and inversely proportional to the  
162 distance between the heating shelf and the core;
- 163 - the paper thermal conductivity is proportional to its density.

164 This model was validated by Crespi et al.<sup>[34]</sup>, taking advantage of an innovative procedure to  
165 visualize the progress of ice sublimation, and used by the same authors for process optimization.

166 Despite the freeze-drying process may be considered a “soft” drying treatment, the final  
167 characteristics of the treated paper and archival material should be a concern. Carlsen<sup>[35]</sup>  
168 compared some properties of paper before and after a freeze-drying treatment for three case  
169 studies, namely a 75% groundwood-25% softwood cellulose based paper, a 100% cotton based  
170 paper and a 100% chemical cellulose based (coated) paper. Three treatments were considered:  
171 air drying and freeze-drying at two different drying temperatures, identified as cold and warm.  
172 Residual moisture, alkali reserve, tearing resistance and folding endurance are the properties

173 considered, in the presence or absence of an accelerated aging step (12 days at 90°C and 50%  
174 relative moisture). Results evidence that each treatment considered in the study affects the  
175 properties of the paper in different ways according to the type of paper. Mechanical strength,  
176 in particular folding endurance, and ageing stability of groundwood paper and coated paper are  
177 affected by the freeze-drying process, while the effect is negligible in case of cotton paper,  
178 whereas fibre-fibre bonds are usually strengthened by freeze-drying, particularly in coated  
179 paper after accelerated drying. Results evidence also that freeze-drying at low temperature  
180 allows maintaining flatter the paper after the treatment, while there are not clear indications  
181 about which operating conditions affect the most ageing stability and mechanical strength.

182 More recently, Fissore et al.<sup>[36]</sup> considered the freeze-drying of several types of archival  
183 materials: paper sheets dating back to the autarchic period were selected, as they are of poor  
184 quality, coated paper, membranous material dating back to various periods, and sheets in  
185 colored folders. The presence of a 16th century linen paste cardboard and fragments of 14th-  
186 16th century parchment completed the experimental panorama. In all cases the freeze-drying  
187 process appeared to be able to dry the object without causing additional damages, although the  
188 case of parchments and membranous materials needs to be more deeply investigated, to address  
189 the interaction of the liquid water with the material, and the associated damages, that cannot  
190 (obviously) be restored by the freeze-drying process.

191 When considering the drying of flooded paper and archival materials there is an additional  
192 issue that must be faced. In fact, cellulose may represent a carbon source for several microbial  
193 classes (*i.e.* bacteria, yeasts and filamentous fungi), resulting in a high bioreceptivity, *i.e.* the  
194 possibility for a material to be colonized by living organisms. Besides, also other paper  
195 components (*e.g.* glue) may be used by microorganisms in their metabolic activity.<sup>[37]</sup> The  
196 occurrence of floodings may be responsible for a combination of temperature and relative  
197 humidity values in the environment that result in a value of water activity suitable for microbial

198 growth, thus enhancing the biodeterioration of paper documents.<sup>[38]</sup>

199 Besides stopping further damage related to the contact between the water and the paper or  
200 archival material, freezing may be effective also to immediately stop microorganism growth  
201 after flooding and freeze-drying appears that to be most obvious treatment to be used for water  
202 removal. Nevertheless, one of the open questions concerning the use of freeze-drying for the  
203 recovery of flooded material is its poor outcome on the microorganism viability. Studies on the  
204 effect of freezing report that increasing the freezing time, or decreasing the freezing rate, can  
205 reduce the viable microbial population.<sup>[39]</sup>

206 Few reports describe the effect of freeze-drying on paper-biodeteriogens; among the other,  
207 it was shown that, concerning filamentous fungi, spores are more resistant than hyphae.<sup>[40],[41]</sup>  
208 In previous works<sup>[38],[42]</sup> the effect of both freezing and lyophilization on different classes of  
209 paper-born microorganism was reported. Modern paper was used, and flooding was simulated  
210 by soaking blocks of paper for 18 hours in distilled water, followed by draining.  
211 Microorganisms were inoculated on a sterile single paper sheet, which was then placed at the  
212 surface or in the middle of the paper block. At first, the role of the freezing stage on  
213 microorganism viability was assessed by carrying out this step in a domestic freezer, with a  
214 final temperature of -20°C, or directly in the freeze-dryer, where a higher temperature decrease  
215 rate could be obtained, about 1°C/min, with a final temperature of -40°C. After the completion  
216 of water freezing, samples were thawed at room temperature, for about 4 hours, in sealed glass  
217 containers and then contaminated sheets were recovered and placed on a suitable agar medium  
218 and incubated at their optimal growth temperature. In order to assess the effect of the freeze-  
219 drying stage, some of the samples, after freezing, were not thawed, but were placed in a pilot-  
220 scale freeze-dryer, where ice sublimation was obtained at 200 µbar and 0°C. In all cases, the  
221 evolution in time of Colony Forming Unit (CFU) for unicellular microorganism and the mean  
222 colony diameter of the pluricellular microorganisms after freezing and thawing or after freeze-

223 drying was compared with that obtained in case no freeze-tawing or no freeze-drying was  
224 carried out. As an example of bacterium and of yeast, *Staphylococcus epidermidis* and  
225 *Rhodotorula mucilaginosa*, respectively, were considered in this study, and no relevant effect  
226 of both freezing and freeze-drying was observed with respect to the untreated microorganisms.  
227 The study also involved *Alternaria alternata*, a biodeteriogen filamentous fungus, and it was  
228 pointed out that a 48 hours of growth inhibition, followed by a lower growth rate, could be  
229 achieved when freeze-drying was carried out at 0°C.

230 Considering the high contribution of filamentous fungi on paper biodeterioration, an  
231 extensive study was then performed on the effect of freezing and of freeze-drying on *A.*  
232 *alternata*. For both cases, contaminated paper sheets, at different growth times (*i.e.* sporified or  
233 not-sporified colonies), were positioned on the surface (S) and in the middle (M) of the soaked  
234 paper blocks, simulating a flooded contaminated book. The blocks were frozen, in a domestic  
235 freezer at -20°C and then kept there for 13 days. After that, they were thawed, for 3 hours at  
236 25°C, and then incubated on Czapeck (CZ) agar at the optimal growth temperature of the  
237 selected microorganism (30°C). The evolution of the mean diameter of the fungal colonies was  
238 then measured and compared to that on an untreated control, *i.e.* an inoculated sample where  
239 not freezing and thawing was carried out, aiming to evidence the effect of the freezing stage on  
240 the microorganism viability. As an example, the results for not-sporified colonies at different  
241 time instants are reported in Figure 1. Diameter of the colonies are smaller, after freeze-thawing,  
242 with respect to the control sheet and for both positions (S and M) aerial mycelium was observed,  
243 more abundant on the surface sheet, along with the absence of sporification. From the results  
244 obtained, it is possible to affirm that *A. alternata* not-sporified is more sensitive than sporified  
245 one to freezing.

246 After the freezing, the frozen samples were freeze-dried in a pilot-scale freeze-drier at 200  
247 µbar at 0°C and 20°C in order to evaluate the influence of the drying temperature on process

248 duration and on the microorganism viability. The ending point of the primary drying stage, as  
249 evaluated through the midpoint of the pressure *vs.* time curve measured by the  
250 thermoconductive gauge<sup>[43]</sup>, appears to be lowered from 17 h to 12 h when the temperature of  
251 the heating shelf during primary drying was 20°C instead of 0°C. In both cases, at the end of  
252 the drying phase, contaminated sheets were recovered and reconditioned in sterile conditions,  
253 positioned on CZ agar plates, and incubated at 30°C. The obtained results, related to both not-  
254 sporified and sporified colonies of *A. alternata* are reported in Figure 2 and 3 respectively, and  
255 compared with the untreated control sheet. The drying, conducted at 0°C, caused a growth  
256 inhibition of not-sporified *A. alternata* up to 48 hours (Figure 2), in both the positions of the  
257 contaminated sheets. After that, at 96 hours of incubation, the colony diameter of the dried  
258 mycelium was significantly lower than that of the control. Differently from the freezing (*see*  
259 Figure 1), the influence of drying on fungal metabolism was evident as mycelium sporification  
260 appeared and at 96 hours the colonies were completely covered with spores. In the presence of  
261 sporified *A. alternata* sheets (Figure 3), a lag phase of 48 hours was also observed, following  
262 which the growth started again with a rate comparable to that of the control, regardless of the  
263 position of the contaminated sheets. Also, in this case at 96 hours it is possible to observe the  
264 growth of new mycelium on sporified colonies. Thus, a strong influence of the freeze-drying  
265 process, independently from the colonies age, is evident.

266

267

### 268 **3. Freeze-drying of waterlogged wood**

269

270 A particularly challenging case study is represented by the freeze-drying of waterlogged  
271 archaeological wood. This is due to the fact that wood is a hygroscopic and porous material,  
272 where the microstructure is closely related, among the others, to moisture distribution.<sup>[44],[45]</sup>

273 During drying we may observe, as a consequence of the removal of water, the formation of new  
274 hydrogen bonds between the cell walls polymers and this results in a certain degree of shrinking.  
275 Moreover, in case of waterlogged wood, also the degradation of the cell walls due to contact  
276 with water has to be accounted for.<sup>[46],[47]</sup> In fact, internal mechanical stresses may arise due to  
277 the fact that the shrinkage of the external, dried, layer may be hampered by the inner part of the  
278 object, that remains saturated. If these stresses overcome the mechanical resistance of the  
279 material, cracks may appear. Besides, as the cell walls are degraded due to contact with water,  
280 they may collapse due to capillary forces and to the effect of the high surface tension of liquid  
281 water.<sup>[48]-[51]</sup> In this framework it should be clear that the dimensional stabilization is a key issue  
282 when drying waterlogged wood. This may be obtained by full impregnation of the object to be  
283 dried with water-soluble agents, in case of freeze-drying, or by replacing water with a low  
284 surface tension solvent, in case of air drying.<sup>[52]-[55]</sup>

285 Broda et al.<sup>[56]</sup> considered drying of waterlogged archaeological elm coming from a  
286 medieval bridge found at the bottom of a lake in Poland. Several drying treatment were  
287 considered in the study, namely (i) air drying (at room temperature, for two weeks), (ii) oven  
288 drying (at 50°C or 104°C, for one week), (iii) freeze-drying, without any pre-treatment, (iv)  
289 CO<sub>2</sub> supercritical drying and (v) air drying after dehydration with 96% ethanol (4 weeks)  
290 followed by acetone exchange (3 days). They evidenced that freeze-drying, and CO<sub>2</sub>  
291 supercritical drying, were the best method to stabilize wood dimensions, with a shrinkage lower  
292 than 10%. This was the result of the higher value of wall cell area, and of the fact that freeze-  
293 dried and critical point dried materials retain the typical open porous structure of wood. Besides,  
294 freeze-drying, as well as CO<sub>2</sub> supercritical drying, removes the effect of surface tension, thus  
295 minimizing shrinkage.<sup>[53],[57],[55],[58],[59]</sup>

296 Gregory et al.<sup>[60]</sup> compared several drying treatments for wood shipwrecks in marine  
297 environments. They investigated both (i) conservation methods, involving the impregnation

298 with water soluble molecules, *e.g.* PEG or sugars, that replace the free water and then solidify  
299 at room temperature, fixing the structure of the cells and preventing shrinkage or collapse, and  
300 (ii) the use of controlled air drying or freeze-drying to remove the liquid phase after  
301 impregnation. In case the molecular mass of the impregnating agent is larger than 6-800 g/mol,  
302 it can penetrate only the lumen of the cells and, thus, it acts as a void filling agent that prevents  
303 the collapse of the structure. Lower molecular weight agents (*e.g.* sugars like sucrose, mannitol,  
304 sorbitol and lactitol, or low molecular weight PEG<sup>[61]</sup>) may enter the cell walls, replacing bound  
305 water and preventing shrinkage. Each of these molecules has its own advantages and  
306 disadvantages. PEG impregnation is a non-expensive, robust and non-toxic method, which can  
307 be used on wood in all states of conservation; it produces heavy objects, show surface generally  
308 needs to be treated to recover the original appearance. Besides, PEG is corrosive and, thus, it  
309 should not be used in presence of metallic elements. Sucrose and lactitol are inexpensive as  
310 well, and their low molecular weight allows good penetration in the wood. They are not  
311 corrosive, but they may promote microbial attack both in the treatment and conservation stages,  
312 and, in case of lactitol, the formation of the trihydrate crystals must be avoided as the volumetric  
313 expansion will result in the destruction of the material.

314 Air drying is a very cheap method, but the final result may be highly variable, and severe  
315 cracks or shrinkage may occur as a consequence of the drying conditions. On the other hand,  
316 in the freeze-drying process no liquid phase exists and, thus, capillary forces are avoided.  
317 Anyway, freeze-drying of the original object is not recommended as when the free water in the  
318 cell lumen turns into ice, the expansion may result in cracks in the objects. Besides, shrinkage  
319 or cracks may appear when the bound water is removed from the cells wall. Therefore, beside  
320 carrying out the freeze-drying process it is recommended the pre-impregnation of the object  
321 with a solution containing 35-45% of the selected agent, with a slightly higher value in case of  
322 PEG, 0.55g/g.<sup>[62]</sup> Lowering of the freezing point has to be considered in this framework and,

323 thus, the use of PEG with a molecular weight higher than 1500 g/mol is suggested as the eutectic  
324 temperature, in this case, is about -23°C.<sup>[63]</sup> Moreover, PEG with a smaller molecular weight  
325 are not solid at room temperature. The process allows retaining in an excellent way the quality  
326 features of the original object, although drying time may be very long, as shown also by other  
327 authors, *e.g.* Stelzner et al.<sup>[64]</sup>. In some cases a mixture of low and high molecular PEG solutions  
328 was proposed, taking advantage of their different role.<sup>[65]</sup>

329 The possibility of using sugar solution instead of PEG was investigated by several authors.  
330 Babinski et al.<sup>[66]</sup> used aqueous mixtures of lactitol and threalose and of mannitol and threalose  
331 as impregnating agents before freeze-drying. They pointed out that even at a low uptake (about  
332 10%) of these mixtures (they considered a ratio of 9:1 w/w lactitol and threalose, and a 9:1 w/w  
333 mannitol and threalose) a significative reduction of shrinkage could be achieved after drying.  
334 Moreover, when stored at room temperature (18°C) in a humid environment (50% relative  
335 humidity) the wood treated with the mannitol-threalose mixture absorbs less humidity than that  
336 treated with the lactitol-threalose mixture.

337 Similarly, mannitol, sorbitol and threalose were investigated by Jones et al.<sup>[67]</sup> as an  
338 alternative to PEG. The most remarkable anti-shrinkage effect was obtained by using a 20%  
339 sorbitol solution in the pre-impregnation step.

340 Low molecular weight chitosan, medium molecular weight alginate and cellulose  
341 nanocrystals were studied by Walsh-Korb et al.<sup>[68]</sup>, evidencing that alginate, with chitosan,  
342 appear to be suitable molecules for consolidation of archaeological wood, even when the  
343 cellulosic component is highly reduced. In fact, after freeze-drying alginate was able to find a  
344 network structure promoting the dimensional stability of the sample, differently from the other  
345 molecules considered in the study, suggesting that the resistance of alginate to the effect of  
346 sugars and polymers in the sample is higher.

347

348

#### 349 **4. Freeze-drying of historical wet textiles**

350

351 A particular case study in the field of drying waterlogged archaeological finds is represented  
352 by textile materials. In this case pre-treatment is usually required, aiming to clean the material  
353 before drying, and the medium used to rinse it, as the method used to rinse it, plays a key role  
354 in the final quality, besides, obviously, the type of drying method used. Telleman and coworkers  
355 carried out an extensive investigation to optimize the recovery of historical wet textiles coming  
356 from a shipwreck off Texel, in Netherlands.<sup>[69]</sup> This was a quite unique find of textiles as,  
357 usually, textiles do not survive in seawater, and when they do, these finds consists often of small  
358 fragments, seldom of silk. This motivated the experimental investigation shown in Ref. [69],  
359 where four different rinsing medium were tested, namely (1) soft tap water (as it is highly  
360 available, at low cost), (2) deionized water (purer than tap water), (3) seawater (as the fragments  
361 survived for a long time in it), and (4) a mixture of water and ethanol (30-70%, aiming to  
362 prevent further mould damages). For each medium, three rinsing method were tested, trying to  
363 reduce mechanical stress to the minimum: (i) the textiles were submerged in the liquid bath,  
364 some centimeters deep, or (ii) they were supported on a rigid plate and rinsing was obtained  
365 through a gentle stream of liquid obtained from a squeezable bottle, or, as a further alternative,  
366 (iii) the textiles were placed on a rigid support, inclined at a certain angle, and they were put  
367 into contact with a sponge soaked with the liquid. Drying was conducted in four different ways:

- 368 - using air at room temperature (21°C), with humidity at 51%;
- 369 - accelerating the speed of the air drying by putting the textiles in contact with an  
370 absorbent material;
- 371 - reducing the speed of the air-drying treatment by using an air stream at higher moisture  
372 levels (80-85%);

373 - carrying out a freeze-drying process. In this case freezing was carried out in a separate  
374 equipment, at -25°C, and drying was carried out at room temperature and 2 mbar, till  
375 total ice sublimation.

376 As far as the rinsing treatment is considered, the authors evidenced that the best treatment, *i.e.*  
377 the one consisting of a fine stream of water, is also that responsible for the greatest loss of  
378 material, due to mechanical action. As far as the drying method is concerned, at micro-level it  
379 seems that the four methods are equivalent, but the freeze-dried textiles remained more flexible,  
380 less crumpled and distorted, with respect to those air dried. Such results may be strictly related  
381 to the specific features of the materials treated in this study and, in particular, to their highly  
382 degraded fibers and, thus, further studies are needed to get more general conclusions.

383

384

## 385 **5. The use of freeze-drying to create artworks**

386

387 In the field of cultural heritage, a peculiar case study is represented by the use of freeze-drying  
388 to create artworks. In this case, the low temperature drying process is used to remove water  
389 from an otherwise perishable material. The most representative work in this field is probably  
390 the “*Imitatio Christi*” by the Italian artist Roberto Cuoghi.<sup>[70]</sup> He was invited to present his work  
391 at the Italian Pavilion in the 57th International Art Exhibition of Venice and, as the title of this  
392 masterpiece suggests, he was inspired by the 15<sup>th</sup> century treatise “*De Imitatione Christi*” and,  
393 in particular, by the chapter focused on the transitory nature of the human conditions. The  
394 “*Imitatio Christi*” is thus a journey to seek the true face and body of Jesus Christ after death.  
395 The artist prepared the bodies with agar-agar and then they were subjected to a decomposition  
396 process, like if they were human bodies, by yeasts and molds present in the environment.  
397 Finally, in order to stop this degradation process, these materials were firstly dried through

398 contact with natron, a sodium carbonate already used by the Egyptians in the mummification  
399 processes, and finally by freeze-drying, to (almost) completely remove the water. By this way  
400 it was possible to preserve the original shape of the objects, provided that the operating  
401 conditions of the freeze-drying process were selected. No specific pieces of information are  
402 available on the freeze-drying process used in this case, but it may be supposed that very  
403 cautious operating conditions were used, to avoid collapse of the agar-agar based objects,  
404 despite the very long drying time that could have resulted from this choice.

405

406

## 407 **6. Avoiding freeze-drying in the conservation of frozen stored materials**

408

409 The last section of this paper is devoted to another peculiar case study, always related to the  
410 freeze-drying process, but in which the occurrence of freeze-drying is undesired. You might  
411 think that freeze-drying is possible only at very low pressure, but actually, it may occur in a  
412 frozen object even at atmospheric pressure, provided that (i) the water vapor pressure in the  
413 object is below the water vapor partial pressure in the environment surrounding the object itself  
414 and (ii) energy is provided in some way, as ice sublimation is an endothermic process.<sup>[71]</sup> This  
415 is called “atmospheric” freeze-drying, a valuable alternative to the traditional batch freeze-  
416 drying process in terms of final product quality.<sup>[72]</sup> Besides, atmospheric freeze-drying may be  
417 carried out continuously, with energy savings up to 35%.<sup>[73]</sup> As drying is carried out at low  
418 temperatures, final product features may be similar to those obtained at the end of the vacuum  
419 freeze-drying process, as shown, among the others by Stawczyk et al.<sup>[74]</sup> in the food field,  
420 investigating drying of apple cubes. Unfortunately, the process is quite slow, mainly due to the  
421 fact that the rate controlling step is the mass transfer in the dried material, and several solutions  
422 were proposed to speed up the process, *e.g.* mixing the material with an absorbent to keep the

423 air humidity very low<sup>[75]</sup>, or using power ultrasound, *i.e.* acoustic waves with frequencies  
424 between 20 and 100 kHz and a power of over 1 W cm<sup>-2</sup>.<sup>[76],[77]</sup>

425 The occurrence of atmospheric freeze-drying in case of frozen stored materials may be, in  
426 some cases, undesired. This is the issued faced by Bruttini and Samadelli in the conservation  
427 of the mummy of Similaun.<sup>[78],[79]</sup> In 1991 an iceman mummy, nicknamed Ötzi, was found in  
428 the Similaun glacier (Tyrolean Alps), on the border between Austria and Italy.<sup>[80]</sup> The body,  
429 dating back to the copper age, *i.e.* over 5000 years ago, was well preserved, and after the  
430 discovery it was moved to the Museum of South Tyrol, where it was placed in a refrigerated  
431 cell, where the temperature was set at  $-6\pm 0.1^{\circ}\text{C}$  (a sort of average temperature of the glacier  
432 where it was found) and humidity was set at  $98\pm 1\%$  through ice panels placed on the internal  
433 surfaces of the cell walls. Despite the humidity conditions created in the chamber, atmospheric  
434 freeze-drying occurred in the mummy and a weight loss of 150 g/month was measured in a few  
435 months of observation. This could be the result of energy sources that could not be avoided,  
436 *e.g.* radiation from the inspection windows and the illumination lights. Bruttini and Samadelli  
437 investigated theoretically the phenomenon by using a detailed multidimensional model.<sup>[15]</sup> The  
438 complex geometry of the mummy was simplified: the bust was assumed to be an elliptic  
439 cylinder, the legs and the arms were considered cylindric and the heat spherical. They modelled  
440 the various radiative heat fluxes that may exist in the conservation chamber, *i.e.* from the ice  
441 on the walls of the chamber, from the inspection window and from the ice on the surface of the  
442 body. They evidenced that the role of the inspection light is almost negligible, being most of  
443 the weight loss due to the inspection window. Besides, they optimized the design of the  
444 conservation cell, being able to increase the total humidity to a value of  $99.42\%\pm 0.15\%$ ,  
445 improving also the temperature stability in the chamber ( $-6.03\pm 0.02^{\circ}\text{C}$ ). The final result was a  
446 significantly lower value of weight loss, 4 g/month, that was considered compatible with the  
447 mummy conservation.<sup>[78],[79]</sup>

448

449

## 450 **7. Conclusions**

451

452 The cultural heritage sector appears to be a field of application of freeze-drying processes no  
453 less important than the pharmaceutical and food sectors. This is due both to the high value of  
454 the objects to be dried, both from a commercial sense, and to their intrinsic value, and to the  
455 large quantities of material that must be treated, especially in the case of flooded books and  
456 archive materials. Unfortunately, to date, the freeze-drying of these materials remains a poorly  
457 studied process, and applied in a "quick and dirty" way. This is due to the extremely  
458 heterogeneous nature of the materials to be dried, which can vary from books, to wooden finds,  
459 to fabrics, even to frozen mummies. In each of these cases the geometrical features of the  
460 objects may be different, as well as the quality issues that must be faced. Despite this, the  
461 methodologies developed for the pharmaceutical and food fields both to monitor the process  
462 and to design it off-line with mathematical models can be successfully used in this case too, as  
463 highlighted in the few studies where this was done. The authors hope that modern analytical  
464 process technologies will be increasingly applied to guarantee the quality of the dried object,  
465 as well as the concept of design space for the identification of operating conditions, to guarantee  
466 the conservation and transmission of cultural heritage to future generations.

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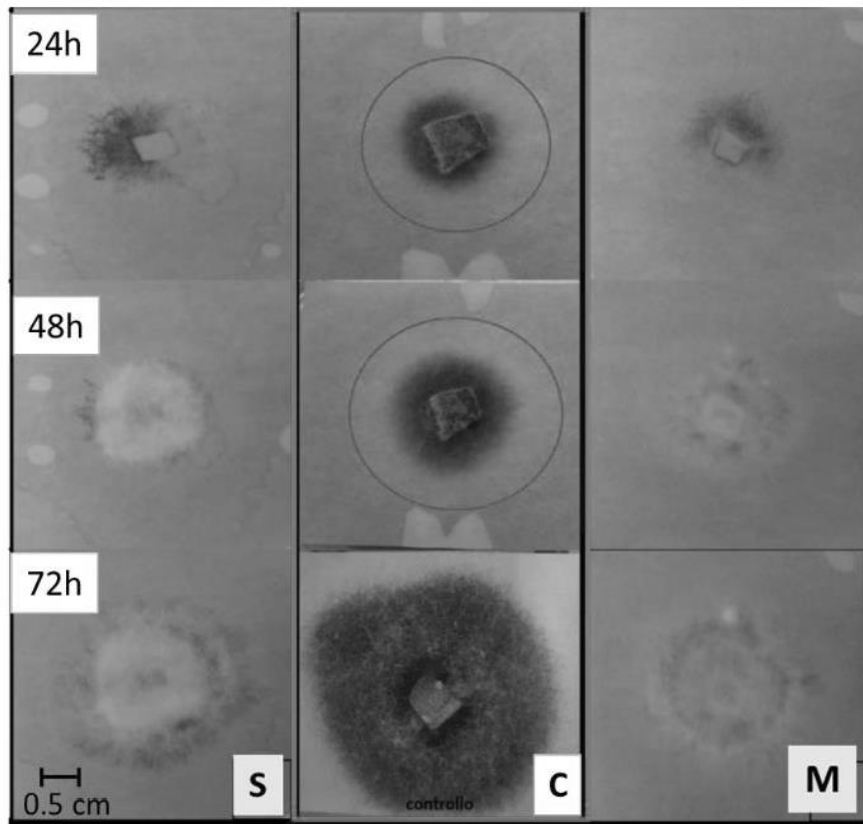
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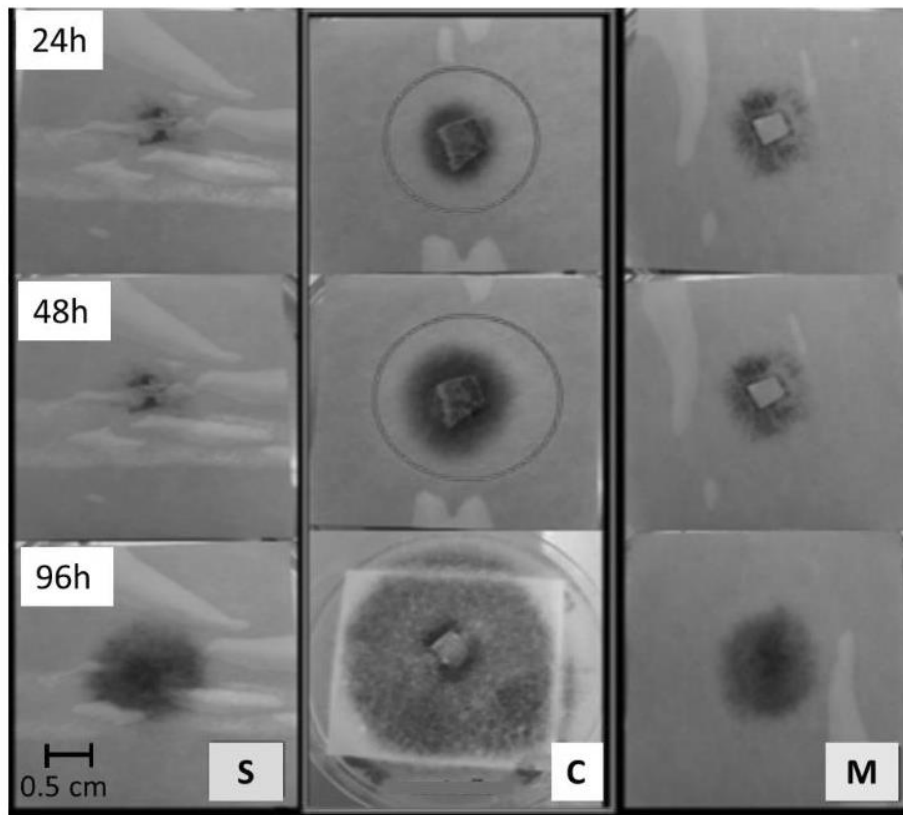
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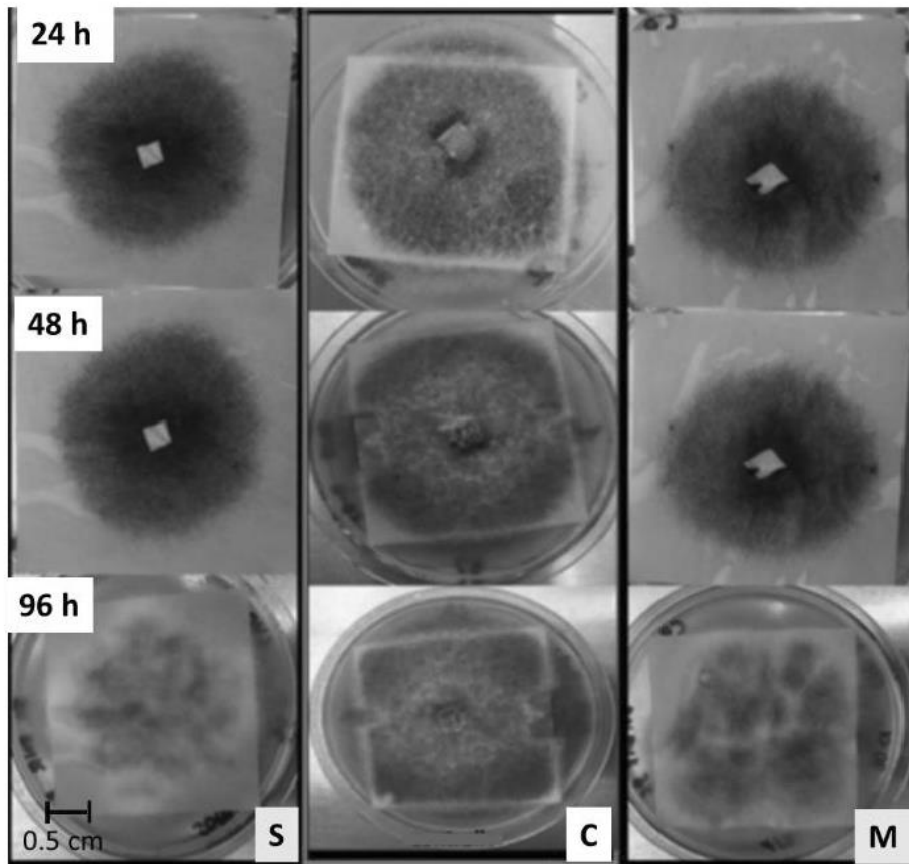
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**Figure 1.** *A. alternata* (not-sporified, after freezing at  $-20^{\circ}\text{C}$  in a domestic freezer) growth on CZ medium at different times of incubation. C-control sheet, S-contaminated sheets on the surface, M- contaminated sheets in the middle of paper block.



**Figure 2.** *A. alternata* (not-sporified, after drying at 0°C) growth on CZ medium at different times of incubation. C- control sheet, S-contaminated sheet on the surface, M- contaminated sheet in the middle of paper block.



**Figure 3.** *A. alternata* (sporified, after drying at 0°C) growth on CZ medium at different times of incubation. C- control sheet, S-contaminated sheet on the surface, M- contaminated sheet in the middle of paper block.