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Freeze-drying and cultural heritage: An overview

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Abstract

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

Keywords

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

1. Introduction

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

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

The role of the freezing stage is particularly important as the temperature decrease velocity may affect the size of the ice crystals and this has an impact on both the drying rate and on the final product features. Small ice crystals may be helpful in preserving the original material shape, but the porous structure arising from the ice removal will present low diameter holes (as the holes correspond to the ice crystals), thus decreasing the permeability to vapor flux and resulting in a longer drying time.

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

Monitoring the primary drying, that is the most critical step of the overall process in this framework, is a must to check if desired features are obtained in the final product. When referring to pharmaceutical products, usually formulated as aqueous solutions either poured into vials or processed as bulky product filling trays, the goal is usually to maintain product temperature below a value corresponding to the collapse temperature of the dried product, in order to maintain its elegance and the possibility of a fast reconstitution.[6] This requires the use of temperature probes as well as of *ad hoc* designed process analytical technologies.[7],[8] The other key issue is the detection of the ending point, thus avoiding any extra time in the process or extracting the product from the dryer before the completion of ice sublimation[9] In case of food products, also nutritional properties have to be accounted for, as product temperature in the process may affect, *e.g.*, the total antioxidant capacity, the phenolic content, the amount of ascorbic acid and others.[10] Less attention was paid to the secondary drying stage, due to the very low value of humidity, although the role of the operating conditions on the duration of this stage and on final humidity was also assessed.[11],[12] The operating conditions resulting in the desired final product features may be found either by experimental investigation, possibly using a suitable design of experiments, or by mathematical modeling and *in silico* simulation of the process. In the first case, several experiments are needed to assess the effect of shelf temperature and chamber pressure on product temperature, drying duration and on some product features, *e.g.* residual moisture. This approach is certainly time consuming~~,~~ and needs the availability of non-negligible amounts of materials to be dried, that may be an issue in the case of pharmaceutical products. As an alternative, it is possible to minimize the experimental effort by carrying out *ad hoc* experiments to get the parameters through which it is possible to model the heat transfer from the equipment to the product inside the drying chamber and the mass transfer from the product to the drying chamber. The following *in silico* simulation of the evolution of the temperature and of the residual humidity in the object during the freeze-drying process allows getting the best operating conditions that minimize the drying time beside preserving final product quality. Both simplified models[13],[14], where a specific geometry of the moving interface between the frozen and the dried layers is assumed, and detailed multi-dimensional models[15] were proposed to this purpose, showing that they could be used to calculate off line the design space of the primary[16],[17] and of the secondary drying[12],[18] stages, *i.e.* the set of operating conditions that allows obtaining the target features in the final product.

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

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

2. Freeze-drying of flooded papers and archival materials

Flooding of archives and libraries is, unfortunately, a common event. This may be due to natural events as well as to the intervention of fire extinguishing systems or to breakdowns of fire-fighting systems or of plumbing systems in the building where the archive or the library is located. Water seriously damages the books and the archival materials: it causes mechanical distortions, due to swelling and adhesion of leaves, it may seriously corrupt the content of the archival material, through inks and dyes migration and solubilization of water-soluble components, and it may promote microbiological infections. As a consequence, it is necessary to stop this sequence of events as soon as possible, and freezing of water is the elective choice as an emergency intervention.[20] After that, the traditional approach consists of defrosting the material and drying it manually, with the action of air at a moderate temperature[21] and, in some cases, by interleaving it with absorbent paper. This way of working, although it allows us to obtain a high-quality product, requires a lot of time and, therefore, is expensive. Furthermore, the time that elapses between the accident and the moment in which the material is again available to the user can be particularly high. This motivated the study of alternative drying methods, *e.g.* vacuum drying[22], microwave drying[23], freeze-drying[24]-[26], allowing for a faster ice removal. In all cases it must be highlighted that no drying techniques may repair the damage caused by water in the time interval between the accident and the moment in which water is turned into ice. The goal of the drying process is "just" to remove water as soon as possible without causing additional damages.[27] In this framework a pivotal role may be played by freeze-drying mainly due to the low operating temperatures. One of the first studies in the field are those by Flink and Hoyer[28] and Flink[29] who used freeze-drying to dry the Kleinschmidt collection, damaged after a fire extinguished in the Greenland Regional Library in Godthab in 1968. They evidenced the excellent results obtained for paper documents: each page separated easily and in no case did the ink run, while in case of a photograph album the prints tended to stick together after drying, mainly due to their chemical composition. Ref. [30] presents the Records and Archives Management Programme (RAMP) study, presenting a broad summary of data about freeze-drying of water-soaked paper, comparing the vacuum freeze-drying (a dry process) and the vacuum drying by evaporation (a wet process), and providing useful guidelines for practitioners in the field. More recently Capolongo and Barresi[31] investigated the use of freeze-drying to recover water-damaged library materials comparing the results obtained in case of standard freezing and in case of evaporative freezing. In the first case water freezing was carried out is a conventional freezer, at -20°C, while in the second case water freezing was the result of the sudden pressure decrease: it caused the evaporation of part of the liquid water, resulting, at the same time, in the decrease of product temperature and, finally, in water freezing. At first, they set up a method to produce reproducible water-soaked samples, moving from the method of Fisher[32], and then pointed out that no excessive damages occurred in samples for both freezing method. Unfortunately, over-drying could not be avoided, and re-conditioning of brittle and rigid paper was recommended to avoid potential mechanical damage.

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

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

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

Despite the freeze-drying process may be considered a “soft” drying treatment, the final characteristics of the treated paper and archival material should be a concern. Carlsen[35] compared some properties of paper before and after a freeze-drying treatment for three case studies, namely a 75% groundwood-25% softwood cellulose based paper, a 100% cotton based paper and a 100% chemical cellulose based (coated) paper. Three treatments were considered: air drying and freeze-drying at two different drying temperatures, identified as cold and warm. Residual moisture, alkali reserve, tearing resistance and folding endurance are the properties considered, in the presence or absence of an accelerated aging step (12 days at 90°C and 50% relative moisture). Results evidence that each treatment considered in the study affects the properties of the paper in different ways according to the type of paper. Mechanical strength, in particular folding endurance, and ageing stability of groundwood paper and coated paper are affected by the freeze-drying process, while the effect is negligible in case of cotton paper, whereas fibre-fibre bonds are usually strengthened by freeze-drying, particularly in coated paper after accelerated drying. Results evidence also that freeze-drying at low temperature allows maintaining flatter the paper after the treatment, while there are not clear indications about which operating conditions affect the most ageing stability and mechanical strength.

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

When considering the drying of flooded paper and archival materials there is an additional issue that must be faced. In fact, cellulose may represent a carbon source for several microbial classes (*i.e.* bacteria, yeasts and filamentous fungi), resulting in a high bioreceptivity, *i.e.* the possibility for a material to be colonized by living organisms. Besides, also other paper components (*e.g.* glue) may be used by microorganisms in their metabolic activity.[37] The occurrence of floodings may be responsible for a combination of temperature and relative humidity values in the environment that result in a value of water activity suitable for microbial growth, thus enhancing the biodeterioration of paper documents.[38]

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

Few reports describe the effect of freeze-drying on paper-biodeteriogens; among the other, it was shown that, concerning filamentous fungi, spores are more resistant than hyphae.[40],[41] In previous works[38],[42] the effect of both freezing and lyophilization on different classes of paper-born microorganism was reported. Modern paper was used, and flooding was simulated by soaking blocks of paper for 18 hours in distilled water, followed by draining. Microorganisms were inoculated on a sterile single paper sheet, which was then placed at the surface or in the middle of the paper block. At first, the role of the freezing stage on microorganism viability was assessed by carrying out this step in a domestic freezer, with a final temperature of -20°C, or directly in the freeze-dryer, where a higher temperature decrease rate could be obtained, about 1°C/min, with a final temperature of -40°C. After the completion of water freezing, samples were thawed at room temperature, for about 4 hours, in sealed glass containers and then contaminated sheets were recovered and placed on a suitable agar medium and incubated at their optimal growth temperature. In order to assess the effect of the freeze-drying stage, some of the samples, after freezing, were not thawed, but were placed in a pilot-scale freeze-dryer, where ice sublimation was obtained at 200 μbar and 0°C. In all cases, the evolution in time of Colony Forming Unit (CFU) for unicellular microorganism and the mean colony diameter of the pluricellular microorganisms after freezing and thawing or after freeze-drying was compared with that obtained in case no freeze-tawing or no freeze-drying was carried out. As an example of bacterium and of yeast, *Staphylococcus epidermidis* and *Rhodotorula mucilaginosa*, respectively, were considered in this study, and no relevant effect of both freezing and freeze-drying was observed with respect to the untreated microorganisms. The study also involved *Alternaria alternata*, a biodeteriogen filamentous fungus, and it was pointed out that a 48 hours of growth inhibition, followed by a lower growth rate, could be achieved when freeze-drying was carried out at 0°C.

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

After the freezing, the frozen samples were freeze-dried in a pilot-scale freeze-drier at 200 μbar at 0°C and 20°C in order to evaluate the influence of the drying temperature on process duration and on the microorganism viability. The ending point of the primary drying stage, as evaluated through the midpoint of the pressure *vs*. time curve measured by the thermoconductive gauge[43], appears to be lowered from 17 h to 12 h when the temperature of the heating shelf during primary drying was 20°C instead of 0°C. In both cases, at the end of the drying phase, contaminated sheets were recovered and reconditioned in sterile conditions, positioned on CZ agar plates, and incubated at 30°C. The obtained results, related to both not-sporified and sporified colonies of *A. alternata* are reported in Figure 2 and 3 respectively, and compared with the untreated control sheet. The drying, conducted at 0°C, caused a growth inhibition of not-sporified *A. alternata* up to 48 hours (Figure 2), in both the positions of the contaminated sheets. After that, at 96 hours of incubation, the colony diameter of the dried mycelium was significantly lower than that of the control. Differently from the freezing (*see* Figure 1), the influence of drying on fungal metabolism was evident as mycelium sporification appeared and at 96 hours the colonies were completely covered with spores. In the presence of sporified *A. alternata* sheets (Figure 3), a lag phase of 48 hours was also observed, following which the growth started again with a rate comparable to that of the control, regardless of the position of the contaminated sheets. Also, in this case at 96 hours it is possible to observe the growth of new mycelium on sporified colonies. Thus, a strong influence of the freeze-drying process, independently from the colonies age, is evident.

3. Freeze-drying of waterlogged wood

A particularly challenging case study is represented by the freeze-drying of waterlogged archaeological wood. This is due to the fact that wood is a hygroscopic and porous material, where the microstructure is closely related, among the others, to moisture distribution.[44],[45] During drying we may observe, as a consequence of the removal of water, the formation of new hydrogen bonds between the cell walls polymers and this results in a certain degree of shrinking. Moreover, in case of waterlogged wood, also the degradation of the cell walls due to contact with water has to be accounted for.[46],[47] In fact, internal mechanical stresses may arise due to the fact that the shrinkage of the external, dried, layer may be hampered by the inner part of the object, that remains saturated. If these stresses overcome the mechanical resistance of the material, cracks may appear. Besides, as the cell walls are degraded due to contact with water, they may collapse due to capillary forces and to the effect of the high surface tension of liquid water.[48]-[51] In this framework it should be clear that the dimensional stabilization is a key issue when drying waterlogged wood. This may be obtained by full impregnation of the object to be dried with water-soluble agents, in case of freeze-drying, or by replacing water with a low surface tension solvent, in case of air drying.[52]-[55]

Broda et al.[56] considered drying of waterlogged archaeological elm coming from a medieval bridge found at the bottom of a lake in Poland. Several drying treatment were considered in the study, namely (i) air drying (at room temperature, for two weeks), (ii) oven drying (at 50°C or 104°C, for one week), (iii) freeze-drying, without any pre-treatment, (iv) CO_2 supercritical drying and (v) air drying after dehydration with 96% ethanol (4 weeks) followed by acetone exchange (3 days). They evidenced that freeze-drying, and CO_2 supercritical drying, were the best method to stabilize wood dimensions, with a shrinkage lower than 10%. This was the result of the higher value of wall cell area, and of the fact that freeze-dried and critical point dried materials retain the typical open porous structure of wood. Besides, freeze-drying, as well as CO_2 supercritical drying, removes the effect of surface tension, thus minimizing shrinkage.[53],[57],[55],[58],[59]

Gregory et al.[60] compared several drying treatments for wood shipwrecks in marine environments. They investigated both (i) conservation methods, involving the impregnation with water soluble molecules, *e.g.* PEG or sugars, that replace the free water and then solidify at room temperature, fixing the structure of the cells and preventing shrinkage or collapse, and (ii) the use of controlled air drying or freeze-drying to remove the liquid phase after impregnation. In case the molecular mass of the impregnating agent is larger than 6-800 g/mol, it can penetrate only the lumen of the cells and, thus, it acts as a void filling agent that prevents the collapse of the structure. Lower molecular wight agents (*e.g.* sugars like sucrose, mannitol, sorbitol and lactitol, or low molecular weight PEG[61]) may enter the cell walls, replacing bound water and preventing shrinkage. Each of these molecules has its own advantages and disadvantages. PEG impregnation is a non-expensive, robust and non-toxic method, which can be used on wood in all states of conservation; it produces heavy objects, show surface generally needs to be treated to recover the original appearance. Besides, PEG is corrosive and, thus, it should not be used in presence of metallic elements. Sucrose and lactitol are inexpensive as well, and their low molecular weight allows good penetration in the wood. They are not corrosive, but they may promote microbial attack both in the treatment and conservation stages, and, in case of lactitol, the formation of the trihydrate crystals must be avoided as the volumetric expansion will result in the destruction of the material.

Air drying is a very cheap method, but the final result may be highly variable, and severe cracks or shrinkage may occur as a consequence of the drying conditions. On the other hand, in the freeze-drying process no liquid phase exists and, thus, capillary forces are avoided. Anyway, freeze-drying of the original object is not recommended as when the free water in the cell lumen turns into ice, the expansion may result in cracks in the objects. Besides, shrinkage or cracks may appear when the bound water is removed from the cells wall. Therefore, beside carrying out the freeze-drying process it is recommended the pre-impregnation of the object with a solution containing 35-45% of the selected agent, with a slightly higher value in case of PEG, 0.55g/g.[62] Lowering of the freezing point has to be considered in this framework and, thus, the use of PEG with a molecular weight higher than 1500 g/mol is suggested as the eutectic temperature, in this case, is about -23°C.[63] Moreover, PEG with a smaller molecular weight are not solid at room temperature. The process allows retaining in an excellent way the quality features of the original object, although drying time may be very long, as shown also by other authors, *e.g.* Stelzner et al.[64]. In some cases a mixture of low and high molecular PEG solutions was proposed, taking advantage of their different role.[65]

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

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

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

4. Freeze-drying of historical wet textiles

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

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

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

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

5. The use of freeze-drying to create artworks

In the field of cultural heritage, a peculiar case study is represented by the use of freeze-drying to create artworks. In this case, the low temperature drying process is used to remove water from an otherwise perishable material. The most representative work in this field is probably the "*Imitatio Christi*" by the Italian artist Roberto Cuoghi.[70] He was invited to present his work at the Italian Pavilion in the 57th International Art Exhibition of Venice and, as the title of this masterpiece suggests, he was inspired by the 15th century treatise "De Imitatione Christi" and, in particular, by the chapter focused on the transitory nature of the human conditions. The "Imitatio Christi" is thus a journey to seek the true face and body of Jesus Christ after death. The artist prepared the bodies with agar-agar and then they were subjected to a decomposition process, like if they were human bodies, by yeasts and molds present in the environment. Finally, in order to stop this degradation process, these materials were firstly dried through contact with natron, a sodium carbonate already used by the Egyptians in the mummification processes, and finally by freeze-drying, to (almost) completely remove the water. By this way it was possible to preserve the original shape of the objects, provided that the operating conditions of the freeze-drying process were selected. No specific pieces of information are available on the freeze-drying process used in this case, but it may be supposed that very cautious operating conditions were used, to avoid collapse of the agar-agar based objects, despite the very long drying time that could have resulted from this choice.

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

The last section of this paper is devoted to another peculiar case study, always related to the freeze-drying process, but in which the occurrence of freeze-drying is undesired. You might think that freeze-drying is possible only at very low pressure, but actually, it may occur in a frozen object even at atmospheric pressure, provided that (i) the water vapor pressure in the object is below the water vapor partial pressure in the environment surrounding the object itself and (ii) energy is provided in some way, as ice sublimation is an endothermic process.[71] This is called "atmospheric" freeze-drying, a valuable alternative to the traditional batch freeze-drying process in terms of final product quality.[72] Besides, atmospheric freeze-drying may be carried out continuously, with energy savings up to 35%.[73] As drying is carried out at low temperatures, final product features may be similar to those obtained at the end of the vacuum freeze-drying process, as shown, among the others by Stawczyk et al.[74] in the food field, investigating drying of apple cubes. Unfortunately, the process is quite slow, mainly due to the fact that the rate controlling step is the mass transfer in the dried material, and several solutions were proposed to speed up the process, *e.g.* mixing the material with an absorbent to keep the air humidity very low[75], or using power ultrasound, *i.e.* acoustic waves with frequencies between 20 and 100 kHz and a power of over 1 W cm^{-2}.[76],[77]

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

7. Conclusions

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

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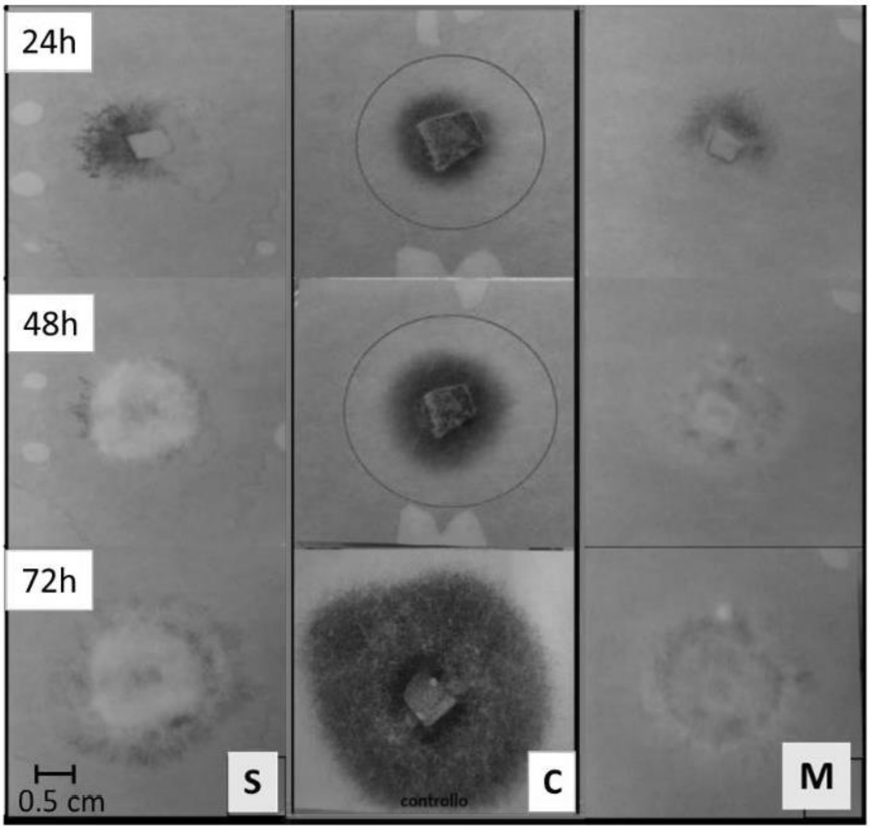

Figure 1. *A. alternata* (not-sporified, after freezing at -20°C in a domestic freezer) growth on CZ medium at different times of incubation. C-control sheet, S-contaminated sheets on the surface, M- contaminated sheets in the middle of paper block.

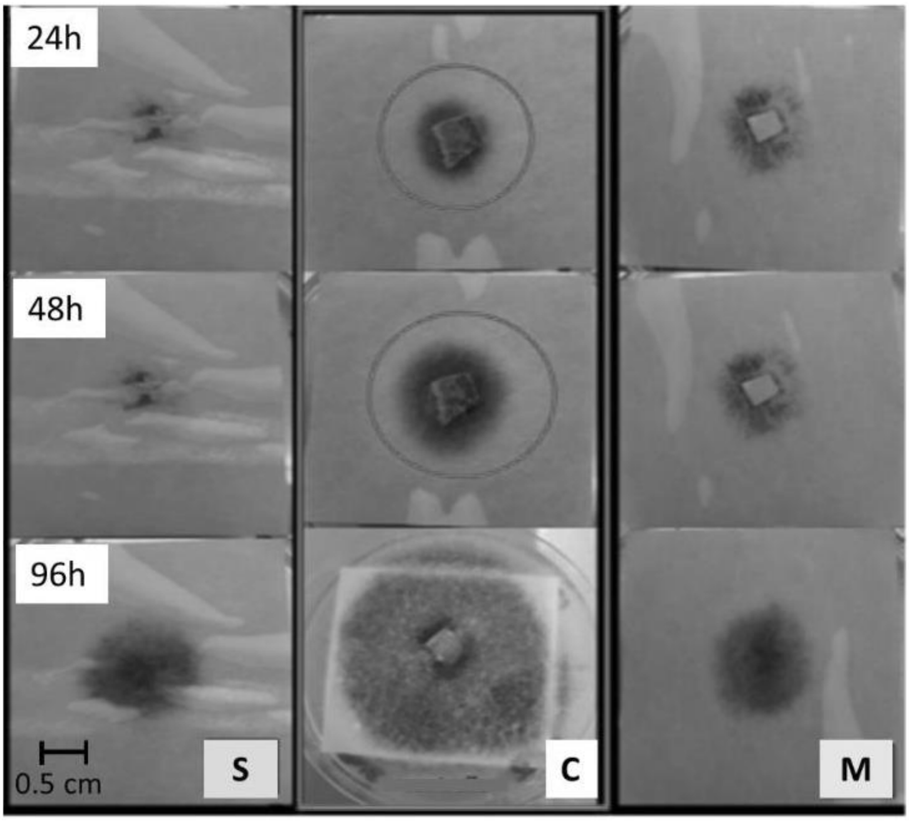

Figure 2. *A. alternata* (not-sporified, after drying at 0°C) growth on CZ medium at different times of incubation. C- control sheet, S-contaminated sheet on the surface, M- contaminated sheet in the middle of paper block.

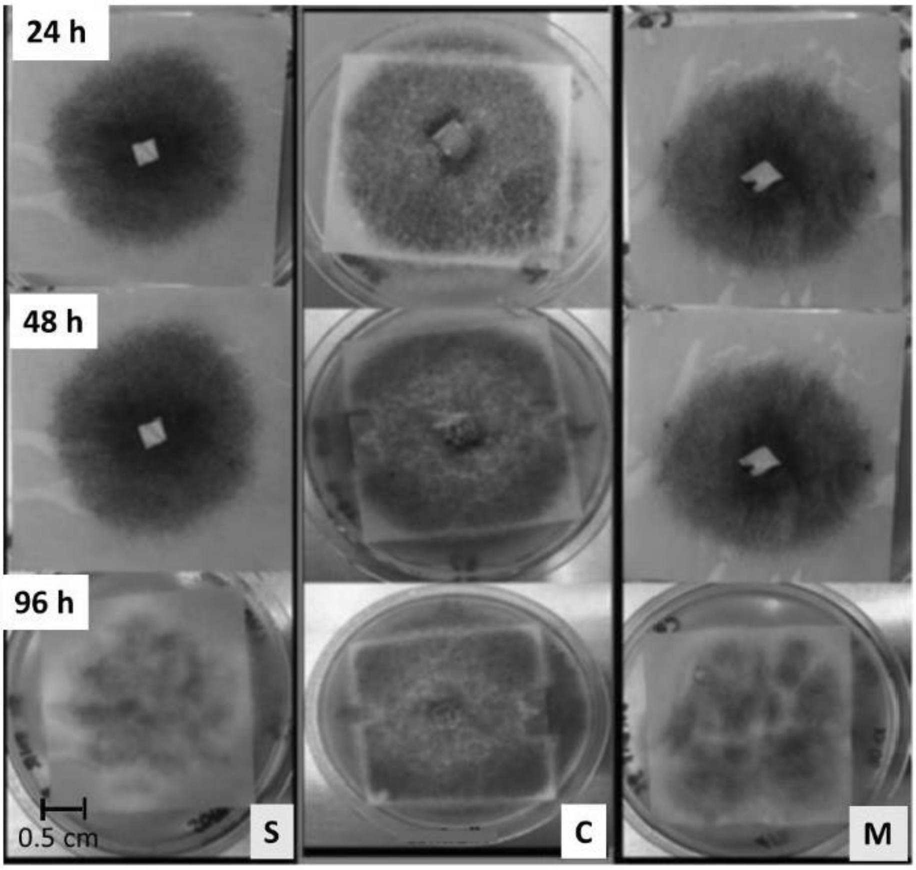

Figure 3. *A. alternata* (sporified, after drying at 0°C) growth on CZ medium at different times of incubation. C- control sheet, S-contaminated sheet on the surface, M- contaminated sheet in the middle of paper block.