



Review

Water-polysaccharide interactions and their properties in freezing conditions

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ABSTRACT

Freezing processes involving polysaccharides are widespread, spanning from organisms resisting cold environments, to emerging applications for cryopreservation in biomedical and food technologies. Yet, many fundamental questions on these processes remain unanswered. The challenge arises both from the complexity of polysaccharides and their structure and from water, a simple molecule with unique properties; such complexity can only be understood using a strongly interdisciplinary approach spanning through physics, chemistry, materials science and engineering. This review explores the current understanding of the complex phenomena underpinning the behavior of water when interacting with polysaccharides, with a special interest at freezing conditions, when ice forms. Herein, we draw a comprehensive picture on the most relevant findings, connecting scientific knowledge from diverse fields, from chemistry to biotechnology, materials science and engineering, all linked by the interest in water-polysaccharide interactions, especially at interfaces.

After introducing the key concepts related to the structure of water at the interface with polysaccharides, such as bound water, non-freezing water and quasi-liquid layer, the review compares the main techniques used to study the polysaccharide-water interaction in freezing conditions, discussing the relevant findings for a variety of polysaccharides.

1. Introduction

Polysaccharides are biopolymers that have been naturally selected through evolution to fulfill crucial biological functions, such as providing structural support, nutrition, and managing surface interactions. Their interface with external bodies is mediated by water molecules associated with the polysaccharide at both molecular and supramolecular levels. Water is known to be the “solvent of life”, at least on planet Earth, a complex fluid, whose role is still to be fully unraveled in assisting life. Yet, the common picture of water as a pure passive solvent matrix which acts solely as a vehicle for solute motion is not representative of its complexity (Ball, 2017). It is becoming increasingly clear that water plays an active role in the organization of life activity and morphology, being the field of interaction of biological matter with the environment (Hartmann et al., 2025). Therefore, the analysis of polysaccharide-water interaction is extremely complex, due to a variety of reasons: (i) water itself, despite being a rather simple molecule, displays complex behaviors, particularly when phase transitions are involved, such as evaporation or freezing (Fitzner et al., 2015; Hayton

et al., 2023); (ii) polysaccharides, especially when bioderived, possess a complex structure, which rises analytical challenges for defining structural characteristics and solving structure-properties relationships both at the nano- and meso-scale, especially in the context of bio-activity (Fernandes & Coimbra, 2023; Ji et al., 2023). Moreover, transport phenomena at polysaccharide-water interfaces, such as wetting, tribology and ice nucleation, represent complex investigation challenges, due to the rich underpinning physics.

One particular condition of interest, representing a major focus in this review, is the interaction of polysaccharides with water in freezing conditions. The investigation of water at interfaces in freezing conditions has been and still is of great interest, placing great challenges from characterization techniques to theoretical models; also, it retains significant importance for the description of fundamental phenomena at interfaces, which are crucial in biology and in materials science, but also in the explanation of atmospheric phenomenon including that of lightning and frost heave (Wettlaufer & Dash, 2000).

In nature, we may observe several examples showcasing how organisms have evolved to use polysaccharides, cold-stress induced

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proteins and vernalization (Trischuk et al., 2014) to protect themselves from freezing damage in cold climates (see overview in Fig. 1). These examples remarkably span on the entire spectrum of living organisms, from simple bacteria to algae, up to plants and vertebrates (Storey & Storey, 2017), and can serve as bio-inspiration to develop new sustainable functional materials or processes based on polysaccharides. Among other cryoprotectant strategies, the mostly known and commonly cited examples of natural substances helping against freezing are the so-called “antifreeze proteins” (AFPs), found commonly in Arctic fishes (T. Sun et al., 2014) and insects (Liou et al., 2000). Differently than colligative antifreeze activity of solutes, where the effects are dependent on concentration, AFPs depress the freezing point even at very low concentration. As an example, *Tenebrio molitor* AFP depresses the freezing point by 2 °C at 10 μM (compared to salt, more effective by a factor 105 on a molar base and 300 on a weight base) (Bar Dolev et al., 2016). Pioneering studies (DeVries et al., 1970; DeVries & Wohlschlag, 1969) have shown that the freezing point depression of the blood of marine polar fishes, such as the Antarctic fish *Pagothenia borchgrevinki* (Fig. 1a), is due to dissolved salts and sugars, but also circulating glycoproteins (AFGPs), polypeptides with sugar groups. AFPs and AFGPs belong to the wider class of so-called ice-binding proteins (IBPs), a more inclusive descriptive term used to indicate proteins that bind ice and modify its structure (Duman, 2015; Gibson, 2010; Vance et al., 2019).

Wood frogs (*Rana sylvatica*) can survive being frozen solid (Fig. 1b). They accumulate glucose, a simple sugar, in their tissues, which acts as a

cryoprotectant, by preventing excessive dehydration of cells during freezing. Glucose can rise from 5 mM (a concentration similar to the one of healthy human blood, typically in the range 4–5.5 mM) to 40 mM within the first hour post-nucleation and reach levels of 150–300 mM in core organs of fully frozen animals (Al-attar & Storey, 2022). Glucose helps wood frogs survive having up to 65 % of their total body water frozen in winter months: during this time, their heart stops beating, and they cease breathing. Upon thawing, frogs can resume normal functions within hours. Looking at smaller scales, tardigrades (Møbjerg et al., 2011), also commonly known as water bears, can survive extreme dehydration and freezing by entering a state called cryptobiosis. They also produce trehalose, which helps protect their cellular structures during desiccation and freezing.

Nature displays multiple strategies for facing diversified challenges of cold habitats, providing countless examples for bioinspired research. Recently, it has been shown that polar bear fur (Fig. 1c) displays ice-phobic properties, and specifically low adhesion to ice, comparable to fluorinated materials (Carolan et al., 2025). The sebum (hair grease analysis) has revealed that lipids include cholesterol, diacylglycerols, and anteisomethyl-branched fatty acids. As such, polar bear recalls to the so-called synthetic SLIPS (superhydrophobic liquid infused porous surfaces) or LIS (liquid infused surfaces), where the presence at the interface of a liquid immiscible to water, typically an oil, facilitates ice detachment (Lv et al., 2014). Differently, penguin body feather ice-shedding functionality was found to be due to surface morphology,

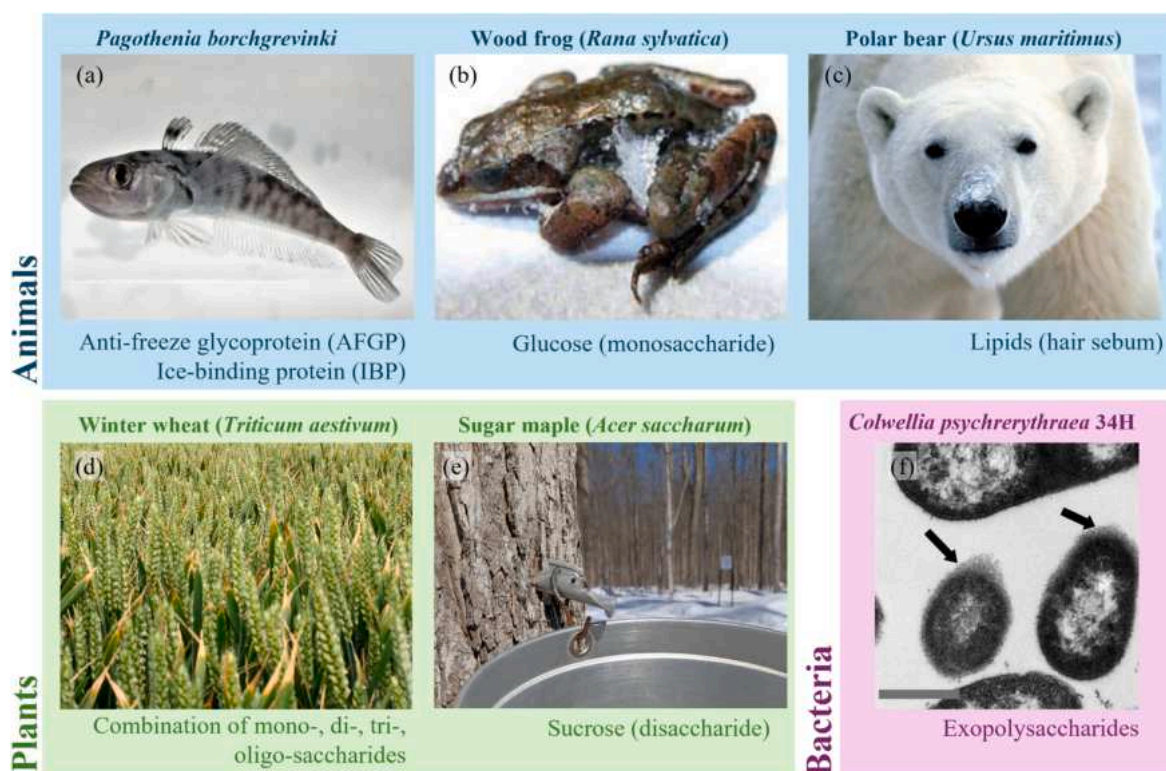


Fig. 1. Overview of ice protection strategies in nature. Animals: (a) Antifreeze proteins (AFPs) belonging to the wider class of so-called ice-binding proteins (IBPs) depress the freezing point of the blood of marine polar fishes; the AFP freezing depression is not colligative and due to specific interactions. *Pagothenia borchgrevinki* from McMurdo Sound, 2018, by Kenneth Zillig. (b) The wood frog can survive being frozen solid, by accumulating glucose in concentrations up to 150–300 mM, as a cryoprotectant; glucose prevents excessive dehydration of cells during freezing (Storey & Storey, 2017). (c) Polar bear hair shows low adhesion to ice due to sebum (hair grease), with lipid analyses revealing the presence of cholesterol, diacylglycerols and anteisomethyl-branched fatty acids. Image source: <https://flickr.com/photos/38485387@N02/3582475670/> License: CC BY-NC-ND 2.0. Plants: (d) At low temperatures, winter wheat increases the production of various sugars and sugar alcohols, including sucrose and trehalose (disaccharides), raffinose (trisaccharide), fructans (oligo- and polysaccharides) to increase freeze tolerance. Image source: <https://flickr.com/photos/ndrwfgg/173181035> License: CC BY. (e) Sugar maple produces sugar, primarily sucrose, to lower the sap freezing point and to protect cells from frost damage. The freeze-thawing process in spring enables extraction of sap (~2 % sugar), from which maple syrup is produced. Image source: Carlo Antonini, 2025. Bacteria: (f) psychrophilic microorganisms, such as the Antarctic bacterium *Colwellia psychrerythraea* 34H, produce exopolysaccharides (EPSs). EPSs, produced as cold adaptation response, form an organic network within the ice, modifying the structure of brine channels and enabling the microorganism to survive under extreme environmental conditions. Scalebar is 300 nm. Adapted with permission from (Carillo et al., 2015). Copyright © 2015, American Chemical Society.

rather than lubrication (Wood et al., 2023): the preen oil covering the penguin body feather are helpful to facilitate water-shedding, but is not required to reduce ice adhesion, which is promoted by the wire-like structure of the feather barbs, decorated with nanoscale wrinkles. Moving to plants, a similar process is used by winter wheat (Fig. 1d), which increases its freeze tolerance by accumulating sugars and other osmolytes in its cells during cold acclimation. Specifically, as temperatures drop, these cold-acclimated plants upregulate the synthesis of various sugars and sugar alcohols, including sucrose and trehalose (disaccharides consisting of two glucose units), raffinose (a trisaccharide composed of galactose, glucose, and fructose), fructans (fructose-based oligo- and polysaccharides). Sugar maple trees (*Acer saccharum*) use sugar, primarily sucrose, to lower the sap freezing point and to protect cells from frost damage. Interestingly, the maple sap production reaches a high between March and April, as it relies on alternating freeze-thaw cycles. These day-night temperature fluctuations above and below zero cause the sugar-rich sap exudation (Fig. 1e), a process controlled by root water uptake and freezing point depression due to sap sugar content (Graf et al., 2015). Also, the composition of maple sap changes throughout the tapping season, with sugar content generally decreasing as the season progresses (it typically ranges from 1 to 5 %).

Besides the production of mono- and disaccharides, which impact on a colligative base, other non-colligative cryoprotective strategies common in psychrophilic microorganisms (i.e. diatoms species and many bacteria), comparable to the above-mentioned IBPs, involve the production of exopolysaccharides (EPSs) (Casillo et al., 2017) (Fig. 1f). Some EPSs can be produced as a cold adaptation response, forming an organic network within the ice, modifying the structure of brine channels and concurring to a number of different purposes assisting the microorganism in survival under extreme environmental conditions (Nagar et al., 2021). EPSs help preserving cell membrane from stress, retaining water and preventing desiccation, buffering against salinity and pH, sequestering metal ions, providing nutrition and cell adherence to surfaces. Although an increased interest is growing in investigating the EPS properties, many challenges arise from their analytical characterization, which remain still highly unknown in most cases, and, therefore, a clear understanding of structure-property functions is still far from being achieved.

All the above studies highlight the complexity of ice protection strategies evolved by different organisms. Among these, still, the role of polysaccharides and their ice protection mechanisms are still to be fully unraveled. By now, we cannot draw a clear picture of how structural polysaccharide features can control interactions with ice, although broadly exploited in many applications involving freezing, such as food cryoprotection (X. Sun et al., 2022): recent studies are just now starting to scratch the surface to identify correlation between physicochemical properties and IRI activity (X. Zhang et al., 2025). This investigation can generate a great impact, as they underpin a variety of processes and fields, spanning from cryopreservation for food and crop security, to cell therapies including cancer and fertility treatments, vaccine storage and delivery (Murray & Gibson, 2022). Also, polysaccharides can play a crucial role for the development of sustainable materials and coatings with bioinspired engineered surface functionalities (C. Xie, 2019), diverse wetting and adhesion properties (Tagliaro et al., 2023), selective liquid-sorption and separation, adsorption of contaminants in water such as metal ions, drugs or toxic chemicals (Sharma et al., 2024). In this manuscript, we aim to critically review the most important findings related to polysaccharide-water interactions, especially in freezing conditions, to draw a unified interdisciplinary framework across chemistry, biotechnology, materials science and engineering. In Section 2, we focus on the studies related to experimental investigation of hydrated polysaccharide water states. In Section 3 we describe water behavior on surfaces in freezing conditions. In Section 4, we report the main findings related to water-polysaccharides interactions at freezing conditions, categorized by polysaccharide type, and finally provide concluding remarks in Section 5.

2. Characterization of hydrated polysaccharide water states

In water, a large dipole moment between oxygen and hydrogens atoms generates a network of hydrogen bonds with energy of attraction between water molecules (hydrogen bond enthalpy) of $23.3 \text{ kJ}\cdot\text{mol}^{-1}$ (Chaplin, 2010). The network is highly dynamic with an average lifetime of picosecond for each bond. With a 104.6° angle between hydrogens formed by the presence of two pairs of electrons on the oxygen atom, the shape of water molecules drives the formation of a tetrahedrally coordinated structure, scaffold of a possible five- and six-membered rings organization which was experimentally observed for the first time at the beginning of the 2000 by scanning tunneling microscope (STM) on metal surfaces at low temperatures (Verdaguer et al., 2006). In icing conditions, this structure of ordered rings, which results in a consequent lowering of density, determines the formation of empty space within the network, in which defects may enable molecules to occupy the free space. Therefore, the bulk water structure can be described as an intermediate entity between ice-like loose structure and liquid-like random tight packing. Thermodynamically, water solvation balances between an enthalpic term governed by hydrogen bonding, electrostatic, and van der Waals water-water and water-solute bonding, and an entropic term due to formation and disruption of hydrogen-bonded networks affected by the environment (Ball, 2017).

Water-polysaccharide interactions are particularly complex and interesting since the local organization of water molecules is greatly affected by the hydrophilic and flexible polymer chains decorated by polar groups. Although they share a similar structure, even minor changes impact on polysaccharide interactions with water. At the same time, water is involved in the folding process of secondary structures, as it competes with intramolecular hydrogen bonds and affects the relative rotamer populations (Djalali et al., 2024). In the cases of cellulose, chitin and mannan, which favor intramolecular hydrogen bond, a rigid and elongated structure is the one that maximizes the degrees of freedom, resulting in solid materials, while xylan and hyaluronic acid, predicted to favor hydrogen bonds with water, are indeed soluble polymers (Almond & Sheenan, 2003). Therefore, each different hydrated polysaccharides have specific interactions with water producing differentiated water states (F. Jiang et al., 2025). Common characterization techniques for polysaccharide water states are infrared (IR, Raman) spectroscopy, nuclear magnetic resonance (NMR) and differential scanning calorimetry (DSC), investigating different aspects of matter, creating sometimes difficulties in obtaining consistent and comparable results across techniques. Vibrational spectroscopy provides information on the hydrogen bond network present in the sample, while DSC highlights water phase transitions, and solid-state NMR characterizes water based on its dynamic properties. Novel techniques are emerging for exploring specific features of water-polysaccharide interactions. Thermoporosimetry (Maloney et al., 2023) provides a different approach to calculating water states, contrast X-ray micro-computed tomography (Ferreira et al., 2023) can map liquid percolation, while wide angle X-ray diffraction and small angle X-ray scattering (Okugawa et al., 2023) can detect fiber packing dependence on water content.

By using the most correct technique-associated terminology, water states investigated by NMR are referred to as *mobile*, *intermediate* and *rigid*, by FTIR *bound*, *weakly-bound* and *free*, by DSC *freezing* and *non-freezing*. These key concepts, defined by experimental observation, do not always allow to define a one-to-one correspondence of water states belonging to different characterization approaches. Moreover, there is a great variety in terminology, which may further generate confusion. Generally, the scientific literature refers to mainly three possible states of water identified with a wide variety of names: (i) *free-water* (also named as *bulk* (El Nokab et al., 2022), *normal* (Jhon & Andrade, 1973), *multimer* (Grossutti & Dutcher, 2016), *mobile* (El Nokab and Van Der Wel, 2020)), referring respectively to water states which are resembling that of bulk water, (ii) *intermediate-water* (or *weakly bound* (Kramer & Carvalho, 2021), *loosely bound* (Mccrystal et al., 1999)), referring to

water molecules with an intermediate behavior between “free” and “bound” and (iii) *bound-water* (Cocusse et al., 2022) (or *ice-like* (Grossutti & Dutcher, 2020), *non-freezing* (Espinosa-Andrews et al., 2023), *tightly bound* (Jing et al., 2023), *solid* (Sterling & Masuzawa, 1968), *glass-like* (Li, Mao, et al., 2024), *stiff* (Agles & Bourg, 2023), *rigid* (F. Wang et al., 2016)), accounting for the first molecular layers of water molecules at the interface with the polysaccharide. Additional naming refers to water molecule features at the boundary between intermediate and bound, comprising *weakly/loosely bound* (M. Chen et al., 2023), *freezing-bound* (Miwa et al., 2010; Xia et al., 2020), *non-freezing bound* (Yoshida et al., 1993) are also used.

Because of their variety in structure, each polysaccharide possesses unique interactions with water, making general consideration solely related to functional groups hard to define. The same polysaccharide could even provide different interaction with water depending on its physical form. In the case of cellulose, the presence of *intermediate* water was reported to decrease with the increase in crystallinity, suggesting that the adsorption sites of cellulose are mainly present in the amorphous regions. It was calculated that water binds to the three hydroxyl groups of every glucose unit of the amorphous regions (H. Hatakeyama & Hatakeyama, 1998). In cellulose nanofibers, every hydroxyl group interacts with two water molecules (Salem et al., 2023).

2.1. IR Spectroscopy

By attenuated total reflection infrared (ATR-IR) spectroscopy, it is possible to investigate the interaction of infrared light with polysaccharides in the form of films, powders or soft networks (hydrated structures and hydrogels), providing insight into the strength and arrangement of chemical bonds. Of particular interest for the analysis of water states, it is possible to focus the investigation on the region of the ν (OH) O—H stretching band between 3800 and 3000 cm^{-1} , which reflects environmental changes in hydrogen bonding. It was reported that the covalent O—H bond is weakened by the presence of hydrogen bonding, thus obtaining a decrease in the ν (OH) frequency (Ping et al., 2001). Moreover, the angle at which the hydrogen bonding is acting impacts on its strength, being maximum when the O—H...O bond angle lays on a straight line. Therefore, also the frequency is affected by the distortion in linearity of the hydrogen bonding. Lower linearity results in a decrease in ν (OH) frequency (Disalvo & Frias, 2013). Summarizing, differences in the amount, in the geometry and in the energy of hydrogen bonding all impact on the frequency of the ν (OH) IR absorption band, providing semiquantitative information on the structural arrangement of water hydrogen bonding.

Fig. 2a shows the ATR-IR spectra of the ν (OH) region measured in hydrated polysaccharides. The peak at 3800-3000 cm^{-1} shows the presence of three different contributions. At 3250 cm^{-1} , we observe the collective in phase ν (OH) of tetrahedrally coordinated water molecules. The O—H groups vibrating at this wavenumber are attributed to atoms involved in geometrically ordered hydrogen bonds typical of ice-like water (four hydrogen bonds per molecule), identified as *bound-water*. At 3400 cm^{-1} , O—H vibrations are assigned to water in a disordered tetrahedral structure (three hydrogen bonds per molecule), conceptually assimilable to *intermediate-water*, while at 3600 cm^{-1} water O—H bonds are involved in a highly disturbed hydrogen bond network where water clusters contain two or less hydrogen bonds per molecule (Grossutti & Dutcher, 2016), attributable to *free-water*.

This picture of water states allows to identify differences among linear and branched polysaccharides, with a higher degree of bound water reported for branched polysaccharides (Grossutti & Dutcher, 2020). Differently, an AFM-FTIR (atomic force microscope-FTIR) combined analysis on cotton fibers was able to recognize only two ν (OH) at 3440 and 3300 cm^{-1} (Fig. 2b), described by the authors as vibration modes relative to air–water (hydrophobic) and water–cellulose (hydrophilic) interfaces (Igarashi et al., 2020); based on the above discussion, these two modes may be attributed to intermediate and bound-

water, respectively.

2.2. DSC

With a different approach, water states can be investigated by means of DSC, identifying water phase transitions. DSC thermograms carry information related to the change in heat capacity related to freezing (exothermic) and melting (endothermic) phenomena of water. In a typical DSC curve of a hydrated polysaccharide, for example chitosan (see Fig. 2c, case of medium molecular weight 190–310 kDa, with the degree of deacetylation 0.75–0.85 %), during the cooling phase an exothermic peak due to freezing is observed at T between -15 and -20 °C, while upon heating, an endothermic melting peak around 0 °C is observed. Temperatures and intensities of these phase changes can be highly impacted by the variation of water content, presence of salts, and functional groups (Mlčoch & Kučerík, 2013). The enthalpic contribution of melting is ascribed to the amount of water that is available for the freezing process to occur, while water molecules not available for freezing will not concur on the increase of enthalpy. Therefore, by knowing the total amount of water in the sample, assessed in a separate experiment by thermogravimetric analysis (TGA) or gravimetric analysis, it is possible to compare differences in intensity and temperature of melting, enabling the assessment of non-freezable water. The content of the freezable water in the sample, called W_f , can be calculated as:

$$W_f = \Delta H_f / \Delta H_0$$

where ΔH_f is the melting enthalpy of freezable water, ΔH_0 is the standard enthalpy of fusion of pure water with values of 324 (J/g) (Tagliaro et al., 2024). By knowing the total water content of the sample, defined as W_c , the amount non-freezing water, W_{nf} , is therefore determined by:

$$W_{nf} = W_c - W_f$$

One option is to identify non-freezing water with water molecules that are involved in chemical bonds with the polysaccharide and are thus not available for freezing, and assume that freezing-water corresponds to bound-water, as it was frequently done in literature (H. Hatakeyama & Hatakeyama, 1998). However, there is no scientific agreement that the amount of non-freezable water is the same as the amount of water interacting with polymer molecules. Water may be hindered from freezing because of porosity effects. At the eutectic point, when all liquid water is frozen, the solid fraction also crystallizes, but in the case of a gel, crystallization may not occur because of an entangled network (Belton, 1997). The idea of water restricted in its mobility in a porous medium was further confirmed by consideration related to the glass transition temperature, T_g . The T_g of a polysaccharide varies in a very broad range of temperatures depending mainly on the crystallinity and the water content. For example, the T_g of cellulose at 70 % crystallinity may vary from -60 °C at 20 % of water content to 40 °C at 5 % of water content (Szcześniak et al., 2008). During a DSC measurement, when we approach the polymer T_g , water mobility is further restricted in the pores, with the consequent effect of inhibiting water freezing (Kocherbitov, 2016). As such, in this case non-freezing water is both at interfaces and in nanopores.

Fig. 2d shows a DSC analysis of cellulose with 0.8 g/g water content. At decreasing temperature, we observe a first peak between -15 °C and -20 °C and a second peak between -43 °C and -53 °C (H. Hatakeyama & Hatakeyama, 1998). The formation of a second peak was attributed to the freezing of an irregular structure of ice formed under the influence of the hydroxyl group of cellulose. Moreover, since the T of freezing of peak one is slightly lower than that of pure water, -18 °C (X. Zhao et al., 2020) in supercooling conditions, this may also suggest that the freezing of freezable water reflects the influence of cellulose matrix. Comparing cellulose samples with increasing amount of water, we first observe the formation of peak one at water contents between 0.15 (g/g) and 0.25 (g/

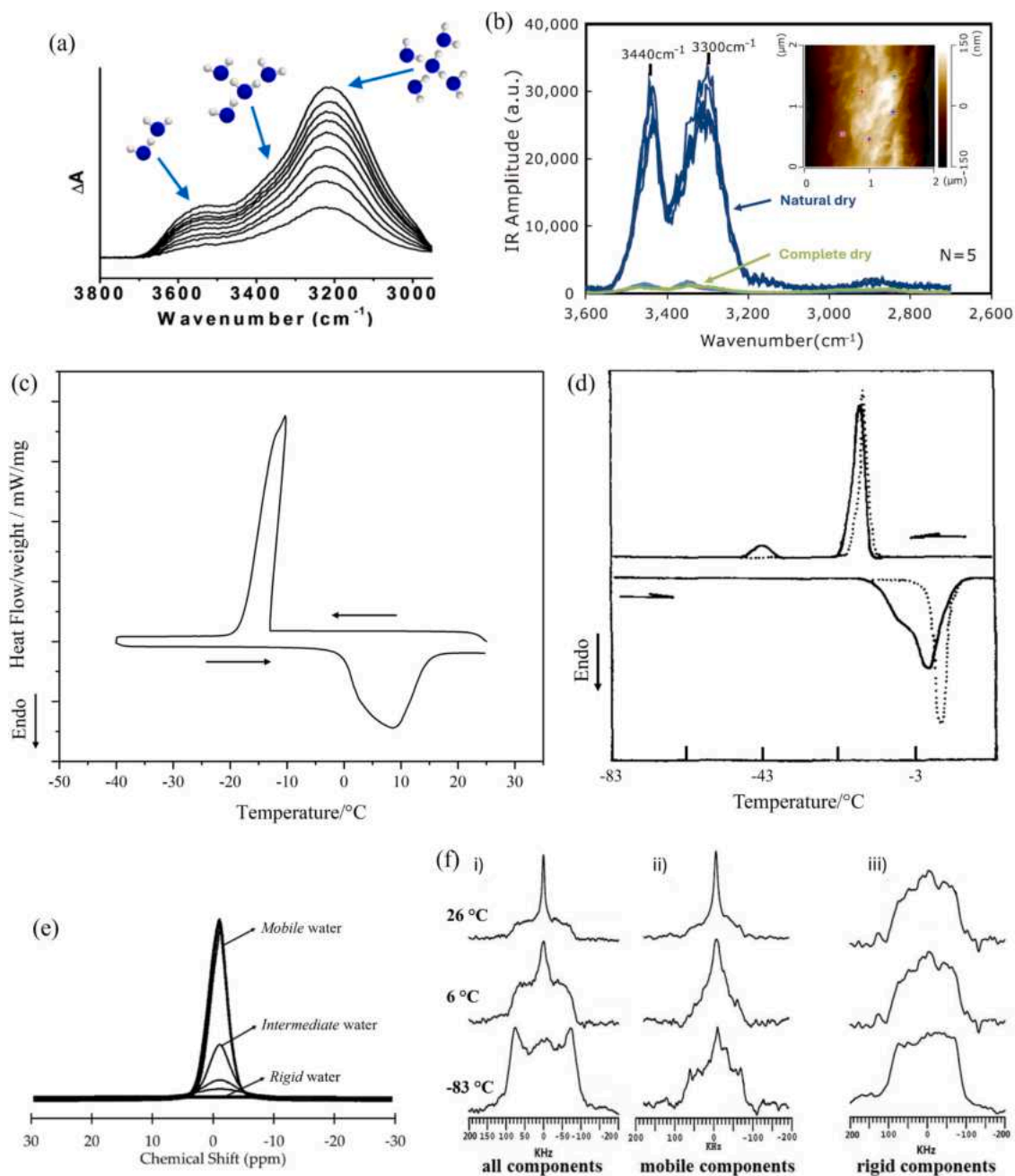


Fig. 2. (a) ATR-IR analysis of $\nu(\text{OH})$ stretching vibrations in hydrated polysaccharides at increasing amount of water. Three water states are visible: 3250 cm^{-1} (tetrahedrally coordinated water molecules or ice-like water, identified as *bound-water*), 3400 cm^{-1} (disordered tetrahedral structure of water, with three H-bonds per water molecule, identified as *intermediate-water*), 3600 cm^{-1} (highly disordered water structure, with one or two H-bonds per water molecules, identified as *free-water*). Reprinted with permission from (Grossutti & Dutcher, 2016) Copyright © 2016, American Chemical Society. (b) AFM-FITIR on cotton fibers showing two water states at 3440 and 3300 cm^{-1} described by the authors as vibration modes relative to air-water (hydrophobic) and water-cellulose (hydrophilic) interfaces, assimilable to intermediate and bound water (Igarashi et al., 2020). This is an unofficial adaptation of an article that appeared in an ACS publication. ACS has not endorsed the content of this adaptation or the context of its use. (c) DSC of chitosan hydrogel, prepared as described in (Tagliaro et al., 2024), showing an exothermic peak of freezing $\sim -18^{\circ}\text{C}$ and an endothermic peak of melting around 9°C with a shoulder at lower temperatures. (d) DSC of cellulose showing two freezing peaks ($\sim -18^{\circ}\text{C}$ and $\sim -43^{\circ}\text{C}$, attributable to the freezing of an irregular ice structure) and one peak of melting with a pronounced shoulder at lower temperatures, due to the melting of two types of water. Adapted from (H. Hatakeyama & Hatakeyama, 1998) Copyright © 1998, with permission from Elsevier. (e) ^2H NMR of a representative hydrophilic polymer, poly(2-methoxyethyl acrylate), showing different dynamics of water molecules: rigid (water molecules with restricted mobility, giving quadrupole interactions and broadening of the peak), intermediate and mobile (water molecules with total freedom of movement, giving weak quadrupole interactions and narrower peaks). Adapted from (Bag & Valenzuela, 2017). License: CC BY 4.0. (f) ^2H -Solid state NMR of hydrated chitosan with identification of mobile and rigid components at different temperatures (26, 6 and -83°C). Adapted from (F. Wang et al., 2016). License: CC BY 4.0.

g) depending on the cellulose crystalline structure (respectively cellulose I and cellulose II). Regarding the cooling part of the DSC curve, we observe a peak around 0 °C with a pronounced shoulder at lower temperatures. This shape describes therefore the melting of two types of water.

The scenario gets even more complex if we analyze polysaccharide solution/systems with a T_g well below 0 °C. In some cases, it is possible to observe what is known as “cold crystallization”. When the amorphous structures frozen under the T_g are heated in a DSC measurement above its T_g , kinetic energy is supplied to the system, which builds an imperfect crystal rearrangement producing a small exothermic peak (Xin et al., 2019). In the case of hyaluronic acid, a cold crystallization peak was observed in a temperature range from –50 to –20 °C, with T_g in a temperature range from –70 to –100 °C and water content from 0.4 to 2.0 (Průšová et al., 2013; Yoshida et al., 1993). In these cases, the melting peak of the hyaluronic acid-ice system occurs in two or three steps in the temperature range from –30 to 3 °C. Other polymers, which show cold crystallization are cellulose, methylcellulose, carboxymethylcellulose, alginate, pectin, xanthan gum, and others (T. Hatakeyama et al., 2010).

These findings suggest that by DSC we may be able to define three states of water: freezable water, a second type of freezable water, which freezes at lower T, and non-freezable water (T. Hatakeyama et al., 2016). Taking into considerations of porosity and T_g effects, able to restrain the mobility of water, it is not straightforward to identify a direct correspondence to IR results.

2.3. Solid state NMR

NMR is a unique analysis technique providing insight into the dynamic behavior of water molecules. The first investigation of liquid NMR on polysaccharide solutions did not identify evidences of mobility alteration of water molecules (Belton, 1997). Indeed, in the solution state, small soluble molecules undergo rapid thermal isotropic motions, which average out all orientation dependent nuclear magnetic interactions. Only by magic angle spinning (MAS) solid state NMR information on solid but also gel-like samples is available. ^1H and ^2H MAS solid state NMR characterize solvent molecules within a supramolecular assembly, unraveling their dynamical properties and distinguishing water molecules based on differences in their chemical shift, relaxation properties and (quadrupolar) order parameters (El Nokab et al., 2022). Fig. 2e shows generic ^2H NMR (Bag & Valenzuela, 2017) spectra for a hydrophilic polymer, poly(2-methoxyethyl acrylate), as a representative example. Depending on the broadening of the signal, it is possible to differentiate contributions related to rigid or mobile water fractions. Rigid water components, whose mobility is restricted by interaction with the polysaccharide, expresses strong quadrupole interactions with a broadening of the peak, while mobile components, derived from water molecules experiencing complete freedom in their mobility, expresses weak quadrupole interactions with a narrowing of the peak. These investigations allowed the definition of different states of water: *bound* water (rigid, highly restricted), *intermediate* bound (restricted) and *free*-water (mobile, not restricted) (H. Zhao et al., 2019). The variation of the dynamic characteristics allowed to define three/four water states for cellulose (Radloff et al., 1996) and chitosan (Fig. 2f) (F. Wang et al., 2016) by ^2H -Solid State NMR. Water states can vary when polysaccharides are investigated in gels and at high moisture content. Time-Domain Nuclear Magnetic Resonance (TD-NMR) can be complementary used to probe the molecular dynamics of water in polysaccharides. The relaxation times (T_1 and T_2) are directly associated with proton mobility, providing insights into the specific chemical and physical environments of water molecules based on their confinement within different sizes of pores (J. Li & Ma, 2022). TD-NMR has been successfully applied to unveil specific water adsorption/retention aspects in cellulose composites (Falourd et al., 2024).

3. Water on surfaces in freezing conditions

Icing on surfaces is a complex multiscale event, which includes nucleation, occurring in nanoseconds at the nanoscale, and freezing of supercooled drops, with time scales from milliseconds to seconds (Tagliaro et al., 2021) at the millimeter scale. Computational modeling of interfaces can explain physical phenomena where experimental investigation remains challenging, yet, one modeling tool cannot accurately model all spatial and temporal scales. Continuum modeling has been applied for investigation of impacts and freezing of supercooled drops and predict fracture mechanics, while molecular modeling for studying the intermolecular forces occurring at the interface between the ice and surface. Differently, by molecular modeling, it was possible to investigate the spatial region of interface, defining in theory the so called *quasi-liquid layer*, composed of water molecules arranged in semi-ordered layers, existing in neither a fully liquid- nor solid-like state. Using molecular dynamics, the ice nucleation rate can be studied in its dependence to water-surface interactions and surface morphologies (Pruppacher et al., 1998). A good ice-nucleation agent has available hydrogen bonds, a crystalline structure matching to the surface of ice, and/or active sites. Active sites are defined as surface property inhomogeneities, including: (i) morphological, e.g. corrugations, cracks, or cavities (Cox et al., 2012), (ii) chemical, e.g. the presence of foreign ions, hydrophilic/hydrophobic sites (Cox et al., 2015b; Lupi & Molinero, 2014) and (iii) electrical, e.g. opposite charges (Glatz & Sarupria, 2016). However, these principles are still under discussion, since a crystallographic similarity to ice does not seem to be always a requirement, because amorphous surfaces also showed ice nucleation properties (Bi et al., 2016; Pedevilla et al., 2016). An increase in the water density profile above the surface was observed to improve nucleation in hydrophilic surfaces (Cox et al., 2015a; Goertz et al., 2007), concurring to the idea that a fine tuning of surface hydrophilicity may be the key to the ice nucleation control and, therefore, to the control of the *quasi-liquid layer*.

Different characterization techniques provided experimental evidence of the *quasi-liquid layer*, although considerable challenges are faced for imaging a water layer in cryogenic conditions. In the early 2000, first attempts by STM successfully imaged water monomers adsorbed on metal surfaces at –257 °C (Morgenstern & Rieder, 2002) and the growth of hexagonal honeycomb water structure (Mitsui et al., 2002) at –173 °C. The STM analysis encounters difficulties related to conductivity for imaging at higher temperatures because of the formation of multiple water monolayers, which cause considerable insulation (Verdaguer et al., 2006). Other studies of water interactions with metal oxides focused on the ability to induce water dissociation by Scanning probe microscopy (SPM) in combination with X-ray photoelectron spectroscopy (XPS) (Verdaguer et al., 2006). The imaging investigation of the *quasi-liquid layer* faces significant problems because the presence of liquid water is incompatible with Ultra High Vacuum Systems (UHV) and the insulating effects hinder the analysis of multiple water layers, therefore limiting imaging to water-rich substrates such as polysaccharides. Environmental Scanning Electron Microscope (E-SEM), which does not need UHV, opens new possibilities for water-substrate interface investigation at cryogenic conditions, but still only few attempts have been made for the experimental observation of the *quasi-liquid layer*. By E-SEM, by controlling temperature and humidity, in situ observation of ice formation allowed the study of ice formation mechanisms and morphological change during ice growth (Krausko et al., 2014). Ice formation on materials such as PTFE and Nylon-6 were investigated by E-SEM with identification of Wenzel and Cassie ice (Lo et al., 2021), defined in analogy to liquid wetting state. By advanced optical microscopy techniques, two types of *quasi-liquid layer* (drop-type and thin-layer-type) were found on both basal and prism faces of ice crystals (Sazaki et al., 2022), formed by the wetting transition of *quasi-liquid layer* on ice crystal surfaces in supersaturated or undersaturated water vapor. The two types were found to be respectively 20- and 200-

times less fluidic than bulk water, determined by the surface tension-to-shear viscosity ratio. Optical microscopy offers also the possibility of studying the ice crystal formation on different substrates (Pach et al., 2018), but lack the spatial resolution for making assumptions on *quasi-liquid layer* thicknesses.

If considerable difficulties in imaging water interfaces at icing conditions persist, the evaluation of *quasi-liquid layer* thickness still faces uncertainties. Spectroscopic measurements suggest that the length scale is up to hundreds of nm, whereas molecular dynamics calculations indicate thicknesses of the order of 10 nm. For example, *quasi-liquid layer* on silicon was calculated to be 30 nm at $-7\text{ }^{\circ}\text{C}$ (Ochshorn & Cantrell, 2008), while on Ge from 1 nm below $-10\text{ }^{\circ}\text{C}$ to 15 nm around $0\text{ }^{\circ}\text{C}$ (Sadchenko & Ewing, 2002). Additional studies calculated *quasi-liquid layer* ranging from 0.5 nm at $-73\text{ }^{\circ}\text{C}$ to 1 nm at $0\text{ }^{\circ}\text{C}$ (Shi et al., 2022). Besides differences in the absolute calculation, it appears clear that the *quasi-liquid layer* thickness increases with temperature (Sadchenko & Ewing, 2002). This was confirmed by ATR-IR spectroscopy, where the interface of ice on Ge was experimentally measured to vary from 1 nm at $-13\text{ }^{\circ}\text{C}$ to 18 nm at $0\text{ }^{\circ}\text{C}$ (Walker et al., 2013). Previous studies measured 2 nm at $-7\text{ }^{\circ}\text{C}$ to 6 nm at $0\text{ }^{\circ}\text{C}$ (Uda et al., 2007). Interestingly, thicker layers were also measured at increasing NaCl concentration allowing the observation up to 160 nm around $0\text{ }^{\circ}\text{C}$ (Walker et al., 2013). These observations prove that the perturbation of the water molecules at the interface may greatly vary up to hundreds of nm, reaching considerable thickness at temperatures around $0\text{ }^{\circ}\text{C}$ and in the presence of solutes. These findings greatly expand our understanding of interfaces and pave the way to the investigation of hydrophilic/water-rich substrates, improving our possibilities to control surface phenomenon by the control of the *quasi-liquid layer*. The technical complexity of producing direct experimental data on *quasi-liquid layer* greatly limits the possibility of investigation. To the best of our knowledge, there are no studies which addressed direct analysis of *quasi-liquid layer* on polysaccharides, but only indirect evidence which will be discussed in the next sections. Although, the formation of a *quasi-liquid layer* was quantified up to 5 nm around $0\text{ }^{\circ}\text{C}$ (Walker et al., 2013) in the case of an AFP solution, which is a very interesting system for investigating water-proteins interactions at freezing conditions.

The ice-AFPs interaction opens a whole new field of investigation of surfaces at freezing conditions and provides valuable comparison to ice-polysaccharide interactions, because broadly investigated since more than 50 years. AFPs lower the freezing point of aqueous solutions without affecting the melting point (thermal hysteresis, TH) (Meister et al., 2013), up to $5\text{ }^{\circ}\text{C}$. A direct protein binding to the growing ice surface generates alteration of water dynamics extending up to $20\text{ }\text{\AA}$ from the protein surface. Therefore, AFP act as ice nucleators producing a higher number of small crystals at the expense of bigger ones (ice recrystallization inhibition, IRI), and can modify the morphology of ice crystals (dynamic ice shaping, DIS) (He et al., 2018). The most recognized structure-property feature of these proteins is their amphiphilic nature, which has been connected to formation of an ice binding site and a non-ice binding site (He et al., 2018), a perfect arrangement for better interacting with growing ice. Another interesting feature is the presence of amino acids such as Threonine or Alanine (i.e. in the AFP ex1 wfAFP-1), which may alter the crystalline structure of forming ice (T. Sun et al., 2014). Despite the structural diversity of AFP, common features of the ice-binding sites include hydrophobicity, no charge, flatness, rigidity (Davies, 2014). In the case of AFGPs, the sugar unit was also reported to be an active ice-binding site (Groot et al., 2016). In a hypothetical ice-binding mechanism, water is organized by the ice-binding sites into a constrained ice-like arrangement which freezes below $0\text{ }^{\circ}\text{C}$ (Bar Dolev et al., 2016; Davies, 2014). The well-established freezing protection action of AFPs is already being exploited in the preservation of frozen foods, as cryoprotective agent in fertility treatments, in biomedical application i.e. cryosurgery (Baskaran et al., 2021), and to produce icephobic materials (Esser-Kahn et al., 2010; Gwak et al., 2015; Kasahara et al., 2020).

The investigation of the AFP interaction with ice inspired the exploration of other classes of materials exhibiting similar interactions with ice i.e. polymers and polysaccharides. The specific investigation of cryoprotective aspects of polysaccharides by the techniques (i.e. splat and sandwich assay) has been addressed in the following chapters. The idea of exploiting the AFP ability to control ice growth over surfaces resulted in the development of new strategies to produce icephobic materials. AFPs were applied to a glass (Kasahara et al., 2020), aluminum (Gwak et al., 2015) or polymeric (Esser-Kahn et al., 2010) surfaces, opening to new investigation strategies of the interface. Icephobic materials, also referred to as passive surfaces/coatings, are developed (in combination to active strategies i.e. thermal heating, antifreeze liquids and mechanical vibrations) to induce weak adhesion of ice to surfaces and low energy or natural debond of ice for aerospace applications (Irajzad et al., 2019). In this field, the investigation of the ice interface is commonly addressed by the measurement of ice adhesion (force of detachment/area of ice-surface contact). Icephobic materials can be produced varying both the chemistry and the structure of the surface. If superhydrophobic surfaces (obtained by a combination of roughness and chemical repellence) (Amirfazli & Antonini, 2016) are considered the benchmark for water rapid shedding and roll-off before freezing occurs (He et al., 2021), in high humidity conditions, water can penetrate in the asperities of highly structured materials causing mechanical interlocking and stitching irreversibly ice to surfaces (J. Chen et al., 2012; He et al., 2021; Tagliaro et al., 2024; Varanasi et al., 2010). Therefore, smooth surfaces are usually more desirable in the aim of lowering ice adhesion. Smooth fluorinated polymers can achieve very low ice adhesion lower than 100 kPa (Hernández Rodríguez et al., 2024), but are now raising concerns for environmental and health reasons (Evich et al., 2022). Other surfaces promoting ice slippage thanks to liquid infusion, SLIPS (P. Kim et al., 2012; Wong et al., 2020) can display extremely low ice adhesion $<30\text{ kPa}$. Alternative strategies are devoted to investigating hydrophilic surfaces for controlling ice accretion and adhesion force. Materials based on hygroscopic polymers (J. Chen et al., 2013) and polymers with hydrophilic pendants (Dou et al., 2014) showed icephobic performance, being able to reduce ice adhesion down to $(55 \pm 15)\text{ kPa}$ and $(27.0 \pm 6.2)\text{ kPa}$, respectively. In this area, polysaccharides appear as very promising raw materials combining hydrophilicity and water adsorption capacity, aspects that may provide a fine control of the ice interface, providing, at the same time, environmental benefits because biodegradable and non-toxic (J. Chen et al., 2014). The effective understanding and exploitation of multiple water states, as detailed in the previous section, may greatly impact on our ability of engineering efficient strategies for icephobic materials.

By the implementation of investigation techniques from different areas of materials science naming (i) *quasi-liquid layer* modeling and characterization, (ii) cryopreservation analysis by TH, IRI, DIS and (iii) icephobicity properties and ice adhesion, we foresee great benefit in the overall understanding of water at the interface at freezing conditions.

3.1. Ice for polysaccharide templating

Ice formation and growth can be exploited to prepare specific material structures, results of water-surface interactions at freezing conditions. Therefore, freeze-drying could be regarded as a relevant model system to probe the interaction of water with surfaces, but still our understanding allows for limited exploitation only for material processing. Freeze-drying, or its variant freeze-thawing-drying, is a widely used process used both for high-value products in pharmaceutical, food and biomedical industries, and for fabrication of porous materials from solutions and suspension by ice-templating (Deville, 2013; Pawelec et al., 2014), specifically polysaccharides (Antonini et al., 2019; Gelas & Budtova, 2024; Legay et al., 2023; Pourhaghgouy & Zamanian, 2015). Ice templating enables the creation of three-dimensional porous structures, taking advantage of ice natural tendency to exclude impurities or solutes during crystallization (Pawelec

et al., 2014). During freezing, ice is “self-cleaning” as it does not allow incorporation of particles, so that spaces between ice grains are enriched with the solutes or suspended materials (Deville, 2013). The process is versatile and can generally be used with a variety of materials, including colloids, ceramics and particles (Deville, 2017). The end product is a porous material, with typical pore sizes ranging from 1 to 100 μm (or even 1 mm), that can be either isotropic or anisotropic, with high interconnectivity between the pores (i.e. open porosity).

The thermodynamic process is schematically presented in Fig. 3a: a colloidal suspension or solution containing the desired material (such as a polysaccharide) in liquid water (state 1) is frozen by lowering temperature, reaching a frozen state (2) at environmental pressure. At these point, two options are possible: in freeze-thawing-drying, the block is first thawed to room temperature (3), then the solvent is evaporated (4); differently, in freeze-drying the pressure is decreased below the triple point (for water, $p = 611 \text{ Pa}$, $T = 273.16 \text{ K}$) and temperature increased, so that direct ice sublimation takes place. Fig. 3b shows the example of a suspension of cellulose nanofibrils (CNFs) in water, which in the wet state appears as a paste. If water is removed by direct drying, capillary forces lead to a material collapse, resulting in a dense, non-porous material (Fig. 3b). To get to a porous material, freeze-drying/freezing-thawing-drying are necessary. First, by cooling the cellulose suspension ice nucleation and crystal growth take place; during this stage, ice crystals begin to form at nucleation sites. The ice formation follows specific crystallographic patterns, with growth typically occurring faster along the a-axis than the c-axis. Directional growth is crucial if anisotropic aligned porous structures are desired. As the ice crystals grow, the forming ice rejects the suspended fibers, concentrating them in the spaces between the growing ice crystals. This rejection mechanism is key to the process, as it creates the template for the final porous structure. The fibers become concentrated in the interstitial spaces between ice crystals, forming the walls of what will eventually become pores. After complete freezing, the ice is removed, either through sublimation (direct conversion from solid to vapor) under vacuum conditions (freeze-drying) or by thawing and drying. This step preserves the structure created by the ice crystals, leaving behind a porous network where the ice once was (see SEM image in Fig. 3c).

The resulting final porous structures can be controlled through various processing parameters, including: (i) the freezing rate, affecting pore size and orientation, (ii) the solid loading (concentration of particles and fiber), influencing the final porosity and density; (iii) the temperature gradient direction, controlling the pore alignment, and (iv)

additives, that can modify the ice crystal growth and ultimately the final structure. Nonetheless, the correlation between process parameters and ice-templated material properties (pore size, distribution, specific surface area, etc.) are limited, and based on empirical rule-of-thumbs, rather than robust design principles. Research on the topic is thus desirable to create predictive physics-based models.

4. Properties of hydrated polysaccharides in freezing conditions

Our understanding of water states and investigation of interfaces in freezing conditions for different applications in the previous section has set the background for interpretation of experimental results on polysaccharide-based materials. In this section, our ambition is to comprehensively review what is known on the properties of polysaccharide-based materials in freezing conditions, merging knowledge from different disciplines and applications. The discussion is structured and categorized on the base of the polysaccharide type, based primarily on literature availability. Among all polysaccharides, we have shortlisted those that were systematically investigated across different disciplines. Outside our discussion, it is worth mentioning Fucopol which is a high molecular weight exopolysaccharide containing fucose, known for being applied as cryoprotectant (Carvalho & De, 2009; Guerreiro et al., 2020).

4.1. Cellulose

Cellulose, a water insoluble (T. Hatakeyama et al., 2016) polymer of glucose (Czihak et al., 1999), is the most abundant polysaccharide (Hou et al., 2021) and renewable biopolymer in existence (T. Li et al., 2019), primarily found in plants, but also bacteria, as a structural material (Czihak et al., 1999). More in detail, cellulose is a linear-chain polymer, formed by the repetition of β -D-glucopyranose units linked through a 1,4-glycosidic bond (Wohler et al., 2022). The presence of hydroxyl groups makes cellulose mainly hydrophilic; however, because of the ordered crystalline stacking of glucose units, cellulose remains insoluble and often requires proper chemical modification (Klemm et al., 2005). Different orientation in the crystalline planes determines increased hydrophilicity by exposing (110) (dominant) and (010) planes, while hydrophobicity increases in (200) planes. It has been calculated that the two crystalline hydrophilic planes of cellulose expose about 5.4 OH groups/ nm^2 that are potentially accessible to the environment, while in fibrils 3.6 OH groups/ nm^2 are involved in weak bonds of microfibrils

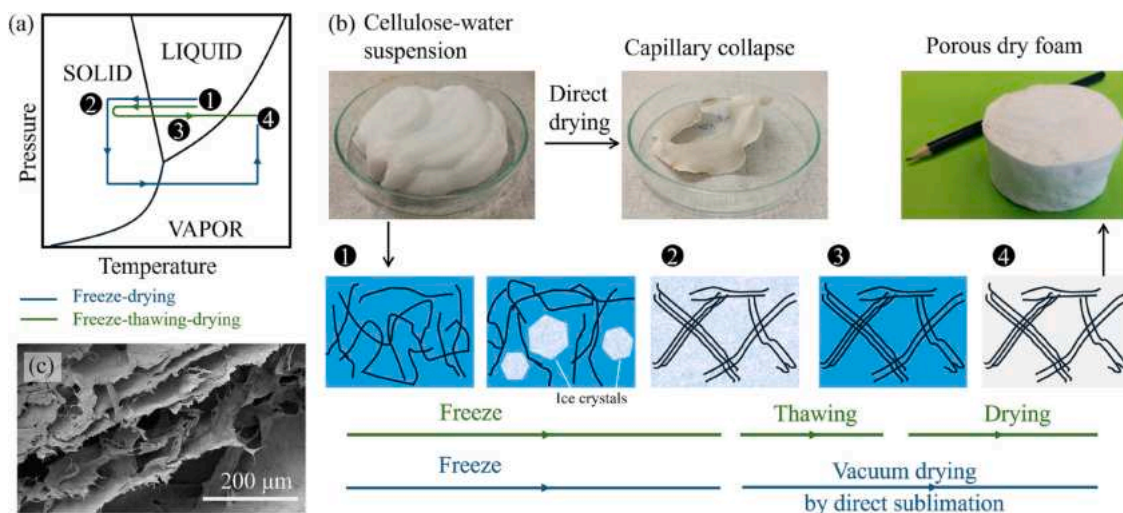


Fig. 3. Schematic of the freeze-thawing-drying and freeze-drying processes. (a) Phase diagram of water, showing the states and processes of polysaccharide suspension. Numbers correspond to states illustrated in (b). If a cellulose nanofibril-water suspension (state 1) is directly dried, capillary collapse occurs. However, following freeze-thawing-drying (state 2, 3, and 4, respectively) or freeze-drying processes, a porous dry foam can be obtained. (c) SEM image of a porous dry cellulose nanofibril foam, where the characteristic cellulose walls creating during the freeze-thawing-drying process can be observed.

(Wohlert et al., 2022). Cellulose is found in nature as assembly of highly crystalline nanofibrils, frequently referred to as I_α and I_β depending on the origin of the cellulose (Klemm et al., 2005), separated by amorphous regions (T. Li et al., 2019). Therefore, it is possible to isolate cellulose nanofibrils (CNFs) (also referred to as nanofibrillated, microfibrillated cellulose), through mechanical delamination of wood pulp or through enzymatic (Henriksson et al., 2007; Pääkko et al., 2007) and chemical treatments (Saito et al., 2007), obtaining long fibers comprising

amorphous and crystalline domains; also, it is possible to isolate cellulose nanocrystals (CNCs) through acid hydrolysis and sonication, resulting in the complete elimination of the amorphous regions (Klemm et al., 2011).

Great mechanical properties of toughness and strength have been connected to the intricate intermolecular interaction forces which take place in CNFs. Hydrogen bonds work both inter- and intra-molecular, holding the cellulose polymer chains together side-by-side, with the

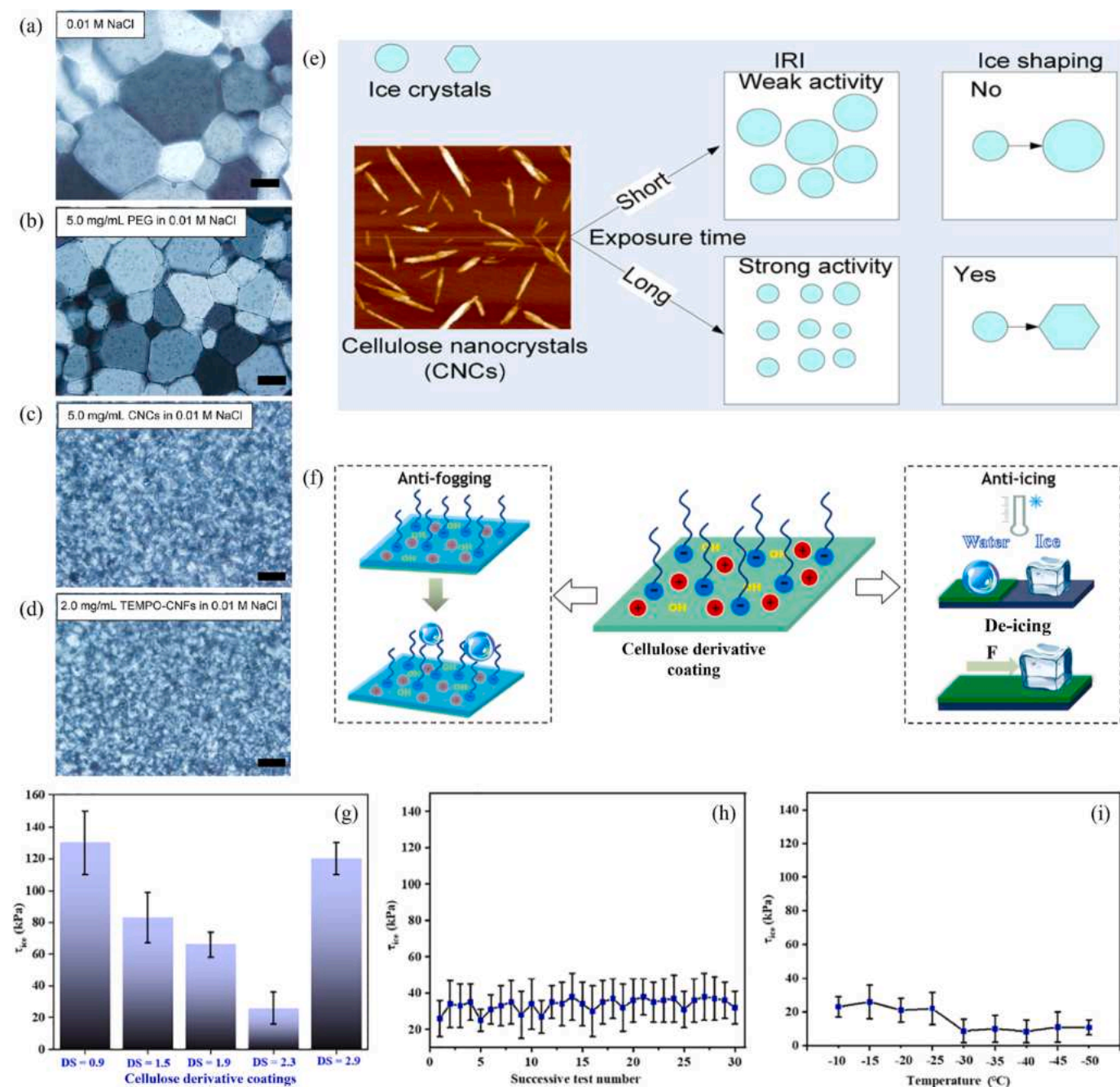


Fig. 4. Representative polarized light microscopy images of ice wafer after 30 min annealing at -8 °C in 0.01 M NaCl: (a) blank, (b) 5.0 mg/ml polyethylene glycol (PEG), (c) 5.0 mg/ml CNCs, (d) 2.0 mg/ml 2,2,6,6-tetramethylpiperidine-1-oxyl oxidized cellulose nanofibrils (TEMPO-CNFs); (scale bar = 50 μ m; NaCl = blank control; PEG = negative control). Adapted with permission from (T. Li et al., 2019). Copyright © 2019, American Chemical Society. (e) Time-dependent ice recrystallization inhibition and ice shaping: no ice recrystallization inhibition is observed after a short exposure time, but both ice recrystallization inhibition and ice shaping activity can be noticed after a long exposure time. Reprinted with permission from (M. Li et al., 2022). Copyright © 2022, American Chemical Society. (f) Schematic diagram of anti-fogging, anti-icing and de-icing of cellulose derivative coating (adapted); (g) ice adhesion strength at -20 °C on the cellulose derivative coatings with different DS (DS = degree of substitution, average number of hydroxyl groups which were modified in each anhydroglucose unit); (h) ice adhesion strength on the cellulose derivative coating with DS = 2.3 at -20 °C repeated 30 times; (i) ice adhesion strength on the cellulose derivative coating with DS = 2.3 at different temperatures. Adapted from (Cheng et al., 2022) Copyright © 2022, with permission from Elsevier.

formation of fibrils, also due to van der Waals weak bonds at longer interaction range and to physical entanglement (Li, Chen, et al., 2021). Because of the cooperative nature of hydrogen bonds, a molecular-level toughening mechanism was observed at lowering dimensions of fibrils, involving a cascade of breaking and reforming weak bonds under tension, leading to energy dissipation and, therefore, enhancement of fracture toughness (Zhu et al., 2015). Because of its high water-binding capacity, CNFs are generally considered difficult to dry and to subsequently rehydrate (Onyianta et al., 2023), hindering their applicability at industrial levels. Water-nanocellulose interactions were used to characterize the degree of fibrillation in CNFs from various fiber sources, finding increased water retention at higher defibrillation energy (X. Zhang et al., 2023).

CNFs and CNCs were investigated in their ice nucleating properties showing an activity comparable to mineral dust particles (Hiranuma et al., 2019). In this study, CNFs resulted in higher ice nucleating activity than CNCs below -20°C , although the difference was not maintained at higher T. The same authors observed experimentally that ice nucleation can be promoted by CNF particles in simulated supercooled atmospheric clouds (Hiranuma et al., 2015). Other investigations were performed on 2-hydroxyethyl cellulose showing low ice nucleation activity and no influence between samples with low and high molecular weight. At the same supercooling rate, the difference in the nucleation rate in the presence of modified cellulose was reported to be less than 1 order of magnitude compared to pure water, while significantly different from the remarkable effect of AgI and other particles (X. Zhang & Maeda, 2022). Differently, the comparison between CNCs decorated with chitosan and AgI nanoparticles showed opposite results in ice nucleating performances in PBS solutions (Hou et al., 2021). In this study, the presence of decorated CNCs promoted the growth of needle-like ice crystals for application in cancer therapy for targeting and intensifying tumor eliminations through cryoablation, a therapeutic technique that exploits formation of ice crystals to disrupt undesired tissue. They also calculated by molecular dynamics simulation that the icy cluster formation in a pure water model is up to 500 times slower than the case of water nucleation on the (-110) crystalline plane of cellulose.

CNCs were also studied for their IRI activity (Li, Dia, & Wu, 2021, Li, Chen, et al., 2021, Li et al., 2022, Li et al., 2023b, Li et al., 2023c, Li et al., 2023a; T. Li et al., 2019; Li, Li, Dia, et al., 2020; Li, Li, Zhong, & Wu, 2020; Li, Li, Zhong, & Wu, 2020), which is the effect of preventing large ice crystals growth at the expense of smaller ones.

Ice recrystallization is a specific case of Ostwald ripening (general physical process describing the growth of larger particles at the expense of smaller ones) concerning ice crystals. Therefore, IRI is the suppression of this process. The inhibition mechanism and the factors involved in IRI are still poorly understood, although the control on ice crystal growth is of tremendous interest in food storage and cryopreservation (Rahman et al., 2019). From Fig. 4a-d, it is possible to appreciate IRI activity of CNCs (suspended in 0.01 M NaCl solution) from the lowering in crystals dimensions of an order of magnitude in comparison with the bare solution 0.01 M NaCl and the polyethylene glycol (PEG) negative control (T. Li et al., 2019). The reported IRI effect was anyway below that of polyvinyl alcohol (PVA), usually referred as positive control. In the same study, bare CNFs and CNFs which underwent a 2,2,6,6-tetramethylpiperidinyloxy (TEMPO) oxidation also showed IRI activity, but much reduced for bare CNFs. After this first result, other studies focused on study of different parameters influence on the CNCs IRI activity, including temperature (M. Li et al., 2023c), concentration of CNCs (M. Li et al., 2023a) which may imply depletion effects, significantly more in sandwich assays rather than splat assays (M. Li et al., 2023a), surface charge density (T. Li, Zhong, et al., 2020), sucrose concentration (Li, Dia, & Wu, 2021), observation time scale (M. Li et al., 2022), fibril length (T. Li, Li, Zhong, et al., 2020), and addition of other polymers (M. Li et al., 2023b). In summary, both CNC diffusion rate and ice crystal coverage affect IRI activity (M. Li et al., 2023c).

Moreover, CNCs also demonstrated ice-binding properties, which

result in the formation of angular and elongated ice crystals formation (Gaukel et al., 2014). The CNC ice-binding properties were proved to be highly dependent on the observation time. In a 25 % sucrose solution, no CNC IRI activity was observed on a short time scale (up to 2 h), while a positive effect was recorded at longer time of exposition (up to 72 h), when after slow adsorption kinetic the adequate surface coverage of ice crystals was reached (Fig. 4e).

Because of its demonstrated IRI activity, CNCs are promising additives in the food industry (Li, Dia, & Wu, 2021, 2022, 2023b, 2023a, 2023c; Su et al., 2023). For example, carboxymethyl cellulose was added in frozen dough as inhibitor of ice crystal formation and recrystallization (Su et al., 2023) and CNCs (M. Li et al., 2022) as stabilizer in ice cream. Furthermore, ice recrystallization inhibitors are emerging also in the field of cryoprotection, for cell cryopreservation. However, the IRI activity does not always correlate with the cryoprotection ability, as is the case of AFGPs and polyvinyl alcohol, which are not effective cryoprotectants despite being excellent IRI. The IRI has been correlated to their dynamic ice shaping ability, which causes the formation of ice crystals with needle-like shape, that can pierce and kill cells (T. Li, Li, Dia, et al., 2020). Moreover, CNC dispersions aggregate at high ionic strength, losing IRI activity, and significantly limiting their use in physiological conditions (T. Li et al., 2019).

Finally, different kinds of modified cellulose were investigated to develop anti-icing and de-icing coatings (Cheng et al., 2022; Shibraen et al., 2016). Anti-icing typically refers to ice formation inhibition or delay, and de-icing to ice detachment. An anti-fogging and anti-frosting material was prepared by layer-by-layer assembling of quaternized cellulose and carboxymethyl cellulose, due to the electrostatic interaction between positive charges of quaternized cellulose and the negative charges of carboxymethyl cellulose. Best anti-fogging and anti-frosting properties were found for carboxymethyl cellulose, and were attributed to its water sorption ability. The performance was better than similar assemblies of quaternized cellulose with synthetic poly-electrolytes, namely poly(acrylic acid) and poly(styrene sulfonate), which did not show similar properties (Shibraen et al., 2016). Anti-fogging, anti-bioadhesive and anti-icing properties were obtained with the synthesis of a cellulose derivative decorated with hydrophilic hydroxyl groups, imidazolium cations, and hydrophobic perfluorooctanoate anions (Fig. 4f). The anti-icing and de-icing effect of these transparent and hard membranes was attributed to high water contact angle and to the reduced heat transfer between the substrate and water, allowing a decrease of ice nucleation temperature (reduced to -30°C with freezing time after 2 h at -25°C) and ice adhesion strength down to 10 kPa (Fig. 4g-i) (Cheng et al., 2022).

4.2. Chitin, Nanochitins and Chitosan

Chitin is a structural, highly insoluble polysaccharide, the second most abundant in the world, present in shrimp and crab shells, fungal *mycelia*, and in the yeast cell walls with a chemical structure of 2-acetamido-2-deoxy- β -D-glucose (Kumar, 2000; Pillai et al., 2009). From its partial deacetylation, a polysaccharide soluble in acidic water, called chitosan, is derived (Rinaudo, 2006).

From chitin, different forms of nanochitins can be obtained, such as chitin nanoparticles, nanofibers and nanocrystals (Bai et al., 2022; Correa-González et al., 2024), also able to stabilize emulsions (Perrin et al., 2014; Y. Zhang et al., 2015) and with possible IRI activity due to their amphiphilic nature.

Only very recently it was demonstrated that rod-like chitin nanocrystals, prepared by HCl hydrolysis, showed IRI activity at concentrations below 10 mg/g (in sucrose solution and PBS), together with ice-shaping at a concentration of 1 mg/g. In this study (Correa-González et al., 2024), chitin nanocrystals were found to express higher IRI and ice-binding properties than CNCs, but no TH (Fig. 5a). Besides, no structure-properties explanations were formulated to explain these properties.

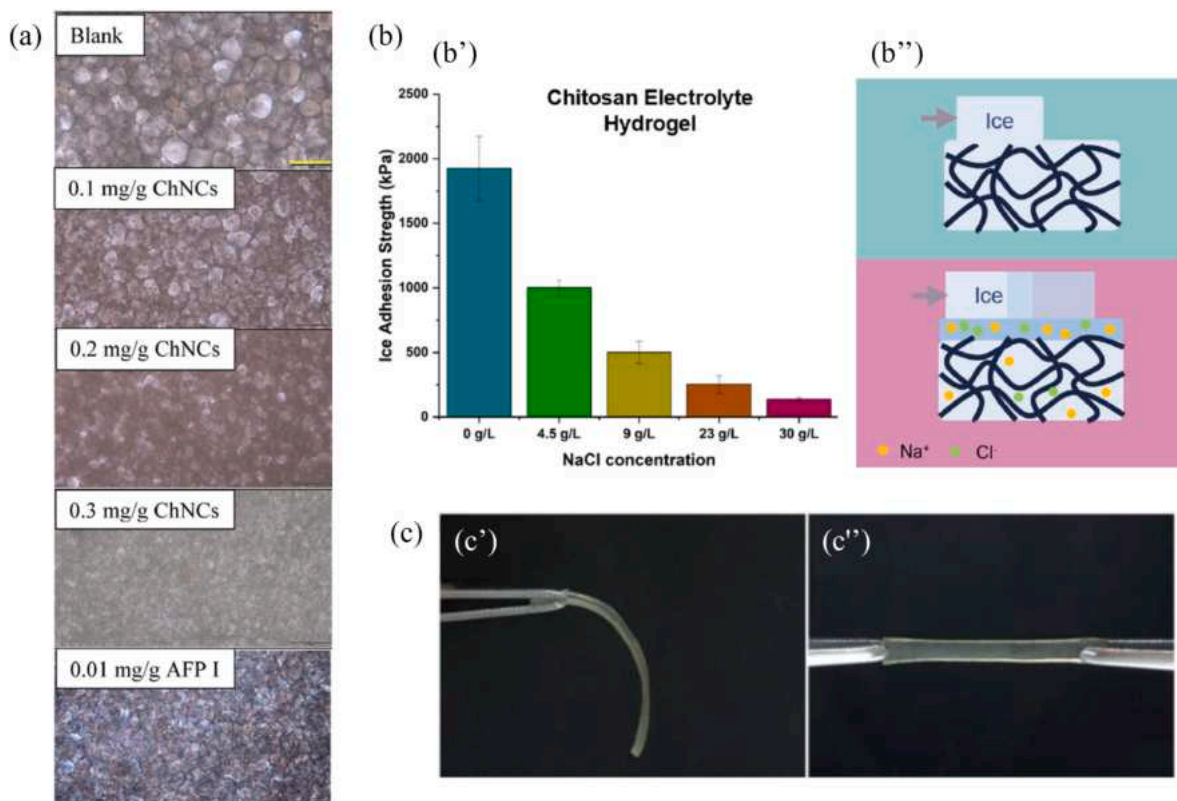


Fig. 5. (a) Representative polarized light microscopical images of ice wafer images of blank (3.0 % sucrose solution), 0.1–0.3 mg/g chitin nanocrystals (ChNCs), and 0.01 mg/g AFP I (the scale bar is 50 μ m). Adapted from (Correa-González et al., 2024) Copyright © 2023, with permission from Elsevier. (b) Low ice adhesion chitosan hydrogel: (b') ice adhesion strength on chitosan hydrogels infused with NaCl at different concentrations (0 g/L, blue; 4.5 g/L, green; 9 g/L, yellow; 23 g/L, orange; 30 g/L, magenta) at -10 °C; (b'') representation of colligative mechanism for obtaining low ice adhesion. Reprinted from (Tagliaro et al., 2024). License: CC BY 4.0. (c) Chitosan-based hydrogel infused with sodium citrate can remain (c') soft and can be (c'') stretched even after 24 h at -20 °C. Adapted from (Yang et al., 2021) Copyright © 2020, with permission from Elsevier.

Although studies on chitin and chitosan as cryoprotectant are still at an embryo stage, a variety of chitosan-based materials were studied as hydrogels or porous/adsorbent matrices resistant to freezing, for applications as sensors (Yang et al., 2021), flexible electronics (Yang et al., 2022) and low ice adhesion surfaces (Tagliaro et al., 2024). In these studies, chitosan is commonly used as a supportive matrix for holding high amounts of water together with anti-freezing agents. Chitosan-based hydrogels showed low ice adhesion properties down to 140 kPa (Tagliaro et al., 2024) when NaCl solutions were added (Fig. 5b) and to 29 kPa (Kwon & Jin, 2023) when infused with commercial lubricants. Moreover, chitosan-based sensors which can maintain their flexibility and electrical conductivity at low temperature were formulated in the presence of sodium citrate (down to -20 °C, Fig. 5c) (Yang et al., 2021) and glycerin (down to -25 °C) (Gao et al., 2022). Based on available literature, the anti-freezing properties of chitosan-based materials seem to be ascribed to colligative properties of solutions, whereas additional studies are required to investigate more in detail the ice-binding properties of nanocrystals and open investigations on chitosan water states in hydrogels to unravel the chitosan potential to hold multiple water states and solutes.

4.3. Hyaluronic acid

Hyaluronic acid is a glycosaminoglycan composed of repeating disaccharide units of D-glucuronic and N-acetyl-D-glucosamine linked through β -1,4 and β -1,3 glycoside bonds (Vigetti et al., 2014). The repetition of these units forms coiling chains, whose molecular weight and length can vary up to 1000 kDa and 10 nm, respectively (Vasvani et al., 2020). Due to the presence of many carboxylic, hydroxyl and

amide groups, different chemical modifications can be performed. In addition, since at physiological pH the carboxylic moieties are dissociated and interact with other cations, such as Na^+ , hyaluronic acid is also called “hyaluronan”. The presence of hydrophilic and negatively charged moieties provides also the ability of binding water molecules and to form viscous networks, especially at high molecular weights (Abatangelo et al., 2020). This polysaccharide is naturally present in all vertebrates, especially in the extracellular matrix of adult soft connective tissues (Vigetti et al., 2014), to which provides support due to the water binding, and provide cell signaling functions (Dovedytis et al., 2020).

Hyaluronic acid is widely used in the pharmacological field, due to its biocompatibility, physiological activity, and viscoelastic properties, but also due to biodegradability, non-toxicity and large-scale production (T. W. Lee et al., 2021). In terms of cryoprotection properties, sodium hyaluronate with different molecular weights was applied for the cryopreservation of red blood cells (X. Liu et al., 2021). The aspects involved in the properties of cryopreservation have been addressed to the water binding capacity and the increased viscosity of solutions which can promote vitrification under fast freezing, reducing ice crystals formation and cell cryoinjuries (Fig. 6a). In particular, with a medium molecular weight (135 kDa) and a concentration of 1 mg/ml, a recovery of (63.2 ± 3.5) % was obtained, with improvements up to more than 90 % with an addition of only 5 % v/v of glycerol (X. Liu et al., 2021). Other studies report a post-thaw recovery of mouse NIH-3 T3 cells up to 90 %, when compared to 37 % of recovery in presence of DMSO, the most common cryoprotectant (T. W. Lee et al., 2021). Also, low molecular weight hyaluronan was tested as cryoprotectant in encapsulated cells (Gurruchaga et al., 2018) and in human dermal fibroblast monolayers

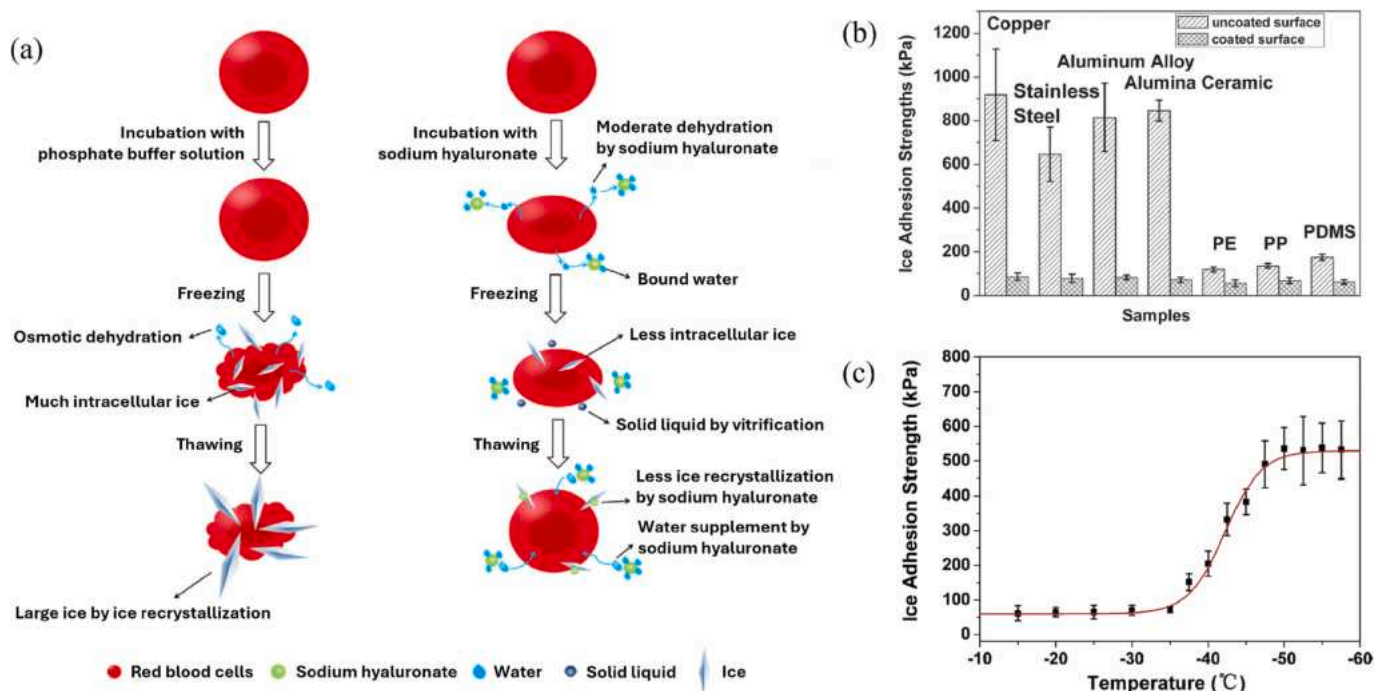


Fig. 6. (a) Schematic representation of the mechanism of medium molecular weight sodium hyaluronate in cryopreservation of red blood cells: water binding ability and solution viscosity increase can inhibit ice crystal formation and promote vitrification under fast freezing, leading to reduced cell cryoinjuries. Adapted from (X. Liu et al., 2021). License: CC BY 4.0. (b) Average ice adhesion strengths on different materials uncoated and coated with hyaluronic acid measured at -15°C with a probe speed of 0.5 mm s^{-1} , and (c) ice adhesion strength on the aqueous lubricating layer of hyaluronic acid surface at different temperatures (the increase of ice adhesion at $\sim -42^{\circ}\text{C}$ is ascribed to the disappearance of the aqueous lubricating layer due to the phase transition of the liquid water to the ice). Reprinted with permission from (J. Chen et al., 2014) © 2014 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

(Ujihira et al., 2010). Because in these studies no IRI effect was observed, it was proposed that the inhibition of signal pathways and the direct interaction with the primary receptor of hyaluronan (transmembrane CD44) may be responsible for effective cryopreservation (Gurruchaga et al., 2018) because of reduced apoptosis (T. W. Lee et al., 2021).

Hyaluronic acid was also applied in the lyophilization of unilamellar liposomes used in carrier-mediated therapies that require particles of nanometer size. In detail, hyaluronan was anchored to unilamellar liposomes for increased cryopreservation during the process of lyophilization, enhancing structural stability of liposomes and reducing stress related to rehydration (Peer et al., 2003).

Finally, a de-icing coating was created by combining the water absorbing property of hyaluronic acid with dopamine, which acted as both adhesion promoter and crosslinking agent. (Fig. 6b). Depending on the coating thickness, ice adhesion strength varied down to 61 kPa with film thickness of 20 nm. The authors associate the low ice adhesion to the swelling of the hyaluronic acid-dopamine network with the formation of a lubricating water layer over the surface with ice down to -42°C (Fig. 6c) (J. Chen et al., 2014). We infer that the lubricating layer mentioned corresponds to an interface of disordered water molecules, i. e. *quasi-liquid layer*.

4.4. Tamarind seed polysaccharide (TSP)

Tamarind seed polysaccharide is a galactoxyloglucan extracted from the tamarind seed. It is constituted of glucose, xylose and galactose, but the exact molar ratio between these components depends on different factors, such as the extraction method, and the geographical origin, breed and physiological stage of the seeds. A mixture of nonasaccharides and octasaccharides in a percentage ratio of 3:2, sometimes interrupted by heptasaccharides, hexasaccharides, or hendesaccharides, constitutes the chemical structure of TSP, which was obtained by knowing the

monosaccharide composition, and due to methylation analysis, NMR and MALDI-TOF MS studies (Zhang, Cao, & Juan, Wei, W. ying, & Ying, X. guo., 2020).

It was found that TSP shows IRI properties (X. Sun et al., 2024, 2025; Sun, Guo, Zhan, et al., 2023; Sun, Guo, Kou, et al., 2023) and that its IRI activity depends on its molecular weight, concentration, and ions present in the dispersing medium. In addition, TSP was found to lower the melting temperature of ice and inhibit the ice crystal growth. Raman spectroscopy measurements highlighted the weakening of vibrations related to the tetrahedral arrangement of water molecules in the presence of TSP, revealing a possible destructuring effect on the hydrogen-bond network of water (X. Sun, Guo, Kou, et al., 2023). Molecular dynamic simulations showed that TSP may adsorb at the ice front, inducing structural defects in the first molecular ice layers at the interface with the polysaccharide and also a curvature of the ice front. These findings may support the experimental analysis on the retarding of ice growth (Fig. 7). A stronger IRI effect for low molecular weight TSP ($\sim 20\text{-}200\text{ kDa}$) was observed in comparison to the native TSP ($\sim 2400\text{ kDa}$), ascribed to an enhancement of chain hydration caused by a decrease in aggregation (X. Sun, Guo, Kou, et al., 2023). These low molecular weight TSP fractions are also particularly promising as ice recrystallization retardation agents in ice cream mixes, due to the formation of uniformly shaped ice crystals (X. Sun et al., 2024). Together with depolymerization, degalactosylation of TSP was also found to improve IRI due to the formation of supramolecular self-assembled rod-like fiber. The authors addressed the IRI activity to the supramolecular assemblies rather than to ice binding properties (X. Sun, Guo, Zhan, et al., 2023). Moreover, also the presence of ions can influence the IRI activity of TSP. Indeed, TSP shows a greater IRI activity when highly hydrated and in a lower aggregation state, and salts are actually able to act on hydration and aggregation of polysaccharides. In particular, an inverse dependence from the Hofmeister anion sequence (a classification of ions based on their lyotropic properties) was observed (X. Sun et al., 2025). From an

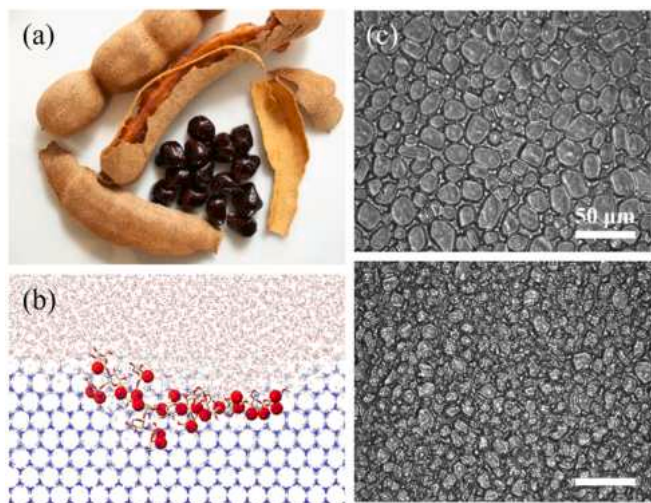


Fig. 7. (a) Picture of tamarind seeds. (b) Molecular dynamic simulation of ice-growth shows how tamarind seed polysaccharide may bind to ice. (c) Micrograph images of ice recrystallization inhibition activity of tamarind seed polysaccharide: reference sample (ice cream mix; top) and ice cream mix containing 3.0 % depolymerized TSP (bottom), after 90 min of ice recrystallization. Adapted from (X. Sun et al., 2024) Copyright © 2023, with permission from Elsevier.

application point of view, TSP was added in frozen dough as low-temperature protective agent (Ren et al., 2023), in grass carp surimi (F. Xie et al., 2024), and in starch (M. H. Lee et al., 2002).

4.5. Alginic acid and Alginate

Alginic acid, or algin, is a natural polysaccharide that can be extracted from brown seaweeds and bacteria, being brown seaweeds the main source when considering commercially available products (Pawar & Edgar, 2012). Alginic acid is a linear polymer constituted by β -D-mannuronic acid and α -L-guluronic acid residues, linked through 1,4-glycosidic bonds, both as homodimers and heterodimers (Guo et al., 2020), with a final molecular weight varying from tens to hundreds of kDa (Abka-khajouei et al., 2022). When the alginic acid binds ions (typically sodium and calcium), the resulting salts are known as alginates. The specific sequence of the monomers is dependent on the source from which the alginic acid is extracted, causing also a wide variation in the physicochemical properties of the polysaccharide. Alginic acid presents many interesting properties, such as biocompatibility, biodegradability, film-forming ability and water solubility, which determines its application in many fields. For instance, water treatment, catalysis, cosmetics, medicine, pharmaceutical industry, tissue engineering, and food industry (Abka-khajouei et al., 2022; Adamiak & Sionkowska, 2023; Guo et al., 2020). In particular, sodium alginate can be used to retard ice recrystallization in frozen food (Li, Bai, et al., 2024; Y. Liu et al., 2020; Regand & Goff, 2003; X. Wang et al., 2022; B. Zhang et al., 2019). Moreover, alginate oligosaccharides also show IRI activity, due to the establishment of electrostatic interactions, hydrogen bonds and hydrophobic interactions with the ice crystal surface. Indeed, these interactions avoid binding of more water molecules and promote solvation, thus inhibiting the growth of the ice crystal (B. Zhang et al., 2019).

Sodium alginate has also good gel forming properties, allowing the production of hydrogels displaying self-healing ability and tunable mechanical properties, depending on the crosslinking method and degree (H. Wang et al., 2025). The possibility of tuning the mechanical properties of such hydrogels allows their applications in different fields, protecting plants from frost damages among others (Hassanisaadi et al., 2024). For instance, sodium alginate hydrogels with anti-icing performance were produced, using dopamine as cross-linking agent (Huang

et al., 2020; Meng et al., 2023). The cross-linking degree was tuned both by changing the ratio between sodium alginate and dopamine (Huang et al., 2020), and by changing the pH in presence of Fe^{3+} ions (Meng et al., 2023). These coatings were effective in both inhibiting ice nucleation, that occurred at temperatures lower than $-23\text{ }^{\circ}\text{C}$ (Huang et al., 2020) and $-26\text{ }^{\circ}\text{C}$ (Meng et al., 2023) and in delaying ice nucleation, of more than 100 min in the coatings with the highest cross-linking degree at $-23\text{ }^{\circ}\text{C}$ (Huang et al., 2020) and $-26\text{ }^{\circ}\text{C}$ (Meng et al., 2023). These studies show that the anti-icing performance improves by increasing the cross-linking degree, thus it was hypothesized that the higher cross-linking degree causes a reduction in the free water fraction, thus hindering ice nucleation. However, the presence of ions used as crosslinking agents may impact on the colligative properties of the solution by decreasing the freezing point.

4.6. Carrageenan

Carrageenan is a collective term that identifies polysaccharides extracted from red algae. Its general structure is constituted by disaccharides containing a 1,3-linked β -D-galactopyranose unit and a 1,4-linked α -D-galactopyranose unit or a 3,6-anhydro- α -D-galactopyranose unit. All these units can be variably sulfated (Kiran-Yildirim et al., 2021), leading to a sulfate content of the polysaccharide between 22 and 38 % (w/w) (Hale et al., 2024). Different types of carrageenan exist depending on the main disaccharides involved, the main being κ -carrageenan, ι -carrageenan, and λ -carrageenan (Kiran-Yildirim & Gaukel, 2020), containing conventionally one, two or three sulfate groups per disaccharide. This classification is anyway not representative of the molecular complexity of carrageenans. Indeed, studies have shown how commercial carrageenans present considerable structural heterogeneity and that characteristics specified by the manufacturer do not necessarily reflect the actual structural composition. Commercial carrageenans were also found to contain different types of cations which influence aggregation (Hale et al., 2024). Carrageenans find applications in the food industry as thickener, water-binding agents and recrystallization inhibitors in frozen food, especially κ -carrageenan (Kamińska-Dwórznicza, Antczak, et al., 2015), but the lack or difficulties in characterization make it more difficult to predict their macroscopic properties. Their desirable characteristics are usually linked to their capability of forming thermally reversible gels in presence of ions (K^+ , Na^+ , Ca^{2+}), for which the gelation process is still matter of debate (Xu et al., 2024). Moreover, because applied in a wide variety of commercial products, they are usually formulated with additives, salts, and other sugars. Considering all these challenges, the correlation between properties and structural aspects is still far from being fully understood.

κ -carrageenan is reported to be the carrageenan showing the most important IRI effect. Studies highlight improvement of IRI activity with the decrease of molecular weight by acidic hydrolysis from thousands to hundreds of kDa (Kamińska-Dwórznicza, Antczak, et al., 2015; Kamińska-Dwórznicza et al., 2016; Kamińska-Dwórznicza, Matusiak, et al., 2015; Leiter, Mailänder, et al., 2017). Carrageenan oligosaccharides also showed IRI activity (B. Zhang et al., 2018, Zhang, Zhao, et al., 2020). The presence of different cations is also reported to greatly affect the IRI activity: it has been proposed that a higher amount of cations favors gelation, but reduces IRI (Funami et al., 2007; Leiter et al., 2018; Leiter, Ludwig and Gaukel, 2017). This effect was reported to be connected to the increased gelation at increased ion concentration, which impact the water molecules availability at the ice-polysaccharide interface (Leiter et al., 2018). These studies have highlighted how IRI activity and ice shaping are correlated; indeed, carrageenan with higher IRI effects resulted in increased ice shaping, obtaining angular and elongated ice crystals (Kiran-Yildirim et al., 2021; Kiran-Yildirim & Gaukel, 2020; Leiter, Ludwig and Gaukel, 2017). Because κ -carrageenan has IRI activity comparable to fish AFP but at higher concentrations and the shapes of the ice crystal resembles that of AFP solutions, studies have

hypothesized a similar binding effect, although no TH was observed (Kiran-Yildirim & Gaukel, 2020).

4.7. Exopolysaccharides from Psychrophilic bacteria

Exopolysaccharides (EPSs) are carbohydrate-based biopolymers which microorganisms (i.e. archaeal, bacterial, and eukaryotic microbes) secrete in their surrounding environment. The acronym EPSs is sometimes also defined as “extracellular polymeric substances” to indicate different compounds including, beside polysaccharides, also nucleic acids, proteins, lipids and polysaccharides, but also inorganic compounds (Flemming, 2016; Parrilli et al., 2022). For clarity, in this review we refer to EPSs as exopolysaccharides. EPS production is fundamental to biofilm formation, whose characteristics determine the microenvironment of biofilm cells by affecting porosity, density, water content, charge, sorption properties, hydrophobicity, and mechanical stability. Because of its essential role in the microbe’s life, the biofilm has been defined as the “city of microbes” (Flemming et al., 2007). The EPS composition is, therefore, closely correlated with the microbial species, but also can change dynamically in response to environmental pressures (Flemming & Wingender, 2010). Indeed, EPSs can allow microbial colonies which inhabit extremely hostile environments (i.e. marine extremophilic bacteria) to counteract various environmental

stresses associated with freezing temperatures, such as source nutrition, osmotic imbalance, dehydration, intracellular ice nucleation, adhesion to ice surfaces, and attenuate the presence of heavy toxic metals (Collins & Margesin, 2019). The case of EPS production in psychrophilic bacteria is, therefore, of particular interest since we assume that certain structural characteristics of polysaccharide have been developed by microbe evolution to survive in specific environment at low temperatures.

Fig. 8a shows transmission electron microscope (TEM) images of *Colwellia psychrerythraea* 34H (Cp34H), a Psychrophilic bacterium considered a model for studying life in permanently cold environments. The synthesis of EPSs, highlighted by arrows in Fig. 8a, was proved to be relevant to cold-adaptation of *Colwellia*, especially in subfreezing environments (Carillo et al., 2015). In 2005, genomic analysis showed that Cp34H developed several strategies to live at cold temperature involving the synthesis of EPSs (Methé et al., 2005). A significant increase in EPS production was detected under colder temperatures (-8 to -14 °C) compared to milder experimental conditions (Marx et al., 2009). Notably, Cp34H EPS proved to have a superior cryoprotectant activity compared to the standard cryoprotectant glycerol, increasing the recovery of bacterial cells. Further studies enabled the complete characterization of the structures of *Colwellia* EPSs including a medium release (Carillo et al., 2015) and a capsular polysaccharide (Casillo et al., 2017) with molecular weight of thousands of kDa (Fig. 8b). The first one was

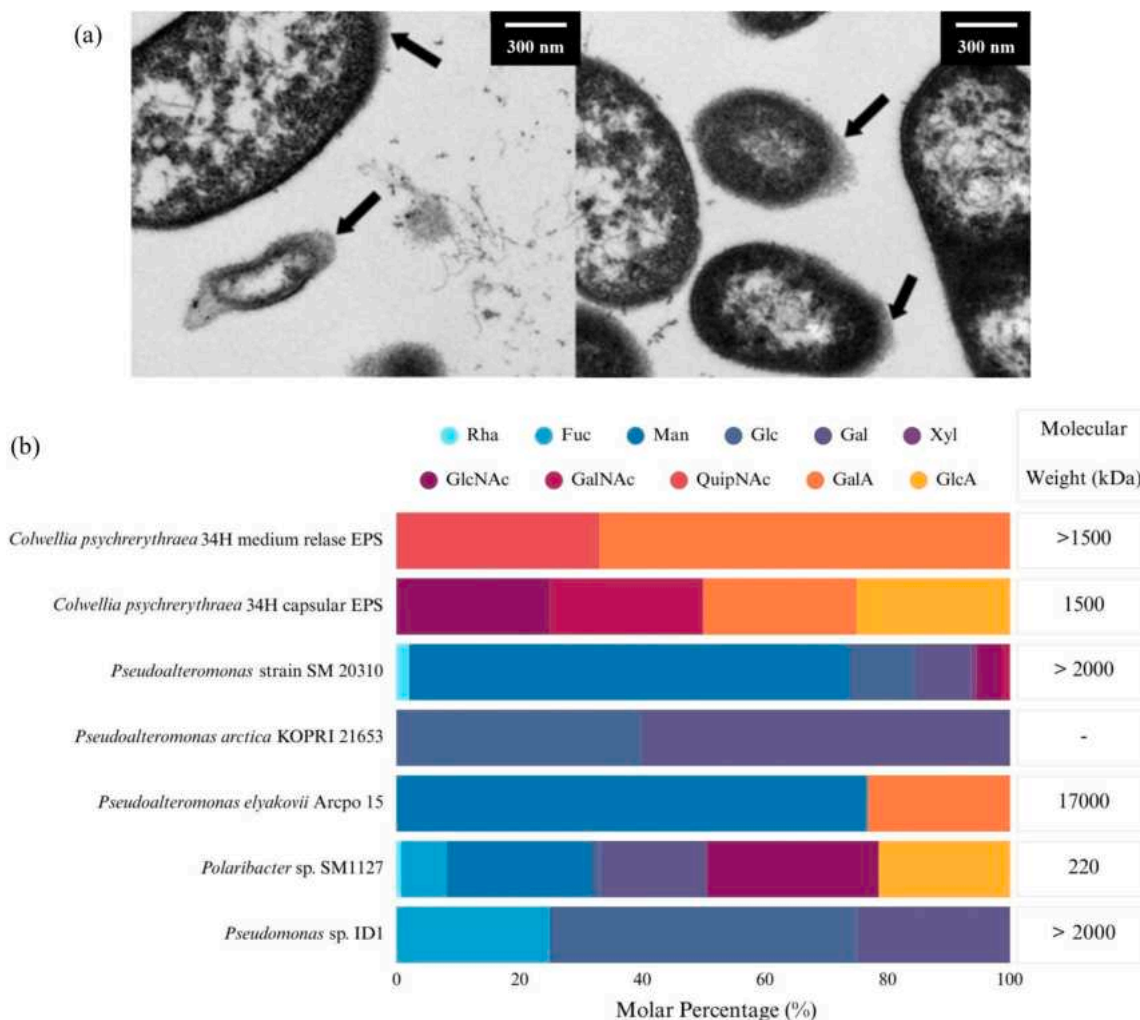


Fig. 8. (a) TEM images of *Colwellia psychrerythraea* 34H. Black arrows point to capsular EPS, strictly associated with the cell surface. Adapted with permission from (Carillo et al., 2015). Copyright © 2015, American Chemical Society. (b) Molecular weight of EPS and molar percentage of glycosidic units of EPSs from Marine and Marine Extremophilic Bacteria tested in cryoprotection activity. Rhamnose (Rha), Fucose (Fuc), Mannose (Man), Glucose (Glc), Galactose (Gal), Xylose (Xyl), N-Acetyl-Glucosamine (GlcNAc), N-Acetyl-galactosamine (GalNAc), N-Acetyl-quinovosamine (QuipNAc), Galacturonic acid (GalA), Glucuronic acid (GlcA).

described as a completely released polysaccharide in the liquid medium, while the second as strictly associated with the cell surface (Fig. 8a): in perspective, a capsular polysaccharide is potentially relevant to design synthetic coatings on a solid surface. In detail, the *Colwellia* medium release EPS is based on a trisaccharide repeating unit of one *N*-acetyl-quinovosamine and two galacturonic acids both linked to an *L*-alanine molecule via amide bond on C6 (Casillo et al., 2017). The capsular EPS shows a tetrasaccharide repeating unit of a glucuronic acid, *N*-Acetyl-glucosamine, a galacturonic acid and *N*-Acetyl- β -*D*-galactosamine, with a *L*-threonine linked via an amide bond on C6 of galacturonic acid (Casillo et al., 2017). The characteristic uronic acid disaccharide motif and the presence of threonine present remarkable similarities with that of AFP (Graether et al., 2000), suggesting interesting possible structure-property correlations. Moreover, molecular dynamics simulations on the capsular EPS reveal a flexible structure characterized by “zigzag” arrangement, where at each corner threonine molecules pointing out the external environments may interact with ice, inhibiting the formation of the first hydration shell and altering ice growth. By assessment of IRI activity, both the medium release and the capsular EPS exhibited an inhibitory effect on ice growth, although less pronounced than that of AFP. Besides, a third capsular EPS extracted from *Colwellia*, for which it was also possible to define the complete structure, did not show antifreeze activity (Casillo et al., 2022).

The assessment of the complete structure of EPSs is extremely difficult because of inherent heteropolysaccharide analytical challenges, but also because of difficulties related to extraction and purification. Other EPSs (Casillo et al., 2018) with only partially resolved structures (Fig. 8b) of three different *Pseudoalteromonas* strains show cryoprotective activity by assessment of post-thaw viability. Comparing the glycosidic composition of these EPSs, we can appreciate that *Pseudoalteromonas* strain SM 20310 (S. B. Liu et al., 2013) and *Pseudoalteromonas arctica* KOPRI 21653 (Sung & Joung, 2007) produce neutral EPSs, whereas the EPS from *Pseudoalteromonas elyakovii* Arcpo 15 (S. J. Kim et al., 2016) a uronic acid-based polysaccharide, therefore bearing a charged carboxylic group. Beside the common characteristic of extremely high molecular weights, given the diversified glycosyl composition of EPSs which displayed cryoprotection, at present it is not yet possible to draw structure-property correlations.

Similarly to the case of *Colwellia*, EPS derived from Antarctic cold-adapted *Pseudomonas* sp. ID1 (Carrion et al., 2015) showed cryoprotective activity. EPS monosaccharide content revealed both neutral sugars and uronic acids together with the presence of amino acids (data not shown in Fig. 8a). Since in this case only a partial structure has been defined, it was not clarified if amino acids are part of the polysaccharide structure. In the case of *Polaribacter* sp. SM1127 (M. L. Sun et al., 2015) the production of EPS containing rhamnose, fucose, mannose, glucose, glucuronic acid and glucosamine, together with a higher amount of the latter two, was associated with cryoprotection and also with great moisture-adsorption, denoting a superior retention compared to hyaluronic acid. Concluding, besides the identification of a common feature of extremely high molecular weight, further investigation of antifreeze EPSs from psychrophilic bacteria is still necessary to assess structural determination and clarify antifreeze biochemical activity. By now, *Colwellia* EPSs are, to the best of our knowledge, the only structurally resolved EPS extracted from psychrophilic bacteria with proved cryoprotective properties.

4.8. Other polysaccharides

In food science, polysaccharides are crucial additives for controlling the ice crystallization for food preservation. Generally, high molecular weight polysaccharides can slow down water diffusion and crystallization kinetics. Xanthan is an extracellular anionic polysaccharide produced by the bacterium *Xanthomonas campestris* (Tegopoulos et al., 2023). It has a backbone equivalent to that of cellulose with sidechains constituted by charged trisaccharides, making xanthan soluble in water

(Giannouli & Morris, 2003). Xanthan can bind a considerable amount of water molecules, highly impacting on rheological properties with a shear thinning behavior (Katzbauer, 1998). Therefore, it has been widely applied as food additive for improving water retention (Ahmed et al., 2021; Ma et al., 2023; Sae-kang & Suphantharika, 2006). Although it has been assessed that xanthan can hold a water fraction up to 0.35 in a non-freezing state by DSC analysis (Tegopoulos et al., 2023), xanthan shows moderate to no ice crystal growth inhibition (Regand & Goff, 2002). When compared to other polysaccharides, namely pullulan, gellan gum, κ -carrageenan and carboxymethyl cellulose, xanthan is less performing in the preservation of frozen fish, showing the formation of big ice crystals (Xiao et al., 2024).

Dextran is an EPS synthesized by lactic acid bacteria with a backbone constituted by *D*-glucose units linked by α -(1 \rightarrow 6) bonds, possible branches of *D*-glucose units and different molecular weights from thousands Da to hundreds of kDa, depending on the producing strain and highly impacting on its properties (Díaz-Montes, 2021). Dextran is used as food additive (Li, Mao, et al., 2024), as thickening and moisturizing agent in cosmetics, but also as cryoprotective agent in combination with other cryoprotectants. Dextran with molecular weight of tens of kDa demonstrated scarce ability to inhibit ice crystal growth (Jain et al., 2014; P. Jiang et al., 2023), whereas when grafted with PVA shows marked IRI and DIS activity, similarly to PVA-grafted hyaluronic acid, pectin and alginate (X. Wu et al., 2022).

Starch is a plant-derived co-polymer composed of two macromolecular complexes, namely amylose and amylopectin. Amylose is a linear polysaccharide constituted by α -(1 \rightarrow 4)-*D*-glucopyranose units, while amylopectin is a branched polysaccharide, with the same backbone of amylose and branches linked through α -(1 \rightarrow 6) bonds. Native starch is used in the food industry for texture improvements (Singh et al., 2007). In its modified forms, usually acetylated, hydroxypropylated, carboxymethylated, hydroxyethylation and cross-linked (Masina et al., 2017), it has been applied for improving the frozen storage and reducing dehydration of food (Han et al., 2005; Hu & Li, 2021; Y. Wu et al., 2025).

For the reader's convenience, the most representative results related to polysaccharide-water interactions at freezing conditions for different polysaccharides are summarized in Table 1.

5. Conclusions

In this review, we have focused on the complex interaction between water and polysaccharides, focusing specifically on freezing conditions, which are relevant in a variety of industrial, biological and biotechnological processes. In nature, we observe how polysaccharides have been developed as an alternative strategy for preventing ice damage and, thus, inspiring scientists to investigate their properties.

We presented methods to investigate water states in polysaccharide materials and discussed the related challenges. Common strategies are dedicated to investigating the bulk and highly hydrated systems, while few studies could provide experimental data on interfaces and surface-related aspects which could greatly expand our knowledge on polysaccharide science.

The structure of water in freezing conditions implies the formation of *quasi-liquid layer*, disordered water interface where water does not behave as free. Due to their high hydrophilicity, the presence of many polar groups and chain mobility, we can infer that polysaccharides are potentially able to impart great structuring to interfacial water, possibly resulting in *quasi-liquid layer*, with a thickness up to hundreds of nm. Indirect experimental evidence is provided by polysaccharide IRI and antifreeze activity broadly applied in cryoprotection and icephobic materials. By now, a clear correlation between IRI and viscosity has been established, but still many open questions remain on how the polysaccharide structure act in limiting ice crystal growth (X. Zhang et al., 2025). Dedicated investigation of polysaccharide-water interfaces in freezing conditions is, therefore, becoming increasingly urgent.

On the basis of the polysaccharide type, we discussed how cellulose,

Table 1

Summary table of representative relevant results on polysaccharide-water interactions in freezing conditions.

Polysaccharide	Description	Application	Property	Main results	References
Cellulose	CNCs decorated with chitosan (nanorod shape with mean diameter 194 ± 50 nm)	Cryoablation	Ice nucleation	Promotion of needle-like ice crystal growth	(Hou et al., 2021)
Cellulose	CNCs (hydrodynamic radius 85 ± 1 nm) and TEMPO-CNFs (hydrodynamic radius, 167 ± 1 nm)	–	IRI	IRI activity at 5.0 and 2.0 mg/ml in 0.01 M NaCl	(T. Li et al., 2019)
Cellulose	CNCs	Ice cream stabilizer	DIS	DIS observed only after a long exposure time (24 and 72 h)	(M. Li et al., 2022)
Cellulose	Cellulose derivative decorated with hydrophilic hydroxyl groups, imidazolium cations, and hydrophobic perfluorooctanoate anions	Icephobic coatings	Ice adhesion and nucleation	Ice adhesion strength of 10 kPa at -30 °C, ice nucleation at -30 °C, freezing after 2 h at -25 °C	(Cheng et al., 2022)
Chitin	Rod-like chitin nanocrystals (length 200–950 nm; width 8–31 nm)	Food science and biomedicine	IRI and DIS	In sucrose solution, IRI at concentrations lower than 10 mg/g and DIS at 1 mg/g	(Correa-González et al., 2024)
Hyaluronic acid	Medium molecular weight sodium hyaluronate (average molecular weight 135 kDa)	Cryopreservation of red blood cells	Vitrification	Water binding capacity and increased viscosity of solutions promote vitrification under fast freezing, reducing ice crystals formation and cell cryoinjuries	(X. Liu et al., 2021)
Hyaluronic acid	Hyaluronic acid hydrogel (molecular weight 96 kDa)	Icephobic coatings	Ice adhesion	Ice adhesion of 61 kPa with 20 nm thickness	(J. Chen et al., 2014)
Tamarind Seed Polysaccharide	Low molecular weight TSP (224 kDa and 90 kDa)	Frozen food industry	IRI and ice melting temperature reduction	Destructuring effect on the hydrogen-bond network of water	(X. Sun, Guo, Kou, et al., 2023)
Alginic acid	Alginate oligosaccharides	Frozen food industry	IRI	Interactions of oligosaccharides with the ice crystal surface inhibit ice crystal growth	(B. Zhang et al., 2019)
Alginic acid	Sodium alginate hydrogels (molecular weight 96 kDa)	Icephobic coatings	Ice nucleation inhibition and delay	Ice nucleation at $T < 23$ °C and with a delay >100 min at -23 °C	(Huang et al., 2020)
Carrageenan	κ -carrageenan	Frozen food industry	IRI and DIS	IRI at 2.5 mg/ml; after 70 h of storage at -8 °C, ice crystals shape similar to that observed in AFP solution, suggesting a similar action mechanism	(Gaukel et al., 2014)
Cp34H capsular exopolysaccharide	Average molecular weight 1500 kDa	–	IRI	Cp34H capsular EPS interacts with the ice surface similarly to AFPs	(Carillo et al., 2015)

chitin, chitosan, hyaluronic acid, TSP, alginic acid, carrageenan and EPS display activity in controlling ice nucleation, growth and adhesion. Extended studies on IRI proved positive effects for CNCs (at 5 mg/ml), chitin (<10 mg/ml), TSP (showing dependence molecular weight and concentration), EPSs from Cp34H, alginate and carrageenan. Indeed, IRI activity has been usually associated with cryoprotection, but this is not always the case. CNCs showing ice-shaping of needle-like ice would alternatively concur in pierce and kill cells, as investigated for cancer therapy treatments. Cryoprotective properties of polysaccharides have been commonly associated with the formation of gels, as for chitin, and to vitrification, as for sodium hyaluronate. Although a complete understanding of polysaccharide cryoprotection mechanism is far from achieved, there are increased proofs that the polysaccharide hydrophilic chemical groups and its structural conformation determines a local effect in water organization at the interface.

Similarly to AFPs, the effect exerted by polysaccharides is non-colligative and, therefore, connected to their chemical nature, and have an impact on ice growth and shape. Still, while AFPs induce strong TH (lowering temperature freezing and leaving unaltered temperature melting) over 5 °C, polysaccharide solutions usually result in a slight increase in freezing temperature of few degrees (Guerreiro et al., 2024), corresponding to different kinetic effects. These interesting properties were exploited for the design of anti-icing, de-icing, anti-fogging and anti-frosting materials based on polysaccharides, as in the case of cellulose, chitosan, hyaluronic acid and alginate. Currently, the understanding of how the polysaccharide structure directly impacts ice formation, including aspects related to functional groups, branching, conformation and inter- intra-molecular bonds is very limited. In this review, we have collected and discussed all relevant studies up to now, hopefully stimulating the research community to address the open questions and the knowledge gaps.

Polysaccharides display a great potential in applications involving

the presence of water at freezing temperature, including food, cryopreservation, and surface engineering, but a solid fundamental understanding requires dedicated strategies of investigation to solve unanswered structure-property questions. As a future perspective, we foresee great advances in multimodal investigation dedicated to quasi-liquid layer, including coupled high-resolution microscopies (e.g., cryo-TEM, cryo-STM) with molecular spectroscopies (e.g., surface-sensitive IR, Raman, NMR). Future studies should aim at developing polysaccharide-dedicated simulation techniques to model water mobility and confined dynamics. Essential structure-function questions related to hydroxyl group density, branching length, or presence of other functional group influence icing phenomena remain currently unanswered. Moreover, standardization of assays for dynamic ice shaping, thermal hysteresis and ice recrystallization inhibition would concur our understanding of polysaccharide ice interactions.

CRedit authorship contribution statement

Federica Marelli: Writing – review & editing, Writing – original draft, Conceptualization. **Daniele Pontoriero:** Writing – original draft. **Carlo Antonini:** Writing – review & editing, Supervision, Conceptualization. **Irene Tagliaro:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization.

Declaration of competing interest

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

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Data availability

Data will be made available on request.

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