# Potential food applications of high-pressure effects on ice–water transitions

Pressure depresses the freezing point of water and the melting point of ice, as well as enabling various high-density forms of ice to be obtained. These effects of pressure on the solid–liquid phase diagram of water have several potential applications in food technology, including pressure-assisted freezing, pressure-assisted thawing and non-frozen storage at low temperature (under pressure). Studies that have been published in these and in related areas are reviewed, and the potential applications and limitations are highlighted.

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High pressure has interesting effects on the solid–liquid phase diagram of water (Fig. 1). These effects have been studied in detail by Bridgman[2], but little attention has been paid to the potential food applications until recent years. The application of high pressure reduces the freezing and melting points of water to a minimum of –22°C at 207.5 MPa (Tables 1 and 2), as pressure opposes the volume increase occurring on the formation of type I ice crystals (the type we are familiar with). The homogeneous ice-nucleation temperature (i.e. the temperature at which crystal nucleation occurs in the absence of impurities or other ice-nucleation sites) is similarly reduced, from –40°C to a minimum of –92°C at 209 MPa (Ref. 4). At higher pressures other ice polymorphs may be formed (Fig. 1). Ice I is unique in having a lower density than liquid water, resulting in a volume increase of ~9% on freezing at 0°C, increasing to ~13% at –20°C (Table 1), which may cause significant tissue or textural damage.

The formation of other ice polymorphs generally involves a similar or smaller decrease in volume (increase in density) relative to the liquid state (Table 1), which may result in reduced tissue damage compared with ice I. Ice VI may be formed at room temperature at pressures of ~900 MPa. If such pressures became readily available, high-pressure 'frozen' storage (type VI ice) might be an economical alternative to conventional freezing for some products, as cooling would not be necessary. However, no experiments have yet been carried out in this area, owing to the high pressures required. (It is sometimes possible to obtain certain ice forms, such as ice III or ice VI, outside their regions of stability, especially with the aid of nucleating agents specific to each form[5].)

Pressure shifting between the ice polymorphs I, III and V (in the range 160–460 MPa, at –25°C) has been envisaged as a means of disrupting microorganisms, with shifts between type I and III ice (in the range 160–225 MPa) proving the most successful[6,7]. However, this is unlikely to be suitable for food applications as the tissue or textural damage could also be considerable (as the volume change, $\Delta V$, is >17%; see Table 1).

Some of the triple points of water are given in Table 2. The knowledge of these may prove useful, as Bridgman[2] reported that the rate of a solid-to-solid transformation seems to increase rapidly in the vicinity of a triple point; for example, the transformation of ice III to ice V was 'explosive' at –17°C (the triple point), but 'almost impossible' at –35°C, whereas the transformation of ice V to ice VI is slow at –17°C (Ref. 2).

It should be noted that the presence of solutes in foods (particularly those of low molecular weight) will result in a shift in the water phase diagram (e.g. melting-point depression). In the case of surimi, the depressed melting-point curve was found to be almost parallel to that of pure water as a function of pressure[8].

The primary applications of pressure in relation to the water phase diagram are the increased freezing rates obtained using pressure-assisted freezing (the pressure-induced melting-point depression enables the sample to be supercooled to –20°C, resulting in rapid and uniform nucleation and growth of ice crystals on releasing the pressure), the increased thawing rates and also the possibility of non-frozen storage at subzero temperatures. These aspects will be considered in more detail.

## High-pressure-assisted freezing

The optimum freezing rate for food products or living cells is highly system-dependent. Slow freezing generally results in larger ice-crystal sizes, which may cause extensive mechanical damage, whereas ultra-rapid freezing (e.g. cryogenic freezing) may cause lethal intracellular ice crystallization[9] or mechanical cracking[10].

Slow freezing (>30 min) may cause problems owing to structural damage, accelerated enzyme and microbiological activities as well as potentially increased oxidation rates, resulting from the increasing substrate concentration and the insolubility of oxygen in ice[9,11]. However, the increasing solute concentration (reducing diffusion rates) tends to reduce these effects. In cases where rapid freezing is desirable, cryogenic freezing (where the temperature change, $\Delta T$, is very large) often

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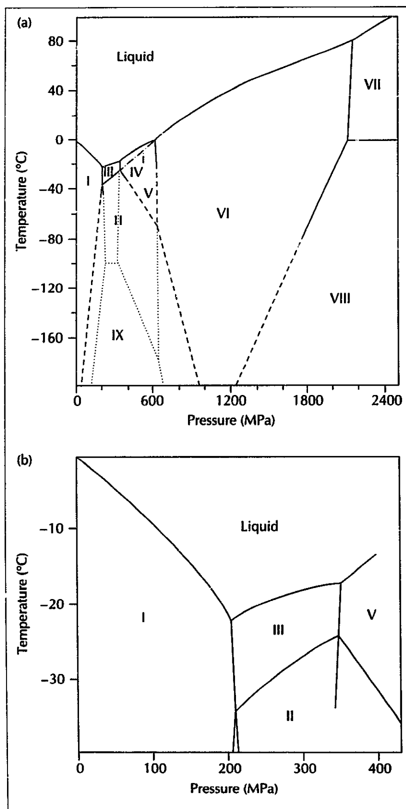


**Fig. 1**
The equilibrium solid–liquid phase diagram of water.
(a), General (redrawn from Hobbs[1], by permission of Oxford University Press); (b), detail of the region of greatest interest (redrawn from Bridgman[2]).

induces cracking, especially in large samples, because of the initial volume decrease (due to cooling) and the subsequent volume increase (due to freezing) as the sample cools and freezes from the outside in. Products of high density (i.e. of high water content) and low porosity are generally more susceptible to freeze cracking than those with low density and high porosity[10].

For each degree K of supercooling (i.e. closer to the homogeneous nucleation temperature) there is an increase of about tenfold in the ice-nucleation rate[12]. The use of high pressure facilitates supercooling, and promotes uniform and rapid ice nucleation and growth throughout the sample on pressure release, producing smaller ice crystals, rather than a stress-inducing ice front moving through the sample. Pressure-shift freezing generally involves cooling the (unfrozen) sample under pressure (usually up to 200 MPa) to −21°C or above (without freezing) before the pressure is released. (Higher pressures and lower temperatures could give rise to other ice forms.)

## Food research applications: microscopy

High pressure is being increasingly used to aid the cryo-fixation of samples for electron microscopy. Microcrystalline (<20 nm) or amorphous cryo-fixation is required to avoid structural damage. This is only possible for samples of a thickness of ~10 μm without the aid of antifreezing agents, which may produce artefacts[13]. The use of high pressure (~200 MPa) reduces the critical cooling rate, or increases the maximum sample thickness, by depressing the freezing point of water. Liquid nitrogen is generally used to obtain rapid cooling, with the result that ice II or ice III may be obtained in addition to amorphous water. Samples must be stored below the devitrification temperature (−133°C) to avoid the formation of ice I; thus, pressurization must be maintained until the centre of the specimen is cooled below −133°C (Ref. 13).

## Commercial food applications

High pressure is still a relatively new technology in food science; applications of pressure-assisted freezing are still under development and, so far, only a few preliminary studies have been published.

A few early studies were carried out with regard to the medical applications of high-pressure technology, but pressure-assisted freezing of chick skin cells and human conjunctiva resulted in their reduced viability as compared with samples that had undergone conventional freezing[14]. This may be due to intracellular ice formation (resulting from more-rapid freezing), or simply to a combination of the harmful effects of pressure and freezing.

Freezing under a gaseous (nitrogen) pressure of ~0.3–10 MPa (50–1500 Psig) at −20 to −25°C resulted in an improvement in the quality of subsequently air-dried fruit, vegetables and meat products relative to samples frozen at −20°C without pressure[15]. Freezing under gaseous pressure resulted in reduced textural damage and shrivelling, more-rapid dehydration and more-uniform rehydration, owing to tiny gas bubbles being trapped in the tissues. Pressure-frozen fruit (e.g. strawberries, pineapples and bananas) possessed a softer texture and could be eaten without thawing[15].

Pressure-shift freezing has been carried out successfully on tofu[16], by cooling it to −18°C under 200 MPa. The pressure-shift-frozen, room-temperature-thawed

**Table 1. Thermodynamic properties of the phase transitions of water**[a]

| Phase transition | $T$ (°C) | $P$ (MPa) | $\Delta V$ ($cm^3 \cdot g^{-1}$) | $\Delta H$ ($kJ \cdot kg^{-1}$) |
|---|---|---|---|---|
| Liquid → ice I | –20 | 193.3 | +0.1313 | –241 |
| | –15 | 156.0 | +0.1218 | –262 |
| | –10 | 110.9 | +0.1122 | –285 |
| | –5 | 59.8 | +0.1016 | –308 |
| | 0 | 0.1 | +0.0900 | –334 |
| Ice I → ice II | –35 | 212.3 | –0.2177 | –42.5 |
| Ice I → ice III | –30 | 211.5 | –0.1919 | +14.6 |
| | –20 | 206.3 | –0.1773 | +23.4 |
| Ice II → ice III | 25 | 330.6 | +0.0148 | +68.2 |
| Ice II → ice V | –25 | 350.2 | +0.0401 | +66.5 |
| Ice III → ice V | –25 | 341.1 | +0.0546 | –3.64 |
| | –20 | 345.5 | +0.0547 | –3.72 |
| Liquid → ice III | –22 | 207.5 | –0.0466 | –213 |
| | –20 | 246.2 | –0.0371 | –226 |
| | –17 | 346.3 | –0.0241 | –257 |
| Liquid → ice V | –20 | 308.0 | –0.0828 | –253 |
| | –15 | 372.8 | –0.0754 | –265 |
| | –10 | 442.4 | –0.0679 | –276 |
| | –5 | 533.7 | –0.0603 | –285 |
| | 0 | 623.9 | –0.0527 | –293 |
| Ice V → ice VI | –20 | 624.4 | –0.0381 | –0.76 |
| | 0 | 626.0 | –0.0389 | –0.83 |
| Liquid → ice VI | –10 | 518.0 | –0.0960 | –264 |
| | 0 | 623.9 | –0.0916 | –295 |
| | 10 | 749.5 | –0.0844 | –311 |
| | 20 | 882.9 | –0.0751 | –320 |
| | 30 | 1038.9 | –0.0663 | –330 |

[a] After Karino *et al.*[3]; data obtained from Ref. 2
$T$, Transition temperature
$P$, Transition pressure
$\Delta V$, Volume change
$\Delta H$, Enthalpy change

product had a homogeneous structure very similar to that of an untreated product, whereas air-blast-frozen samples dripped, deformed and had a rigid core following thawing[16].

High-pressure air (300–800 MPa) has also been used to sterilize pre-sorted food materials following liquid-nitrogen freezing and before freeze drying, resulting in more rapidly rehydrated dried products, which retained both nutrients and taste[17]. The effects of this procedure are unclear. Perhaps pressure blanching occurs at very low temperatures (owing to the formation of different ice polymorphs, as discussed above). The improved dehydration and rehydration could be the result of the incorporation of air, as demonstrated by the aforementioned work of Haas *et al.*[15]

Preliminary studies carried out at the Berlin University of Technology, Germany, have shown that high-pressure treatment and freezing can influence the dehydration and subsequent rehydration of potatoes, green beans and carrots (Refs 18, 19 and H. Koch and M.N. Eshtiaghi, unpublished). The results are very product-dependent. Studies of high-pressure freezing and thawing of food gels (e.g. agar, starch and whey protein) and the effects on the gel structure are also in progress (M.T. Kalichevsky, unpublished).

## Practical aspects

Because of the large heat of fusion ($\Delta H$) of ice formation (Table 1), freezing has a warming effect, and therefore additional cooling of the sample is required to enable complete freezing to occur. This may limit the sample size that can be successfully pressure-shift frozen, as incomplete freezing could result in subsequent sample deterioration, arising from the growth of ice crystals and the melting of smaller ice crystals. Some form of agitation within the pressure vessel would aid heat transfer.

Pressurization itself induces a temperature increase ($\Delta T$), which increases with increasing initial temperature and increasing pressurization rate, until adiabatic

**Table 2. Triple points of water**[a]

| Triple point | Pressure (MPa) | Temperature (°C) |
|---|---|---|
| Liquid–ice I–ice III | 207.5 | −22.0 |
| Ice I–ice II–ice III | 212.9 | −34.7 |
| Ice II–ice III–ice V | 344.3 | −24.3 |
| Liquid–ice III–ice V | 346.3 | −17.0 |
| Liquid–ice V–ice VI | 625.9 | +0.16 |

[a] Data taken from Ref. 2

heating is observed[20]. The $\Delta T$ per MPa also decreases with increasing pressure[21]. The calculation of $\Delta T$ is complicated by the fact that the constants required are pressure- and temperature-dependent. The adiabatic $\Delta T$ is proportional to the thermal expansion coefficient of the sample ($\alpha_p$, in $K^{-1}$) and inversely proportional to the heat capacity ($C_p$, in $Jg^{-1}K^{-1}$) as shown by Eqn 1 (after Nakahara[21]):

$$\Delta T / \Delta P = (T \cdot V \alpha_p) / C_p \qquad (1)$$

where $T$ is the temperature in degrees Kelvin, and $V$ the molar volume. Estimates of $\Delta T$ for various liquids, calculated using Eqn 1, are shown in Table 3.

Table 3 indicates that $\Delta T$ for water at ambient temperature is generally relatively small, owing to its low compressibility and high heat capacity. The data for ethylene glycol in water are of interest, as ethylene glycol is often used as the pressure-transmitting medium at low temperatures. It should be noted that pressure treatment of samples containing large concentrations of organic liquids, such as fats or alcohol, could involve considerably greater temperature variations. The equation predicts a fall in temperature on pressurizing water below 4°C (the temperature of the maximum density of water), because of the anomalous negative thermal expansion coefficient. However, under high pressures the behaviour of water becomes less anomalous[2]; for example, the 'sharpness' of the density maximum decreases with increasing pressure[25].

When freezing occurs on pressure release a temperature decrease is generally observed in the pressure medium, while the temperature of the water-containing sample increases rapidly to its melting point, owing to the release of the latent heat of crystallization as ice is formed (for example, see Kanda *et al.*[16]). The sample temperature then remains at its melting point (below 0°C in the presence of solutes) until freezing is complete.

## High-pressure thawing

Thawing generally occurs more slowly than freezing, potentially allowing further damage to the sample. Faster thawing reduces the loss of liquid retention properties and can improve colour and flavour preservation in fruit[9]. The earliest studies of pressure-assisted thawing appear to have been in the medical field, possibly resulting in improved survival rates for slowly frozen human conjunctiva[14]. To our knowledge, subsequent studies have not been carried out in this area.

A frozen food at −10 or −18°C may be thawed by pressure treatment at 110 or 200 MPa, respectively[26]. The presence of sugar or salt reduces the pressure required to thaw ice[27]. Heating is also required to supply the heat of fusion (which results in cooling on thawing) and also to prevent recrystallization on depressurization. The thawing rate depends only on the conduction of heat, as pressure is transmitted uniformly through the sample. In fact, a small temperature increase is expected on depressurization below 4°C (Table 3), favouring thawing. It is uncertain whether this is observed in practice, as ice crystallization often occurs in this region. Takai *et al.*[8] claim to have observed such a temperature increase in thawed surimi blocks, on depressurization, but the temperature change observed by them is larger than predicted and may also have been caused by the formation of a small amount of ice.

Conditions of −10°C and 120 MPa were found to be suitable for thawing frozen beef in a third of the time required at atmospheric pressure, whereas at lower temperatures and higher pressures, toughening and surface whitening were observed[26]. In addition, Deuchi and Hayashi[27] observed that high-pressure thawing of beef results in reduced drip. Reduced drip, but no increase in the thawing rate, was also observed for pressure-thawed tuna muscle block[28]. However, colour changes and reduced solubility of sarcoplasmic proteins (due to protein denaturation) were also observed with increasing thawing pressure (from 50 to 150 MPa)[28]. Takai *et al.*[8] observed greatly enhanced thawing rates for surimi, depending on the temperature of both the sample and the medium and also on the pressure used; they also noted protein denaturation and discolouration of the fish meat at higher pressures.

No improvement in the texture of a 1.7% agar gel frozen at −20°C on pressure thawing was observed relative to a sample thawed at ambient pressure[27], indicating that, in this case, the structural damage occurs largely on freezing and not on thawing. Pressure treatment of 200 MPa for 30 min at 5°C was found to be sufficient to completely thaw ices prepared at temperatures in the range −10 to −30°C. A reduced treatment time produced a 'state of sherbet' (small ice crystals in liquid water), apparently indicating the uniform nature of pressure thawing[27]; however, this may also have been caused by recrystallization in a fully thawed sample on depressurization, owing to insufficient warming. The uniform action of pressure was clearly shown by three thermocouples in a block of ice, which all read the same temperature at different locations during pressure treatment[29].

The use of pressure facilitates rapid thawing without the necessity of heating the sample above 5°C (Ref. 8). Limitations arise as a result of pressure-induced protein denaturation (producing toughening) and meat

Table 3. Calculation of $\Delta T$, the temperature change per 100 MPa pressure increase[a]

| Sample | | $T$ (°C) | $10^3 \cdot \alpha_p$ ($K^{-1}$) | $C_p$ ($Jg^{-1}K^{-1}$) | $10^6 \cdot V$ ($m^3g^{-1}$) | $\Delta T$ (°C/100 MPa) |
|---|---|---|---|---|---|---|
| Water (liquid) | | 60 | 0.60 | 4.184 | 1.016 | 4.8 |
| | | 25 | 0.26 | 4.180 | 1.005 | 1.9 |
| | | 0 | –0.07 | 4.218 | 0.999 | –0.45 |
| | | –10 | –0.29 | 4.252[b] | 0.999 | –1.8 |
| Ice | | –10 | 0.16 | 2.032 | 1.088 | 2.2 |
| Ethanol | | 20 | 1.094 | 2.50 | 1.267 | 17.34 |
| Acetone | | 25 | 1.43 | 125 | 7.41 | 25 |
| Hexane | | 25 | 1.36 | 195 | 13.2 | 27 |
| Ethylene glycol (% in water) | 100 | 25 | 0.85[b] | 2.43 | 0.793 | 8.3 |
| | 52 | 20 | 0.538 | 3.26 | 0.924 | 4.5 |
| | 34 | 20 | 0.455 | 3.62 | 0.941 | 2.9 |
| | 20 | 20 | 0.343 | 3.90 | 0.971 | 2.5 |

[a] After Nakahara[21]; additional data from Refs 22–24
[b] This is an extrapolated estimate
$T$, Temperature
$\alpha_p$, Thermal expansion coefficient, defined as the change in volume per degree Kelvin divided by the volume at 0°C
$C_p$, Heat capacity
$V$, Specific volume (in $m^3g^{-1} \times 10^6$)
$\Delta T$, Change in temperature on increasing the pressure by 100 MPa, as predicted using Eqn 1

discolouration. However, these obstacles may be overcome by optimizing the thawing conditions used. It is likely that pressure-assisted thawing will have many applications in food and medical fields, especially in cases where significant sample deterioration occurs during thawing. Apparently, Japanese R&D personnel are aiming to commercialize small high-pressure units for thawing fish in sushi bars, and also for other commercial applications where quality and freshness are of primary importance.

## Low-temperature non-frozen storage under pressure

The earliest studies of low-temperature food storage under pressure appear to have been carried out by Charm *et al.*[30] In this case, fish quality and enzyme (horseradish peroxidase and red-crab trypsin) stability were studied on storage at –3°C under 24 MPa, which was sufficient to prevent freezing. In general, enzyme activity was inhibited by pressure below a certain critical temperature, which was dependent on the enzyme (above this temperature, pressure increased enzyme activity). Cod fillet stored under these conditions for 36 days had a perceived storage time of 7 days at 1°C under ambient pressure, as compared with 6 days for a similar period of frozen storage. Samples stored at 1°C under ambient pressure were unacceptable after 9 days. In the case of pollock fillet, storage for 12 days at 1°C and 34.2 MPa gave a perceived storage time of 7 days compared with a product stored at ambient pressure (also at 1°C). The authors pointed out that a significant energy saving could be made using pressure storage rather than freezing[30]. Product deterioration due to freezing and thawing effects is also avoided. Prolonged non-frozen storage under pressure (at –15°C) has also been proposed for red blood cells, but this does not appear to have been tested experimentally[31].

More recent work in this area has been carried out in Japan. The studies of Deuchi and Hayashi[27,32,33] have shown that the mechanical properties of agar gels could be maintained by non-frozen storage at –20°C and 200 MPa (Ref. 27). Strawberries and tomatoes maintained their fresh taste and colour on storage for a few days or weeks under 50–200 MPa at –5 to –20°C. Raw pork could also be successfully stored, avoiding the drip losses occurring after thawing. The microbial counts of most microorganisms in ground beef (coliforms, enterobacteriaceae, Gram-positive and Gram-negative psychrophiles, enterococci and lactic acid bacteria) were reduced by low-temperature storage under pressure (200 MPa, –20°C), in some cases more than by freezing. Yeasts and some bacteria were inactivated. Enzymes that are inactivated by freezing (catalase, β-amylase, cathepsin and lactate dehydrogenase) generally had reduced activity after non-frozen storage, but were not inactivated[32,33].

Ooide *et al.*[34] have carried out studies on muscle from chicken and carp stored at –8 or –15°C under 170 MPa for 50 days. They showed that it is possible to preserve meat texture at low temperatures without extensive protein denaturation, but that enzyme activity under these conditions may be a potential problem. Enzymatic degradation of compounds related to nucleic acids (i.e. ATP, ADP, IMP and AMP) was only slightly slower under high-pressure conditions than under refrigerated storage, whereas it was significantly reduced by freezing[34].

Low-temperature non-frozen storage under pressure therefore appears to be applicable to prolonging the shelf life of certain foods, as compared with refrigeration, while avoiding the damage caused by freezing. Continuing (though reduced) enzyme activity is likely to limit potential storage times (unlike freezing).

## Other related areas of interest

Watanabe *et al.*[35–37] have carried out several studies on ice-nucleation bacterial cells, which are the most effective heterogeneous ice-nucleating agents known. According to these authors, a high-pressure treatment of ≥300 MPa at 5°C or 20°C was sufficient to sterilize the ice-nucleating bacteria *Erwinia ananas* and *Xanthomonas campestris*, but storage for 1 day at 0°C was necessary before recovery of ice-nucleation activity after pressurizing *E. ananas* at 20°C. Subsequent freeze drying could result in a sterile ice-nucleation-active preparation, whereas thermal sterilization resulted in the loss of ice-nucleation activity[35,36].

The presence of ice-nucleation bacteria enables large ice crystals to be formed at temperatures above –5°C (often above –2°C). Food applications that have been envisaged include freeze texturization and freeze concentration. Studies have been carried out on the freeze concentration of egg white[36] (ice was separated out with a sieve or by centrifugation), milk and fruit juices[36,37]. In the case of fruit juice, the original flavour was maintained, and strawberry jam could be made without the use of heat, by adding the juice concentrate to the pulp[37]. Lemon-juice concentrate (prepared by freeze concentration using ice-nucleation bacteria) had a fresh flavour[37].

*Saccharomyces cerevisiae* (yeast) cells became both barotolerant (to 150 MPa for 60 min) and thermotolerant (to 51°C for 10 min), while the amount of non-frozen water at –50°C was almost doubled as a heat-shock response after treatment at 43°C for 30–60 min. This was associated with the production of heat-shock proteins[38]. If such proteins could be obtained inexpensively, they could be used, in addition to other cryoprotectants and sterilized ice-nucleation bacterial preparations (described above), to provide an additional tool for the control of freezing rates and ice-crystal sizes, perhaps analogous to the controlled freezing that occurs naturally in some animals and insects[39].

## Conclusions

The intention of this brief review is to highlight areas of potential interest in the high-pressure and low-temperature area, as well as to provide a resumé of work that has already been carried out in this area. The greatest potential appears to be in high-pressure-assisted freezing and thawing and possibly storage under pressure, which deserve further study with regard to food, pharmaceutical and possibly medical applications.

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## Review

# Bacterial ice nucleation and its potential application in the food industry

**Jingkun Li and Tung-Ching Lee**

Extensive investigations of bacterial ice nucleation by strains of *Pseudomonas*, *Erwinia* and *Xanthomonas* have indicated that highly homologous proteins, each encoded by a single gene, are involved in the ice-nucleation active sites of either intact bacterial cells and/or cell-free ice nucleators. The application of these bacterial ice nucleators to the freezing of some model food systems and real foods, such as salmon, egg white protein and cornstarch gels, elevates nucleation temperatures, reduces freezing times and improves the quality (e.g. the flavor and textural properties) of frozen foods; this suggests that there may be profound potential for energy savings and quality improvement in the food industry. The use of bacterial ice nucleators is a unique application of biotechnology, as it directly improves freezing processes. Further research is needed to gain a better understanding of the basic mechanisms, the practical applications and the safety of this particular material in the commercial freezing of food products.

Freezing is one of the best methods available in the food industry for preserving food products with high quality. Many factors affect the economic viability of the freezing process and the quality of the food products. Overall, the efficiency of this process and the resulting food quality are affected by two important factors: supercooling (the cooling of liquid below its freezing point without freezing) and nucleation (the initiation of the crystallization of liquid water into solid ice)[1]. There are potentially two types of ice nucleation. Homogeneous ice nucleation takes place only in extremely purified water, where an ice nucleus is formed by the random accumulation of water molecules. Heterogeneous ice nucleation is predominant in the freezing of real food systems, and occurs when water molecules aggregate in a crystalline arrangement on nucleating agents such as suspended foreign particles, surface films or walls of containers. The type of ice nucleation is determined by the properties of the solutes and the freezing rate, which consequently affect crystal size and crystal structure within the food and, therefore, product quality[2].

Bacterial ice nucleation by strains of *Pseudomonas*, *Erwinia* and *Xanthomonas* has been both acknowledged and investigated since the early 1970s, and it has been recognized as one of the major causes of frost injury in plants[3]. Many studies have contributed to the understanding of ice-nucleation mechanisms[4], and the elimination of ice-nucleation injury in plants[5]. The unique activity of ice nucleation at higher subzero temperatures (in the range –2 to –5°C), however, makes these microorganisms very useful in such processes as the production of artificial snow and the freezing of some food products (Refs 6, 7 and J.M. Ryder, PhD thesis, University of Rhode Island, Kingston, RI, USA, 1987), where ice nucleation is a limiting step.

The aim of this article is to present both basic and up-to-date knowledge of bacterial ice nucleation, and to discuss the application of this fascinating phenotype to the study of freezing foods as well as the potentially profound impact it may have on energy savings and quality improvement in the food industry.

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### Ice-nucleation-active bacteria

Strains of *Pseudomonas syringae* were first observed to catalyze ice formation in supercooled water in 1974


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