



Review article

A review of methods for reducing ice crystal size in food products

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ABSTRACT

Freezing is recognized as one of the most popular and comprehensive preservation techniques in the food industry. By reducing water activity, freezing leads to the formation of ice crystals and increases the concentration of solutes in the remaining unfrozen water, which in turn helps extend the shelf life of food products. Nevertheless, this process can negatively affect the texture of food, primarily due to the formation of ice crystals. The extent of this damage is strongly dependent on the size, shape, distribution and location (intracellular or extracellular) of the ice crystals within the food tissue. This article presents a range of innovative freezing strategies designed to mitigate the detrimental effects associated with ice crystals formation. In fact, these methods aim to regulate the nucleation process and reduce the freezing temperature, thereby minimizing tissue damage and enhancing the overall quality of frozen food. The strategies reviewed include rapid freezing, the application of nucleating agents and antifreeze proteins, high-pressure freezing, ultrasonic-assisted freezing (UAF), and the use of electric and magnetic waves. In addition to the review of these emerging technologies, the article reports on an experimental case study evaluating the effects of microwave assisted freezing on the microstructure of button mushrooms, a representative plant tissue. Finally, results from the study demonstrated that reduced ice crystals size and better preserved cellular structure. This improved microstructural integrity translated into superior quality of the mushrooms upon thawing.

Keywords: Nucleating agents; Antifreeze proteins; High-pressure freezing; Ultrasound-assisted.

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1. Introduction

Freezing is one of the most widely used and comprehensive methods for food preservation in the food industry. This process, by reducing water activity in food, leads to the formation of ice and an increase in the concentration of the remaining (unfrozen) dissolved substances in the water, which in turn extends the shelf life of food products. However, the freezing process can significantly affect the quality of food, particularly its texture. These effects primarily depend on the size, shape, distribution, and location (intracellular or extracellular) of ice crystals within the food tissue. In recent years, innovative freezing technologies have increasingly been recognized as the most satisfactory methods for the long-term preservation of fruits and vegetables, as these techniques have the ability to preserve the material and maintain the similarity of products processed with rapid freezing. The damage caused by freezing is heavily dependent

on the size, shape, and distribution of ice crystals. As a result, a range of strategies for controlling ice crystals in food have been developed, including controlling nucleation, attempting to reduce the freezing temperature, and damage-free freezing. In this regard, nucleation control, which is a key factor in determining the nucleation point and growth of ice crystals, allows for the control of ice crystal size. The aim of this review article is to examine and analyze the various methods that have been proposed for reducing ice crystal size in food products and to highlight the positive impacts of these techniques on the quality and texture of food products after thawing. This article addresses a range of innovative strategies for controlling ice crystals in food, including increasing freezing speed, using nucleating agents (NA), using antifreeze proteins (AFP), high-pressure freezing (HPF), ultrasound-assisted freezing (UAF), and the use of electric and magnetic waves. Finally, a case study of button mushroom freezing, as a plant tissue affected by microwave waves, is also

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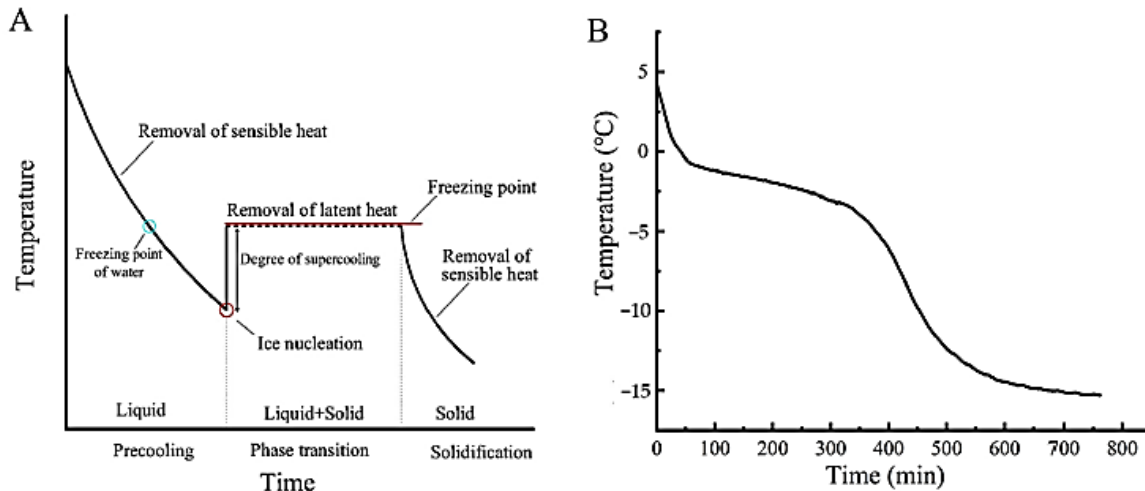


Fig. 1. Schematic representation of the typical time–temperature profile of water during the freezing process (A), illustrating the characteristic stages of phase transition. Subfigure (B) shows the freezing curves recorded at the centers of large yellow croakers stored in a -20 °C refrigerator. The data used to construct Figure 1B were obtained experimentally by the authors (Tan, Mei, & Xie, 2021).

examined (X.-F. Wu, Zhang, Adhikari, & Sun, 2017).

2. Water molecule

The basis of many new freezing technologies lies in the physical properties and unique characteristics of water. Plant and animal tissues typically contain over 80% water. The water molecule is composed of two light hydrogen atoms and one relatively heavy oxygen atom. The difference in weight, which is approximately 16 times greater between the hydrogen and oxygen atoms within the water molecule, provides the ability for hydrogen to move and rotate. When a covalent bond is formed between two dissimilar atoms, due to the difference in electronegativity between the two atoms, the charge distribution (*electron density*) in the bond will not be uniform. The electron density will be greater on the more electronegative atom. As a result, the bond becomes polar with two poles, positive and negative. In the case of the bond between hydrogen and oxygen atoms in water (*H-O*), the electron density around the oxygen atom is greater than around the hydrogen atoms. This results in a polar bond, and the water molecule is not linear but takes a V-shape due to this polarity (Xanthakis, Le-Bail, & Ramaswamy, 2014).

Due to this asymmetrical structure, water molecules orient themselves in an electric field, with the hydrogen atoms, which have a partial positive charge, aligning towards the negative pole or cathode and the oxygen atom, which has a partial negative charge, aligning towards the positive pole or anode (Fennema, 1996). The property observed in polar molecules like water is called dipole moment. During freezing, food loses both sensible, latent heat and its temperature drops below the freezing point, causing a portion of the water present in the food to undergo a phase change and transform into ice crystals (Fig. 1).

3. Crystallization

Self-crystallization involves two stages: nucleation (initiation) and crystal growth. The interaction between these two stages determines the crystal characteristics, such as size, distribution and shape of the crystals (Hartel, 2001). The nucleation stage is considered the most important stage in the formation of new crystals

because it can affect the size, shape and distribution of ice crystals. Therefore, if the nucleation and crystal growth process can be controlled in such a way that smaller and more numerous nuclei are formed, it can reduce damage to the food tissue. The formation of large extracellular ice crystals can lead to significant damage to the product tissue (Evans, 2009). Important quality indicators of food materials that are affected by freezing and thawing include water holding capacity reduction, formation of ice crystals which can damage the structure, changes in color and changes in texture (Reid, 1993). Currently, innovative freezing technologies are undoubtedly the most satisfactory method for long-term preservation of fruits and vegetables. In these methods, the nutrient content is largely preserved and the product is similar to fresher than thermally processed foods. However, the major changes resulting from freezing are related to tissue damage. As mentioned, nucleation and growth of ice crystals are one of the main factors in reducing the quality of fruits and vegetables during freezing. Therefore, a set of strategies has been developed to control ice crystals in food, including controlling nucleation, attempting to reduce the freezing temperature, minimizing chemical and physical changes, and freezing without harmful effects. In this regard, controlling nucleation, which is a key factor in determining the nucleus and growth of ice crystals and is temperature-dependent, enables the relative rates of nucleation and ice crystal growth to be controlled. With appropriate manipulation, the heat transfer rate can be optimized (Vardanjani, Hamdami, Dalvi-Isfahan, & Le-Bail, 2024).

4. The effect of freezing rate on ice crystal size

Recently, refrigeration of food has become a powerful tool in the industrial world for improving quality of life. Freezing food is a complex process that involves heat transfer, ice nucleation, ice growth, ice distribution and changes in the physical and chemical properties of food. In the early 20th century, many people were experimenting with mechanical and chemical methods for preserving food. Rapid freezing was introduced as an industrial process to improve the state of freezing, to the extent that a combination of ice, wind and low temperatures in the north pole was observed to almost immediately freeze freshly caught fish. More

importantly, when such fish are frozen at high speeds, they do not differ much in taste and texture from their fresh state. Machost (1930) formed the first cold chain for food in the world; therefore, rapid freezing has been widely accepted as a commercial method for the long-term preservation of perishable foods, which improves the health and comfort of all industrialized countries. Freezing rate greatly affects the quality of frozen foods, in which the predominant water content must be rapidly frozen in the crystalline structure to prevent destruction of cell tissues and quickly prevent spoilage by microbiological and enzymatic processes (Cheng, Sun, Zhu, & Zhang, 2017). In this method, the internal temperature of the product passes through the critical zone in a short period of time. The freezing rate is determined by the ratio of the temperature difference between the sample and the cold environment over the freezing time. The size of ice crystals depends on the effective interaction between freezing rate and degree of supercooling. The relationship between the local freezing rate (u) and the average diameter of ice crystals was obtained based on experiments performed on the freezing of cylindrical gelatin (Eq. (1))

$$dp = a \times u^b \quad (1)$$

In Eq. (1) u is the local freezing rate. The coefficient values of $a = 2.89 \times 10^7 \text{ m}^2/\text{g}$ and $b = 0.45$ have been determined by fitting experimental results with a power law. In this equation, the unit of coefficient a is equivalent to mass transfer unit, and logically, it can be concluded that with increasing water permeation, the size of ice crystals becomes larger. Rapid freezing can lead to preserving the quality and freshness of frozen food products by reducing their crystal size. The freezing rate of food is determined by the ratio of the difference between the initial and final temperature of the food material to the freezing time (Cheng et al., 2017). The freezing rate has a direct effect on the number, size, shape, and distribution of ice crystals. Generally, reducing the number of crystals leads to a decrease in the severity of damage caused by freezing. Large ice crystals that form during slow freezing can damage the cellular structure of food materials and cause changes in their texture and flavor (Silvestrelli & Parrinello, 1999). Rapid freezing can be achieved through various methods, such as using very low temperatures where the food material is frozen at high speeds, immersion freezing, or using cryogenic gases such as liquid nitrogen or carbon dioxide. In addition to improving the quality of food, rapid freezing can help reduce bacterial growth and food spoilage while preserving nutritional content and freshness. Regarding the effects of heat transfer, the greater the difference between the surrounding environment temperature and the freezing temperature (ΔTS), the higher the heat transfer rate (G) will be, which leads to an increase in the rate of ice crystal growth. Eq. (2) describes this relationship:

$$G = \beta (\Delta TS)^n \quad (2)$$

In this equation, β and n are empirical constants.

TEM (Transmission Electron Microscopy) offers powerful magnification and provides valuable insights into surface, shape, size, and structural features (Pérez-Bermúdez et al., 2023). In a study conducted by Meziani et al. in 2012, researchers investigated the effects of various freezing treatments (-20°C , -30°C , -40°C , and liquid nitrogen) on both fresh and thawed sweet doughs. The results showed that the freezing rate significantly affected the integrity of the dough network, as different temperatures influenced the size and

distribution of ice crystals. Specifically, the study found that smaller ice crystals were unstable, which could lead to their recrystallization into larger crystals during the thawing process. This recrystallization caused considerable damage to the cells within the sweet doughs.

Wolkerz et al. (2007) found that the nucleation temperature is a key factor in the formation of intracellular ice crystals, such that a lower nucleation temperature leads to more and smaller intracellular ice crystals. Although rapid freezing processes have been shown to improve the quality of frozen food samples by reducing the size of ice crystals, these types of processes require a high amount of energy and may not be economically feasible. Therefore, in recent years, research has been conducted using innovative methods to determine the best approach for increasing the number of crystals and reducing their size. The goal is to achieve high-quality frozen food products while minimizing energy consumption and cost (Wolkers, Balasubramanian, Ongstad, Zec, & Bischof, 2007).

5. Nucleating agents

Nucleating agents are insoluble materials that are effective in nucleation. The presence of these materials leads to heterogeneous nucleation, increasing the rate of ice crystal formation and a decrease in freezing time. In addition, the presence of these materials reduces the equilibrium freezing point compared to pure water, resulting in less supercooling, which can play an effective role in creating smaller crystals. Furthermore, these materials are insoluble and their use can result in non-homogeneous nucleation at higher temperatures compared to homogeneous nucleation and can promote uniformity and reduce the costs associated with the freezing process. Biological nucleating additives, by increasing the nucleation temperature, cause a decrease in the degree of supercooling and a change in the pattern of ice formation in frozen food products and are used in processes such as freeze-drying, cryopreservation, and freeze concentration. Most studies on nucleating agents have focused on bacteria. Bacteria species that produce nucleating agents include *Erwinia herbicola*, *Erwinia ananas*, *Pseudomonas fluorescens*, *Pseudomonas syringae*, and *Xanthomonas campestris*. Although not all natural strains show ice nucleation activity, strains producing ice nucleation agents (INA+) and inactive strains have been identified. The physiological role of these substances in bacteria is a host-protective role against freezing. Based on the chemical structure of these substances, nucleating agents in these bacteria are classified into three main groups: glycoproteins, lipoglycoproteins, and proteins. The structural properties of these materials and their structural similarity to ice crystal networks, surface charge, and high hydrophobicity are the main factors improving ice nucleation (Fernández et al., 2007). Despite these advantages, some problems in the use of these materials are observed due to the pathogenic properties of some strains of *Pseudomonas* and *Erwinia*. However, these problems can be solved by using non-pathogenic strains or activating active cells using heat treatments (D.-W. Sun & Li, 2003).

6. Antifreeze proteins

Antifreeze proteins (AFP) are peptide compounds that are effective in reducing the freezing point. They were first discovered in the blood of fish in the southern polar regions in the early 1970s, and the presence of these substances has protected fish in very low polar temperatures (Hew & Yang, 1992). These proteins are divided into two categories: thermal hysteresis proteins (THP) and ice structural proteins (ISP) and are found in various species, including

fish, bacteria, fungi, and plants. The function of antifreeze proteins is to prevent and control ice growth up to a certain point, so that the concentration of these proteins prevents freezing (Venketesh & Dayananda, 2008).

The results of the mechanism of action of this protein indicate that by binding to ice crystals, the freezing point of ice is reduced and its melting point is increased. This is due to the prevention of ice crystal growth by the protein upon binding to the ice crystals (Hassas-Roudsari & Goff, 2012). The mechanism of action of these proteins involves their ability to recognize ice crystals and bind to them after their formation, preventing their growth within a temperature range below zero degrees Celsius. When water freezes, a water molecule forms hydrogen bonds with three neighboring molecules, taking on a hexagonal structure. However, in the presence of antifreeze proteins, the ice crystals take on a pyramidal or cylindrical structure, thereby preventing longitudinal ice growth (Deng, Andrews, & Laursen, 1997). Due to their mechanism of preventing ice crystal growth, these types of proteins can be useful in the food industry during cold storage. By reducing the crystal size present in food tissue, antifreeze proteins can lead to a reduction in cell damage, a decrease in drip loss and the preservation of the nutritional value of the product (Wathen & Jia, 2005). Antifreeze proteins can be incorporated into food by mixing them with food ingredients, injecting them into plant tissue, soaking, spraying, vacuum purification, or creating them in a recombinant form. In a study that investigated the effect of antifreeze proteins on meat, the results indicated that injecting glycoproteins into livestock before slaughter led to a reduction in ice crystal size within the meat tissue. Additionally, the presence of these proteins led to a decrease in drip loss due to the prevention of freezing. Furthermore, these proteins can be used in the ice cream industry to create small crystals that result in a desirable soft texture in ice cream (Regand & Goff, 2006).

7. High pressure freezing

When water freezes under atmospheric pressure, its volume increases, resulting in ice having a lower density than liquid water. At high pressures, the freezing point of liquid water can be reduced to below zero degrees Celsius and the degree of supercooling (the degree of extreme cooling) can be increased to facilitate the formation of small ice crystals after pressure release. As soon as the pressure is reduced, ice nuclei are immediately formed and rapidly grow, resulting in the formation of very small and uniform ice crystals. This property can be utilized to facilitate the formation of small crystals in various industrial applications (Tironi, De Lamballerie, & Le-Bail, 2010). During the phase transition of ice under high pressure, a significant increase in volume is not observed, which reduces tissue damage in food materials during transportation. Figure 1 of Salzmann (2019) illustrates the phase diagram of ice, showing the thermodynamically stable regions of liquid water, hydrogen-disordered and hydrogen-ordered ice phases, as well as the six types of polymeric ice (I-VI) at different pressures and temperatures. The density of ice varies depending on the type of ice. During freezing at pressures between 0 to 209 MPa, water usually transforms into ice. If the pressure is continuously increased, the sixth type of ice can be obtained. Therefore, their volume does not increase during freezing and the problem of mechanical damage to food tissues can be reduced.

Fernández et al., 2007 found that a large number of small ice crystals immediately formed after expansion and were uniformly distributed throughout the gelatin samples during the high-pressure freezing process (Fernández et al., 2007). The fine structure of the

samples was preserved and the total freezing time was strongly influenced by the initial amount of ice nuclei. The pressure and temperature conditions prior to expansion, cooling rate and amount of ice nuclei were the main factors affecting ice crystal formation. More ice will be produced momentarily as pressure increases at a certain temperature prior to expansion (Otero & Sanz, 2006). Additionally, after expansion, the pressure environment plays a significant role in removing heat from the sample and the ratio of the pressure environment to the sample is also an important factor. The main advantage of high-pressure freezing is the instantaneous and homogeneous formation of ice crystals throughout the entire volume of the product, resulting in an extraordinary cooling effect upon pressure release.

Other advantages of using high-pressure freezing in the food industry include the inactivation of microorganisms with minimal heat treatment, preservation of freshness, taste, texture, color and minimal loss of vitamin C during freezing with pressure changes. This technology may be useful for freezing large-sized food materials that require uniform ice crystal distribution and have distinct thermal gradients, where damage from freezing using conventional methods such as immersion and air cooling may occur. Many studies have demonstrated the superiority of high-pressure freezing over conventional freezing methods such as immersion and air cooling in preserving the fine structure of plant and animal food materials (Luscher, Schlüter, & Knorr, 2005; Molina-García et al., 2004). High-pressure freezing is used to prevent damage caused by crystallization. The promising features of high-pressure freezing are:

- Reduction in freezing point and latent heat of phase change
- Short freezing time and its advantages (such as reducing ice crystal size)
- Inactivation of microorganisms and enzymes and structural changes without significant changes in sensory and nutritional quality.

High-pressure freezing results in instantaneous and uniform ice crystal formation throughout the entire volume of the product and is an innovative and promising technology for improving product quality (Buggenhout, Messagie, Van Loey, & Hendrickx, 2005; B. Li & Sun, 2002). Although the method of high-pressure freezing causes damage to the texture and denaturation of food protein structures, it has been very successful for various food materials such as meat, fish, fruits, vegetables and gels (D. Li, Zhu, & Sun, 2018).

8. Ultrasound-assisted freezing (UAF)

Ultrasound is widely used in various fields such as medicine, chemistry and food science. Generally, ultrasound can be divided into two categories based on the frequency and intensity: high-frequency ultrasound with low intensity and low-frequency ultrasound with high intensity (Awad, Moharram, Shaltout, Asker, & Youssef, 2012). The former is mainly used for non-destructive analysis and process control of food due to its considerable influence on food and food packaging properties (Cheng et al., 2017). On the other hand, low-frequency ultrasound with high intensity, also known as power ultrasound, can be used in various food processing fields such as emulsification, homogenization, sterilization, pasteurization, extraction, drying, freezing, etc. (Hengl et al., 2014).

UAF can preserve nutritional value of food products. Given these advantages, UAF has been recognized as an effective and innovative method in the food industry. Several physical phenomena generated by ultrasound in a liquid medium are responsible for the improved effect of ultrasound, including cavitation, microstreams, and fracture of large ice crystals. In the food industry, ultrasound is

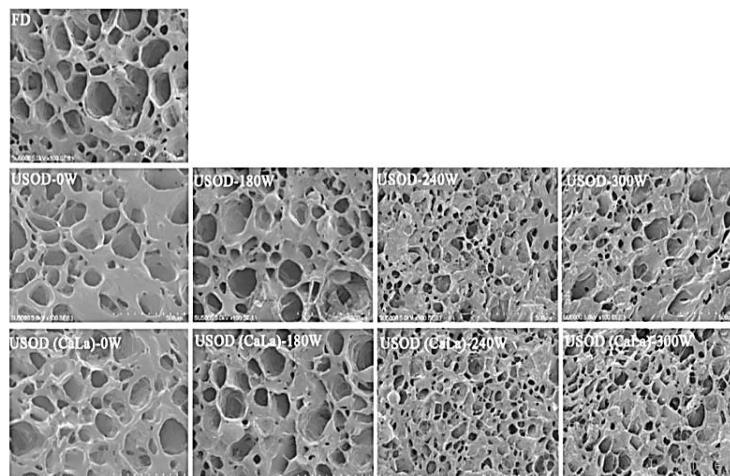


Fig. 2. Observation of Scanning Electron Microscopy (SEM), distribution of frozen peach pores under various ultrasonic treatments. This figure is adapted with gratitude from [Chu et al., 2021](#), with kind permission under the Open Access Creative Commons licence Attribution (CC BY) license <https://creativecommons.org/licenses/by/4.0/>.

widely used to inactivate microorganisms and inactive enzymes in food materials and to improve the quality of final products. Theoretical studies suggest that cavitation bubbles generated by ultrasound are responsible for the nucleation process. During a sonication process, when a sound wave collides with a liquid medium, longitudinal waves are created, resulting in regions with variable density and expansion, causing molecules to distance themselves and overcome intermolecular forces. These regions, with variable pressure, lead to the formation of cavitation (voids) and gas bubbles in the medium. When ultrasound waves are uniformly distributed throughout the liquid, the size of the bubble reaches an equilibrium state. Otherwise, the bubble begins to grow and the bubble wall is unable to maintain it, leading to bubble collapse. This phenomenon is called unstable or transient cavitation.

This process occurs by reducing the resistance of heat and mass transfer at the interface between the liquid and the ice and, therefore, increases the intensity of freezing ([Mortazavi & Tabatabaie, 2008](#)). The collapse of cavitation bubbles can create positive pressure (5 GPa or more) in nanoseconds, which leads to high cooling rates and acts as a driving force for instantaneous nucleation ([Chemat & Khan, 2011](#)). In addition, these cavitation bubbles can act as nuclei for ice nucleation until they reach a critical size; therefore, ultrasound-assisted freezing can promote primary nucleation, which involves the crystallization process in a solution without pre-existing crystals, which has been confirmed by various experiments. Furthermore, it is expected that nucleation will occur immediately after the collapse of cavitation bubbles. However, some studies have shown that ice significantly nucleates, causing a delay between cavitation and nucleation ([Dodds et al., 2007](#)). Additionally, the pressure gradient created by cavitation bubbles is also considered as a potential driving force for ice nucleation. Meanwhile, microstreams that can be generated due to motion can also be considered as another potential driving mechanism for ice nucleation during the ultrasound-assisted freezing process.

Mechanisms of cavitation and micro-flow effects are two fundamental principles for accelerating ice nucleation. As a result, the number of nuclei produced increases, leading to control of the size and shape of ice crystals that become small and uniformly distributed. Moreover, large randomly growing ice crystals can be

broken due to the change in sound pressure during the collapse of cavitation bubbles, which ensures the dominant presence of small and uniform ice crystals. Additionally, micro-flow has been shown to enhance heat and mass transfer as well as bubble collapse near the solid-liquid interface, which creates turbulence and disturbance at the interfacial layers ([Kiani, Zhang, Delgado, & Sun, 2011](#)). Therefore, the resistance to heat and mass transfer in the solid-liquid interface decreases and the freezing rate increases. [Chu, Wei, Ding, Mei, & Xie \(2021\)](#) observed that ultrasonic wave radiation increases nucleation in supercooled aqueous solutions ([Fig. 2](#)). If ultrasonic waves are used in the initial stages of freezing (subcooling) in a pulsed manner, it can be highly effective in the nucleation process, ultimately leading to improved freezing quality. Many factors affect the effectiveness of ultrasonic-assisted freezing, such as ultrasonic intensity and frequency, sample position, coolant temperature, coolant properties and flow rate ([Chu, et al., 2021](#)). To date, ultrasound has been used to assist the freezing process of solid and liquid food samples. [D.-W. Sun & Li, 2003](#) investigated the microstructure of frozen potato tissue by ultrasonic immersion. Their results indicated that the freezing rate increased and cellular destruction was prevented, resulting in the production of small intercellular ice crystals throughout the potato sample and its fine structure was well-preserved ([D.-W. Sun & Li, 2003](#)). It was found that under ultrasonic waves at 25 kHz and 288 or 360 watts, the freezing time was significantly reduced and a large number of small ice crystals were produced inside the frozen gel ([Cheng et al., 2017](#)). [Kiani et al. \(2011\)](#) investigated the mechanism of ultrasound (25 kHz, 0.25 W/cm²) for freezing both liquid (sucrose solution) and solid (agar gel) samples. In their study, it was found that ultrasound was more effective in initiating ice nucleation in liquid samples and its effect was reduced in solid samples ([Kiani et al., 2011](#)). Also, there was a linear relationship between temperature during ultrasonic irradiation and the freezing point of the liquid sample. Furthermore, ultrasound, due to its uniform nature, had a more pronounced effect on the heat transfer rate during the freezing process of liquid samples. In general, in both high-pressure and ultrasound-assisted freezing, samples are usually immersed in a cooling liquid medium and can be considered as special techniques to improve immersion freezing. One of the most important disadvantages of ultrasound-

assisted freezing is its heat generation during the cavitation stage, which leads to a decrease in the freezing rate and a failure to repeat the nucleation process. Therefore, optimization of the ultrasound power and duration of its application is required for freezing (Kiani, Sun, Delgado, & Zhang, 2012).

9. Distorted electric and magnetic freezing

Electric freezing was first discovered by Dufour, 1862. However, the first attempt to induce nuclear fusion in supercooled water droplets using a high-voltage electric field was carried out by Rau, 1951. Nowadays, three electromagnetic technologies, including high-voltage electric field, magnetic field, and radio frequency (including microwave waves) have emerged for food freezing.

9.1. Electromagnetic freezing

Electric fields can be divided into two categories: oscillating electric fields (FEF) and static electric fields (SEF). Oscillating electric fields are mainly used for heating pure water (dielectric heating), while static electric fields are mainly used for freezing products. The mechanism of oscillating electric fields is the rotation of water molecules in response to an applied electric field, as water molecules, due to their inherent electric dipole moment, behave as a dielectric material. Oscillating electric fields at radio frequency or microwave frequencies have been considered a superior method for rapid thawing of frozen materials (S. Wu et al., 2018).

For frozen foods, oscillating electric fields are capable of suppressing ice formation and increasing the cooling rate, which is likely attributed to the redistribution of water molecules in the electric field. Jackson and colleagues (1997) investigated the effect of oscillating electric fields on the purity and homogeneity of ethylene glycol solutions and found that this field at a frequency of 2.45 GHz was able to reduce the amount of ice formed (Gholami, Ahmadi, & Farris, 2017). A study by W. Sun, Xu, Sun, Ying, & Xu, 2006 demonstrated that an electric field at 50 kHz could minimize the size of ice crystals (W. Sun, Xu, Sun, Ying, & Xu, 2006). On the other hand, freezing under a static high-voltage electric field has also been used to increase ice nucleus temperature and improve the quality and microstructure of frozen food. In general, water molecules are highly polar due to their characteristic dipolar structure. Due to this dipole moment, in the presence of an electric field, the dipole polarization of water can be realigned and water molecules move in the direction of the electric field. In liquid water, clusters of molecules exist that are connected to each other by hydrogen bonds and appear solid-like. As a result, the structure of water clusters can be changed, which has been observed in molecular dynamics simulations conducted by some researchers (Zangi & Mark, 2004).

Ice nuclei are associated with the accumulation of small stable water molecules that propagate during the static electric field process. However, in early studies, there were different opinions about the final changes in the water structure during the static electric field process. On the one hand, in the static electric field freezing process, water molecules are well-aligned and transformed into mixed solid-ice structures, but they are still in the liquid phase. In addition, the study by Orłowska, Havet, & Le-Bail, 2009 showed that with increasing voltage, the nucleation temperature increases. According to this study, the strength of the static electric field also has a positive correlation with the degree of cooling. In general, the structure of food can be preserved when the ice nucleus and cooling temperature are controlled (Orłowska, Havet, & Le-Bail, 2009). The

study by Dalvi-Isfahan et al. (2019) investigated the effect of static electric field (SEF) application on the quality characteristics of frozen sheep meat. The results showed that although the electric field had no significant effect on the color, firmness, and freezing rate of the meat, the size of ice crystals and the level of drip loss decreased with increasing static electric field. In addition, they attributed the main factor for these changes to the acceleration of nucleation and crystal growth, in which the formation of a larger number of small nuclei led to the formation of a larger number of small ice crystals in the frozen meat tissue. The study by Xanthakis, Havet, Chevallier, Abadie, & Le-Bail, 2013 showed that the size of ice crystals inside pork tissue decreased from $32.79 \pm 4.04 \mu\text{m}$ to $14.55 \pm 8.20 \mu\text{m}$ when the intensity of the electric field increased from 0 to 12 kV. All of these studies have provided strong evidence that static electric field freezing has a positive effect on the freezing process of food, including ice nucleation initiation and reduction of damage to the frozen food structure. In addition to the main state of the samples that should be frozen, factors affecting ice nucleation initiation by static electric field freezing include electrode specifications such as electrode shape and materials, and electric field parameters. Even if the freezing process is performed at the same high voltage, the actual magnitude of the electric field dissipated may differ in different systems. Therefore, to optimize the static electric field freezing process, attention must be paid to these factors, and appropriate parameters for each system must be determined.

9.2. Freezing using magnetic fields (MF)

Magnetic fields have been developed for achieving rapid freezing, especially for products that have magnetic properties (Aleksandrov, Barannikov, & Dobritsa, 2000). Similar to electric fields, magnetic fields can also be divided into two categories: oscillating magnetic field (OMF) and static magnetic field (SMF). Water with diamagnetic properties and no inherent magnetic dipole torque is identified. Therefore, it is sensitive to the development of magnetic dipole torque under a magnetic field. When discussing the cooling of samples in magnetically disturbed freezing, special attention must be paid to initiating a magnetic torque and maintaining it thereafter. Since hydrogen bonds between water molecules become stronger under a strong magnetic field, the melting point and thermal conductivity of water also increase simultaneously and are usually well distributed and stable (Inaba, Saitou, Tozaki, & Hayashi, 2004). The mechanism of the effect of magnetically disturbed freezing on water molecules is different from the mechanism of the effect of electron-disturbed freezing. Freezing using a magnetic field is also capable of reducing the degree of cooling, which is the main advantage of freezing using a magnetic field. The rotation of the magnetic field is a periodic transverse field that rotates around the central axis. Several magnetic poles are placed at equal distances and are sequentially connected to a multi-phase AC power source that can produce a rotating magnetic field. Therefore, in addition to the effect of the static magnetic field on the degree of freezing, rotational magnetic freezing can control ice growth and lead to the formation of uniform ice crystals without crystalline grouping (Suzuki et al., 2011; Woo & Mujumdar, 2010).

9.3. Freezing using radio frequency (RF)

Radio frequency belongs to the electromagnetic field and has recently been used for freezing purposes. Radio frequency waves can create torque in water molecules by changing their equilibrium relationships within the ice cluster and therefore, the dipole rotation

of water can be utilized to control the size of ice crystals during freezing. [Anese et al. \(2012\)](#) used low-voltage pulse radio frequency (2 kv) to assist in the cryogenic freezing of pork with liquid nitrogen and compared the results with air blast freezing and liquid flow freezing. The results showed that the frozen pork with radio frequency had better cellular structure, fewer intercellular cavities and less cell damage. At the same time, small ice crystals were formed and distributed well in the intracellular region. The better microstructure of the frozen pork using radio frequency was due to its ability to reduce the freezing point and, consequently, promote ice nucleation. As previously mentioned, a similar effect on the freezing point can also be achieved using radio frequency during freezing.

9.4. Freezing using microwave waves (MWF)

Microwave or "micro-wave" is a combination of two words: "micro" meaning small and "wave" meaning a form of electromagnetic radiation characterized by short wavelengths and high frequencies. Microwave is a type of electromagnetic wave, which is essentially a type of radio wave with very high frequencies. The higher the frequency, the shorter the wavelength of the radiation. The frequency of these waves can range from 300 MHz to several gigahertz per second. The range of these waves is short, typically a few meters, but they can penetrate materials relatively well. As the frequency increases, the penetration depth increases, but the range of the waves becomes shorter. Similar to the principles of electron-beam and ultrasound-assisted freezing, the inherent electric dipole moment of water molecules can be altered by microwaves and therefore, microwave-assisted freezing can impact ice formation. In this freezing process, the matrices of food are affected by their main component, which is water. This technology integrates the benefits of microwave energy to overcome the shortcomings of other food processing technologies, with advantages such as energy conservation, improved product quality and reduced time and operational costs.

The final quality of frozen products depends on the phase transition or crystallization process of water into ice. The size of ice crystals is vital for the final quality of frozen food because it can cause irreversible damage to the cellular structure, which in turn reduces the texture and color quality of the product. This technology exploits the physical properties and characteristics of water molecules. Typically, the geometry of a water molecule is assumed to be an ideal tetrahedral with a bond angle of 104.5 degrees between two hydrogen atoms in liquid water. Due to its electronegativity, the oxygen atom attracts the hydrogen atoms more strongly than they attract each other. This asymmetrical electron density distribution between the atoms results in a slightly positive charge on each hydrogen atom and a slightly negative charge on the oxygen atom. These properties are responsible for the polar properties with significant dipole moment and polarizability of water molecules. Their polarity forces them to interact with each other and form a network through multiple hydrogen bonds between neighboring molecules. Water molecules, in addition to being polar, also interact with each other through hydrogen bonding due to their asymmetric charge distribution. These properties promote the idea that water can be considered as a purely electrostatic system ([Xanthakis et al., 2014](#)). Each water molecule can form up to four hydrogen bonds. Oxygen can form a hydrogen bond with two hydrogen atoms, each of which is available to form a hydrogen bond. It is important to note that hydrogen bonds can be considered weak compared to other bonds. Therefore, in the liquid state, water structures are constantly

changing as hydrogen bonds break and reform. Due to the weak hydrogen bonding and polarity, if a water molecule is placed in an electric field, it will not only respond to the field through its dipole moment interaction but also the dipole polarization of the molecule will increase in the presence of the field. As mentioned above, water molecules react when electric or magnetic fields attempt to disrupt their equilibrium. Electrical and magnetic disturbances are factors that can rearrange the hydrogen bonding network of water. Reports indicate that electric fields are more effective in altering the network structure of water due to the intrinsic electric dipole moment of the water molecule, while much stronger magnetic fields are required to exert the same force. Generally, since the majority of food structures are composed of water, a number of water molecules are always in close proximity to each other, creating an unstable core. However, since the free energy of the system is higher, the possibility of forming stable cores is provided by reducing the temperature and entropy (disorder) in the system. Therefore, when a water molecule is placed in an electric or magnetic field that attempts to disrupt their equilibrium, the dipole moment interaction will increase. Water molecules will react and cause changes in the direction of the water molecules and the hydrogen bonding network will be disrupted. This factor can also lead to the production of smaller ice crystals during freezing in food materials ([Atani, Hamdami, Dalvi-Isfahan, Soltanizadeh, & Fallah-Joshaqani, 2022](#)).

In addition, it seems that the electromagnetic disturbance during the process caused premature nucleation, which led to a decrease in the supercooling degree. Generally, in conventional freezing processes, two parameters that are important for minimizing the size of ice crystals are the degree of supercooling and the freezing rate. However, in these conditions, the results of this study do not follow the general theory of conventional freezing. In the presented method, although a lower degree of cooling and slower crystallization were followed, it resulted in a significant decrease in the size of ice crystals. The results of the study by [Xanthakis et al. \(2014\)](#) on selected samples of frozen pork under the influence of microwave waves showed that the presence of these waves resulted in a decrease in the degree of supercooling, the freezing rate and an increase in the freezing time of the samples. Moreover, the results of the analysis of the microstructure of the pork samples indicated a 62% reduction in the average size of the ice crystals formed under the influence of microwave waves compared to those formed when microwave waves were not used for freezing. The authors attributed this to the heat generated by the microwave waves, which caused limited temperature oscillation during the nucleation and crystal growth, preventing repeated melting and immediate regeneration of ice crystals, thereby hindering crystal growth and resulting in the formation of multiple, smaller crystals.

Similarly, [Jackson et al. \(1997\)](#) used a cryoprotectant (*Ethylene Glycol*) along with 2.45 GHz microwave radiation to investigate ice crystal size and formation in tissues, and their results showed an increase in protection against freezing of biological substrates. Therefore, the final quality of frozen food can be improved with less damage to the microstructure and tissue, which is achieved by reducing the average size of ice crystals and the degree of supercooling. One of the most significant drawbacks of microwave-assisted freezing is the heat generated, which leads to a decrease in the freezing rate ([Ekezie, Sun, Han, & Cheng, 2017](#)).

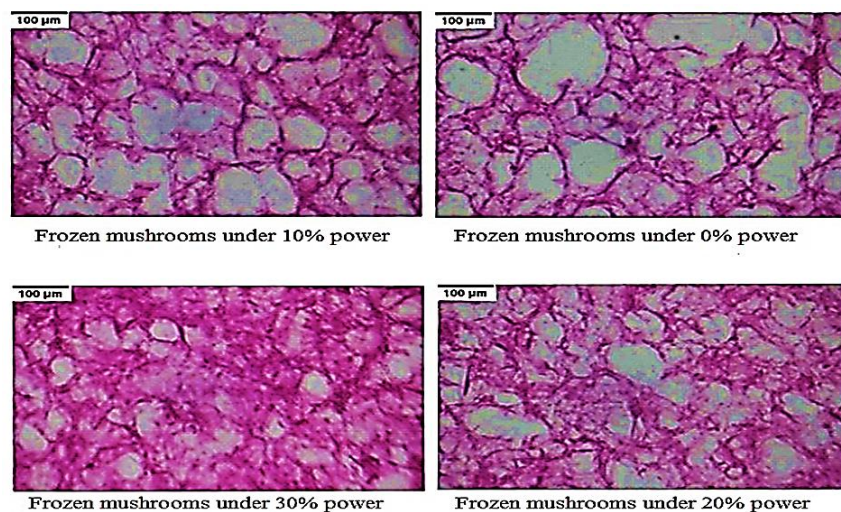


Fig. 3. Micrographs of frozen button mushrooms under various microwave powers (0%, 10%, 20% and 30%). This figure is adapted from Vardanjani et al., 2024, with kind permission under the Open Access Creative Commons licence Attribution (CC BY) license <https://creativecommons.org/licenses/by/4.0/>.

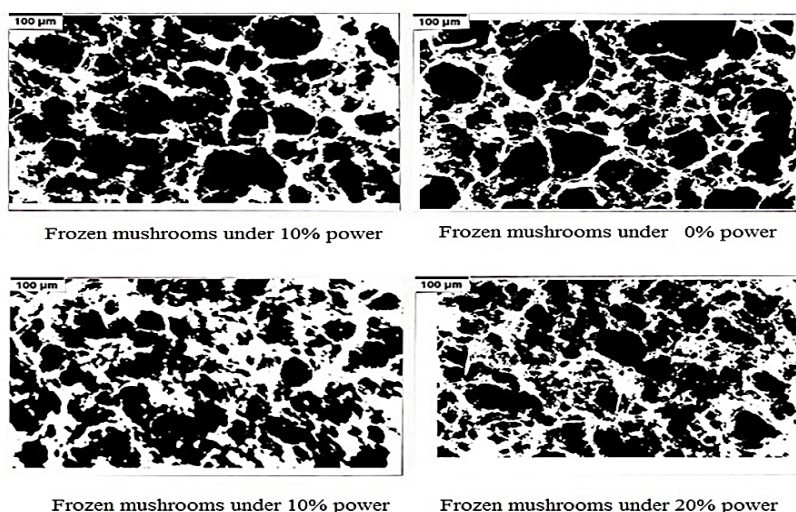


Fig. 4. Output Images from ImageJ Software for the Determination of Size and Shape of Frozen Mushroom Ice Crystals. This figure is modified/adapted from Vardanjani et al., 2024, with kind permission under the Open Access Creative Commons licence Attribution (CC BY) license <https://creativecommons.org/licenses/by/4.0/>.

10. Study of the freezing of button mushroom as a plant tissue under the influence of microwave waves

Fig. 3 shows changes in the microstructure of frozen mushrooms under different microwave powers. It shows micrographs obtained from frozen button mushroom samples, illustrating the microscopic changes in tissue due to freezing at power levels (0%, 10%, 20% and 30%). The results indicate a direct effect of increasing microwave power on reducing the size of ice crystals. Colored areas represent voids and intercellular spaces considered as residual effects of ice crystals. Interstitial spaces are the most suitable locations for ice crystal formation and depending on the size and quantity of ice

crystals and the extent of damage inflicted on the mushroom cell structure, the shape and size of these voids can vary. Freezing food samples leads to an increase in the size of white-colored voids compared to unfrozen samples. Reducing the size of ice crystals present in the food material tissue leads to an improvement in product quality during thawing. To examine the effect of different power levels on the microstructure of mushrooms, parameters such as ice crystal size and the percentage distribution of crystals were used. These quality indicators result in improved tissue quality of the mushroom sample and reduced damage to the food material during phase change (Jha, Chevallier, Xanthakis, Jury, & Le-Bail, 2020). To thoroughly investigate the effects of microwave waves on the microstructure of frozen mushroom samples, microscopic images were analyzed using image analysis software. For this purpose, it

was necessary to first obtain separable images. Then, with the help of these separated images, the size, distribution and shape of ice crystals within the mushroom tissue were observed and analyzed. Fig. 4 displays the distribution of ice crystals that have been separated using image analysis software, indicating a reduction in size and distribution of ice crystals due to an increase in microwave power. The results showed that the diameter of ice crystals in the control sample and those treated under different microwave powers had a decreasing trend with an increase in microwave power. As observed, higher microwave power led to the formation of smaller ice crystals, which are represented as black spots in Fig. 4.

11. The Size of Ice Crystals

Fig. 5 illustrates the effect of different microwave power levels on the freezing of mushroom samples. As evident from the figure and the obtained graph, increasing microwave power levels have led to a reduction in the size of ice crystals, which is statistically significant ($p < 0.05$). In the study by Vardanjani et al., 2024, after recording temperature changes, it was shown that a thermal effect occurs due to the friction of water molecules under the influence of microwaves, which is generated through their torque around the microwave field. Therefore, it seems that temperature fluctuations during both nucleation and ice crystal growth phases affect the crystal size. Repeated fluctuations during the formation of ice nuclei and crystal growth may be responsible for frequent melting and instantaneous reconstruction of ice crystals, ultimately resulting in the creation of multiple, smaller ice crystals. The results indicated a 26% reduction in the average ice crystal size due to increased microwave power levels (Vardanjani et al., 2024). The findings demonstrate the impact of using microwave and electric fields in reducing the size of ice crystals in both plant and animal tissues. Larger crystals can damage cell membranes because the pressure from expansion during phase change leads to the formation of cracks within the tissue and loss of water upon thawing, resulting in reduced product quality after thawing. The results of ice crystal dimension measurements show a significant difference between control group data and those exposed to high microwave power levels. In this study, profiles of recorded actual temperatures clearly indicate temperature fluctuations under microwave levels. Therefore, it appears that the effect of microwave application during freezing is related to temperature fluctuations in both nucleation and crystal growth phases. Limited temperature oscillation during ice nucleus formation and crystal growth may be responsible for repeated, rapid melting and reconstruction of ice crystals, ultimately leading to the formation of multiple, smaller ice crystals (Xanthakis et al., 2014).

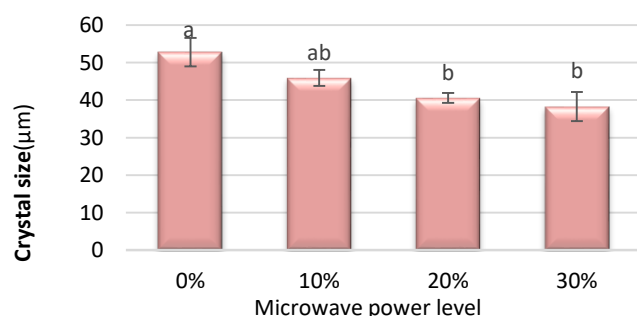


Fig. 5. The equivalent diameter of ice crystal at various microwave power levels. The data used in this chart has been adapted from Vardanjani et al., 2024.

12. Conclusion

Emerging freezing technologies, such as rapid freezing, nucleating agents, antifreeze proteins, high-pressure freezing, and assistance using ultrasound, electric, and magnetic waves, can be utilized industrially to improve the food freezing process. These methods contribute to the reduction in ice crystal size and distribution by enabling more precise control over process parameters. Utilization of these technologies not only enhances the quality of the final product but also improves the shelf life, stability, and nutritional value of foodstuffs, while reducing waste associated with freezing damage and structural damage. In this way, these methods possess the capability to significantly optimize industrial freezing processes and play a significant role in reducing costs and improving efficiency within the food industry.

Conflict of interest

The authors declare that there is no conflict of interest.

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