Effects of Magnetic Fields on Freezing: Application to Biological Products

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ABSTRACT

Magnetic freezing is nowadays established as a commercial reality mainly oriented towards the food market. According to advertisements, magnetic freezing is able to generate tiny ice crystals throughout the frozen product, prevent cell destruction, and preserve the quality of fresh food intact after thawing. If all these advantages were true, magnetic freezing would represent a significant advance in freezing technology, not only for food preservation but also for cryopreservation of biological specimens, such as cells, tissues, and organs. Magnetic fields (MFs) are supposed to act directly on water by orientating, vibrating and/or spinning molecules to prevent them from clustering and, thus, promote supercooling. However, many doubts exist about the real effects of MFs on freezing and the science behind the potential mechanisms involved. To provide a basis for extending the understanding of magnetic freezing, this paper presents a critical review of the materials published in the literature up to now, including both patents and experimental results. After examining the information available, it was not possible to discern whether MFs have an appreciable effect on supercooling, freezing kinetics, ice crystals, quality, and/or viability of the frozen products. Experiments in the literature frequently fail to identify and/or control all the factors that can play a role in magnetic freezing. Moreover, many of the comparisons between magnetic and conventional freezing are not correctly designed to draw valid conclusions and wide ranges of MF intensities and frequencies are unexplored. Therefore, more rigorous experimentation and further evidence are needed to confirm or reject the efficacy of MFs in improving the quality of frozen products.

Keywords: magnetic fields, freezing, supercooling, food preservation, cryopreservation

1. INTRODUCTION

Freezing is one of the most popular and widely used methods for preservation of biological products. However, the ice crystals formed in the process, especially if they are large, can severely damage the frozen material. The size, shape, and distribution of the ice crystals depend on freezing kinetics and, therefore, it is important to optimize the process to minimize injuries.

It is well known that freezing takes place in three key steps: precooling, phase transition, and tempering. In the precooling step (A-B in Figure 1), sensible heat is removed from the product and its temperature is lowered. After reaching the freezing point (T_F), phase transition is not usually triggered immediately, but cooling continues. Therefore, at the end of the precooling step, the product remains unfrozen below its freezing point, that is, the product is supercooled. At a certain degree of supercooling (ΔT), ice nucleation suddenly occurs and, thus, the product temperature rapidly increases due to the release of latent heat (B-C in Figure 1). During the phase transition step, also known as freezing plateau (C-D in Figure 1), temperature remains almost constant at T_F while the latent heat of crystallization is removed and ice crystals grow in the product. The larger the degree of supercooling (difference between freezing and nucleation temperatures), the larger the amount of ice instantaneously formed at nucleation and, therefore, the shorter the phase transition time. Once most of the water has transformed into ice, sensible heat is removed during the tempering step (D-E in Figure 1) while the product is cooled down to the freezer temperature.

The final size, shape, and distribution of the ice crystals formed throughout a product depend on the rates of ice nucleation and subsequent crystal growth. The larger these rates, the smaller, the rounder, and the more homogeneously distributed the ice crystals. Ice nucleation is an activated process driven by supercooling and, according to Burke and others (1975), the rate of ice nucleation increases roughly tenfold for every degree of supercooling. Crystal growth takes place only once nucleation has occurred, through addition of water molecules to the nuclei already formed, and its rate mainly depends on the efficiency of latent heat removal (Reid 1983; Petzold and Aguilera 2009; Kiani and Sun 2011). For decades, the efforts to optimize freezing processes have been focused on improving the efficiency of heat removal. Thus, different strategies such as lowering the refrigerating medium temperature, enhancing the surface heat transfer coefficient, or reducing the size of the products to be frozen have been applied (Reid 2000; Fikiin 2003). Only recently, attention has been paid to ice nucleation and several technologies, such as high pressure, ultrasounds, and electric and magnetic fields, for example, have been proposed to control the nucleation phenomenon (Otero and Sanz 2011; Kiani and others 2014; James and others 2015b). All these technologies are applied as adjuncts to existing freezing systems to either inhibit or promote supercooling and, in this way, to control ice formation. Among them, only magnetic fields (MFs) have been already implemented and marketed for industrial food freezing (Ryoho Freeze Systems Co. 2011; ABI Co. 2012). Thus, since 2000, ABI Co., Ltd. (Chiba, Japan) commercializes a system called 'CAS (Cells Alive System) freezing' that uses static and oscillating MFs, while Ryoho Freeze Systems Co., Ltd. (Nara, Japan) sells, since 2003, 'Proton freezers' that combine static MFs and electromagnetic waves. Both CAS and Proton freezers are patented systems that apply magnetic fields during freezing, although in quite different ways (Owada and Kurita 2001; Owada 2007; Owada and Saito 2010; Fujisaki and Amano 2012). In theory, they act directly on water by orientating, vibrating and/or spinning molecules to prevent them from clustering and, thus, promote supercooling, but the science behind these mechanisms is not clear. In fact, the precise mechanisms that cause the effects observed in water exposed to magnetic fields are not yet completely elucidated and they remain as an open question (Colic and Morse 1999; Pang 2006; Zhao and others 2015).

According to commercial advertisements, both CAS and Proton freezers are able to generate tiny ice crystals throughout the frozen product, prevent cell destruction, and preserve the quality of the fresh product intact after thawing (ABI Co. 2007; IFP Ltd. 2015). Since the earlier 2000s, many magnetic freezers have been sold to food processors, restaurants, hotels, hospitals, and research centers in and outside Japan (Kelly 2008; James and others 2015a). Magnetic freezing has awakened much interest on the Internet and there exist innumerable websites with amazing videos on CAS and Proton freezers (ccnishio 2010; monodzukuri 2011; jasbcocinca 2011; sou seki 2013; Ikeda 2014a, b). These videos usually show delicate products magnetically frozen, such as fruits, vegetables, seafood, and even flowers, that retain their fresh appearance and original taste, flavor, and texture after thawing. If MFs were responsible for all these advantages, magnetic freezing would represent a significant advance in freezing technology, not only for food preservation but also for cryopreservation of biological specimens, such as cells, tissues, and organs. Surprisingly, scientific studies on the effect of magnetic fields on freezing of water and biological products are very scarce and, to date, clear evidence of the promised effects has not yet been found (Woo and Mujumdar 2010; Xanthakis and others 2014). Moreover, results published in the literature are often apparently contradictory probably because some factors that play a role in magnetic freezing are not considered. Finally, it is important to note that the existing scientific papers have been written by experts in quite different disciplines (physics, food science, cryobiology, for example) and, therefore, they are sometimes focused on particular aspects of their area of study, while other important aspects of the process are neglected.

To provide a basis for extending the understanding of magnetic freezing, this paper presents a critical review of the material published in the literature up to now. As water seems to be the target of magnetic freezing, a brief description of the water molecule and its particular characteristics is first introduced. Taking into account that the magnetic properties of a substance arise from the spin and orbital angular momentum of electrons, special attention has been paid to the electron configuration of water. Based on this configuration, the magnetic properties of water are then detailed and, also, the effects observed in water exposed to magnetic fields. Moreover, some hypotheses to explain the mechanisms that produce these effects are commented to establish a theoretical basis for speculating about the operating mode of magnetic freezing. After these hypotheses, existing patents on magnetic freezing are described. Then, in an attempt to back the theory under these patents up with some form of evidence, experimental data on the effects of MFs on freezing of water, food products, and biological specimens are compiled. Particular care has been taken to critically evaluate the reasons of the apparent discrepancies observed by different authors. Finally, some key considerations are analyzed that reveal several research needs for clarifying the effects of magnetic fields on freezing of biological products. This review contributes to increasing knowledge on magnetic freezing and stimulating future research on this innovative technology for preservation of biological products.

2. THE WATER MOLECULE

Water is a small molecule composed of two hydrogen atoms covalently bonded to one atom of oxygen. Water bonding and geometry can be roughly explained according to the valence bond theory, the hybridization of atomic orbitals, and the Valence-Shell Electron-Pair Repulsion (VSEPR) model. Figure 2.a shows the electron configuration of the constituent atoms of the water molecule. Hydrogen atom has one electron (Z = 1), while oxygen has 8 electrons (Z = 8). Two of them are core electrons located in the first s atomic orbital, immediately adjacent to the nucleus, while the other six are valence electrons. The Lewis formula for water (Figure 2.b) shows that oxygen, the central atom of water, is surrounded by four separate regions of high electron density: two of them correspond to two lone pairs of electrons and the other two correspond to two σ-bonding pairs of electrons. For this configuration, the VSEPR model predicts a tetrahedral distribution of electron clouds with an ideal bond angle of 109.5º that results in a V-shaped water molecule. This geometry can be explained by sp^3 hybrid orbitals (Figure 2.c). Thus, 2s and 2p orbitals in oxygen would hybridize to form four sp^3 orbitals oriented at a bond angle of 109.5º. The valence electrons on oxygen would fill two of these hybrid sp^3 orbitals (two lone pairs of electrons), while each of the other two hybrid sp^3 orbitals would be occupied with one unpaired electron. Bonding of water would occur through the overlap of these latter two hybrid sp^3 orbitals on oxygen with 1s orbitals on the two hydrogen atoms. As bonding pairs of electrons experience less repulsive force than lone pairs of electrons, the perfectly symmetrical shape ideally adopted by the electron pairs would be distorted. Thus, the water geometry shown in Figure 2.d, with an angle of 104.5º between the hydrogen atoms, could be explained.

To understand water properties, it is also important to note that the oxygen in the water molecule attracts electrons more strongly than hydrogen due to its large electronegativity. Therefore, shared electrons in the covalent bond will be closer to oxygen and, thus, the oxygen region of the water molecule has a slightly negative charge, while each hydrogen atom has a slightly positive charge. Water is, therefore, a polar molecule. The electrostatic attraction between the partial positive charge near the hydrogen atoms and the partial negative charge near the oxygen makes water molecules interact with each other and form intermolecular hydrogen bonds. Hydrogen bonds in water are much weaker than covalent bonds (about

23 kJ mol^{-1} compared to the O–H covalent bond strength of 492 kJ mol^{-1}), but they are the strongest kind of dipole-dipole interaction. Thus, these H-bonding forces allow for a strong interaction between water molecules and this intermolecular interaction is responsible for most of the anomalous properties of water: volume expansion on freezing, high freezing point, high specific and latent heats, high surface tension, among others. Each water molecule can form up to four hydrogen bonds and, thus, the structure of liquid water is usually represented either as a continuous three dimensional network of hydrogen bonds or as a mixture of clusters of molecules with different degrees of hydrogen bonding in an equilibrium. Water clusters can have different number of molecules (dimers, trimers, tetramers, and so on up to hundreds of molecules) and adopt different structures: linear, rings, prisms, or cages, among others (Chaplin 2000; Pang 2006; Tigrek and Barnes 2010). However, rotation and other thermal motions in water molecules cause individual hydrogen bonds to break and re-form on a 10^{-12}-10^{-9} s time scale. Therefore, any specific structure in liquid water is continuously changing.

3. EFFECTS OF MAGNETIC FIELDS ON WATER

First of all, it is important to note that a real understanding of the magnetic properties of matter cannot be achieved by classic physics, but only by quantum electrodynamics. However, this is out of the scope of this paper and, therefore, in this review, very simplified concepts are used to explain water magnetization.

As outlined in the Introduction, the magnetic properties of a substance arise from the orbital motion and spin of electrons in the atoms. Nuclei also possess spin, but electron-field interactions are thousands of times stronger than nuclear ones. Therefore, electrons, not nuclei, primarily determine magnetic susceptibility. The orbital motion of electrons around the nucleus creates tiny atomic current loops that induce a magnetic moment along the axis of rotation. In the same way, the spinning of electrons also produces a magnetic moment. The net magnetic moment of an atom is the vector sum of both orbital and spin moments of all the electrons in the atom.

In water, all the orbitals are fully occupied by pairs of electrons (Figure 2.c). Paired electrons orbit in opposite directions and, therefore, orbital moments are cancelled. The same occurs with the spin moments of paired electrons. Thus, water has no net magnetic moment in the absence of an externally imposed magnetic field. When an external magnetic field is applied, the orbital motion of electrons is altered in such a way that the induced magnetic fields oppose to the external field. This may be viewed as an atomic version of Lenz's law, which is a direct consequence of the energy conservation law. Thus, the effect is to ‘repel’ the external field and water is, therefore, a diamagnetic substance.

The magnetic force exerted on a substance is proportional to the square of the strength of the external magnetic field and the magnetic susceptibility. Magnetic susceptibility of water is rather low ($\chi_v = -9.035 \cdot 10^{-6}$) and, therefore, weak MFs will have little effect on water, while strong MFs can exert considerable force. The effects of strong MFs (≥ 10 T) can be macroscopically visualized by levitating water droplets in air, that is the magneto-Archimedes levitation (Beaugnon and Tournier 1991; Ikezoe and others 1998), or by visibly deforming the water surface (Kitazawa and others 2001). Weaker MFs also produce effects on water, although not so evident. For example, magnetic fields of the order of one third of a tesla can still create a microscopic depression of about 25 µm in the water surface (Chen and Dahlberg 2011). Moreover, some properties such as the surface tension force, the viscosity, the refractive index, the electric conductivity, and the enthalpy of vaporization, among others, seem to be affected after exposing water to magnetic fields, even at intensities as low as 1 mT (Semikhina and Kiselev 1988; Hosoda and others 2004; Pang and Deng 2008a, b; Toledo and others 2008; Cai and others 2009; Szcześ and others 2011; Pang and others 2012). In recent years, analyses of magnetized and non-magnetized water by spectroscopic techniques confirm that MF exposition produces changes (shifts of some peaks and appearance of new bands) in infrared, Raman, visible, ultraviolet, and X-ray spectra (Iwasaka and Ueno 1998; Pang and Deng 2008a, b). Some authors attribute these modifications to changes in water structure by displacement and polarization of molecules and atoms and modification of hydrogen bonding (Rai and others 1995; Iwasaka and Ueno 1998; Pang and Deng 2008a, b), while others identify in the spectra of magnetized water some similarities with the spectra of ozone and hydrogen peroxide (Colic and Morse 1999). Thus, Colic and Morse (1999) suggest that Lorentz forces can produce small currents in water, producing the same effect as electrolysis.

The magnitude of the changes observed in water after MF exposition depends on the magnetic field strength and frequency, the exposition time, and the temperature, but no linear relationship among these factors has been found. A saturation effect is also observed, that is, the MF effects reach a maximum after a given exposition time. Moreover, these effects seem to not disappear immediately after MF removal, but remain for a time, i.e., the so-called memory effect of magnetized water (Semikhina and Kiselev 1988; Pang and Deng 2008a, b).

However, the results reported in the literature have low reproducibility and little consistency. For example, Pang and Deng (2008b) observed that the exposition of water to a 440 mT static magnetic field for 30 min significantly decreased the surface tension force and the viscosity. By contrast, Toledo and others (2008) reported an increase in the surface tension and the viscosity of water after exposition to a 45-75 mT magnetic field for 3 h, while Cai and others (2009) detected a decrease in the surface tension and an increase in the viscosity of purified water circulated at a constant flow rate in a 500 mT magnetic field. The reproducibility of experiments with magnetized water is hampered by many factors related to the sample, the magnetic field applied, and the measurements performed. Some factors related to the sample, such as magnetic impurities and quantity of dissolved oxygen, are difficult to control (Toledo and others 2008). The reproducibility of the magnetic fields applied is not trivial and special care must be taken to guarantee that the magnetic field can penetrate the whole water sample (Pang and Shen 2013). Moreover, it is important to note that when time-varying MFs are applied, electric fields are induced. Electric fields have been proved to efficiently reorient water molecules due to the intrinsic electric dipole moment that water molecule has (Chaplin 2000). Therefore, discerning the effects of time-varying magnetic and electric fields on water is not simple. On the other hand, the MF effects observed in water are usually very weak and effective methods and sensitive instruments must be employed to detect them. Moreover, these effects should be measured during magnetic field application (Hosoda and others 2004) or, at least, immediately after it because they are not permanent, as previously mentioned, but they fade away after some time (Pang and Deng 2008a, b; Pang and Shen 2013). Furthermore, it is important to note that impurities can be dissolved or suspended from the sample containers or from the measurement instruments and they can affect the results (Amiri and Dadkhah 2006; Holysz and others 2007). Therefore, caution is needed in the interpretation of the observed phenomena.

The precise mechanisms that produce these effects in water exposed to MFs are not clear, although many hypotheses have been proposed (Colic and Morse 1999; Vallée and others 2005b; Pang 2006; Cefalas and others 2010; Wang and others 2013). For example, taking into account the polar nature of the water molecule, Wang and others (2013) assumed that the thermal motion of the partially charged atoms of water under the magnetic field gives rise to Lorentz forces. The direction of these forces on the positive charge center of the water molecule is opposite to that on the negative charge center and, therefore, both centers will be relocated, the distance between them will become larger and these changes will weaken the hydrogen bonds. Based on the existence of linear and ring hydrogen bonded chains of molecules in water, Pang and others (2006, 2013) hypothesized that closed hydrogen-bonded chains become ‘ring electric-current’ elements when submitted to a magnetic field due to the proton transfer in them under the action of Lorentz forces. The magnetic interactions of these ‘ring electric-current’ elements with each other or with the externally applied magnetic field would result in the reorientation and formation/breaking of hydrogen bonds. Consequently, the distribution of water molecules would be modified and, therefore, also the physical and chemical properties of the magnetized water. By contrast, experiments by Colic and Morse (1999) and by Vallée and others (2005a, b) suggest that the gas bubble/water interface is the primary target of the MF action because no MF effects are observed when water is degassed. According to these authors, MF exposition leads to the destabilization of the air nanobubbles, naturally present in non-degassed water, by disturbing the ionic balance between the negative ions adsorbed on the bubbles and the shell of counter ions. These changes at the gas/water interface can modify the water cluster size and the reactivity of bulk and interfacial water.

Computational techniques, such as Monte Carlo and Molecular Dynamics (MD) simulations, have been also employed to elucidate the effects of magnetic fields on water systems at the molecular level (Zhou and others 2000; Chang and Weng 2006; Toledo and others 2008). These studies show that hydrogen bonds can be affected by magnetic fields and, consequently, also the interactions between water molecules (Zhou and others 2000; Chang and Weng 2006). Therefore, new clusters arrangements can be formed that, obviously, can affect water properties. Again, the conclusions by different authors are, in some way, divergent. For example, using Monte Carlo computer simulation, Zhou and others (2000) found that 100-200 mT MFs increased the mean distance between water molecules, while field strengths smaller than 50 mT did not produce appreciable effects. Thus, depending on its strength, an external magnetic field could cause the weakening of hydrogen bonds and the diminishing of the hydrogen bond average number among water molecules. However, using MD simulation, Chang and Weng (2006) found that the number of hydrogen bonds increased by approximately 0.34% when the magnetic field strength increased from 1 to 10 T. Thus, according to these authors, magnetic fields enhance the bonding among water molecules and stabilize the structure of liquid water. At this point, it is interesting to note that hydrogen bonds can be inter- and intra-cluster. In this sense, the results obtained by Toledo and others (2008) suggest that magnetic fields weaken the intra-cluster hydrogen bonds, breaking large water clusters and forming smaller clusters with stronger inter-cluster hydrogen bonds.

Despite the low reproducibility of the experimental data and the controversial theories explaining water behavior in a magnetic field, all the studies conclude that MFs affect the hydrogen bond networks. Rearrangements in hydrogen bonding can substantially impact on some water properties that govern freezing kinetics, such as the freezing point, the specific heat capacity, or the thermal conductivity. However, data about the effect of magnetic fields on these water properties are especially scarce. Inaba and others (2004) measured the MF effect on the freezing point of water by using a high resolution and supersensitive differential scanning calorimeter working in a magnetic bore. They found that exposition to static MFs increased the freezing temperature of water and the temperature shift was proportional to the square of the MF intensity. At 6 T, the freezing point increased by $5.6 \cdot 10^{-3}$ °C; therefore, they concluded that magnetic fields strengthen hydrogen bonding and make the solid phase more stable than the liquid state. Moreover, Monte Carlo simulations by Zhou and others (2000) predicted significant changes in the internal energy and specific heat when water is exposed to 100 mT magnetic fields or larger. These predictions were later confirmed by Pang and others (2012) who observed a decrease in the specific heat of water when exposed to 440 mT static MFs for 30 min. Some MD simulations of ice growth from water exposed to magnetic fields have been recently published (Zhang and others 2010; Hu and others 2012). Thus, Zhang and others (2010) investigated freezing of confined water in a 10 T static MF. According to the authors, confinement induced a bilayer crystalline ice which resembled none of the structures of existing ice polymorphs, while MF exposition significantly increased the freezing temperature of the confined water. Thus, at 10 T, an anomalously high freezing temperature of 67 °C was found. Hu and others (2012) studied the microscopic mechanism for ice growth from supercooled water when external electric (0-10^9 V/m) and magnetic (0-10 T) fields were applied simultaneously. They found that the ice growth on the primary prismatic plane could be accelerated when fairly low electric (10^6 V/m) and magnetic (10 mT) fields were applied. By contrast, the growth on the basal plane was hardly affected unless the fields increased up to 10^9 V/m and 10 T. Moreover, when studying electric and magnetic fields separately, they found that electric fields could play a significant role in the hydrogen-bonding structures of liquid water, but the effect of magnetic fields, even at 10 T, was only marginal.

4. PATENTS ON MAGNETIC FREEZING

In recent years, many patents have been developed that try to take advantage of MF effects on water properties to improve freezing of foods, ice cubes, feedstuffs, living cells (blood, animal tissues and organs, for example), flowers, chemical and pharmaceutical products, among others (Hirasawa and others 2000, 2001; Owada and Kurita 2001; Kino 2002; Owada 2007; Sato and Fujita 2008; Kim and others 2009; Owada and Saito 2010). All these patents claim that the application of MFs during freezing inhibits water crystallization and allows large supercooling. When freezing occurs, either by lowering temperature well below the freezing point or by ceasing MFs, small ice crystals are formed throughout the whole volume of the product. In this way, damage produced is supposed to be substantially reduced. However, the extremely low strength of the frequently applied MFs casts doubt on the effects that these weak MFs can have on a substance with a low magnetic susceptibility such as water. Moreover, the mechanisms adduced in the patents to explain the expected improvements are frequently vague, not scientifically proved and, according to Kobayashi and Kirschvink (2014), *'do not agree with basic biophysics'*.

Most of the inventors state that MFs act by aligning the electronic and nuclear spins of the atoms in the direction of the magnetic field. The hydrogen nuclei of water molecules have an odd number of protons and, therefore, a net magnetic moment. When a magnetic field is applied, hydrogen nuclei will behave like mini-bar magnets and will align with the external magnetic field. Moreover, the spinning nuclei will precess around the direction of the field. According to the patents, this will enhance the thermal vibration of the hydrogen nuclei, supercooling, and heat transfer throughout the product (Hirasawa and others 2001; Owada and Kurita 2001; Ino and others 2005; Owada 2007; Owada and Saito 2010). Moreover, hydrogen bonds of water molecules will be also affected and some authors consider that the cluster size of free water will tend to decrease (Owada and Kurita 2001; Owada 2007; Sato and Fujita 2008; Owada and Saito 2010). According to Sato and Fujita (2008), when water clusters are fragmented in this way, small ice crystals are formed and the quality (appearance, flavor, and fragrance) of the products to be frozen is hardly deteriorated. On the other hand, some authors claim that decreasing the cluster size of free water makes it possible to increase the amount of non-freezable bound water and this involves a better preservation of the product freshness (Owada and Kurita 2001; Owada 2007; Owada and Saito 2010). They consider that small water clusters are capable of forming hydrogen bonds with the polar groups of the tertiary structures of proteins and carbohydrates and attach compactly to them. Thus, MFs would reduce the amount of freezable water and, therefore, the amount of ice crystals. Moreover, MFs would also prevent tertiary structures from being oxidized by promoting a hydration structure on them.

Published patents make use of both static and oscillating MFs (Table 1), either separately or combined (Owada and Kurita 2001; Hirasawa and others 2001; Ino and others 2005; Owada 2007; Owada and Saito 2010). Moreover, these fields can be continuous (Hirasawa and others 2001; Mihara and others 2012) or pulsed (Miura and others 2005; Sato and Fujita 2008). Static MFs can be generated by permanent magnets or by electromagnets, although the former is preferred because operating costs are lower. Oscillating MFs are generated by coils supplied with AC current. When static and oscillating MFs are combined, overlapped in the same direction or not, the expected effects on freezing are enhanced and operating costs can be reduced (Hirasawa and others 2001; Owada and Kurita 2001; Owada 2007; Owada and Saito 2010). When used separately, oscillating MFs are preferred to static MFs due to their versatility (Sato and Fujita 2008). Moreover, as previously mentioned, oscillating MFs induce oscillating electric fields. These induced electric fields can also enhance the impediments for ice formation. Thus, water molecules, that have an electric dipole, will be orientated in the oscillating direction of the induced electric field. The vibration and friction of water molecules will generate minute heat that also inhibits water crystallization. According to Hirasawa and others (2001), the combined action of oscillating magnetic and induced electric fields allows reaching large supercooling. Moreover, Owada and Kurita (2001) claimed that this electric field will generate free electrons that are gained by the water and oxygen molecules in the freezing device. According to the authors, hydroxyl-radicals are then produced that destroy the cell membrane of microbes and, therefore, reduce the microbial load in the frozen product. Owada and Saito (2010) also remarked that free electrons will avoid the oxidation of the product to be frozen.

The existing patents on magnetic freezing are not especially strict on the selection of the optimal values of the process parameters. Thus, the MF strength and frequency ranges included in the demands are particularly wide (Table 1). The MF strength can vary between 0.1 mT and 2 T, while the MF frequency ranges between 50 Hz and 10 MHz. However, the conditions tested by the inventors are considerably more limited: MF strengths between 0.1 mT (Mihara and others 2012) and 800 mT (Sato and Fujita 2008) and MF frequencies between 50 Hz (Owada 2007) and 200 kHz (Mihara and others 2012). Most of the authors claim that the MF strength and frequency are not particularly restricted, but according to Mihara and others (2012) and Ino and others (2005), the optimal values should be selected based on the specific characteristics of the product to be frozen (size, type, impedance). The wave shape (sinusoidal, square, or triangular, for example) in oscillating MFs is not frequently described, but some inventors prefer rectangular waves (Ino and others 2005). Other parameters of the freezing process such as the cooling rate or the freezer temperature are even less limited and can range between 0.1 and 1 °C/min and between –2 °C and –100 °C, respectively (Table 1).

The uniformity of the magnetic fields applied during freezing is a relevant issue. According to Owada (2007), if MFs are not uniformly applied, their effects are not evenly exerted on the frozen product and, therefore, the product quality can be affected. Owada (2007) found that the uniformity of oscillating MFs increases by disposing a plurality of coils along the sample holder. Sato and Fujita (2008) also designed a number of feasible embodiments that allow a uniform application of magnetic fields on the product.

In most of the published patents, the preferred embodiments combine permanent magnets and/or electromagnetic coils with other devices to improve the freezing process. Thus, the combination of magnetic with electric fields (Owada and Kurita 2001; Hirasawa and others 2001; Owada 2007; Owada and Saito 2010) and/or other energy-generating devices that irradiate ultrasonic waves, microwaves, far infrared rays, ultraviolet light, α-rays, and negative ions, among others, is frequently described (Hirasawa and others 2000; Owada and Kurita 2001; Kino 2002; Toyoshima 2005; Owada 2007; Sato and Fujita 2008; Owada and Saito 2010; Fujisaki and Amano 2012).

Oscillating electric fields are, generally, applied to produce the vibration of water dipoles and, in this way, to inhibit crystallization (Hirasawa and others 2001). According to the revised patents, the electric field strength and frequency range between 10-1000 kV/m and 50 Hz-5 MHz, respectively (Owada and Kurita 2001; Owada 2007; Owada and Saito 2010). Owada (2007) claimed that the growing of ice crystals can be substantially reduced by applying an electric field of variable frequency in stages. Thus, in the temperature range between –2 °C and –10 °C, a frequency of 250 kHz is particularly effective in decreasing ice crystals size, while the optimal frequency in the temperature range between –30 °C and –60 °C is 3 MHz. Moreover, as previously mentioned, Owada and Kurita (2001) remarked that oscillating electric fields also enhance the production of hydroxyl-radicals that have an anti-microbial effect on the product.

The effect of other energy-generating devices is more ambiguous. According to Sato and Fujita (2008), the use of negative ions, α-rays, ultrasonic waves, microwaves, far infrared rays, and ultraviolet light allows a better fragmentation of water clusters by MFs. Moreover, Owada (2007) stated that the addition of ionic air (negative ions) to the cold wind also improves heat transfer. However, no evidence of such statements is provided.

Especially interesting is the combination of static MFs with electromagnetic waves to induce nuclear magnetic resonance in the hydrogen atoms of water molecules and, in this way, achieve large supercooling (Hirasawa and others 2000; Kino 2002; Toyoshima 2005). Hirasawa and others (2000) designed a freezing device with a static MF in its inner space and an electromagnetic wave generator. When the product to be frozen is located inside the freezer, the hydrogen nuclei of water molecules exhibit a precessional motion around the direction of the static MF as mentioned. The precession frequency (ω) depends on the MF strength (B) and the gyromagnetic ratio (γ, 42.6 MHz/T for the hydrogen nucleus) and, according to the Larmor equation, it is $\omega = B \cdot \gamma$. When the product is irradiated with electromagnetic waves, either continuously or intermittently, at this same frequency, nuclear magnetic resonance is induced in the hydrogen nuclei of water molecules. According to the inventors, the energy of electromagnetic waves absorbed by the hydrogen nuclei avoids water freezing at temperatures as low as –40 °C. Unfortunately, the authors don't provide experimental evidence of this statement. Moreover, it is important to note that it is difficult to obtain a uniform magnetic field inside the freezer and, therefore, the resonance frequency is not constant in the whole volume of the device. Later patents in the literature tried to overcome this problem. Thus, Kino (2002) presented different solutions based on either varying magnetic fields or applying broadband electromagnetic waves to generate nuclear magnetic resonance in wide regions inside the freezer.

Apart from these energy-generating devices, some patents also include other elements to improve the freezing process such as sound-waves generators, far-infrared-ray absorbers, and pressure regulators. Owada and Kurita (2001) proposed superimposing sound waves, in the audio-frequency range, to the cold wind in contact with the product to stir up the boundary layer of air that inhibits heat transmission. Moreover, far-infrared-ray absorbers can be arranged on the inner walls of the freezer to absorb the radiant heat of the product, and thus, accelerate the cooling rate (Owada and Kurita 2001; Owada 2007; Owada and Saito 2010; Fujisaki and Amano 2012). Pressure regulators are employed to adjust gas pressure (above or below the atmospheric pressure) inside the freezer (Sato and Fujita 2008; Owada and Saito 2010). By decreasing pressure with a suction pump, it is possible to reduce the temperature inside the freezer and also to eliminate oxygen, and thus, avoid product oxidation. Once temperature is low enough, increasing pressure limits water evaporation and prevents drying of the product. Moreover, increasing pressure with gases with low or no oxygen content contributes to avoid product deterioration.

Other common devices and elements, usually employed in conventional freezers to improve heat transfer, are also included in the preferred embodiments described in most of the patents: heat insulators to maintain the freezer temperature (Owada and Kurita 2001; Toyoshima 2005;

Ino and others 2005; Owada 2007), ventilators to circulate cold air in the freezer (Owada and Kurita 2001; Ino and others 2005; Owada 2007; Sato and Fujita 2008), air sanitizers to avoid product contamination (Hirasawa and others 2000; Owada and Saito 2010), honeycombs to promote a uniform flow of cold air (Owada 2007), and air dehumidifying devices to avoid frost formation both on the freezer and on the frozen product (Sato and Fujita 2008).

Patented equipment includes solutions for both batch and continuous freezing. Continuous freezers can be straight belt, multi-pass belt, or spiral type and the product to be frozen is continuously conveyed through them. Therefore, continuous freezers require more complex embodiments to apply uniform MFs, either static or time varying, during the complete freezing process (Ino and others 2005; Owada 2007; Sato and Fujita 2008; Fujisaki and Amano 2012).

On the other hand, it is important to note that MFs can be applied not only during freezing but also during frozen storage and subsequent thawing. Thus, Ino and others (2005) patented a system in which oscillating MFs are applied during the complete freezing-storage-thawing process. According to the authors, this method allows better preservation of the food quality.

5. EXPERIMENTAL DATA ABOUT THE EFFECTS OF MAGNETIC FIELDS ON FREEZING

As commented in the Introduction, if all the advantages described in the above patents were true, magnetic freezing could lead to a significant advance in freezing technology. For this reason, the interest of the scientific community on magnetic freezing is increasing. Several authors, from different universities and research centers, have performed experiments to assess the effect of MFs on freezing of a wide variety of products such as water (Semikhina and Kiselev 1988; Aleksandrov and others 2000), aqueous solutions (Rohatgi and others 1974; Iwasaka and others 2011; Mok and others 2015), nanofluids (Jia and others 2016), foods (Watanabe and others 2011; James and others 2015c), cells (Kaku and others 2010; Lin and others 2014), tissues (Lee and others 2012b), organs (Samanpachin and others 2009; Niino and others 2012), and even organisms (Naito and others 2012; Morono and others 2015). The experiments have been carried out in both commercial equipment (Yamamoto and others 2005;

Kaku and others 2010; Lee and others 2012a; James and others 2015c) and lab prototypes specially designed for the trials (Rohatgi and others 1974; Suzuki and others 2009; Lou and others 2013). However, no consistent results have been found.

According to the published patents, the main benefit of MFs on freezing is to achieve large supercooling in the sample before ice nucleation occurs (Hirasawa and others 2000, 2001; Owada and Kurita 2001; Kino 2002; Owada 2007; Owada and Saito 2010; Mihara and others 2012). Thus, when crystallization is triggered, the nucleation rate is high and many tiny ice crystals are instantaneously formed. Moreover, Owada and Kurita (2001), Owada (2007), and Owada and Saito (2010) claimed that MFs not only enhance supercooling but also the cooling rates during the freezing process. Therefore, finding evidence of MF effects on freezing seems to be easy. It would be reduced to detect larger supercooling, shorter phase transition and total freezing times, and reduced damage in the frozen product when MFs are applied during freezing. However, all these advantages have not yet been proved because replicating magnetic freezing experiments and obtaining consistent results is a really hard task.

Apart from the aforementioned difficulties associated to MFs, there are other obstacles associated to freezing that also hamper the reproducibility of the experiments. First, there are many factors involved in the tendency of a system to supercool, including temperature, rate of cooling, volume, type of container, particles in the liquid, etc. Even when all these factors are controlled, different degrees of supercooling occur in repeated experiments (Heneghan and others 2002) due to the stochastic nature of the heterogeneous nucleation of supercooled water. Second, locating temperature sensors to register thermal evolution in the samples is also difficult, especially in biological products due to their inherent variability. Finding completely equal samples (size, shape, and composition) and locating temperature sensors at exactly the thermal center is sometimes impossible. Third, when comparing magnetic and conventional freezing, special care must be taken to keep all the freezing parameters (freezing temperature, air velocity, air convection, sample location in the freezer, etc.) fixed except the magnetic field. Ideally, the experiments should be performed in the same freezer with and without MF application. Even in this case, a common problem when oscillating MFs are applied is the heat produced in the coils that hamper comparisons between different MF conditions (no MF and MF of different intensities). Finally, when using commercial magnetic freezers, it is important to know exactly the characteristics of the magnetic fields applied (static, oscillating, or both; intensity and frequency values; combination with electric fields, electromagnetic waves, etc.) to obtain reproducible results. This is not always easy because manufacturers usually do not provide these technical data. For example, ABI equipment frequently includes permanent magnets and coils to combine static and oscillating MFs. The user can program different ‘CAS conditions’ (0-100% CAS) at the control panel, but the MF intensities and frequencies associated to these ‘CAS conditions’ are not specified. Measurements with devices not usually employed in freezing research (teslameters and oscilloscopes, for example) are needed to correctly describe commercial equipment and, for this reason, many authors do not report these data (Kim and others 2013a, b; Ku and others 2014; Choi and others 2015). Therefore, the experimental conditions of assays published in the literature must be analyzed carefully and the results should be treated with caution before drawing conclusions. All these problems make it difficult to find evidence of the effects of MFs on supercooling, freezing kinetics, ice crystals, quality and/or viability of the frozen products.

5.1. Freezing of water and aqueous solutions

There exist several papers in the literature that describe freezing experiments of water and aqueous solutions exposed to both static and oscillating MFs (Table 2).

If static MFs are strong enough, it is even possible to solidify levitating water under containerless condition. Thus, Tagami and others (1999) froze levitating water globules, as large as 6 mm in diameter, in a hybrid magnet able to generate up to 23 T in a 52 mm bore. These authors observed that water could be supercooled down to –10 °C before ice crystallization occurred. Taking into account the sample size and the containerless condition, this degree of supercooling is not unexpected. Unfortunately, as levitation under containerless condition cannot be achieved with no MF application, the only effect of a strong static MFs cannot be evaluated in levitating water globules.

The effect of weaker static MFs on water supercooling is not clear. Thus, Zhou and others (2012) froze tap water in several static MFs, with strengths up to 5.95 mT, and observed that the degree of supercooling increased with MF intensity. They found that freezing water in a 5.95 mT static MF increased supercooling by 1.2 °C compared with no MF application. By contrast, Aleksandrov and others (2000) froze drops of distilled water in more intense MFs (71-505 mT) and noted that supercooling decreased with increasing MF strength. They reported that supercooling was negligible at 505 mT and, accordingly, the freezing plateau time was the longest. In this sense, Mok and others (2015) also observed an effect of static MFs on the length of the freezing plateau. These authors froze 0.9% NaCl saline solutions at the midpoint between two neodymium magnets, either in attractive (480 mT) or repulsive (50 mT) position, and compared the phase transition times with those corresponding to no MF application (control). Compared to the control, the phase transition time in the repulsive MF was reduced by 32%, while it increased by 17% in the attractive MF. The authors suggested that the unidirectional and outward field forces produced when the magnets were located in attractive and repulsive positions, respectively, affect freezing kinetics in a different way, but the mechanisms implied are not clear.

The effect of oscillating MFs on supercooling of water and saline solutions has been also studied by several authors. Semikhina and Kiselev (1988) submitted bidistilled water to weak oscillating MFs (up to 0.88 mT and at frequencies between 10^{-2} and 200 Hz) for 5 h and observed an increase in supercooling due to MF exposition. These authors found that ΔT depended on both the MF intensity and frequency. Moreover, for a given MF intensity, they noted that there is an optimal MF frequency that produces maximal supercooling. Experiments by Mihara and others (2012) and Niino and others (2012) seem to confirm the effect of MF frequency on supercooling. These authors compared the supercooling reached in physiological saline solutions frozen without MF (control) and with a 0.12 ± 0.02 mT MF at different frequencies from 50 Hz up to 200 kHz. They observed an increase of supercooling in the samples frozen with MFs at frequencies ≥ 200 Hz and, for a 0.12 mT MF, they reported that maximal supercooling (close to 20 °C) was reached for a MF frequency of 2 kHz. It is interesting to note that this frequency is rather close to the corresponding Larmor frequency (~5 kHz). By contrast, at 50 Hz, no significant differences were observed compared with the control. In this sense, several authors have proved that, for weak magnetic fields, 50-60 Hz frequencies and lower have no effect on supercooling. Thus, Watanabe and others (2011) demonstrated that

0.5-10 mT MFs at 50 Hz have no apparent influence on supercooling of pure water and 1- molal NaCl solutions. Accordingly, Naito and others (2012) did not find any effect of a 0.5 mT MF at 30 Hz on supercooling of both distilled and saline water.

Although one of the most claimed advantages of magnetic freezing is the small size of the ice crystals formed, only a few research papers study the effect of MFs on ice crystals. Rohatgi and others (1974) froze 2.8-14.9% NaCl solutions in several static (100 mT-5 T) and oscillating (600 mT, 60 Hz) MFs and compared the shape and spacing of the ice dendrites with those obtained with no MF application. They studied two different freezing systems: one that avoids thermal gradients in the sample (small drops of NaCl solution in an organic liquid at –20 °C) and other that allows a unidirectional heat flow (the sample is poured into a tygon tube mounted on a cold copper plate at –70 °C). Rohatgi and others (1974) observed that MFs, either static or oscillating, promote side branching of the dendrites and increase their spacing in the droplets system, but no effect was detected in the unidirectional freezing system. It is important to note that, in the droplets, nucleation and crystal growth are free in the whole volume of the sample, whereas in the unidirectional freezing system, nucleation only occurs in the vicinity of the cold copper plate. Therefore, the authors concluded that MFs influence ice nucleation in NaCl solutions, but do not have an appreciable effect on the growth phenomenon. Iwasaka and others (2011) froze aqueous solutions under pulsed train MFs of up to 325 T/s at 6.5 mT and observed the ice crystal formation at real time with an optical microscope. When freezing DMEM cell culture medium, they reported that the exposition to pulsed MFs enhanced the movement of ice crystals and floating particles probably due to the induced electric field. According to the authors, stirring of the small ice crystals promotes their assembling and, as a result, samples exposed to pulsed MFs had broad areas with uniform ice crystals, whereas the non-exposed samples showed grid patterns. Real time measurements of light transmission during freezing also showed clear differences in 0.1 M NaCl solutions exposed or not to pulsed MFs. These differences could be related with differences in the nucleation rate and the size and shape of the ice crystals formed. In this sense, Mok and others (2015) observed that the ice crystals formed in NaCl solutions frozen under static MFs had more irregular shapes than those produced without MF. Moreover, they detected different patterns depending on the force directions in attractive or repulsive MFs.

5.2. Freezing of food products

Experiments of static magnetic freezing on food are very scarce (Table 3). To the best of our knowledge, only Lou and others (2013) froze whole carps in an air-blast freezer while applying static MFs with intensities up to 1.08 mT. The temperature-time curves at the center of the samples did not show any MF effect on the precooling rate. Moreover, no supercooling previous to nucleation was observed with or without MF application. However, the authors reported that MFs substantially reduced the freezing plateau time, while increasing the tempering stage and the total freezing time. Unfortunately, these freezing experiments were performed only in duplicate, the weight of the samples was rather variable (600-800 g), and no statistical analysis of the data was performed to find significant differences that support the conclusions inferred by the authors.

Regarding oscillating MFs, several patents include experimental data that show advantageous effects of MFs on freezing kinetics, ice crystals and/or quality attributes of frozen foods. Thus, in US7237400 B2 patent, Owada (2007) claimed that the application of an oscillating MF (0.5-0.7 mT, 50 Hz), combined or not with a 1 mT static MF, reduces the time for lowering the core temperature of chicken and tuna samples from 0 °C to –20 °C by 20-50%. According to the inventor, cells in the thawed samples were hardly destroyed and color, flavor, and taste were similar to those of the original raw food. Similar results were described in other patents. Thus, Sato and Fujita (2008) froze packed Chinese noodles, spinaches, packed pasta, lumps of pork, and tofu blocks in a freezer equipped with a MF generating device (200-300 mT, 60-100 Hz) and a cold atmosphere with low water vapor content. After thawing, they reported that the quality was satisfactorily maintained. By contrast, remarkable quality losses were observed when these products were frozen in the same freezer with no MF generating and dehumidifying devices at similar cooling rates. Lamentably, it is not clear from these results whether the observed effects are due to the MF, the dehumidifying device, or both. Ino and others (2005) froze sweet potatoes and observed that the mean gap area left by the ice crystals after thawing was considerably smaller when the samples were frozen in oscillating MFs (≤ 100 mT, ≤ 10 MHz) compared with conventional quick and slow freezing. Also, color, taste, aroma, smoothness, and hardness (mouthfeel) were better preserved. Unfortunately, no information about the freezers temperature or the cooling rates achieved during magnetic, slow, and quick freezing was provided and, therefore, it is not possible to discern whether the improvements observed are produced by the magnetic field or by other experimental conditions.

All these results that show significant benefits on freezing kinetics and/or the quality of magnetically frozen products contrast sharply with the experimental results reported in peer-reviewed research papers that describe no or very slight improvements. Thus, as observed for water and aqueous solutions, some papers in the literature clearly prove that weak MFs, at frequencies of 50-60 Hz or lower, have no effect on supercooling, freezing kinetics and/or quality of food products (Table 3). In this sense, Suzuki and others (2009) and Watanabe and others (2011) did not find any difference in the degree of supercooling and the precooling, phase transition, tempering, and total freezing times when freezing radish, tuna, sweet potato, yellow tail, and agar gel in a 0.5 mT MF at 50 Hz compared with no MF application. Moreover, similar ice crystals (size and shape), sensory evaluation, drip losses, color, texture, and microstructure were observed in all the products. The same occurs when weak static and oscillating MFs are combined. Thus, James and others (2015c) did not detect any MF effect on supercooling when freezing garlic bulbs in an ABI freezer (0.1-0.4 mT MFs at frequencies of 50 Hz or lower). James and others (2015a) also carried out several studies on magnetic freezing for commercial customers at the Food Refrigeration and Process Engineering Research Center (Grimsby, United Kingdom). The trials were all performed in ABI freezers and included fruit, vegetables, meat, and fish products. The results are not in the public domain, but the authors declared that they had not found clear and repeatable effects of CAS freezing in any product. Yamamoto and others (2005) observed longer freezing plateaus when freezing chicken breasts in an ABI freezer (1.5-2 mT at 20, 30, and 40 Hz) compared with no MF application. However, the authors remarked that these longer freezing times are reasonable because the electric power of conventional and electromagnetic freezers is the same and magnetic freezing requires part of this power for generating the magnetic field inside the freezer. After one week of storage at –30 °C, no differences were detected in drip and cooking losses and fracture properties (rupture stress and strain) of the chicken breasts. Surprisingly, after six months of storage, samples frozen in the electromagnetic freezer were significantly softer than those conventionally frozen. In these samples, microscopic observations showed large spaces within the muscle fibers, whereas these spaces in the magnetically frozen samples were small and scattered throughout the muscle fibers. Kim and others (2013a, b), Ku and others (2014), and Choi and others (2015) reported reduced total freezing times and improved quality attributes in beef, pork, and chicken samples frozen in a magnetic freezer (ABI Co., Ltd.) at –55 °C compared with samples frozen in an air blast freezer at –45 °C. However, as freezer temperatures were not the same, it is not possible to deduce whether the improvements detected were produced by the magnetic field or by the lower temperature applied.

Increasing frequency of oscillating MFs up to the Larmor frequency seems to have no effect on supercooling and freezing kinetics of food products in contrast with the results obtained by Mihara and others (2012) and Niino and others (2012) in physiological saline solutions. Thus, Watanabe and others (2011) combined a 20 mT static MF with a 0.12 mT oscillating MF at 1 MHz (NMR frequency) to freeze tuna and agar samples and did not detect any influence of the MFs applied.

According to several patents, combining magnetic and electric fields enhance MF benefits on freezing kinetics and food quality. Thus, Owada (2007) observed that adding an electric field (15 kV/m, 50 Hz-5 MHz) to a 5-7 mT oscillating MF (50 Hz), combined or not with a 1 mT static MF, reduced even more the time for lowering the core temperature of chicken and tuna samples from 0 °C to either –20 °C or –40 °C. In particular, it was 50% or more reduced compared with conventional freezing. According to the authors, after 3 months of frozen storage, cells were not destroyed and the products maintained the same color, flavor, and taste as the original raw food. Owada and Kurita (2001) combined static (10 mT) and oscillating (0.5 mT, 50 Hz) magnetic fields with an electric field (600 kV/m) to freeze tuna, sardine, pork, juices, wines, oranges, and cakes. They compared the freezing curves at the core of the samples with those obtained in a conventional quick freezer and observed identical gradual drop in temperature between 10 °C and –2 °C, that is, during the precooling step. Therefore, MFs combined with electric fields did not improve heat transfer in the unfrozen product. Freezing curves did not reveal supercooling in any of the samples, with or without MF application, but the length of the freezing plateaus and the total freezing times were substantially shorter in the samples located in their invention. Regarding food quality, the authors observed substantial drip losses, discoloration, and offensive odors in the samples frozen in the conventional device after 4 months of storage at –50 °C. By contrast, the quality of the samples frozen in their invention did not decay and freshness was preserved at a high standard. Unfortunately, most of the quality attributes were subjectively assessed and no instrumental measurements were performed. Only bacterial counts were objectively evaluated and they showed a marked decrease in samples frozen in their invention. However, experimentation is not clear and some doubts arise about the products employed in these microbial analyses and the number of replicated experiments, among others. Lamentably, to the best of our knowledge, no peer-reviewed research papers are available in the literature concerning the combination of magnetic and electric fields to corroborate or contradict the experimental data presented in the patents by ABI Co., Ltd. (Owada and Kurita 2001; Owada 2007; Owada and Saito 2010).

5.3. Freezing of biological specimens: cells, tissues, organs, and organisms

In the last years, magnetic freezing has received much attention in the field of cryopreservation (Mochimaru and others 2008; Mihara and others 2009; Samanpachin and others 2009; Sankai and others 2010; Kaku and others 2010; Lee and others 2012a; Nakagawa and others 2012). Nowadays, small biological specimens, such as cells and tissue fragments, can be preserved with varying degrees of success by conventional freezing protocols. Thus, for example, in gynecology, freezing techniques are currently being used for sperms, ova, and fertilized eggs. While small specimens can be suitable for freezing, preservation of large tissues and organs is considerably harder due to the difficulty of penetration of cryoprotective agents and the generation of thermal gradients. Efficient cryopreservation of biological materials is still an unresolved matter and innovative freezing techniques able to avoid cell damage generate much interest. For this reason, and despite most patents have been developed for food freezing, many papers exist in the literature that assess the effectivity of magnetic freezing for cryopreservation.

Most of the studies on magnetic freezing have been performed with time varying MFs, but some information exists about the effect of static MFs on cryopreservation of biological specimens (Table 4). Thus, Lin and others (2013a, b) studied the effect of static MFs on the survival rate, morphology, and functionality of slowly frozen human erythrocytes. They reported that freezing coupled with a 200, 400, or 800 mT static MF significantly increased the survival rate of erythrocytes by 6, 10, and 20%, respectively. Moreover, static MFs did not affect the cell morphology and functionality after thawing. Lin and others (2013a, b) observed that static MFs reduced the membrane fluidity probably due to the alignment of the phospholipid bilayer. According to the authors, phospholipids in the cell membranes exhibit a highly diamagnetic anisotropic susceptibility and can be oriented by the torque force of a static MF. They suggested that the cryoprotective effect of static MFs is not due to MF effects on water molecules, but to enhanced biophysical stability of the cell membrane that reduces dehydration damage during freezing. Similar results and conclusions were found by Lin and others (2014) after freezing dental pulp stem cells in 400 mT and 800 mT static MFs.

As regards time varying MFs, many papers have been published that conclude that cryopreservation of biological specimens is substantially improved when oscillating MFs are applied during freezing (Mochimaru and others 2008; Mihara and others 2009; Samanpachin and others 2009; Kaku and others 2010; Lee and others 2012a; Naito and others 2012; Morono and others 2015). However, when analyzing these papers in depth, some doubts arise that question the validity of the conclusions drawn.

On the one hand, in most of these papers, the MFs employed are not precisely described. The authors are, usually, experts in medicine, surgery, or cryobiology who collaborate with ABI Co., Ltd. (Chiba, Japan) to employ CAS freezers for the experiments. Most of the times, the characteristics of the equipment (MF intensity and frequency, presence or not of permanent magnets, combination with electric fields or not, among others) are not relevant for their analysis and, therefore, this information is not reported in the papers. For example, Kyono and others (2008, 2010) reported that they froze whole ovaries of cynomolgus monkeys and rabbits *'in a magnetic field environment'* and Lee and others (2010) only mentioned that their experiments had been performed in an ABI programmable freezer supplied with a *'slight'* MF. Kawata and others (2010) reported the intensity of the electric current needed to generate the MFs in the CAS freezer, but not the MF intensity achieved. In other examples, the MF intensity is mentioned, but not the frequency (Mihara and others 2009; Samanpachin and others 2009; Morono and others 2015) and, even, sometimes the reported MF intensity is wrong. Thus, according to the authors, freezing experiments by Kaku and others (2010) were performed using MFs of 0.005, 0.01 and 0.15 mT, while Lee and others (2012a, b), Kamada and others

(2011), and Abedini and others (2011) employed MFs of 0.01 mT. It is important to note that, as observed by Wowk (2012), some of these MF intensities are lower than that of the Earth's natural MF (0.025-0.06 mT). After publication of Wowk's considerations, Kaku and others (2012) rectified the MF intensity reported in their previous studies (Kaku and others 2010; Abedini and others 2011; Kamada and others 2011) and stated that the MF intensity and frequency really employed were 0.1 mT and 60 Hz.

On the other hand, in most of the studies, magnetic and conventional freezing experiments are not performed at identical conditions (cooling rate, air velocity, target temperature) and, therefore, MF application cannot be studied as an independent factor. Only a few papers perform comparisons in the same freezer with and without MF application (Kaku and others 2010; Niino and others 2012; Naito and others 2012). Thus, Kaku and others (2010) froze human periodontal ligament (PDL) cells and teeth in a programmable ABI freezer at –0.5 °C/min with and without MF application (0.1 mT, 60 Hz). They reported that the proportion of thawed PDL cells, surviving after 48 h of culture, rose from 40% at zero MF to above 70% for 0.1 mT magnetic field. After storing the frozen teeth for one year at –150 °C, they thawed the samples, extracted PDL tissue from them and studied cell proliferation and microstructure. Cells from the teeth frozen with no MF application did not proliferate and the PDL tissue was severely destroyed. By contrast, in non-frozen and in magnetically frozen samples, proliferation of PDL cells started after 10 days and they became confluent after 40 days. Moreover, the histological examination of the magnetically frozen samples proved that the structure of the PDL tissue was well retained and transmission electron microscopy only showed slight damage. Niino and others (2012) froze 4-5 mm portions of swine ovaries and liver in a lab prototype with and without MF application (1.2 ± 0.2 mT, 2kHz). No effect of MF application was observed in ovaries, but tissue destruction was minimized in liver samples when MFs were applied. The authors considered that these divergences could be due to differences in the structure and composition of the tissues. Naito and others (2012) maintained *Drosophila* adult flies in a freezer, with and without MF application (0.5 mT/30 Hz), at temperatures between 0 °C and –10 °C for 1-96 h. At these conditions, it is not clear whether the flies were frozen or not, but the authors observed that the survival rate was significantly increased when MFs were applied. The question that remains unresolved is whether these results are produced by a magnetic effect on the flies or by a temperature increase due to the heat generated in the coils during MF application.

After studies by Kaku and others (2010), Niino and others (2012), and Naito and others (2012), most of the authors consider that the efficiency of MF application to improve the preservation of biological specimens is well proved and, unfortunately, they do not provide more evidence. Thus, most of the experiments are planned to compare magnetically frozen with non-frozen samples (Lee and others 2010; Kamada and others 2011; Abedini and others 2011) or to compare ABI freezing and conventional freezing protocols (Mihara and others 2009; Samanpachin and others 2009; Lee and others 2012a, b; Nakagawa and others 2012; Lin and others 2013c; Morono and others 2015).

In general, experiments show that magnetically frozen samples after thawing are quite similar to the unfrozen specimens. Thus, Kamada and others (2011) found no differences between the collagen type I gene expression of non-frozen PDL cells and that of cells frozen in a 0.1 mT MF, although the expression of alkaline phosphatase messenger RNA was slightly decreased after magnetic freezing. The efficiency of magnetic freezing was also proved in teeth cryopreservation. Thus, Kamada and others (2011) and Abedini and others (2011) realized successful transplantations of magnetically frozen rat incisors and human molars, respectively. The proper periodontal regeneration of these teeth was confirmed by both authors. Unfortunately, in all these experiments, comparisons were only made with non-frozen cells or teeth, but not with conventionally frozen samples.

Usually, the ABI freezing protocol produces better results than conventional procedures. Thus, experiments performed in human stem cells, both dental pulp (Lee and others 2010, 2012a) and embryonic cells (Lin and others 2013c), show that magnetic freezing is a reliable and effective method for stem cells cryopreservation. Lee and others (2012a) proved that the viability, attachment efficiency, and proliferation rate of magnetically frozen dental pulp stem cells were significantly greater than that of conventionally frozen cells. Moreover, the thawed cells did not differ morphologically from unfrozen cells and maintained their stem cell-specific markers, differentiation ability (osteogenic and adipogenic), and DNA stability (Lee and others 2010, 2012a). Lin and others (2013c) confirmed that magnetically frozen embryonic stem cells presented better attachment efficiency than conventionally frozen ones. Moreover, cells could be subcultured while expressing pluripotent markers, differentiated into the three characteristic germ layers in vertebrates (ectoderm, mesoderm, and endoderm), and maintained a normal karyotype. However, it is important to note that the freezing protocols in magnetically and conventionally frozen stem cells were completely different. The magnetic freezing procedure was based on the instructions of the freezer manufacturer (Lee and others 2012a), that is, ABI Co., Ltd. (Chiba, Japan). In brief, the cells were placed in the programmable freezer (0.1 mT/60 Hz) and maintained at –5 °C for 15 min. Then, they were cooled at –0.5 °C/min down to –32 °C and, finally, they were stored at low temperature in a conventional freezer. By contrast, in the conventional protocol, cells were slowly frozen in an isopropanol-jacketed freezing container placed in a –80 °C freezer overnight and, then, they were stored at low temperature in a freezer just as magnetically frozen cells. It is well known that the freezing protocol (temperature, time, cooling rate) has a substantial effect on the effectiveness of cryopreservation (Baust and others 2009; Benson and others 2012). Therefore, this brings up the question of whether the results observed by Lee and others (2012a) and Lin and others (2013c) are only due to the application of MFs during freezing or they are also affected by the freezing protocol.

Similar doubts arise with other studies that compare magnetic and conventional freezing of tissues (Lee and others 2012b), organs (Samanpachin and others 2009; Lee and others 2012b; Nakagawa and others 2012), and even whole organisms, either unicellular (Morono and others 2015) or multicellular (Mihara and others 2009). Concerning tissues, Lee and others (2012b) reported that the explant viability and adherence of dental pulp tissue magnetically frozen according to the ABI protocol was significantly larger than that of conventionally frozen samples. Cell morphology was better preserved and the integrity and structure of the tissue was retained. About organs, Samanpachin and others (2009) observed lower damage in mouse testis frozen in a programmable ABI freezer (0.1-0.2 mT at –30 °C) compared with that of testis frozen in liquid nitrogen. Similar observations were made by Nakagawa and others (2012) in mouse brains and rat brains and pancreas. As regards whole organisms, Morono and others (2015) observed that, after 6 months of storage, microbial counts in conventionally frozen (–20 °C, –80 °C, or in liquid nitrogen) subseafloor sediments were significantly lower than those in magnetically frozen sediments (0.1-0.8 mT at –60 °C). The same results were found for

Escherichia coli after freezing and thawing. Therefore, they concluded that magnetic freezing could be highly useful for the preservation of environmental samples. Mihara and others (2009) froze whole rats, either at –30 °C in a programmable ABI freezer (0.1-0.2 mT) or at –80 °C in an ultracold freezer. After 24 h of storage at –80 °C, they thawed the rats and prepared histological sections of the brain, heart, lung, pancreas, small intestine, liver, kidney, and ovary. Just as Niino and others (2012), they observed that the extent of tissue breakdown was different between organs, but magnetic freezing reduced tissue breakdown, especially in the brain, pancreas, small intestine, and ovary.

6. KEY CONSIDERATIONS AND NEEDS FOR FUTURE RESEARCH

The experimental data compiled in this review have failed to back up the claims reported in patents on magnetic freezing with evidence. Some papers reveal positive results, but others show no effect of MF application. As stated before, many difficulties associated to both MFs and freezing hamper the reproducibility and replicability of the results. Moreover, although there exist a number of reports on the effects of magnetic freezing, it is important to note that many of them have not been peer-reviewed and, in consequence, the quality of these papers is sometimes questionable. Therefore, there exists an urgent need to perform high-quality scientific research to prove whether magnetic freezing is effective in enhancing supercooling and/or improving the quality of frozen products or it is only a commercial fraud. If some effect is confirmed, the following step would be to investigate on the mechanisms that produce such effect.

To perform this research with success, it is essential to carry out well-defined experiments that can be replicated and confirmed by different laboratories. The effects of magnetic fields on freezing should be first evaluated in the simplest matrix, that is, in pure water and, then, more complex samples (saline solutions, food models, real foods, and biological specimens) could be studied. Thus, the impact of added components and their potential interactions can be differentiated. When designing the experiments, special care must be taken to correctly characterize the sample and the freezing equipment and, also, to identify all the factors that can have an influence on the observed results. It is necessary to study both static and time varying

MFs and, in the latter case, the effect of induced electric fields should be also evaluated. The low strength of the MFs applied in ABI freezers (usually lower than 1 mT) casts doubt on the effects that these extremely weak MFs can have on freezing of water and biological products. Therefore, a wide range of MF intensities and frequencies should be tested to verify or discard MF effects on freezing. Moreover, the underlying mechanisms of Proton freezers, that is, the combination of static MFs with electromagnetic waves at the Larmor frequency, should be also investigated. Combinations with electric fields and other energy-generating devices must not be forgotten.

To compare magnetic and conventional freezing, magnetic field exposition should be isolated as an independent factor while maintaining all the other factors (target temperature, freezing rate, air convection, etc) fixed in the experiments. Moreover, the experiments should be carefully replicated to take into account the variability of response due to the sample and the process variations. Only the adequate experimental design and correct sampling will allow a rigorous statistical analysis of the data to draw valid conclusions.

If real effects of magnetic fields on either supercooling, freezing kinetics, ice crystals, quality, or viability of the frozen products were confirmed, the mechanisms involved in the improvements observed should be investigated. To do so, it is necessary to evaluate magnetic effects not only in water but also in other molecules that could be affected by magnetic fields. Thus, as mentioned before, Lin and others (2013a, b, 2014) have already pointed out the role that phospholipids could play in the cryoprotective effect of static MFs. Moreover, Kobayashi and Kirschvink (2014) suggested that the presence of ferromagnetic materials in biological tissues, principally biologically-precipitated magnetite (Fe_2O_4), could be relevant to inhibit ice nucleation. Other mechanisms such as electrostatic effects on heat transport, for example, should not be neglected (Kobayashi and Kirschvink 2014).

Finally, experiments should not be limited to study the effects immediately after freezing but also after prolonged storage time. It is well known that recrystallization phenomena occur during frozen storage and they can produce detrimental effects on food quality and viability of biological specimens. Depending on the rate and extent, recrystallization can nullify all the benefits derived from rapid ice nucleation. To date, only Yamamoto and others (2005) and Choi and others (2015) have published some data about the effect of storage time on the quality of magnetically frozen foods. More information is needed to evaluate the stability of ice crystals after magnetic freezing.

7. CONCLUSIONS

Although it has more than ten years since magnetic freezers were introduced in the market, evidence of the efficacy of magnetic fields in improving the quality of frozen products has not yet been found. Data in the literature are frequently confusing and apparently contradictory and, therefore, much more research is needed to confirm the potential benefits of MFs on freezing. After examining the magnetic properties of water, the low strength of the MFs applied in ABI freezers (usually lower than 1 mT) casts doubt on the effects that these extremely weak MFs can have on water crystallization, but other mechanisms could be affected. Many doubts also arise concerning the working principles of Proton freezers. Although the scientific community has frequently questioned the science behind magnetic freezers, it sounds strange that manufacturers have not yet presented conclusive evidence to dissipate any doubt as far as their efficacy is concerned. In any case, investigations on magnetic freezing should cover not only MF strengths and frequencies currently used in commercial freezers but also much more wide ranges to have a complete view of the potential effects of magnetic fields on freezing of biological products. Only after finding positive results, mechanisms involved in such results should be investigated. Although patents on magnetic freezing claim that magnetic fields mainly affect water supercooling, the role of other molecules and different mechanisms should not be neglected.

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Author Contributions

L. Otero compiled information from the literature, analyzed the data, and wrote the manuscript, while A. C. Rodríguez, M. Pérez-Mateos, and P. D. Sanz contributed to the data gathering and revision of the manuscript. M. Pérez-Mateos also contributed to the paper edition.

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Table 1 Patents on magnetic freezing

B: Magnetic field strength; ω: Frequency; *pw*: Pulse width; *E*: Electric field strength; γ: gyromagnetic ratio for hydrogen (42.58 MHz/T)

INVENTORS		MAGNETIC FIELD			COMBINATIONS			FREEZING TEMPERATURE	PRODUCTS TO FREEZE
		STATIC *B*	OSCILLATING *B*; ω	PULSED *B*; ω; *pw*	ELECTRIC FIELDS *E*; ω	ELECTRO MAGNETIC WAVES ω	OTHERS		
Hirasawa and others (2000)	Patented	ω/γ	-	-	-	0.01-100 MHz	Air sanitazer	–40 °C	Food
Hirasawa and others (2001)	Patented	X	X	-	-	-	Air sanitazer	–20 °C	Food
Owada and Kurita (2001)	Patented	0.1 mT-2 T	0.1-10 mT; 50-60 Hz	-	100-1000 kV/m	-	Sound wave generator Ventilators Far-infrared-ray absorber Heat insulators	–30 to –100 °C	Food and food ingredients
	Tested	10 mT	0.5 mT; 50-60 Hz	-	600 kV/m	-		–50 °C	Tuna, sardines, pork, juices, wine, oranges, and cakes
Kino (2002)	Patented	X	X	-	-	γB	Not described	X	Food
Owada (2007)	Patented	0.1 mT-1 T	0.1-100 mT; 50-60 Hz	-	10-500 kV/m; 50 Hz-5 MHz (0.25-3 MHz)^r	-	Ionic air Ventilators Honeycomb Far-infrared-ray absorber Heat insulators	–20 to –40 °C	Foodstuffs, food products, organisms, and other materials
	Tested	1 mT	0.5-0.7 mT; 50 Hz	-	15 kV/m; 250 kHz, 3 MHz, 50 Hz-5MHz	-		–20 °C, –40 °C	Chicken and tuna
Toyoshima (2005)	Patented	X	-	-	-	X	Ventilators Heat insulators	–40 °C	Food
Ino and others (2005)	Patented	-	⩽100 mT; ⩽10 MHz	-	-	-	Ventilators Heat insulators	X	Food
	Tested	-	X	-	-	-		–35 °C	Sweet potatoes
Miura and others (2005)	Patented	-	-	0.1-10 T; 0.1 Hz-1kHz; 10-100 µs	-	-	Not described	–10 to –60 °C	Starch-containing food
	Tested	-	-	5 T 1 Hz 55 µs	-	-		–20 °C	Potato starch gels
Sato and Fujita (2008)	Patented	-	10 mT-1.2 T (30-700 mT)^r; 20 Hz- 25 kHz (40 Hz-1.2 kHz)^r	-	-		Ionic air Ultrasonic waves, microwaves, far infrared rays, ultraviolet light, α-rays Air dehumidifier Air pressure regulator Ventilators	–20 to –60 °C	Food
	Patented	10 mT-1.2 T (30-700 mT)^r	10 mT-1.2 T (30-700 mT)^r; 20 Hz- 25 kHz (40 Hz-1.2 kHz)^r	-	-	-		–20 to –60 °C	Food
									Chinese noodles,

	Tested	800 mT	300 mT; 100 Hz	-	-	-		–40 °C, –50 °C	spinaches, packed pasta, pork lumps, and tofu blocks
Kim and others (2009)	Patented	-	X	-	-	-	Not described	X	Ice cubes
Owada and Saito (2010)	Patented	0.1 mT-2 T (10 mT)^{r}	0.1-10 mT (0.5 mT)^{r}; 50-60 Hz	-	100-1000 kV/m (2-60 kV/m)^{r}	-	Air pressure regulator Air sanitizer Oxygen absorber Sound wave generator Ventilators Far-infrared-ray absorber Heat insulators	–30 to –100 °C	Food products, food ingredients, medical products, medicines, living tissues, and living cells
	Tested	X	X	-	X	-		X	Mackerel and lobster
Fujisaki and Amano (2012)	Patented	1-200 mT (10-15 mT)^{r}	-	-	-	0.3-2 MHz (0.6-1 MHz)^{r}	Ventilators Far-infrared-ray absorber Heat insulators	X	Food products, cooking ingredients, living bodies, and biological samples
Mihara and others (2012)	Patented	-	0.01-0.4 mT (0.2 mT)^{r}; 200 Hz-200 kHz (2 kHz)^{r}	-	-	-	Not described	–2 to –40 °C (–20 to –40 °C)^{r}	Foods, organs, and the like
	Tested	-	0.12 mT; 0 Hz-200 kHz	-	-	-		X	Physiological saline solution
			0.1-0.2 mT; 2 kHz	-	-	-		–30 °C	Rats
			0.8 mT; 2 kHz	-	-	-		–40 °C	Alkaline phosphatase, green fluorescent protein

B: Magnetic field strength; ω: Frequency; *pw*: Pulse width; *E*: Electric field strength; γ: gyromagnetic ratio for hydrogen (42.58 MHz/T)

-: Not employed; X: Not reported value; ()^{r}: Recommended conditions.

Table 2 Experimental data about the effects of magnetic fields on freezing of water and aqueous solutions

FIELD APPLIED	INTENSITY (mT)	FREQUENCY (Hz)	DEVICE	SAMPLE	EFFECTS OF THE FIELDS APPLIED			AUTHOR/ DOCUMENT
					ΔT	FREEZING KINETICS	ICE CRYSTAL	
STATIC MAGNETIC FIELD	10^2-$5\cdot10^3$		Lab prototype	2.8-14.9% NaCl	-	-	Effects on ice nucleation, but not on crystal growth	Rohatgi and others (1974) Research paper
	Up to $2.3\cdot10^4$		Lab prototype	Water globules	-	-	Levitating	Tagami and others (1999) Research paper
	$(0.71$-$5.05)\cdot10^2$		Lab prototype	Drops of distilled water	Lower	Shorter precooling time Longer freezing plateau No effect on total freezing time	-	Aleksandrov and others (2000) Research paper
	Up to 5.95		Lab prototype	Tap water	Larger	Longer precooling time Longer total freezing time	-	Zhou and others (2012) Research paper
	$4.8\cdot10^2$ Unidirectional field forces		Lab prototype	0.9% NaCl	-	Longer freezing plateau	Irregular shapes dependent on the direction of the field forces	Mok and others (2015) Research paper
	$0.5\cdot10^2$ Outward field forces		Lab prototype	0.9% NaCl	-	Shorter freezing plateau	Irregular shapes dependent on the direction of the field forces	Mok and others (2015) Research paper
OSCILLATING MAGNETIC FIELD	Up to $6\cdot10^2$	60	Lab prototype	2.8-14.9% NaCl	-	-	Effects on ice nucleation, but not on crystal growth	Rohatgi and others (1974) Research paper
	Up to 0.88	10^{-2}-$2\cdot10^2$	Lab prototype	Bidistilled water	Larger	-	-	Semikhina and Kiselev (1988) Research paper
	0.5-10	50	Lab	Pure water and	No effect	-	-	Watanabe and others (2011)

			prototype	1m NaCl				Research paper
	0.12 ± 0.02	$50\text{-}2\cdot10^5$	Lab prototype	Physiological saline solution	Larger at frequencies of 200 Hz and higher No effect at 50 Hz	-	-	Mihara and others (2012) Patent Niino and others (2012) Research paper
	0.5	30	Lab prototype	Distilled and saline water	No effect	No effect	-	Naito and others (2012) Research paper
PULSED MAGNETIC FIELD	6.5 (325 T/s) 3 (150 T/s)	10 18	ABI Co., Ltd	DMEM culture cell medium 0.1 M NaCl	-	-	Enhanced movement of ice crystals: More uniform crystals Effects on light transmission	Iwasaka and others (2011) Research paper

-: Not studied

Table 3 Experimental data about the effects of magnetic fields on freezing of food products

FIELDS APPLIED	INTENSITY (mT)	FREQUENCY (Hz)	DEVICE	SAMPLE	EFFECTS OF THE FIELDS APPLIED				AUTHOR / DOCUMENT
					ΔT	FREEZING KINETICS	ICE CRYSTALS	QUALITY	
STATIC MAGNETIC FIELD	0.36 0.72 1.08		Lab prototype	Carps	No effect	No effect on precooling time Shorter freezing plateau Shorter tempering and total freezing times	-	-	Lou and others (2013) Research paper
OSCILLATING MAGNETIC FIELD	$\leqslant 100$	$\leqslant 10^7$	Patented by Kansho Riyo Gijutsu Kenkyusho:KK	Sweet potatoes	-	-	Smaller	Color, taste, aroma, smoothness, and hardness similar to those of raw potato	Ino and others (2005) Patent
	0.5-0.7	50	Patented by ABI Co., Ltd.	Chicken and tuna	-	Time for lowering the core temperature from 0 °C to –20 °C was 20-50% reduced	-	Cells were hardly destroyed. Color, flavor, and taste similar to those of the original raw food	Owada (2007) Patent
	200-300	60-100	Patented by Shounan Jitsugyou Co.	Packed Chinese noodles, spinaches, packed pasta, lumps of pork, and tofu blocks	-	-	-	Quality satisfactory maintained after thawing	Sato and Fujita (2008) Patent
	0.5	50	Lab prototype	Radish, tuna, sweet potato, yellow tail, and agar gel	No effect	No effect	No effect	No effect	Suzuki and others (2009); Watanabe and others (2011) Research paper
PULSED MAGNETIC FIELD (55 µs)	500	1	Lab prototype	Potato starch gels	-	-	-	Larger exudates Lower rupture stress	Miura and others (2005) Patent
STATIC MAGNETIC FIELD								No effect on drip	

+ OSCILLATING MAGNETIC FIELD	1.5-2	20, 30, and 40	Commercial freezer designed by ABI Co., Ltd.	Chicken breasts	-	Longer freezing plateau	-	and cooking losses Softer texture after 6 months of frozen storage	Yamamoto and others (2005) Research paper
	1 ± 0.6	50	Patented by ABI Co., Ltd.	Chicken and tuna	-	Time for lowering the core temperature from 0 °C to –20 °C was 20% to 50% reduced	-	Cells were hardly destroyed. Color, flavor, and taste similar to those of the original raw food	Owada (2007) Patent
	20 ± 0.12	$1 \cdot 10^6$	Lab prototype	Tuna and agar gel	No effect	No effect	No effect	No effect	Suzuki and others (2009); Watanabe and others (2011) Research paper
	n.r.	n.r.	Commercial freezer designed by ABI Co., Ltd.	Beef (loin and round)	-	-	-	Lower drip losses Larger water holding capacity. No effect on sensory evaluation	Kim and others (2013a) Research paper
	n.r.	n.r.	Commercial freezer designed by ABI Co., Ltd.	Beef (loin and round), pork (belly and ham), and chicken (breast and leg)	-	Shorter total freezing time	-	Effects on drip and cooking losses, water holding capacity, and composition depend on the product	Kim and others (2013b) Research paper
	n.r.	n.r.	Commercial freezer designed by ABI Co., Ltd.	Pork (belly and ham)	-	-	-	Effects on drip and cooking losses, water holding capacity, and moisture content depend on the pork cut. No effect on sensory evaluation	Ku and others (2014) Research paper
	0.098 0.155 0.418	≤ 50	Commercial freezer designed by ABI Co., Ltd.	Garlic bulbs	No effect	-	-	-	James and others (2015c) Research paper

	n.r.	n.r.	Commercial freezer designed by ABI Co., Ltd.	Beef (loin and round)	-	-	Smaller	Larger water holding capacity Better overall acceptability after sensory evaluation. Better flavor and taste	Choi and others (2015) Research paper
OSCILLATING MAGNETIC FIELD + ELECTRIC FIELD	0.5 *EF:15 kV/m	50 *EF: 50 Hz-5 MHz	Patented by ABI Co., Ltd.	Chicken and tuna	-	Time for lowering the core temperature from 0 °C to –20 °C was 50% or more reduced	-	Cells were not ruptured Color, flavor, and taste are the same as the original raw food	Owada (2007) Patent
STATIC MF + OSCILLATING MF + ELECTRIC FIELD	10 ± 0.5 *EF:600 kV/m	50 *EF: n.r.	Patented by ABI Co., Ltd.	Tuna, sardine, pork, juices, wines, oranges, cakes	-	No effect on precooling time Shorter freezing plateau Shorter total freezing time	-	Lower drip losses, color and odor changes, and microbial counts No phase separations	Owada and Kurita (2001) Patent
	1 ± 0.5 *EF:15 kV/m	50 *EF: 50 Hz-5 MHz	Patented by ABI Co., Ltd.	Chicken and tuna	-	Time for lowering the core temperature from 0 °C to –20 °C was 50% or more reduced	-	Cells were not ruptured Color, flavor, and taste are the same as the original raw food	Owada (2007) Patent
	1 ± 0.5 *EF: 100-1000 kV/m	50 *EF: n. r.	Patented by ABI Co., Ltd.	Mackerel and lobster	-	-	-	Improved microstructure after thawing	Owada and Saito (2010) Patent

-: Not studied; n. r.: Not reported; * EF: Electric field

Table 4 Experimental data about the effects of magnetic fields on cryopreservation of cells, tissues, organs, and organisms

FIELDS APPLIED	INTENSITY (mT)	FREQUENCY (Hz)	DEVICE	SAMPLE	CONTROL FOR COMPARISONS	EFFECTS OF THE FIELDS APPLIED	AUTHORS
STATIC MAGNETIC FIELD	200 400		Lab prototype	Human erythrocytes	Samples frozen in the same device with no MF application	Higher survival rate No effects on cell morphology or function Reduced membrane fluidity at 400 mT	Lin and others (2013a)
	400 800		Lab prototype	Human erythrocytes	Samples frozen in the same device with no MF application	Higher survival rate No effects on cell morphology and metabolite levels Reduced membrane fluidity	Lin and others (2013b)
	400 800		Lab prototype	Human dental pulp stem cells	Samples frozen in the same device with no MF application	Higher survival rate Higher survival rate	Lin and others (2014)
	300	n.r.	n.r.	Entire porcine ovaries	Samples frozen in the same device with no MF application	More primordial follicles remained intact after thawing Defects in the interstitial tissues were less evident	Mochimaru and others (2008)
OSCILLATING MAGNETIC FIELD	n.r.	n.r.	ABI Co., Ltd.	Entire ovaries of cynomolgus monkeys	No controls All samples are exposed to MFs during freezing	After frozen-thawed ovarian autotransplantation, four of the five monkeys recovered their ovarian functions with hormone production and the menstrual cycle	Kyono and others 2008
	0.1-0.2	n.r.	ABI Co., Ltd.	One entire rat	One rat frozen in an ultracold freezer at –80 °C	Magnetic freezing reduced tissue breakdown, especially in the brain, pancreas, small intestine, and ovary	Mihara and others (2009)
	0.1-0.2	n.r.	ABI Co., Ltd.	Mouse testis	Mouse testis frozen in an ultracold freezer at –80 °C	Magnetic freezing reduced tissue destruction	Samanpachin and others (2009)
	n.r.	n.r.	ABI Co., Ltd.	Entire ovaries of cynomolgus monkeys and rabbits	No controls All samples are exposed to MFs	Cell structure of frozen ovaries well preserved Viable oocytes immediately after thawing	Kyono and others (2010)
	n.r. 0-0.15*	n.r. 60*	ABI Co., Ltd.	Human PDL cells	No controls All samples are exposed to MFs	Survival rate: Up to 96% depending on the MF strength and freezing temperature	Kawata and others (2010)

n.r. 0-0.15*	n.r. 60*	ABI Co., Ltd.	Human PDL cells	Cells frozen in the same freezer with no MF application	Higher survival rate immediately after thawing Higher cell viability after 48 h	Kaku and others (2010)
n.r. 0.1*	n.r. 60*	ABI Co., Ltd	Human teeth	Teeth frozen in the same freezer with no MF application Fresh teeth Dried teeth	PDL cells from magnetically frozen teeth could proliferate as much as those from fresh teeth. In conventionally frozen tooth, cells did not appear	Kaku and others (2010)
n.r.	n.r.	ABI Co., Ltd	Human premolars	Non-frozen premolars	After culture for 5 generations, no significant difference in cell viability between DPSCs isolated from magnetically frozen teeth and those from fresh teeth No differences in morphology, expression of stem cell markers, or osteogenic and adipogenic differentiations	Lee and others (2010)
0.01 0.1*	n.r 60*	ABI Co., Ltd	Human PDL cells	Non-cryopreserved cells	No difference between the expression of collagen type I messenger RNA in magnetically and non-frozen cells The expression of alkaline phosphatase messenger RNA was slightly decreased after magnetic freezing	Kamada and others (2011)
0.01 0.1*	n.r 60*	ABI Co., Ltd	Rat incisors	Freshly extracted incisors Dried incisors	No progressive root resorption in the teeth that were replanted immediately (fresh incisors) or cryopreserved Widespread root resorption and ankylosis in the dried teeth	Kamada and others (2011)
0.01 0.1*	n.r 60*	ABI Co., Ltd	Human teeth	Freshly extracted teeth	Magnetic freezing did not affect the growth rate and characteristics of PDL cells Proper PDL regeneration and appropriate apexogenesis after transplanting magnetically frozen teeth	Abedini and others (2011)
0.01	n.r.	ABI Co., Ltd	Rat teeth Dental pulp tissue	Rat teeth and dental pulp tissue frozen in an isopropanol-jacketed freezing container at –80 °C	Lower concentration of cryoprotectant and shorter pre-equilibration time are required for magnetic freezing Fewer cracks in magnetically frozen samples	Lee and others (2012b)

0.01	n.r.	ABI Co., Ltd	Human DPSCs	Non-frozen cells Cells frozen in an isopropanol-jacketed freezing container at –80 °C	Lower concentration of cryoprotectant is required for magnetic freezing Larger cell viability, proliferation rate, expression of some stem cell markers, and induced osteogenic differentiation and more viable adherent cells after magnetic freezing	Lee and others (2012a)
0.1-0.2	Broad frequency component	ABI Co., Ltd	3-5 mm sections from mouse brain and rat brain and pancreas	Samples frozen in an ultracold freezer at –80 °C	Lower damage in magnetically frozen samples	Nakagawa and others (2012)
1.2 ± 0.2	2000	Lab prototype	Portions of swine ovaries and liver	Samples frozen in the same device with no MF application	No effect of magnetic fields on ovarian tissue destruction Magnetic fields improved liver cryopreservation	Niino and others (2012)
0.5	30	Lab prototype	Drosophila flies	Flies maintained in the same device with no magnetic field application	Higher survival when magnetic fields were applied	Naito and others (2012)
0.1	60	ABI Co., Ltd	Human embryonic stem cells	Cells frozen in an isopropanol-jacketed freezing container at –80 °C	Higher attachment efficiency after magnetic freezing Magnetically frozen cells can be subcultured while expressing pluripotent markers, differentiate into three germ layers, and maintain a normal karyotype	Lin and others (2013c)
0.1-0.8	n.r.	ABI Co., Ltd	Subseafloor sediments *Escherichia coli* cells	Samples conventionally preserved at 4, –20, –80, and –196 °C	Larger microbial counts in magnetically frozen samples	Morono and others (2015)

n.r.: Not reported; *: Data provided in Kaku and others (2012); PDL: Periodontal ligament; DPSCs: Dental pulp stem cells