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Food Preservation Technology at Subzero Temperatures: A Review

Tim Shafel[1], Seung Hyun Lee[2], Soojin Jun[1]*

[1]*Department of Human Nutrition, Food and Animal Sciences, University of Hawaii, Honolulu, HI 96822, USA*
[2]*Department of Biosystems Machinery Engineering, Chungnam National University, 220 Gung-Dong, Yuseong-Gu, Daejeon 305-764, South Korea*

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Abstract

Purpose: Cold storage is the most popular method used to preserve highly perishable foods such as beef and fish. However, at refrigeration temperatures, the shelf life of these foods is limited, and spoilage leads to massive food waste. Moreover, freezing significantly affects the food's properties. Ice crystallization and growth during freezing can cause irreversible textural damage to foods through volumetric expansion, moisture migration induced by osmotic pressure gradients, and concentration of solutes,which can lead to protein denaturation. **Methods:** Although freezing can preserve perishable foods for months, these disruptive changes decrease the consumer's perception of the food's quality. Therefore, the development and testing of new and improved cold storage technologies is a worthwhile pursuit. **Results:** The process of maintaining a food product in an unfrozen state below its equilibrium freezing temperature is known as supercooling. As supercooling has been shown to offer a considerable improvement over refrigeration for extending a perishable product's shelf life, implementation of supercooling in households and commercial refrigeration units would help diminish food waste. **Conclusions:** A commercially viable supercooling unit for all perishable food items is currently being developed and fabricated. Buildup of this technology will provide a meaningful improvement in the cold storage of perishable foods, and will have a significant impact on the refrigeration market as a whole.

Keywords: Electrochemical impedance spectroscopy, Food quality analysis, Ice crystal nucleation, Superchilling, Supercooling

Introduction

As the global population continues to increase, food production and quality maintenance are becoming increasingly crucial issues for many animal scientists, agriculturists, and food scientists. With all the resources that are put into the production and quality control of food, it is important to limit the amount of food that is wasted. It has been estimated that American households discard 211 kg of food waste per year, and most of this waste is perishable foods, such as meats, which are often lost due to poor cold storage (Parfitt et al., 2010). Another study claimed that 10% of the food in American households is wasted, which is approximately $390 per capita or a total of $165.6 billion per year (Buzby and Hyman, 2012). In the USA as well as other developed countries, minimizing food waste is a primary goal and of utmost importance.

The use of cold storage for perishable foods, particularly muscle foods such as beef and fish, is widely accepted as the best way to preserve food freshness and extend shelf life. Cold storage includes both refrigeration and freezing, at recommended ambient temperatures of 4 and -18°C, respectively. Cold storage is popular because the rate of spoilage through microbial, chemical, and enzymatic mechanisms is highly temperature dependent; thus, lowering the temperature significantly slows these reactions. In fact, many researchers have employed the Arrhenius equation (1) as a model for the effect of temperature on the rate of the chemical and biological processes that lead to negative quality changes in foods (Peleg et al., 2012), which is given by:

***Corresponding author:** Soojin Jun
Tel: +1-808-956-8283; **Fax:** +1-808-956-4026
E-mail: soojin@hawaii.edu

$$k = k_0 e^{\frac{-Ea}{RT}} \quad (1)$$

where k is the reaction rate constant (time^{-1}), $k_o$is a reference rate constant at some set condition (time^{-1}), E_a is the reaction activation energy (J/mol), R is the ideal gas constant (J/mol-K), and T is the temperature (K).

Taking the natural log of both sides of equation 1 yields:

$$\ln(k) = \ln(k_0) - \left({}^{E_a}/_{R}\right)\left({}^{1}/_{T}\right) \quad (2)$$

Making it clear that the rate of the reaction, which is spoilage in the case of food, is inversely proportional to the food storage temperature, where E_a describes the extent of the temperature dependence.

At freezing temperatures, the rates of reactions and microbial growth are slowed enough to safely store foods for extended time. According to the U.S. Food and Drug Administration (FDA), fresh beef can be stored in a properly functioning freezer for 6–12 months and fresh fish can be stored for 2–6 months. In contrast, fresh beef and fish can be stored at refrigeration temperatures for only 3–5 days and 1–2 days, respectively. Freezing foods is unequivocally advantageous over refrigeration for increasing shelf life; however, freezing irreversibly affects the food's properties. For instance, ice-volume expansion may rupture the cells within the food, and a growing ice fraction can concentrate solutes in the unfrozen phase leading to osmotic differences between the intracellular and extracellular fluid. These osmotic gradients cause moisture migration, protein denaturation, solute crystallization, and cell lysis. Thus, holding foods at very low temperatures and minimizing the negative effects of ice has been of great interest to food scientists. Various techniques have been employed to reduce the size of and damage from ice crystals in foods, including ultrasonic waves (Delgado et al., 2008; Hozumi et al., 2002; Li and Sun, 2002), quick freezing using cryo-immersion or air blast (Agnelli and Mascheroni, 2001; Chevalier et al., 2000[a,b]; Khadatkar et al., 2004; Streeter and Spencer, 1973), shifts in pressure (Fernández, et al., 2008; LeBail et al., 2002; Otero et al., 2000 and 2012), and the addition of proteins with antifreeze or ice-nucleating properties (Davies and Sykes, 1997; Feeney and Yeh, 1998; Griffith and Ewart, 1995). Additionally, the impact of electric and magnetic fields on ice crystallization has been investigated (Aragones et al., 2011; Kitazawa et al., 2001; Orlowska et al., 2009; Petersen et al., 2006; Shibkov et al., 2002; Woo and Mujumdar, 2010). Recently, a combination of pulsed electric fields and static magnetic fields has been shown to uniformly reduce the size of ice during freezing (Mok et al., 2015).

An understanding of freezing and the quality changes that occur during the storage of meat and fish is meaningful. This review addresses the changes that occur in the quality factors of meat and fish during storage, the mechanism underlying ice crystal nucleation and growth, and two food preservation methods that attempt to minimize or eliminate ice from subzero temperature food storage, superchilling and supercooling. Superchilling is a process where partial freezing occurs, and supercooling is a process where no ice crystals form in the food product. In addition, we discuss the use of electrochemical impedance spectroscopy (EIS) as a tool for acquiring information about meat and fish quality, i.e., the physio-chemical changes in supercooled food materials. The basic details and applications for the aforementioned subzero temperature food storage methods and food quality analysis are addressed in the following sections.

Quality aspects of beef and fish during storage

The end of the shelf life for perishable foods can be defined as the time point when the food is no longer fit or suitable for sale or consumption. Fit for sale or consumption means that the food is of high technological quality according to strong industrial standards, guaranteed safe, authentic, and no adulteration or improper description of the product has occurred (Monin, 1998). The effects that lead to the end of shelf life can be categorized as microbial, chemical, or sensorial in nature. Since unfitness for consumption is a somewhat vague idea, many quality aspects, such as pH, color, texture, microbial load, and the end products of naturally occurring chemical reactions, have been used to describe and quantify the end of shelf life for many foods.

Meat, lean animal muscle tissue, is particularly susceptible to spoilage due to its high moisture content and initial microbial load. Even at refrigeration temperatures, the shelf life of meat is limited by its potential for enzymatic and microbiological spoilage (Ashie et al., 1996). Since fresh meat has a vast and varied microbial flora, it is not surprising that meat spoilage is mainly a function of microbiological activity. Although the microorganisms responsible for the spoilage of meats such as beef and fish

depend on ecological factors, *Pseudomonas* spp. and a few other gram-negative psychrotrophic organisms dominate under aerobic conditions at refrigeration temperatures (Gram et al., 2002). The growth of these spoilage bacteria leads to an increase in their metabolites, which is the primary reason for rendering meat unfit for consumption.

During aging and spoilage of beef, many noticeable changes can be measured and quantified to assess its fitness for use. Those most often measured include color, texture, pH, lipid oxidation, and off-flavors and -odors. Discoloration in beef during storage can be caused by two mechanisms that generate either a brown or green pigment; oxidation of ferrous myoglobin, the iron-protein complex responsible for beef color, to ferric metmyoglobin, which results in surface browning (Mancini and Hunt, 2005) and bacterial production of hydrogen sulfide, which converts myoglobin to green sulphmyoglobin, resulting in green discoloration (Borch et al., 1996). Along with this discoloration, endogenous and microbial-derived enzymes breakdown the beef, leading to tenderization of the muscle and alteration of muscle protein charges, which cause an overall increase in pH (Livisay et al., 1996).

Secondary to microbial spoilage, oxidation of the lipids in beef lead to rancidity, causing undesired flavor and odor changes. These flavors and odors are often characterized by trained sensory panels or instrumentally by gas chromatography. Lipid oxidation occurs through a set of complex auto-propagating reactions and as a function of oxygen concentration, the degree of lipid unsaturation, and the presence of pro-oxidant catalysts. Methods used to analyze the extent of lipid oxidation often involve quantification of the resulting carbonyl products. Ultraviolet-visible (UV-vis) spectroscopy is often used indirectly quantify malondialdehyde (MDA), the most abundant secondary by-product of lipid oxidation, after it reacts with thiobarbituric acid (TBA), resulting in a colored MDA-TBA complex because of its speed and simplicity (Barriuso et al., 2012). The reaction between MDA and TBA is shown in Figure 1.

Fish undergo many of the same unwanted sensorial changes during storage. In addition to the microbial and sensorial analysis used to assess spoilage, quantification of chemical compounds related to flavors and odors is also common. Measurable nitrogenous compounds, such as trimethylamine (TMA-N), and low molecular weight volatile bases (TVB-N) are produced by bacteria and are often used as spoilage indices (Ocaño-Higuera et al., 2009). However, the concentrations of adenosine triphosphate (ATP) and its breakdown products are considered to be the most reliable and useful indicators of spoilage. ATP levels rapidly decrease after a fish is caught. ATP is converted to inosine-5′-monophosphate (IMP), which is perceived as a freshness compound (Özogul et al., 2000). During storage, IMP undergoes degradation, ultimately to hypoxanthine (Hx), which has a bitter flavor, and is considered a marker of spoilage.

The shelf life of beef and fish can be extended through various preservation techniques; however, cold storage is often preferred, as it requires minimal processing. Although storage at low temperatures will decrease the rate of spoilage, freezing the product causes solute changes and structural damage due to ice crystal formation. Therefore, the fundamentals of classical ice nucleation theory must be addressed before ice crystal manipulations can be attempted.

Ice crystal nucleation and growth

When there are changes in the state of a food system, such as temperature or pressure, a new phase may be formed. As a food is cooled to temperatures below its melting point, or equilibrium freezing temperature, the water within that food will undergo a phase change from liquid to solid. The equilibrium freezing temperature of food is somewhat lower than that of pure water due to the dissolved solids in the liquid portion. Once the internal temperature of the food is lowered below its equilibrium freezing temperature, a complete phase change from

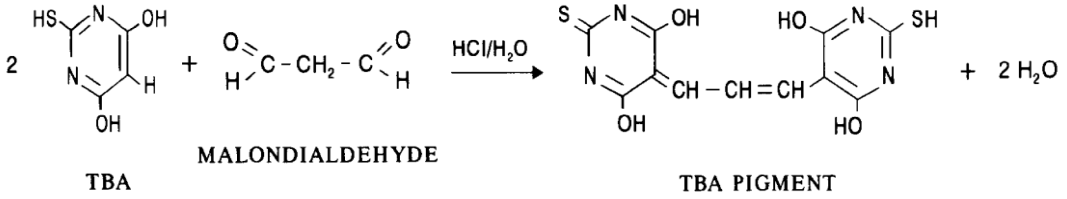

Figure 1. The reaction between TBA and MDA that formsTBA pigment (Fernández et al., 1997).

fresh to frozen should occur. However, the phase change from fresh to frozen does not happen immediately after the food's temperature is below its equilibrium freezing temperature. In fact, sometimes the internal temperature of the food must reach far below its equilibrium freezing temperature before undergoing a phase change. This can be explained through a series of equations describing the phase transformation phenomenon (Walstra, 2003; Kiani and Sun, 2011). The Gibbs free energy change of phase transformation ($\Delta_{tr}G$) is defined as:

$$\triangle_{tr}G = \triangle_{tr}H\left(1-\frac{T}{T_{eq}}\right) \tag{3}$$

where $\Delta_{tr}H$ is the enthalpy change of phase transformation (J/m^3), T is the temperature (K), and T_{eq} is the equilibrium phase transformation temperature.

Since $\Delta_{tr}H$ is a positive quantity, from equation 3, it would seem that a phase change would be favored as soon as $T<T_{eq}$; however, for a phase change to occur, an interface must be created between the phases, adding to the energy requirement. The free energy for a spherical embryo of ice to form (ΔG_{emb}) is given by:

$$\triangle G_{emb} = \frac{3}{4}\pi r^3 \triangle_{tr}G + 4\pi r^2 \triangle_s G \tag{4}$$

where r is the radius of the ice embryo (m) and $\triangle_s G$ is the interfacial free energy (J/m^2). The interfacial free energy is equal to the interfacial tension γ; thus, equation 4 can be written as:

$$\triangle G_{emb} = \frac{3}{4}\pi r^3 \triangle_{tr}H\left(1-\frac{T}{T_{eq}}\right) + 4\pi r^2\gamma \tag{5}$$

A spherical embryo of ice frequently forms below the equilibrium freezing temperature. However, it will simply dissolve if a critical radius is not achieved to reduce the free energy of the system (Damodaran et al., 2008). To find the critical radius for ice nucleation, a derivative of equation 5, in terms of r, must be taken, and the result set to zero, which is the maximum free energy for a spherical embryo of ice. Therefore, the critical radius (r_{cr}) is defined as:

$$r_{cr} = -\frac{2\gamma}{\triangle_{tr}H\left(1-\frac{T}{T_{eq}}\right)} \tag{6}$$

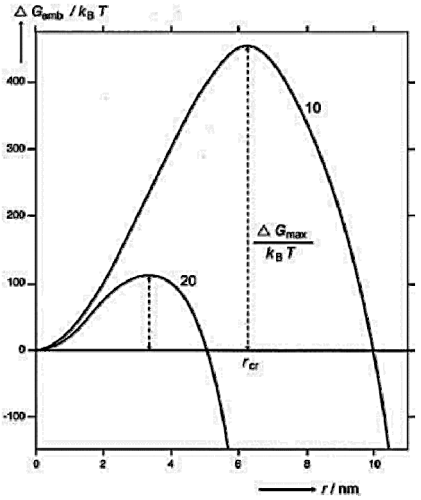

Figure 2. The excess free energy ($\triangle G_{emb}$) of a spherical embryo in a new phase as a function of embryo radius ® for two cases: supercooling by 10 or 20 K. T is the temperature and k_b is the rate constant for nucleation (Walstra, 2003).

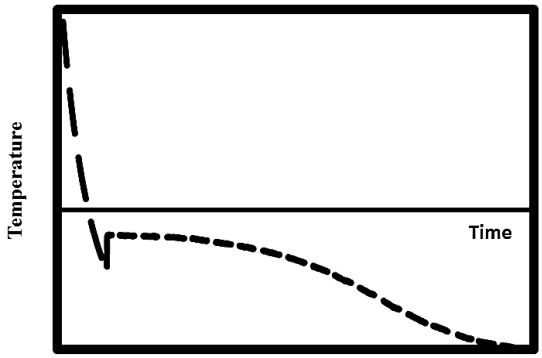

Figure 3. A typical cooling curve of a homogeneous material.

And the maximum free energy change required for ice nucleation (ΔG_{max}) is defined as:

$$\triangle G_{\max} = \frac{16\pi\gamma^3}{3\left[\triangle_{tr}H\left(1-{}^{T}/_{T_{eq}}\right)\right]^2} = \frac{4}{3}\pi r_{cr}^2\gamma \tag{7}$$

From equation 7, it is concluded that the free energy change required for ice nucleation is dependent on temperature, which is correlated with the critical radius of the spherical ice embryo. In Figure 2, ΔG_{max} and r_{cr} will decrease as the temperature decreases below the equilibrium freezing temperature. In addition, r_{cr} is significantly reduced in a heterogeneous system (food), as there are many particulates that act as templates for nucleation.

Figure 3 shows a typical cooling curve of a homogeneous material. In Figure 3, the material is cooled below its equilibrium freezing temperature without freezing,

at which it is in a metastable state known as the supercooled state. Ice nucleation inevitably occurs, denoted by a sudden increase in temperature. This sudden increase in temperature is due to localized release of the latent heat of fusion (H_f) for the phase change. The temperature remains constant as ice crystal growth continues and more latent heat is removed. The rate of heat removal during this step is known to significantly affect ice crystal size (Kiani and Sun, 2011). Once the icecrystals have reached their maximum size (when most water has been transformed to ice), sensible heat will be removed until the temperature of the material is equilibrated with the ambient temperature.

Superchilling

Since the spoilage of fresh foods is highly dependent on the temperature at which they are stored and the growth of ice crystals at freezing temperatures leads to significant irreversible damage to the food's texture, there have been a considerable number of studies aimed at minimizing ice damage. One important technique is known as superchilling. Superchilling is a process that combines low temperature storage with the conversion of some water into ice, which makes it less available for deteriorative reactions (Kaale et al., 2011). The low storage temperature delays the growth of many spoilage microorganisms, and as only some of the water contained in the food product is transformed into ice, less textural damage due to crystal growth should occur. Although it was described as early as 1920 in a patent by le Danois (Stevik et al., 2010), superchilling methods are currently emerging, especially in European countries, as an alternative to freezing to extend the shelf life of perishable foods.

The superchilling process involves rapidly cooling food in a freezer at -20 to -30°C until the food's internal temperature reaches approximately 1~2°C below its equilibrium freezing point. The food is subsequently stored at that temperature with minimal temperature fluctuations. The rapid cooling process ensures the formation of small, uniform ice crystals approximately 1~3 mm from the surface of the product and no ice on its interior (Kaale and Eikevik, 2014). This surface layer of ice acts to protect the food product from oxygen-induced changes, such as lipid and protein oxidation. Furthermore, because only the surface is transformed to ice, cold is accumulated within the product acting as a heat sink, protecting against large temperature fluctuations during storage (Magnussen et al., 2008). However, if processing conditions leading to a stable, superchilled storage temperature are not met, temperature fluctuations will cause changes in ice morphology, namely Oswald ripening, leading to large ice crystals that will significantly damage the food product.

Superchilling has been mostlyused foe fish due to its highly perishable nature, and the notable damage ice causes to the texture of fish. In superchilling, 5~30% of the water in fish will be converted to ice (Wu et al., 2014). Through a combination of partial ice formation and low temperatures during superchilling, researchers have shown significant increases in the acceptable storage length of fish. Olafsdottir et al. (2006) found that superchilled cod fillets were still considered acceptably marketable after 15 days of storage. In addition, fresh salmon fillets were found to maintain their acceptability for 9 to 17 days when preserved by superchilling (Duun and Rustad, 2008; Gallart-Jornet et al.,2007). Overall, it can be concluded that when implementing superchilling technology, there will be an increase in shelf life at least 1.4~4 times that of traditional chilling (Magnussen et al., 2008).

Supercooling

When the internal temperature of a food is below its equilibrium freezing point, before ice nucleation has occurred, it can be considered to be in the supercooled state. The process of supercooling foods is advantageous over superchilling as, unlike superchilling, no ice is present; thus, the food completely maintains its fresh textural integrity. However, little has been done to maintain foods in their supercooled state throughout cold storage. The supercooled state is very unstable, and ice nucleation is stochastic in nature. Strict temperature control is the technique most utilized for supercooling. Even with strict temperature control and little temperature fluctuations during storage, ice crystallization can occur. Furthermore, any physical vibration can stimulate the onset of ice nucleation. The difficult nature of maintaining the supercooled state of a material is why more studies have been reported on superchilling (partial freezing) than on supercooling.

James et al. (2009) investigated the innate ability of various vegetables to be stored in a supercooled state through the use of a near static air environment. They concluded that some vegetables such as garlic and shallots can be stored at a temperature significantly below their equilibrium freezing point for weeks without freezing. In

addition, an attempt was made to extend the supercooling temperature of oranges and other citrus fruits by immersion in a glycol bath at-20°C (Chen, 1986). Using this method, the fruit was found to freeze at a significantly lower temperature; however, after a certain amount of time, the fruit would inevitably freeze. A supercooling study done by Fukuma et al. (2012) showed extension of the supercooled state as well as a lower ice nucleation temperature when different fish samples were cooled slowly, by 0.5°C per day, and found that softening of the fish meat was delayed by this treatment.

A number of studies have examined the supercooling phenomenon under different conditions and treatments, such as pressure shift (Kalichevsky et al., 1995; Martino et al., 1998), irradiation of ultrasonic waves (Inada et al., 2001; Zhang et al., 2001), and the addition of polyvinyl alcohol (Kumano et al., 2011) and antifreeze proteins (Feeney and Yeh, 1998; Li and Lee, 1998). External electric fields and magnetic fields have great potential for freezing by non-thermal mechanisms. The negative effects of an electric field on ice formation due to dipolar vibrations and re-orientation have been reported (Shichiri and Nagata, 1981; Shichiri and Araki 1986; Petersen et al., 2006; Sun et al., 2006, 2008; Orlowska et al., 2009; Woo and Mujumdar, 2010).The diamagnetic properties of water molecules under magnetic fieldshave also been intensively studied. (Aleksandrov et al., 2000; Iwasaka et al., 2011) Therefore, electric and magnetic can be utilized to minimize damage on food tissues. A new freezing system that utilizes a combination of electric and magnetic fields was recently developed in Japan (Iwasaka et al., 2011). However, the mechanisms that control the freezing process are not yet known.

Supercooling treatments have obvious benefits over other storage methods; however, due to the difficulty of maintaininga supercooled state, it may only be suitable for premium products, such as meats and fish (Stonehouse and Evans, 2015).

Quality factor analysis: electrochemical impedance spectroscopy

Many methods have been used to describe the aging and spoilage of perishable foods such as meats and fish. Typical methods include high performance liquid chromatography (HPLC), gas chromatography (GC), electronic nose, texture analysis, and color or pH measurements. Many of these methods rely on physical or chemical measurements, which can be tedious and time consuming. Moreover, some methodologies can get quite complex and require an in-depth knowledge of laboratory procedures and chemical safety. Therefore, the electrical properties of foods have long been investigated as indicators of quality deviations. Specifically, EIS has been used because impedance measurements are simple, rapid, straightforward, and the equipment is affordable (Pliquett, 2010).

When a direct current (I) passes through a material, there is a voltage (V) drop proportional to the resistance (R) of the material as described by Ohm's law:

$$R = {}^{V}/_{I} \tag{8}$$

However, when an alternating current is applied, the effects of inductance and capacitance must be considered. Thus, impedance (Z) includes these effects and is the resistance equivalent in Ohm's law for alternating currents. Reactance is defined as the non-resistive imaginary component of impedance, and it comprises the effects of inductance and capacitance. Impedance can then mathematically be written as (Chang and Park, 2010):

$$Z = R + jX = Z' + jZ'' \tag{9}$$

where R is the resistance (Ω) and X is the reactance (Ω).

Since impedance is dependent on the frequency of the current passing through the material, measurements of impedance over a range of frequencies is the basis of EIS. In EIS, a small sinusoidal excitation signal is applied to the material. The current response after passing through the material will have a shift in phase angle and amplitude, which describe the impedance. A popular way to display impedance information is with graphs of phase angle shifts and amplitude changes vs. applied frequency (Bode plots). However, a Nyquist plot is a better representation because it includes both phase angle shift and amplitude change information within the same graph. A Nyquist plot shows the resistance (Z') vs. the reactance (Z'') over the tested range of frequencies. Figure 4 shows a representative Nyquist plot.

Biological materials, such as foods, can be thought of simply as cells suspended in extracellular fluid, where intra- and extracellular fluids have resistive characteristics and cell membranes can act as small capacitors with reactive characteristics (Bauchot et al., 2000). Therefore, the electrochemical impedance spectrum of a food provides

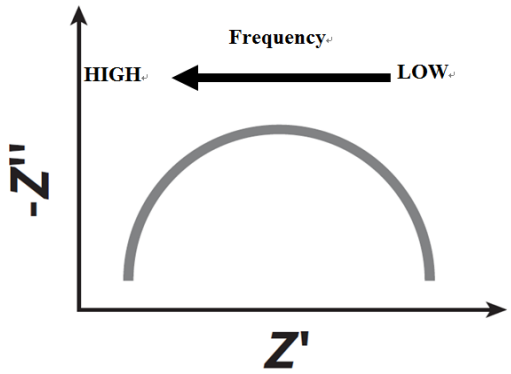

Figure 4. Example of a Nyquist plot obtained by electrochemical impedance spectroscopy. The direction of the frequency applied is indicated on the graph.

information about changes in its microstructure. In fact, many studies have used EIS to show the microstructure of foods during dehydration (Ando et al., 2014; Chee et al., 2014). EIS has also been used to detect the onset of spoilage during the aging of meats (Damez et al., 2008; Guermazi et al., 2011; Niu and Lee, 2000; Oliver et al., 2001; Zhang et al., 2011). Furthermore, investigations of EIS as a technique to differentiate fresh and frozen-thawed products have also proven fruitful (Fernández-Segovia et al., 2012; Fuentes et al., 2013; Vidaček et al., 2008; Wu et al., 2008). From a review of these studies, we concluded that EIS could be a useful tool for understandingthe changes that may occur in a food product during supercooling.

Conclusion

Investigation of the reported quality changes that occur in perishable foods (beef and fish) during storage and unique ways (superchilling and supercooling) to maintain their fresh-like integrity,shows that there is much potential for improvement in cold preservation. Preserving foods at lower temperatures without forming ice is an obvious challenge, and new technologies for cold preservation should be developed. Furthermore, with any new preservation technique, such as electric field and magnetic field applications, it is important to thoroughly prove and measure the quality of the food subjected to that treatment. EIS has shown a promising ability to analyze the structural changes within a food product, such as differentiating between fresh and frozen-thawed, and could be a useful way to deeper understand the impact of a novel cold preservation technique on perishable foods.

Conflict of Interest

The authors have no conflicting financial or other interests.

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