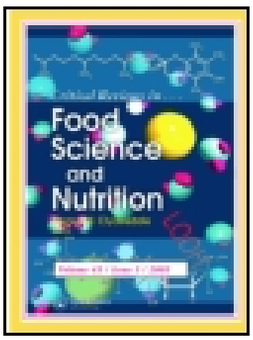

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Improving the Quality and Safety of Frozen Muscle Foods by Emerging Freezing Technologies: A Review

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Abstract

Freezing is one of the most widespread used preservation methods for meats including fish meat. Traditional freezing methods such as air blast freezing and cryogenic freezing could induce some quality deterioration such as damage to cell structure, increased drip loss and poor sensory value. Therefore, novel freezing methods have been developed to minimize the disadvantages of traditional freezing methods. This review describes the enhancement of quality attributes of

muscle tissues frozen by novel freezing technologies, including high pressure freezing, electrically and magnetically assisted freezing, ultrasound assisted freezing and antifreeze protein. These quality attributes include microstructure, moisture loss, colour, tenderness, protein denaturation, lipid and protein oxidation, and microbial counts. In this review, the principles of these emerging freezing technologies are introduced, and the impacts of these technologies on controlling the formation and growth of ice crystals and on complex changes of protein are also discussed. The current review shows that the novel freezing methods have positive effects on promoting the quality of frozen muscle. At a micro level, the majority of the novel methods have some certain ability on controlling the formation and growth of ice crystals, thus creating smaller, and more homogeneous and regular distribution of ice crystals, leading to better microstructure and enhanced quality attributes of frozen meats. Meanwhile, complex changes of protein take place under some of these novel freezing processes, and therefore the possible negative effect of the changes of protein should also be considered for commercial applications of these technologies in the frozen food industry.

Keywords:

freezing, physical-chemical parameters, meat, fish, ice crystals, denaturation of proteins

1. Introduction

Meats including fish meat are an important part of the human diet. In the meantime, meats are highly perishable, and thus quality and safety assurance plays a critical role in the development of the meat industry. As the diverse nutrient compositions of meats often provide an ideal environment for common food borne pathogens and food spoilage organisms to grow and propagate, freezing is a common method to preserve meat and meat products (Sun, 2006).

The deterioration of meat quality, especially caused by both degradation and denaturation of muscle proteins, action of enzyme, oxidation processes and microbial spoilage, would occur if meats are not properly handled (Joo *et al.*, 1999; Benjakul *et al.*, 2003; Rahman, 2007). The biochemical reactions of lipids, heat-labile muscle proteins, and proteolytic enzymes are temperature-dependent, which have been shown to be the key differences among meats during low temperature treatments, which directly translate into the quality issues (Sun, 2006). Nevertheless, since fresh meats including fish meat belong to muscle tissues, which are rich in moisture and protein, their quality would all be affected and even sensitive to cellular damage caused by ice crystals and protein denaturation during freezing and frozen storage.

Traditional freezing methods include air blast freezing, cryogenic freezing and plate contact freezing, in which freezing rate is normally low due to the low thermal conductivity of foods (Sun & Li, 2003). Low freezing rate normally produces large, irregular and unevenly distributed ice crystals, causing severe damage of muscle tissue (Kaale *et al.*, 2013; Cheng *et al.*, 2015). This kind of damage of cell structure caused by ice crystals during freezing and frozen storage may induce an increase of thawing drip loss, denaturation of certain proteins and some other quality losses.

Therefore, novel freezing technologies have been developed to overcome these disadvantages. These technologies mainly include high pressure freezing (HPF), electrically and magnetically assisted freezing (EF & MF), ultrasound assisted freezing (UAF), antifreeze protein (AFP), etc. Several reviews have been published in the past. The early representative review was presented by (Li & Sun, 2002), who reviewed four novel freezing technologies: HPF, dehydrofreezing, antifreeze protein, and ice nucleation protein, while most recent reviews include more techniques, representing the rapid developments in the area. Cheng *et al.* (2015) compared six novel freezing technologies: high pressure freezing, ultrasound assisted freezing, electrically disturbed freezing (EF) and magnetically disturbed freezing (MF), microwave assisted freezing (MWF), and osmo-dehydro-freezing (ODF) while James *et al.* (2015) reviewed 13 novel freezing technologies, covering most of the emerging freezing technologies available. Despite these reviews, no review on the effects of novel freezing techniques on frozen meats including fish meat is available. Therefore, the current review intends to provide critical comparisons of novel freezing technologies on various quality attributes of these muscle tissues. The principles of these novel freezing methods are also briefly discussed. It is hoped that the current review should provide further understanding of these technologies and encourage their future applications in the frozen food industry.

2. Principles of Emerging Freezing Technologies

2.1 High pressure freezing (HPF)

High pressure freezing utilizes different states of water under pressure. As shown in Fig. 1 (Cheng *et al.*, 2015), under high pressures and different temperatures, water usually form multiple kinds of ice (I-VI) by different phases. Therefore, HPF can be classified into three types:

high pressure induced freezing (HPIF, ABEFHI in Fig. 1), high pressure shift freezing (HPSF, ABEFG in Fig. 1) and high pressure assisted freezing (HPAF, ABCD in Fig. 1) (Fernández *et al.*, 2006), with HPSF being the most common option. HPF can not only improve freezing process and the quality of frozen food, but can also have positive effects on food safety due to microbial inactivation (Realini *et al.*, 2011). Further details on the principles of HPF can be found elsewhere (LeBail *et al.*, 2002).

2.2 Electrically and magnetically assisted freezing

In recent years, efforts have been made to apply electromagnetic assisted during freezing for controlling the formation and growth of ice crystals (Wei *et al.*, 2008; Orlowska *et al.*, 2009; Mok *et al.*, 2015) and thus three technologies have emerged: electric field, magnetic field, and electromagnetic field assisted freezing methods.

2.2.1 Electrically assisted freezing (EF)

The principle of applying an electric field to assist a freezing process is mainly based on the fact that the orientating or rotating movements of polar molecules become stronger under the impact of the electric field. Therefore, the effects of water molecules under electric field can influence the free energy, which can control supercooling and ice crystallization (James *et al.*, 2015).

From the thermodynamic point of view, when relative change in the Gibbs free energy for the system became negative, the nucleation process takes place spontaneously. Orlowska *et al.* (2009) studied the change of Gibbs free energy during liquid crystallization under an applied EF, and showed that the free energy of a system under freezing decreases due to the polarization of molecules in the system affected by the strength of the applied field. Therefore ice nucleation

process occurs when the Gibbs free energy of the system is minimized, thus explaining the reason of applying an electric field to lead to the early formation of ice crystals and the enhancement of the nucleation process in the system (Xanthakis *et al.*, 2014a). Therefore, freezing under electric field or electric disturbance, both OEF and SEF show positive effects on the quality of frozen model foods (Hozumi *et al.*, 2005; Sun *et al.*, 2006).

2.2.2 Magnetically assisted freezing (MF)

Similar to electrically assisted freezing, a magnetic field can change the freezing characteristics of water molecules, as diamagnetic material and water molecules are susceptible to the development of magnetic dipole moments under a magnetic field (Cheng *et al.*, 2015; Woo & Mujumdar, 2010).

The mechanism of the influence of MF on food samples is closely associated with the magnetism of water molecules, and the linkage between water molecules in the samples would become closer as more numbers of hydrogen bonds are formed, which makes the network become more stable (Cai *et al.*, 2009), inducing an increase of the thermal conductivity and freezing point of water (Inaba *et al.*, 2004). Therefore, MF seems to have the ability to control the supercooling and the subsequent growth of ice crystals.

A commercial unit for applying magnetic fields in freezing biological tissues was patented and developed a Japanese company (ABI Corporation Ltd, Chiba, Japan), in which induction coils and permanent magnets are used to develop a weak oscillating magnetic field, leading to delay of the formation of ice crystals in supercooled water (Owada & Kurita, 2001; Owada, 2007). Although full scale commercial installations of this novel technology have been reported in Japan and it has been introduced in the market for more than ten years, data in the literature of

the efficacy of MFs in improving the quality of frozen products are frequently confusing and apparently contradictory, therefore, there is still a need for validation on the improvement in quality of real foods (Otero *et al.*, 2016).

2.2.3 Electromagnetic assisted freezing

In the last decade, many research activities have been performed focusing on food processing assisted by electromagnetic wave, in particular microwave methods. The potential of utilizing electromagnetic wave to assist freezing has also been investigated, especially in microwaves (Hanyu *et al.*, 1992; Jackson *et al.*, 1997). Similar to EF and MF, microwave assisted freezing is based on inherent characteristics of water, in which microwaves can induce the dipole rotation, which cause disturbance in ice nucleation, formation and growth. As discussed above, electrical and magnetic impacts could interact with water molecules, rearrange the hydrogen bonds and make the network of water stronger. However, in order to achieve the same effects, more powerful magnetic fields are needed, since it was proved that electric fields were more efficient in altering the conformation of water network compared with the magnetic field, which meant that the impact of electromagnetic wave on water molecules was mostly resulted from the electrical disturbances (Chaplin, 2013).

Microwave is not the only kind of electromagnetic wave that can exert force on water molecules, Chaplin (2013) suggested that, besides microwaves at 915 MHz and 2450 MHz, radiofrequency radiation (RF) at 27.12 MHz or even at extremely low frequency of 3-300 Hz can affect water molecules significantly and lastingly. Therefore, it seems that electromagnetic waves in several frequency bands should be able to assist the freezing process in varying degrees. At present, electromagnetic assisted freezing is at early stage of the development, and only a few

studies on real food (Anese *et al.*, 2012; Xanthakis *et al.*, 2014b) have been published, therefore further studies should be conducted for possible industrial applications.

2.3 Ultrasound assisted freezing

Ultrasound can generally be divided into low intensity diagnostic ultrasound (>1 MHz) and high intensity power ultrasound (20-100 kHz). The medium is not affected by the sound wave propagation under low power ultrasound, and therefore in the food industry, power ultrasound is used for enhancing food processing including freezing (Zhang & Sun, 2015).

Ultrasound assisted freezing is mainly based on the cavitation and micro-streaming effects generated by the ultrasound. It has the advantages of intensifying heat transfer, promoting the formation of ice crystal and fracturing large ice crystals. The cavitation effect would produce and even made cavities or bubbles collapse violently, with microbubbles acting as nuclei for formation of ice crystals and the collapse of cavitation bubbles enhancing the mass and heat transfer. Although studies show that, as a potential tool, ultrasound can be used to make ice crystals in model foods smaller (Kiani *et al.*, 2013), applications of ultrasound assisted freezing are still limited, in particular few investigations are focused on meat freezing (Zhang. & Sun., 2015).

2.4 Antifreeze proteins

In the tissues and blood of the fishes inhabiting polar and northern coastal waters, a family of proteins called antifreeze proteins (AFPs) existed to prevent fishes from freezing, which can decrease the freezing point and have thermal hysteresis activity. It is generally accepted that AFPs can interfere with propagation of water molecule to surface of ice crystal, limiting the spaces for ice lattice to grow and thus reducing the stability of the surface at the ice water

interface (Li & Sun, 2002). Therefore, AFPs can modify and even control the growth and aggregation of ice crystals, during temperature fluctuations to inhibiting nucleation formation and altering growth rate and habit of ice crystals (Clarke *et al.*, 2002).

At present, AFPs have been applied to different kinds of food products successfully including fruit (James *et al.*, 2015) and vegetables, bakery products (Zhang *et al.*, 2007; Zhang *et al.*, 2008; Yeh *et al.*, 2009), and yogurts and ice creams (Soukoulis & Fisk, 2014).

3. Effects on Physical Attributes

For muscle tissues, their physical attributes are quite complex. Some attributes, such as tenderness and colour, can reflect the quality of meats intuitively, and moisture content is an important quality attribute for processing, storage and cooking of meats. Therefore, these attributes influence the commercial value of meat products. Meanwhile, these attributes are usually the combined representations of physicochemical changes at micro level (Leygonie *et al.*, 2012). In the freezing process, the changes in microstructure of frozen samples due to the existence of ice crystals are the main inducement of quality changes, and thus many studies available focus on this aspect.

3.1. Microstructure

It is understood that microstructure can reflect the state of ice crystals in the samples intuitively, and the growth of ice crystals is a critical factor in the freezing process, therefore microstructure is commonly used to assess the quality of the frozen meat products. Scanning electron microscope (SEM), transmission electron microscope (TEM) and optical microscope are the common methods for observing the microstructure.

Among the novel freezing techniques, HPSF is the most studied one. Earlier study (Martino.

et al., 1998; Cheng *et al.*, 2015) showed that HPSF could preserve the microstructure of meat samples better than traditional freezing methods such as air-blast and liquid immersion freezing. For fish samples, Chevalier *et al.* (2000a) froze turbots at 140 MPa (–14 °C) with the pressurization rate of 100 MPa/min and then stored for 2 and 75 days at –20 °C, and found that the ice crystals in pressure-treated samples appeared to be smaller and more regular with a round shape on the surface and in the center as compared with air-blast freezing (–20 °C). Zhu *et al.* (2003) froze Atlantic salmon under pressure at 100 MPa (–8.4 °C), 150 MPa (–14 °C), and 200 MPa (–20.7 °C), respectively, with the pressurization rate of 40 MPa/s, and found that HPSF process appeared to produce a large number of regular and fine endocellular ice crystals distributed throughout the salmon homogeneously, which significantly improved the microstructure of the sample compared with conventional air blast freezing at –30 °C and glycol/water bath freezing at –20 °C. Moreover, with similar experiment conditions, Zhu *et al.* (2004a) treated fresh boneless pork rib portions by HPSF and showed no evidence of shrinkage or deformation of cells in HPSF treated samples, and only tiny and regular ice crystals were created compared with conventional freezing processes. In their research, Zhu *et al.* (2004a) also showed that a higher pressure created a higher degree of supercooling, resulting in even smaller ice crystals. All these studies confirmed that HPSF is an effective method to improve the microstructure of frozen muscle tissues due to the high degree of supercooling at the release of pressure, and thus is an effective freezing technique to control the ice crystals of frozen food quality.

Freezing assisted by a high-voltage static electric field was also studied recently. Xanthakis *et al.* (2013) froze pork assisted by high-voltage static electric fields of 3 kV, 6 kV, 9 kV and 12

kV, and proved that applying high voltages was effective for the formation of small ice crystals. It was observed that with the increase in the power of the electric field, more smaller ice crystals were obtained with the crystal size reduction from 32.79 ± 4.04 μm to 14.55 ± 8.20 μm (Fig. 2), which may be due to that the external electric field had the capability to rotate polar molecules without inducing thermal effect and to make the hydrogen bonds stronger, which in turn had a great promotion on the ice crystallization (Sun *et al.*, 2006).

Besides high-pressure and electric field, electromagnetic assisted freezing has also been studied on real food. Anese *et al.* (2012) froze pieces of pork loin by combining cryogenic fluid flow and radio frequency pulses with a voltage of 2 kV, in which, the sample was frozen rapidly by infiltrating liquid nitrogen in the chamber and simultaneously irradiated with a pilot scale RF field, resulting in significant improvement in the microstructure of pork. As shown in Fig. 3, much less cell disruption and intercellular cavities compared with traditional freezing methods were observed due to a great quantity of smaller intracellular ice crystals. This improvement could be attributed to that RF is able to inhibit the freezing point and then create more nucleation sites. Besides the rotating of the polar molecules with no thermal effect as induced by the low voltage electric field, RF can also interfere with both the kinetics of crystal growth and ice nucleation as water molecules were torqued, which may have made the water molecules out of their equilibrium states in the ice cluster. Meanwhile, Xanthakis *et al.* (2014b) treated pork tender loin with freezing under different microwave intensity levels (40%, 50% and 60%). Although they found that the samples were frozen by a lower degree (a decrease by about 92%) of supercooling and slower freezing rate, sizes of the formed ice crystal decreased significantly by 62% on average. It seems that the limited oscillation of the temperature during the creation of

the ice nucleus and growth of ice crystal caused by microwave might be responsible for regeneration of ice crystals and instantaneous recurring melting, which in turn disturbed the growth of ice crystal and produced the a large number of smaller ice crystals (Fig. 4). These results indicated that applications of electromagnetic waves such as microwave or radio frequency radiation during freezing process should be able to improve the microstructure of the frozen pork and consequently less damage to the muscle tissue occurred.

Besides the use of physical fields, meat freezing can also be enhanced by the addition of biochemical agent. Payne & Young (1995) injected twelve lambs with antifreeze glycoproteins (AFGP) to the final concentrations of 0 μg/kg, 0.01 μg /kg and 100 μg /kg liveweight at 1 h or 24 h before slaughter, and found that the size of ice crystals within the lambs samples with 0.01 μg/kg AFGP at 1 h before slaughter reduced significantly as compared with the control or the 100 μg/kg AFGP samples, and the samples at 24 h before the slaughter allowing for the AFGP to circulate before slaughter produced a greater restraint of ice crystal sizes and crystallization. Therefore, antifreeze protein can have significant positive effects on frozen meat quality.

For data processing and quantifying the quality of the microstructure, many studies using software to analyse images of microstructure for obtaining information such as ice crystal area, equivalent diameter and roundness in both conventional and novel freezing have been carried out. Ngapo *et al.* (1999b) used Genstat 5 statistical software package (Global Lab, Data Translation Inc., Marlboro, USA) to study the combined effects of freezing, thawing rates and frozen storage on the ultrastructure of samples. Su *et al.* (2014) processed images of the slices of frozen shrimp and porcine liver by ImageTool 3.0 (UT-HSCSA, University of Texas, USA) and measured four key parameters of ice crystals: cross-section area, roundness, elongation and equivalent diameter,

to analyse the ice crystals in shrimp and porcine liver frozen under high pressure at 100 MPa (−8.4 °C), 150 MPa (−14 °C) and 200 MPa (−20 °C), and proved that HPSF was beneficial to maintain sample quality compared with conventional liquid immersion freezing at −20 °C and air blast freezing at −20 °C. Moreover, Do *et al*. (2004) took advantage of a micro-slicer image processing system (MSIPS) to measure the three-dimensional structure and distribution of ice crystals formed in frozen beef, and proved that provided MSIPS is an alternative tool to study morphology and distribution of ice crystals under freezing conditions.

3.2 Moisture loss

Since water is the most important component in all foods, meat scientists have always been most interested in macroscopic effects of internal/external quality factors (Forrest *et al.*, 2000; Schäfer *et al.*, 2002). The majority of water in muscle exists either within the myofibrils, between the myofibrils and the sarcolemma, or between cells and muscle bundles.

In the food industry, the loss of moisture under freezing has some negative effects on the quality of frozen meats, as surface drying and thawing loss due to frozen structural damage occur, therefore, minimizing weight loss and preserving meat structure are needed for appealing to the consumer (Campañone *et al.*, 2002; Sun, 2006). Many factors can affect moisture loss in muscle tissues, for instance, original form of meats, adenosine triphosphate losing, pH decreasing, ionic strength, postmortem proteolysis and the steric effects caused by shrinkage of the myofibrils due to conditioning and rigor mortis, which would also influence water-holding capacity (Huff-Lonergan & Lonergan, 2005).

Nowadays, different indicators can be used to evaluate the ability of meat in retaining its liquid portion, including drip loss (DL), cooking loss, total content of moisture and water holding

capacity (WHC) (Leygonie *et al.*, 2012). For measuring these indicators, different techniques including centrifugation, press, gravimetric and cooking are available (Petracci & Baéza, 2011). In recent years, testing technology has been developed rapidly, ElMasry *et al.* (2011) applied near infrared (NIR) hyperspectral imaging to predict WHC non-destructively with reasonable accuracy and the visual results of beef muscles could be identified and classified in a simple way.

It is generally accepted that freezing process would influence the amount of exudation as drip loss and/or thaw loss. During the freezing process, foods would lose moisture due to their surface is exposed to heat and mass transfer with the environment. The water vapor pressure on food surface is different from that in the air bulk, which drives the process of dehydration (Campañone *et al.*, 2002).

Among the freezing methods assisted by physical fields, high-pressure freezing has complex effects on moisture in muscle tissues. As shown in Table 1, on one hand, it seems that when the pressure is below 200 MPa, smaller ice crystals produced by high-pressure freezing can considerably cause less damage to muscle tissue than big ice crystals caused by the conventional processes, thus the drip loss of pressure treated samples would decrease significantly as compared with traditional freezing processes (Chevalier *et al.*, 2000a; Zhu *et al.*, 2004b; Sequeire‐Munoz *et al.*, 2005). On the other hand, it is known that when pressure attains above 200-400 MPa (room temperature), denaturation and aggregation of native myofibrillar proteins are induced, which increases with pressure, and consequently less moisture would retain within tissues (Ma & Ledward, 2004). Fernández-Martín *et al.* (2000) treated meat samples (pork and beef muscles) with HPSF (200 MPa, –20 °C), and found the HPSF samples exhibited high DL compared with air-blast freezing. Moreover, a decrease in water holding capacity occurred in sea

bass after a HPSF process (200 MPa, –18 °C), regardless of whether the fish has been stored for a period of time (Tironi *et al.*, 2010; Tironi *et al.*, 2007), it was also found that after a cooking process, this effect of high pressure on the water holding capacity could be minimized, probably due to the complete protein denaturation at high temperature by cooking. In addition, the extent of protein denaturation due to high pressure is closely related to temperature, pressure level and ionic strength of the tissue of the samples (Montero & Gómez-Guillén, 2005). Fernández *et al.* (2007) also found that in the freezing process, adding salt in the beef pressurised at 650 MPa could decrease the drip loss significantly compared with pressurised fresh beef meat under the same experiment conditions, although both samples were frozen under air blast freezing (–30 °C) before pressurisation, which might be caused by the tissue ionic strength increasing and myofibril swelling.

Meanwhile, in the direction of meat fibres, freezing process would produce large surface fractures, which can increase the water loss as well. Anese *et al.* (2012) found that the DL of cryogenic frozen pork samples was similar with that of air frozen samples, in spite of the smaller ice crystals in the former, the drip loss would be much lower when applying RF pulses on cryogenic freezing, which means that RF could prevent the fracturing probably because the temperature difference between the sample and cryogenic fluid was high.

The use of antifreeze protein can also have positive effects on moisture loss. Payne & Young (1995) treated lamb samples with the injection of antifreeze glycoproteins prior to slaughter and drip losses lower than the control were found. In addition, (Yeh *et al.*, 2009) observed that the DL of the frozen pork treated with an antifreeze cryo-immersion solution (ACIS) contributed by crude rAFP powder dissolved in cryo-immersion solution (CIS) were

lower by approximately 50% than a CIS consisting of food grade NaCl. These results confirm that antifreeze protein is not excreted soon and becomes associated with the muscle tissue in some way, which is probably attribute to the decrease in ice crystal size, as observed from microstructure.

3.3 Tenderness (shear force)

Muscle tenderness (toughness or resistance to cut) is of utmost importance to consumer acceptance and greatly affect consumers satisfaction, which is traditionally measured by trained panellists. On the other hand, the most commonly used objective method for evaluating muscle tissue sensory hardness (tenderness), especially for whole-muscle red meats, is by Warner-Blatzer shear force (WBSF) (Caine *et al.*, 2003; De Huidobro *et al.*, 2005), besides, Kramer shear press for shearing or cutting through muscle fibres and instrumental texture profile analysis for obtaining various texture information are also available (Lyon & Lyon, 2001).

Table 2 summaries the main studies about the effect of high pressure freezing on the tenderness of meats, which were almost all cooked for WBSF tests. It can be seen from Table 2 that in most cases, high pressure treatment would increase WBSF, whether in the process of HPSF or treated by high hydrostatic pressure independently before freezing, this may be due to that such a treatment would cause denaturation, gelation or aggregation of protein, resulting in the either tenderization or toughening the muscle tissues. However, as shown in Table 2, contradictory results were obtained, and no significant differences were observed in the WBSF when samples were treated with high pressure. It seems that these effects depend on the kinds and rigor state of samples, temperature, the duration and level of the pressure treatment, and even air blast freezing process before high pressure would have a certain degree of influence.

Meanwhile, the differences among various kinds of muscle tissues must be taken into account in spite of most samples used in above-mentioned studies are terrestrial animals. For example, presumably resulted from the absence of the trimethylamine system engendering the formaldehyde, which promoted denaturation of protein and destruction of texture of the fish fillets under freezing, carp fillets were less susceptible to tenderness deterioration during frozen storage after high pressure treatment (Sequeire‐Munoz *et al.*, 2005).

However, in the study of radio frequency assisted freezing, firmness of pork meat samples treated by RF cryo-freezing was almost the same as unfrozen samples, and was much less firm than air frozen and cryo-frozen samples, which was consistent with the micrographs of the muscle tissues (Anese *et al.*, 2012). Similarly, all parameters (tenderness, juiciness, flavour and so on) of CIS and ACIS treated pork cut samples, despite these were sensory evaluations, were better than untreated frozen samples significantly (Yeh *et al.*, 2009). These results indicated that for keeping the cellular structure with less effects on proteins, novel freezing methods would play a positive role in improving the muscle tenderness.

3.4 Colour

Besides tenderness, the colour of meats and derived products is also an important aspect for consumer acceptability (Faustman & Cassens, 1990) and a major driver of retail sales and profitability (Jeyamkondan *et al.*, 2000). As for widely consumed meat and some fish rich in myoglobin, their muscle tissue colour is closely related with molecular species of six coordination bonds and redox state of iron ions in myoglobin, which has three forms of deoxy

myoglobin, oxymyoglobin and metmyoglobin, and the ratio between them determines the muscle tissue colour (Bekhit & Faustman, 2005; McKenna et al., 2005), besides, complex microstructure of meat may also have effects on optical properties (Swatland, 2004).

At some stage during freezing process, frozen storage and thawing process, myoglobin denaturation would take place, which leads to an increased sensitivity of stability in muscle colour. As shown in Table 3, in most studies of high pressure assisted freezing (Hansen *et al.*, 2003; Zhu *et al.*, 2004b; Tironi *et al.*, 2007), the effect of this denaturation might be aggravated, because of globin denaturation and/or heme release or displacement, the coagulation of myofibrillar and sarcoplasmic proteins induced by pressure and the changes of water content as drip loss, the high-pressure-treated samples appeared lighter (higher L* in colour values) and turned pale, and a more or less decrease of the a* value (the red-green index) was also observed, which means that the loss of red colour in the pork and fish tissues was partly associated with oxidation of myoglobin, changing ferrous myoglobin to ferric metmyoglobin and other browning processes (Jung *et al.*, 2003). Although the original colours of various meats are quite different, it seemed that there was a similar tendency on L* and a* values under this treatment. As for b* value (the yellow-blue index) of meat samples, different studies obtained different trends from decreasing to increasing (Fernández-Martín *et al.*, 2000; Hansen *et al.*, 2003; Zhu *et al.*, 2004). For fish samples, Chevalier *et al.* (2000b) and Tironi *et al.* (2007, 2010) indicated that b* value in fish was significantly increased in all high-pressure treated systems, which made the appearance of the fish similar to cooked muscle, this phenomenon was resulted from the browning process such as the lipid oxidation (Haard, 1992), and it is well-known that for fish

fillets, lipid oxidation causes discoloration as fish fillets are rich in polyunsaturated fatty acids (PUFAs) that are highly susceptible to oxidation (Ruff *et al.*, 2003). Moreover, when adding salt in the beef treated with air blast freezing plus high pressure at –35 °C, a significant decrease in a* was found and this could attribute to the increase of myoglobin oxidation rate as the myoglobin denaturation was affected by sodium chloride (Fernández *et al.*, 2007), which could be due to that the denaturation would induce an increased susceptibility of myoglobin to autoxidation and a loss of optimum colour presentation subsequently (Leygonie *et al.*, 2012). The effects of high pressure freezing in colour are complex and further research should focus on determining the mechanism of the effects of freezing temperature and pressure.

In addition, radio frequency may have a weaker influence on the colour than high pressure in freezing process, as pork samples frozen in the RF equipment with or without cryo-freezing as well as by air freezing all made a minor increase in a* and b*, which could be associated with slight myoglobin oxidation (Anese *et al.*, 2012). Furthermore, some researches indicated that adding certain biochemical additives such as lactate in frozen pork meat would enhance the colour stability as well (Tan & Shelef, 2002).

4. Effects on Biochemical Attributes

Besides physical parameters, biochemical attributes, especially protein, are needed in the evaluation of muscle tissue quality. The protein denaturation and oxidation occurred under freezing process would have a significant and even critical impact on frozen muscle tissue quality.

4.1 Protein denaturation

To some extent, protein denaturation could be regarded as the most important biochemical

reason for quality change of protein-based foods, as it gives rise to changes of the attributes such as tenderness, drip loss and colour by direct or indirect ways (Joo *et al.*, 1999). Muscle tissue is composed of different proteins, while the myofibrillar proteins make up the major (60-70%) of all the proteins in muscle, which play the main functional and structural role of muscles as they have a tendency to interact with other proteins or non-protein components. Thus losses in functionality protein attributes are commonly assessed by comparing WHC, gelation, viscosity, colour changes and shear force.

It is generally accepted that denaturation of protein can take place during freezing and frozen storage, resulting from mechanical damage to structure and protein directly, the increased strength of intracellular ionic and a concentrated buffer salts following the formation of ice crystals and propagation of water molecules to the extracellular spaces, although some studies suggested that quality loss was not contributed by the denaturation of protein significantly (Mietsch *et al.*, 1994; Ngapo *et al.*, 1999a). Deteriorations caused by enzymatic, functional and biochemical changes of proteins in flavour, colour and sensory are problems related with freezing process and subsequent freezing storage, which should be overcome for the successful freezing of proteins.

Compared with plant-derived proteins, muscle proteins are particularly susceptible to denaturation due to freezing process, as denaturation of protein, freezing-induced enzyme inactivation and correlative functionality losses are often obtained from frozen meat, aquatic product, poultry and other meat products, which result from dehydration, ice crystals, and concentrating of solutes during freezing process (Xiong, 1997a). Protein denaturation caused by freezing process and frozen storage can be measured by capillary gel electrophoresis (CGE),

differential scanning calorimetry (DSC) and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), which can be used to study the patterns of protein exudate. Meanwhile, using modern techniques such as proteomics to assess the drip composition of meat proteins would also be very beneficial (Pazos *et al.*, 2015).

In DSC thermosgrams of traditional meats such as pork and beef, the three characteristic maximum temperatures are at about 58 °C to 58.5 °C (myosin plus sarcoplasmic proteins), 64 °C to 70 °C (collagen), and 81 °C to 81.5 °C (actin) (Fernández-Martín *et al.*, 2000), in the above research, meat samples (pork and beef) were treated with HPSF (200 MPa, –20 °C), and based on analysing the DSC thermograms, they found that the endotherm for actin practically disappeared, myosin and sarcoplasmic proteins were greatly decreased, while the air-blast freezing samples were only slightly different from raw samples. Thus, they concluded that actin was always a pressure sensitive protein, which was easily denatured under pressure with myosin and sarcoplasmic proteins severely affected. Besides, based on comparing results with traditional freezing method, high pressure seemed to be largely responsible for the protein denaturation after treatment. Similar results were obtained in Zhu *et al.* (2004), who reported considerable protein denaturation caused by the HPSF process at 150 MPa and 200 MPa, i.e., sarcoplasmic proteins were much reduced while the peaks for myosin and actin almost disappeared respectively, which meant a high degree of myosin and actin denaturation. On the other hand, collagen, either in combination or singly, was very stable under pressure treatments due to the structure of H-bonded triple a-helix and only fibril formation was suppressed under high pressure (Gekko & Koga, 1983). In the early research (Wagner & Anon, 1985; Xiong, 1997b), myosin was also proved to be the single most abundant protein of the myofibrils and this protein was most

affected by freezing, which should play the important role in the functional attributes of muscle tissue, such as WHC, tenderness and foaming. In researches of seafood muscles, extractability of protein is generally accepted as a measure of solubility, thus salt extractable protein (SEP, mainly myofibrillar proteins) and water extractable protein (WEP, mainly sarcoplasmic proteins) can be obtained from fish samples. In HPSF at 200 MPa (–18 °C) of Norway lobsters, SEP extractability was significantly decreased with no significant difference in WEP extractability among all the samples (Chevalier *et al.*, 2000c). This trend was also obtained in another study (Chevalier *et al.*, 2000b), although both WEP and SEP tended to decline during the storage. Moreover, Chevalier *et al.* (2000a, 2000b) combined SDS-PAGE to analyse protein compositions of WEP and SEP, and founded small new subunit components in electrophoretic profile of WEP, which were the products of degradation process of sarcoplasmic proteins or new water soluble fragments derived from myofibrillar proteins. As for SEP, they found a major reduction in myosin heavy chain, which could be associated with the aggregation of myobrillar proteins and particularly of myosin, and this change of myosin was in accordance with result of DSC in the study of Chevalier *et al.* (2000a). Therefore, it seemed that HPSF treatment produced both aggregations and fragments through the insolubilization process of the proteins, especially myofibrillar proteins in seafood muscle tissues. Comparable results were obtained by Tironi *et al.* (2007), who treated sea bass muscle at 200 MPa, but in their electrophoretic pattern a significant decrease in water extractable protein fraction concentration was found, which might be due to different experimental subjects and processing conditions (pressure level, time and temperature). It is noteworthy that these phenomenon are always accompanied by the abrupt increase in toughness and the alteration in other parameters.

In addition, application of antifreeze protein also has significant effect on decreasing protein loss of frozen lambs (Yeh *et al.*, 2009), meanwhile, other novel freezing methods, such as RF assisted freezing (Anese *et al.*, 2012) would be worthy in delaying the protein denaturation as almost all attributes of frozen pork were improved especially the firmness and drip loss, although no detailed DSC or SDS-PAGE was conducted to study the protein.

4.2 Oxidation of lipids and protein

When muscle foods are frozen and stored at low temperatures, biochemical reactions could still occur, since the remaining unfrozen water are available for these reactions to take place, and oxidative processes of lipids and protein play an important role in these reactions, which would lead to losses in quality of muscle foods.

Lipid oxidations are caused by oxy- and/or lipid free radical generation, which induces the generation of toxic compounds such as cholesterol oxidation products and the malondialdehyde (Morrissey *et al.*, 1998). For the freezing process, the formation and growth of ice crystals could damage cell membranes and organelles, resulting in the release of the contents in cells, especially pro-oxidant such as heme iron and thus the occurring of oxidation (Benjakul & Bauer, 2001; Thanonkaew *et al.*, 2006). Thiobarbituric acid reactive substances (TBARS) method is commonly used as a measuring method for the quality of the by-products of lipid oxidation. Besides, enzymatic hydrolysis would produce free fatty acid (FFA), which can react with protein fractions and induce denaturation primarily via electrostatic, van der Waals forces, hydrogen and hydrophobic forces as well. These reactions may create more hydrophobic regions to replace charged groups or polar, which would make linkages between molecules extensive enough to reduce extractability or induce a reduction in protein solubility in water. And then, off-flavours

from the senses including pungent, rancid and so on would be produced due to these by-products (Leygonie *et al.*, 2012).

Similar with lipid oxidation, the mechanism of protein oxidation is also free radical chain reaction. In proteins, effectively all the amino acyl side chains could be changed due to reactive oxygen species (Shacter, 2000), changes of amino acid side chain groups is one of the main results of protein oxidation, and different amino acids have different oxidation sensitivity (Park & Xiong, 2007). Meanwhile, the relativity between oxidation of lipid and protein in frozen meat storage has been a theme of considerable discussion (Lund *et al.*, 2011). Soyer *et al.* (2010) founded that in frozen chicken, primary and secondary products of lipid oxidation could act as substrates for oxidation of proteins, therefore once the oxidation of lipids started, the protein oxidation would take place as well.

For studying on oxidation stability of lipid and protein in novel freezing process, fish and some other seafood are most commonly used as experimental materials because of polyunsaturated fatty acids (PUFA), particularly the trienoic, pentaenoic and hexaenoic PUFA commonly found in them, render these foods particularly sensitive to oxidative changes (Hsieh & Kinsella, 1989). Although oxidation of protein was mentioned frequently as a major reason for quality loss especially for colour determination in novel freezing treatments, few studies analyse this aspect separately. In the process of HPSF, Chevalier *et al.* (2000b) found that the TBA number of HPSF samples (turbot, 140 MPa, –14 °C) stored 2 days at –20 °C increased significantly (from 0.46 to 0.58) but a decrease in FFA content was observed (from 4.09 to 2.35) compared with the control sample (ABF, –20 °C), and similar changes in TBA number were also obtained in some studies at room temperature (Cheah & Ledward, 1997; Angsupanich &

Ledward, 1998), which could be partially due to the indirect effect of accelerated autoxidation of lipid-pressurized fish tissue on protein aggregations caused by the release of iron from hemoglobin and myoglobin. However, in the study of Sequeire‐Munoz *et al.* (2005), the TBA values of HPSF fillets (carp, 140 MPa, –14°C) remained unchanged during frozen storage, at the same time, FFA levels in the HPSF samples were significantly lower for storage between 15 and 65 days than ABF samples. Therefore, it seemed that HPSF had a retardation affected the release of free fatty acid from hydrolysis of lipid, and as for carp fillets alone, HPSF may take a curtailing effect on the lipid oxidation. It can be inferred that the types of materials may have a certain degree of influences on TBA results from the above studies, as the content of proteins, fat and amines are dissimilar between different kinds of fish, especially in river and marine fish.

5. Effects on Microbial Spoilage

As rich in protein, lipid and other nutrients, meats including fish meat are vulnerable for growth of microbes. Although different microorganisms have been shown to have different sensitivities to different freezing conditions (Archer, 2004), generally spoilage due to microbial growth is efficiently terminated, as during freezing and frozen storage at a comparatively low temperature, the microbes would be in dormant state (Coombs *et al.*, 2017). Vieira *et al.* (2009) reported that beef frozen for up to 90 days after ageing for 3 and 10 days, did not spoil due to microbes, and aerobic lactic acid bacteria were not affected significantly and enteric bacteria were even not detected in any treatment, although the levels of psychrotrophic bacteria showed an increase simultaneously.

As for HPF, some studies reported that high hydrostatic pressure (HHP) could reduce bacterial counts effectively. Fernández *et al.* (2007) reported that a significant decrease for lactic

acid bacteria ($>$ 2.4 $\log_{10}$ cycles) and aerobic total count ($>$ 2 $\log_{10}$ cycles), while Enterobacteriaceae counts below the detection limits of beef samples after HHP treatment (650 MPa, 10 min) were observed for unfrozen samples at 20 °C and frozen samples at –35 °C. Meanwhile, Realini *et al.* (2011) also revealed that Enterobacteriaceae counts of frozen cured pork carpaccio was below the detection limit, although the frozen treatments (400 MPa and 600 MPa/−15 °C and −35 °C/6 min) could not eliminate lactic acid bacteria and psychrotrophs completely. The lower counts observed in these trials may be associated with the decreased water activity of the frozen samples, the inactivation caused by pressure and phase change of water to ice. These results confirm that HHP is effective in hindering the growth and breeding of microbe thus prolonging shelf life of meat and meat derived products. Although many factors would affect the survival of microorganisms during freezing, such as freezing rate, changes of water, composition of freezing matrix, etc. (Archer, 2004), the survival of microorganisms is also affected by different freezing methods. Literature on the microbial count and shelf-life post novel freezing methods is rare for all kinds of meats and most related investigations did not take microbiological analysis as a quality index. Therefore, there is a need for further study to better understand the interaction between novel freezing methods and microorganisms.

6. Conclusions

This review discusses the effects of some innovative freezing processes, including high pressure freezing, electrically and magnetically assisted freezing, and antifreeze protein, on the quality attributes of meats including fish meat and derived products. The general purpose of

these novel freezing methods is to improve the quality of frozen foods and further upgrade their commercial values. In fact, all of these mentioned methods have more or less positive effects on meat samples especially on controlling ice crystals. From the micro perspective, novel freezing methods are able to create smaller, more homogeneous and regular ice crystals, compared with the traditional freezing technologies, such as air blast freezing, cryogenic freezing and so on. The improved sizes and distribution of ice crystals can undoubtedly preserve better microstructure, thus improving related physicochemical properties of these frozen muscle tissues.

Meanwhile, proteins are the most important component in muscle tissues and their states also have direct effects on the quality of muscle tissues. Among the novel freezing technologies discussed, the effects of HPSF on frozen muscle tissues are complex, as based on its ability in controlling the crystallization process, HPSF can improve the sizes and distribution of ice crystals, and thus ensure the intact of tissue structure with little drip loss of frozen muscle tissues. However, the protein denaturation caused by high pressure during freezing process also possesses some disadvantages such as lower tenderness, discoloration and aggravation in lipid and protein oxidation. The balance among the positive and negative effects is closely related to temperature, pressure level and processing time. For other novel technologies, information

available up to now all indicates positive impacts on the quality of frozen muscle tissues with little negative effects on proteins, especially some methods such as electrically assisted freezing or the use of antifreeze protein mainly affect water molecules. Although these novel freezing methods may have great prospect for the meat industry, much more studies and confirmation experiments should be performed.

In addition, it was found that the types of frozen meats in published literatures are not diverse, which mainly focus on pork (the typical meat model) and some aquatic products (commonly used in HPSF), this may be owing to that most researches related to meats are on high pressure freezing, especially the HPSF, which has a long history of research activities. Other techniques such as ultrasound, electromagnetic wave, electrostatic field and antifreeze protein are still at the developing stage, and thus significant efforts should be made and more studies should be carried out to better understand their mechanisms and promote their applications in the food industry.

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Table 1. Main studies on the effect of high pressure freezing for meat drip loss

Food	Conditions	Results	References
Pork and beef	HPSF at 200 MPa (−20 °C)	Samples exhibiting high DL as compared with ABF (pork samples: from 20.2 % for ABF to 31.2 % for HPSF; beef samples: from 25.0 % for ABF to 28.1% for HPSF)	Fernández-Martín *et al.* (2000)
Turbot	HPSF at 140 MPa (−14 °C) and storage at −20 °C	Increase in drip loss after storage as compared with ABF	Chevalier *et al.* (2000a)
Pork	HPSF at 100 MPa (−11 °C), 150 MPa (−16.5 °C), and 200 MPa (−21.5 °C)	Significant decrease ($p < 0.05$) in DL as compared with ABF and LIF (from 0.28 g/g for ABF and 0.29 g/g for LIF to 0.14 g/g at 100 MPa, 0.13 g/g at 150 MPa and 0.11 g/g at 200 MPa, respectively)	Zhu *et al.* (2004b)
Carp	HPSF at 140 MPa (−14 °C) and storage at −20 °C	Significant decrease ($p < 0.05$) in DL after storage as compared with ABF	Sequeire‐Munoz *et al.* (2005)
Sea bass	HPSF at 200 MPa (−18 °C)	Significant decrease ($p < 0.05$) in WHC as compared with ABF (from 97.05% for ABF to 57.24% for HPSF)	Tironi *et al.* (2007)
Beef	ABF plus high pressure at 650 MPa (−35 °C) for 10 min	Salt-added samples showing significantly lower DL values than salt-free samples (from 4.80% for salt-free samples to 2.35% salted samples)	Fernández *et al.* (2007)
Sea bass	HPSF at 200 MPa (−18 °C) and storage at −15 °C and −25 °C	Decrease in WHC after storage as compared with ABF	Tironi *et al.* (2010)

Note: HPSF = high pressure shift freezing, ABF = air blast freezing, LIF = liquid immersion freezing, WHC = water holding capacity, DL = drip loss.

Food	Conditions	Results	References
Norway lobster	HPSF at 200 MPa (−18 °C)	Significant increase ($p < 0.05$) in WBSF values as compared with ABF (from 5.1×10^4 N/m^2 for ABF to 10.3×10^4 N/m^2 for HPSF)	Chevalier, *et al.* (2000c)
Pork and beef	HPSF at 200 MPa (−20 °C)	Significant increase ($p < 0.05$) in WBSF2 values (shear force at the first tip) as compared with ABF	Fernández-Martín *et al.* (2000)
Pork	HPSF at 100 MPa (−11 °C), 150 MPa (−16.5 °C), and 200 MPa (−21.5 °C)	Significant increase ($p < 0.05$) in WBSF values as compared with ABF and LIF (from 23.3 N cm^{-2} for ABF and 25.3 N cm^{-2} for LIF to 38.7 N cm^{-2} at 100 MPa, 49.2 N cm^{-2} at 150 MPa and 53.5 N cm^{-2} at 200 MPa, respectively)	Zhu *et al.* (2004b)
Carp	HPSF at 140 MPa (−14 °C) and storage at −20 °C	No significant difference ($p > 0.05$) between HPSF and ABF samples	Sequeire‐Munoz *et al.* (2005)
Beef	ABF plus high pressure at 650 MPa (−35 °C) for 10 min	No significant difference ($p > 0.05$) in WBSF values with different treatments	Fernández *et al.* (2007)
Cured pork carpaccio	Combinations of pressure (0 MPa, 400 MPa, and 600 MPa) and freezing temperature (−15 °C vs −35 °C) applied for 6 min	Significant increase ($p < 0.05$) in WBSF values as compared with control	Realini *et al.* (2011)

Table 2. Main studies on the effect of high pressure freezing for meat tenderness

Note: HPSF = high pressure shift freezing, ABF = air blast freezing, LIF = liquid immersion freezing, WBSF = Warner-Bratzler shear force.

Table 3. Main studies on the effect of high pressure freezing for meat colour

Food	Conditions	Results			References
		L*	a*	b*	
Pork and beef	HPSF at 200 MPa (−20 °C)	Significant increase ($p < 0.05$)	Significant increase ($p < 0.05$)	Significant increase ($p < 0.05$)	Fernández-Martín *et al.* (2000)
Turbot	HPSF at 140 MPa (−14 °C) and storage at −20 °C	Significant increase ($p < 0.05$)	Significant decrease ($p < 0.05$)	Significant increase ($p < 0.05$)	Chevalier *et al.* (2000b)
Pork	HPSF at 200 MPa (−30 °C)	Significant increase ($p < 0.05$)	Significant decrease ($p < 0.05$)	Significant decrease ($p < 0.05$)	Hansen *et al.* (2003)
Pork	HPSF at 100 MPa (−11 °C), 150 MPa (−16.5 °C), and 200 MPa (−21.5 °C)	Significant increase ($p < 0.05$)	Significant decrease ($p < 0.05$) at 150 MPa or higher	No significant difference	Zhu *et al.* (2004b)
Sea bass	HPSF at 200 MPa (−20 °C)	Significant increase ($p < 0.05$)	Decrease but not significantly ($p > 0.05$).	Significant increase ($p < 0.05$)	Tironi *et al.* (2007)
Beef	ABF plus high pressure at 650 MPa (−35 °C) for 10 min	No significant difference in salt-added samples	Significant decrease ($p < 0.05$) in salt-added samples	No significant difference in salt-added samples	Fernández *et al.* (2007)
Sea bass	HPSF at 200 MPa (−20 °C) and storage at −15 °C and −25 °C	Significant increase ($p < 0.05$)	No significant difference after storage	Be increased after storage	Tironi *et al.* (2010)

Note: HPSF = high pressure shift freezing, ABF = air blast freezing.

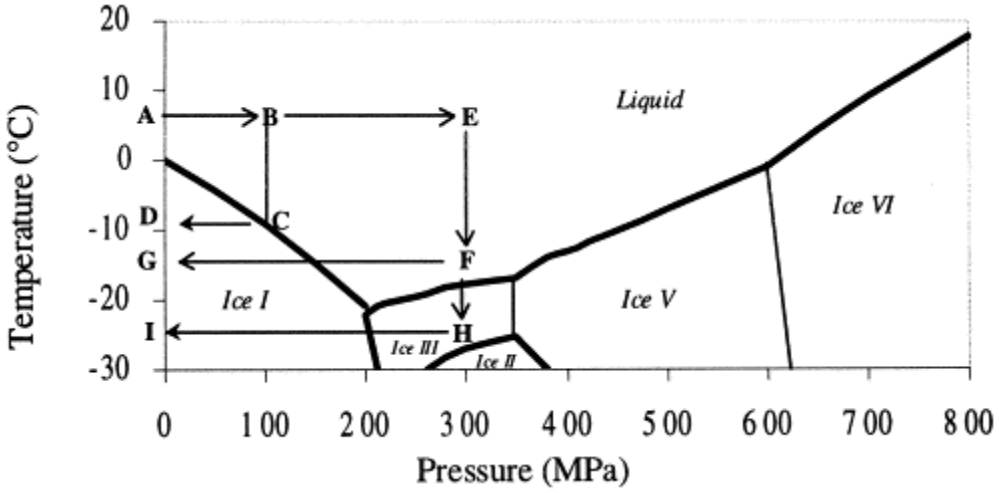

Fig. 1. Phase diagram of water (ABCD for pressure assisted freezing, ABEFG for pressure shift freezing, and ABEFHI for pressure induced freezing) (Cheng *et al.*, 2015).

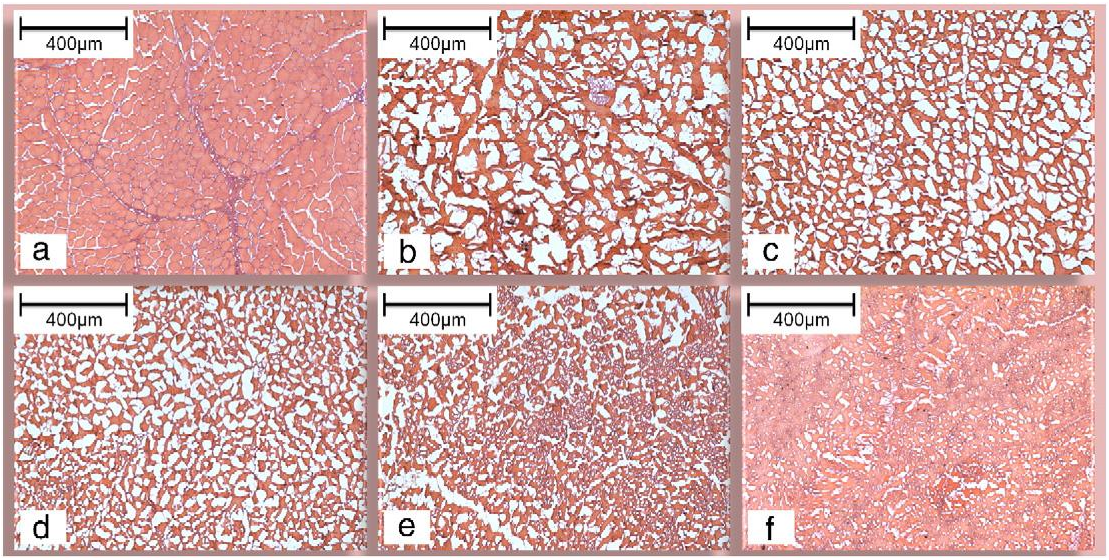

Fig. 2. Micrograph images of frozen pork tenderloin transversal cuts under different magnitudes of static electric fields. (a) Fresh meat, (b) 0 kV, (c) 3 kV, (d) 6 kV, (e) 9 kV, (f) 12 kV (Xanthakis *et al.*, 2013).

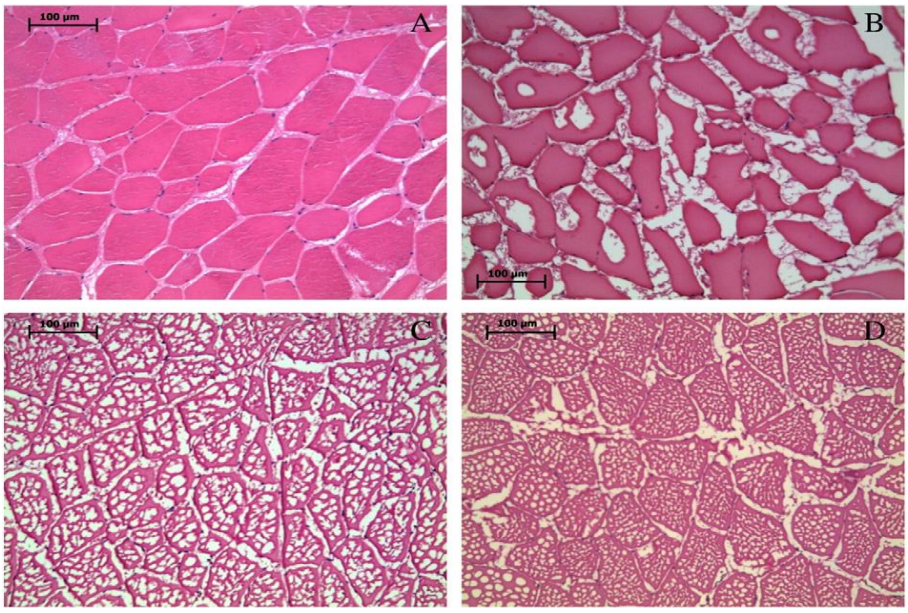

Fig. 3. Micrographs of meat frozen under different conditions. A: control (unfrozen meat); B: air freezing; C: cryo-freezing; D: radio frequency cryo-freezing (Anese *et al.*, 2012).

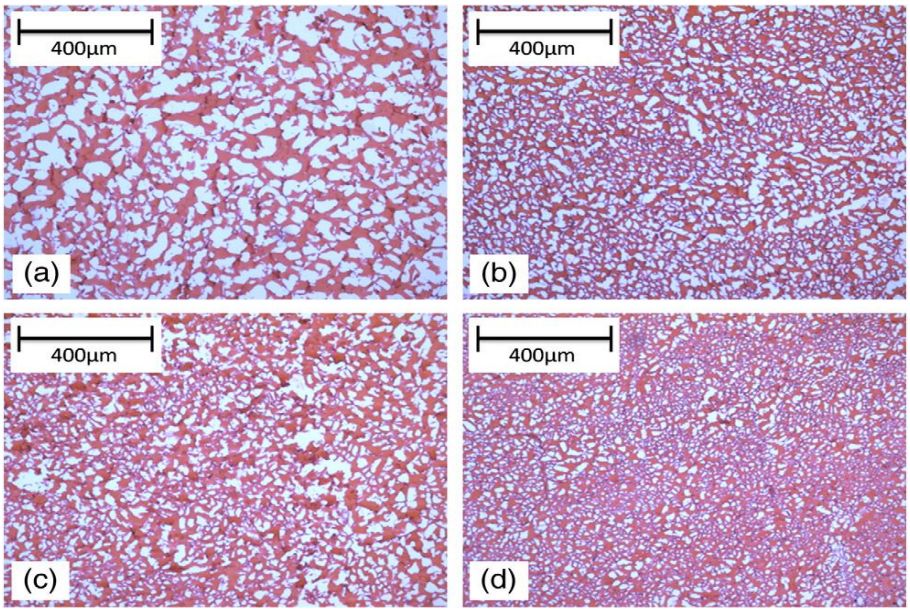

Fig. 4. Micrograph images of frozen pork tenderloin transversal cuts under different levels of microwave power radiation. (a) 0% (conventional freezing), (b) 40%, (c) 50% and (d) 60% (Xanthakis *et al.*, 2014b).