



# Thermal characterization and ice crystal analysis in pressure shift freezing of different muscle (shrimp and porcine liver) versus conventional freezing method

Guangming Su<sup>a</sup>, Hosahalli S. Ramaswamy<sup>b</sup>, Songming Zhu<sup>a,\*</sup>, Yong Yu<sup>a</sup>, Feifei Hu<sup>a</sup>, Menglong Xu<sup>a</sup>

<sup>a</sup> College of Biosystems Engineering and Food Science, Zhejiang University, Key Laboratory of Equipment and Informatization in Environment Controlled Agriculture, Ministry of Agriculture, 866 Yuhangtang Road, Hangzhou, 310058, PR China

<sup>b</sup> Department of Food Science and Agricultural Chemistry, McGill University, Montreal H9X3V9, Canada

## ARTICLE INFO

### Article history:

Received 10 December 2013

Accepted 7 May 2014

Available online 20 May 2014

Editor Proof Receive Date 13 June 2014

### Keywords:

Pressure shift freezing

Ice crystal

Microstructure

Shrimp

Porcine liver

## ABSTRACT

In order to investigate the application of pressure shift freezing (PSF) on different meat products, fresh shrimp and porcine liver were frozen by PSF at 100 MPa ( $-8.4^{\circ}\text{C}$ ), 150 MPa ( $-14^{\circ}\text{C}$ ) and 200 MPa ( $-20^{\circ}\text{C}$ ), as well as by conventional air freezing (CAF) at  $-20^{\circ}\text{C}$  and liquid immersion freezing (LIF) at  $-20^{\circ}\text{C}$ . Temperature profile of test samples were recorded during different freezing processes and phase transformations were calculated. Microstructures of the formed ice crystals were analyzed for cross-section area, equivalent diameter, roundness and elongation. Size, shape and distribution of ice crystals were estimated and compared in different freezing processes. Results clearly showed that PSF is the more advantageous method. The phase transition times (2.97, 1.53 and 1.1 min in shrimp, and 2.47, 1.22 and 0.83 min in porcine liver, when subjecting to 100, 150 and 200 MPa PSF treatments, respectively) induced from the super-cooling attained in PSF treatments was much shorter than that in CAF (148 min in shrimp and 85 min in porcine liver) and LIF (5.9 and 5.5 min in shrimp and porcine liver, respectively). Small, regular and homogeneously distributed ice crystals were obtained in PSF treatments, especially at a higher pressure. The mean cross-section area of ice crystals formed in frozen shrimp was 2852, 1364 and 597  $\mu\text{m}^2$  for PSF treatments at 100, 150, and 200 MPa, respectively, as compared with 151, 100 and 92  $\mu\text{m}^2$  for frozen samples of porcine liver, respectively. The roundness of ice crystals in shrimp formed in PSFs was 0.87, 0.90 and 0.85, respectively, while the roundness of ice crystals in porcine liver was 0.87, 0.90 and 0.89, respectively. The size of ice crystals formed in PSF is pressure dependent, mainly due to a large super-cooling were created in a higher pressure of PSF. However, pressure does not play a major role in the shape of ice crystals. CAF created larger and irregular ice crystals due to the slow freezing rate, and LIF generated relatively smaller but irregular ice crystals, both of which resulted in irreversible damage to muscle tissue and caused degradation of product value. The results achieved in this research provides a better understanding of PSF process of shrimp and porcine liver, and PSF was proved as a promising freezing method for producing frozen products.

**Industrial relevance:** This paper compares different freezing methods and their effect on thermal behavior and microstructure of meat muscle. Pressure shift freezing proved to be superior to conventional freezing regarding high degree of super-cooling, short phase transition time, and small, regular ice crystals. Different meat muscles, including shrimp and porcine liver, were also compared to investigate the effect of PSF on thermal behavior and microstructure. Interestingly, the ice crystals in porcine liver were much smaller than those in shrimp. Results obtained in the paper provide a better understanding of the PSF process and suggest that PSF is a superior method for freezing shrimp and porcine liver.

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## 1. Introduction

The quality of frozen products is highly influenced by the size and quantity of ice crystals formed during the freezing process (Fennema, 1966; Zhu, Ramaswamy, & Le Bail, 2005). For most foods, formation of small ice crystals is critical to minimize texture degradation and

organoleptic quality losses (Martino, Otero, Sanz, & Zaritzky, 1998; Zhu, Bail, & Ramaswamy, 2003). It has been recognized that the freezing rate is critical to the nucleation and growth of ice crystals (Kiani & Sun, 2011). Nucleation is an activated process driven by the degree of super-cooling. The number of nuclei formed is directly proportional to the extent of super-cooling reached in the sample before nucleation (Burke, George, & Bryant, 1975; Otero, Sanz, Guignon, & Aparicio, 2009). Burke et al. (1975) reported that for each degree Celsius enhancement of super-cooling, there is about a ten-fold increase in the ice nucleation

\* Corresponding author. Tel./fax: +86 571 88982373.  
E-mail address: [zhusm@zju.edu.cn](mailto:zhusm@zju.edu.cn) (S. Zhu).

rate. Ice crystal growth takes place after nucleation, requiring only a minimum of subsequent super-cooling (Fennema, 1973). Ice crystal size and distribution throughout a sample depend both on the number of nuclei formed in the earlier phase of the freezing process and on the rate of subsequent crystal growth, which influences the final shape and size of the crystals (Cheftel, Thiebaud, & Dumay, 2002; Fernández, Otero, Guignon, & Sanz, 2006). Therefore, the knowledge of freezing rate and its relationship to the nature and size of ice crystal formation are critical for the understanding of freezing phenomenon affecting the quality of frozen food.

It is generally accepted that slow freezing results in the formation of large intercellular ice crystals while rapid freezing results in fine intracellular ice crystal in frozen foods (Zhu, Ramaswamy, & Simpson, 2004; Zhu et al., 2003). However, in traditional rapid freezing, e.g. cryogenic freezing, intensive nucleation and numerous intracellular ice crystals are induced, but it may cause mechanical cracking of frozen foods (Kalichevsky, Knorr, & Lillford, 1995; Kim & Hung, 1994).

In the last decade, pressure shift freezing (PSF) has drawn the attention of many food researchers, mainly due to its potential for improving the kinetics of freezing and the characteristics of the ice crystals formed (Alizadeh, Chapleau, de-Lamballerie, & Le-Bail, 2009; Castro, Van Loey, Saraiva, Smout, & Hendrickx, 2007; Otero, Sanz, Guignon, & Sanz, 2012; Shim, Hong, Choi, & Min, 2009; Tironi, de Lamballerie, & Le-Bail, 2010; Tironi, Le Bail, & De Lamballerie, 2007; Van Buggenhout, Messagie, Van Loey, & Hendrickx, 2005; Volkert, Puaud, Wille, & Knorr, 2012; Zhu, Ramaswamy, & Le Bail, 2006). PSF has been investigated as an alternative method to the existing freezing processes (Norton & Sun, 2007). The PSF process is based on the principle of water–ice phase transition under pressure, elevated pressure depresses the freezing point of water from 0 °C to −21 °C at 210 MPa (Bridgman, 1912). The PSF process includes several steps (Fig. 1). After sample installation, pressure is increased (AB in Fig. 1) up to the target level. After this, test sample is cooled under pressure until the temperature reaches the desired level (BC in Fig. 1). Then the pressure is rapidly released (CD in Fig. 1). Sample temperature initially decreases, but when the difference between the sample temperature and that corresponding to the current pressure becomes too large, a sudden temperature rise occurs due to latent heat release by ice nucleation. Numerous nuclei produced during this stage (CD in Fig. 1) grow into a massive number of small ice crystals during stage DF (Fig. 1) at atmospheric pressure. Freezing is completed during the stage FG (Fig. 1) at atmospheric pressure.

In recent years, several studies have been carried out on the PSF formed ice crystals in different frozen muscles, such as Atlantic salmon (*Salmo salar*) (Zhu et al., 2003), pork (Martino et al., 1998), sea bass (*Dicentrarchus labrax*) (Tironi et al., 2010), Norway lobster (Chevalier, Sentissi, Havet, & Bail, 2000), and turbot (*Scophthalmus maximus*) (Chevalier, Sequeira-Munoz, Le Bail, Simpson, & Ghoul, 2000). These

studies generally demonstrate that PSF treated muscles had better preserved microstructure compared to the conventional methods. Ice crystal formed in PSF process is fine and uniform throughout the food, which is mainly due to the ice nuclei being formed instantaneously and homogeneously throughout the whole volume of the product resulting from the high super-cooling reached on pressure release (Martino et al., 1998; Otero, Martino, Zaritzky, Solas, & Sanz, 2000; Sanz, Otero, De Elvira, & Carrasco, 1997; Zhu et al., 2004). From the microstructural point of view, damage to cells in the PSF sample is minimized because of the small size of the ice crystals, resulting in a significant improvement of product quality (Chevalier, Le Bail, & Ghoul, 2000; Fuchigami & Teramoto, 1997; Martino et al., 1998; Zhu et al., 2003). Therefore, PSF can be useful to freeze foods especially with large dimensions in which a uniform ice crystal distribution is required and where thermal gradients are pronounced and damage of freeze-cracking would be possible when applying classic freezing methods, including cryogenic freezing (Martino et al., 1998; Otero et al., 2000).

However, there are still many scientific frontiers that need to be investigated, such as limited understanding of physical properties of different muscles when subjecting them to PSF process. Shrimp is a high-value commodity, the behavior of the tiny muscle fibers is susceptible to physical suppression induced by ice crystals formed in freezing process, and it was concluded that the effect of traditional freezing methods could cause severe cell disruption and structural muscle damage (Boonsumrej, Chaiwanichsiri, Tantratian, Suzuki, & Takai, 2007; Sriket, Benjakul, Visessanguan, & Kijroongrojana, 2007). But very few studies have been done on the effect of PSF on shrimp muscle. As well as porcine liver, no published literature in the PSF field has been found. The tissue structure of porcine liver is largely different from above mentioned muscles, featuring deep interlobular fissures and a large amount of interlobular connective tissue, and microsomes constitute the main structural functional units. The investigation of ice crystal formation in freezing processes of porcine liver is critical for the understanding of freezing phenomenon affecting the tissue structure of frozen porcine liver.

Therefore, the objective of this paper was to study the ice crystal formation in shrimp muscle and porcine liver during PSF processes. Temperature and phase transformational changes were monitored in the freezing processes and the ice crystal microstructure (size, location and distribution) were analyzed as compared to classic freezing methods (conventional air freezing and liquid immersion freezing).

## 2. Materials and methods

### 2.1. Chemicals

Ethanol, chloroform, glacial acetic acid, 1-butanol, toluene and paraffin were bought from Sigma. Bright green powder, R.A.L. vert lumière haute pureté, Templemars (France), and mounting medium EUKITT (O. Kindler Co., Germany) were bought from a chemical agency (Fisher Scientific). All chemicals used in this study were of reagent grade.

### 2.2. Sample preparation

Fresh giant shrimp (*Penaeus monodon*) and porcine liver (24–36 h after slaughter) were purchased from local suppliers. Cylindrical samples were cut along the connective tissue using a cylindrical cutter. About 10 g samples were placed in a moisture-impermeable plastic tube (12 mm in diameter, and 75 mm in length) and sealed with plastic plug on the top. Three samples of both shrimp and porcine liver were prepared for further treatments. One sample was prepared for temperature recording and the other two samples were for microstructure analysis. All prepared samples were stored in a cooler (4 °C) before freezing treatment. With regard to samples for temperature recording, one K-type thermocouple (0.3 mm diameter, Omega, Stamford, CT) was installed at the middle region of each sample.

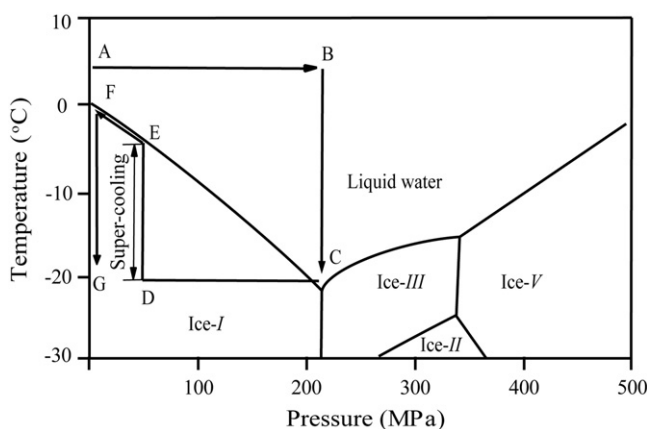


Fig. 1. Water phase transition under pressure and the theory of pressure shift freezing (PSF).

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