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Effects of high pressure freezing (HPF) on denaturation of natural actomyosin extracted from prawn (*Metapenaeus ensis*)

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ABSTRACT

Effects of protein denaturation caused by high pressure freezing, involving Pressure-Factors (pressure, time) and Freezing-Factors (temperature, phase transition, recrystallization, ice crystal types), are complicated. In the current study, the conformation and functional changes of natural actomyosin (NAM) under pressure assisted freezing (PAF, $^{100,150,300,400,500\ MPa}P_{-20\ °C/25\ min}$), pressure shift freezing (PSF, $^{200\ MPa}P_{-20\ °C/25\ min}$), and immersion freezing ($^{0.1MPa}P_{-20\ °C/5\ min}$) after pressure was released to 0.1 MPa, as compared to normal immersion freezing process (IF, $^{0.1\ MPa}P_{-20\ °C/30\ min}$). Results indicated that PSF ($^{200\ MPa}P_{-20\ °C/30\ min}$) could reduce the denaturation of frozen NAM and a pressure of 300 MPa was the critical point to induce such a denaturation. During the periods of B → D in PSF or B → C → D in PAF, the generation and growth of ice crystals played an important role on changing the secondary and tertiary structure of the treated NAM.

1. Introduction

Like drying (Cui, Sun, Chen, & Sun, 2008; Sun & Woods, 1994) and cooling (Hu & Sun, 2000; Mc Donald & Sun, 2001; Sun, 1997; Sun & Brosnan, 1999; Sun & Hu, 2003; Sun & Wang, 2000; Wang & Sun, 2002a,b, 2004; Zheng & Sun, 2004), freezing (Cheng, Sun, & Pu, 2016; Kiani, Zhang, Delgado, & Sun, 2011; Ma et al., 2015; Xie, Sun, Xu, & Zhu, 2015) is commonly used to maintain the quality of perishable foods in the food industry. During the freezing process, denaturation of protein occurs, which can impact on the properties of protein-based food, including water-holding capacity, texture, gelation and color (Huang, Liu, Xia, Kong, & Xiong, 2015). Ice crystals are widely regarded as a crucial factor in affecting the conformation of protein and controlling the quality of frozen protein-based food (Cheng, Sun, Zhu, & Zhang, 2017; Zhang, Sun, Zhu, & Cheng, 2015).

High pressure freezing (HPF) is a novel freezing technique, which has the potential to manage the formation and distribution of ice crystals and thus improve the quality of frozen foods (Cheng, Sun, Zhu, & Zhang, 2017). HPF can be divided into pressure assisted freezing (PAF), pressure shift freezing (PSF) and pressure induced freezing (PIF) (Choukroun & Grasset, 2007), according to the different crystallization paths and ice crystal types (Fig. 1), which is the focus in most of the investigations of HPF, including formation (Smith, Burlakov, & Ramos, 2013; Zhu, Ramaswamy, & Le Bail, 2005a), distribution (Otero, Sanz, Guignon, & Aparicio, 2009; Zhu, Ramaswamy, & Le Bail, 2005b) and their effects on frozen food quality (Alizadeh, Chapleau, de-Lamballerie, & Le-Bail, 2009; Bulut, 2014; Fernández et al., 2007; Su et al., 2014).

Pressure and temperature as the two basic parameters during HPF process, can not only synergistically impact the water and control the crystallization paths and ice crystal types, but can also induce the denaturation of protein. For example, actomyosin from tilapia was aggregated at a pressure of 200 MPa and 4 °C due to the formation of hydrogen and disulphide bonds (Hsu, Hwang, Yu, & Jao, 2007). Baier, Purschke, Schmitt, Rawel, and Knorr (2015) observed distinct changes in the secondary structure of whey proteins while the tertiary structure remained unchanged at 500 MPa (−15/−35 °C). In addition, Volkert, Puaud, Wille, and Knorr (2012) noticed a slight denaturation (about 3%) in milk protein treated by HPF (300 MPa, −16 °C), leading to a change in the sensory property

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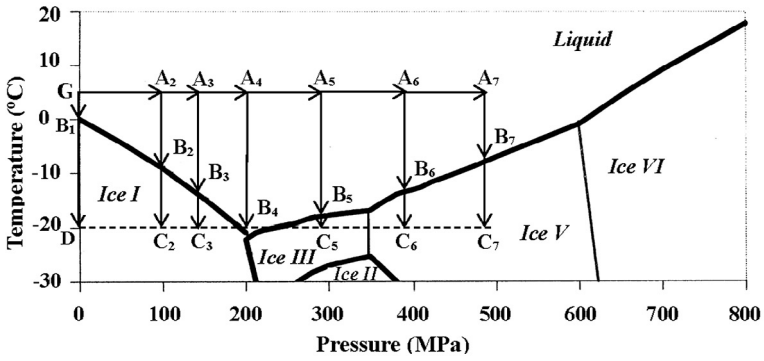
(i)



(ii)

No. (groups)	Treatment	Process	Phase transiting	Category
0	untreated	-	-	-
1	$^{0.1MPa}P_{-20°C/30min}$	$G \rightarrow B_1 \rightarrow D$	$L \rightarrow L + S(I) \rightarrow S(I)$	IF
2	$^{100MPa}P_{-20°C/30min}$	$G \rightarrow A_2 \rightarrow B_2 \rightarrow C_2 \rightarrow D$	$L \rightarrow L+S(I) \rightarrow S(I)+S(I)$	PAF
3	$^{150MPa}P_{-20°C/30min}$	$G \rightarrow A_3 \rightarrow B_3 \rightarrow C_3 \rightarrow D$	$L \rightarrow L+S(I) \rightarrow S(I)+S(I)$	PAF
4	$^{200MPa}P_{-20°C/30min}$	$G \rightarrow A_4 \rightarrow B_4 \rightarrow D$	$L \rightarrow S(I)$	PSF
5	$^{300MPa}P_{-20°C/30min}$	$G \rightarrow A_5 \rightarrow B_5 \rightarrow C_5 \rightarrow D$	$L \rightarrow L+S(III) \rightarrow S(III)+S(I)$	PAF
6	$^{400MPa}P_{-20°C/30min}$	$G \rightarrow A_6 \rightarrow B_6 \rightarrow C_6 \rightarrow D$	$L \rightarrow L+S(V) \rightarrow S(V)+S(I)$	PAF
7	$^{500MPa}P_{-20°C/30min}$	$G \rightarrow A_7 \rightarrow B_7 \rightarrow C_7 \rightarrow D$	$L \rightarrow L+S(V) \rightarrow S(V)+S(I)$	PAF

(iii)

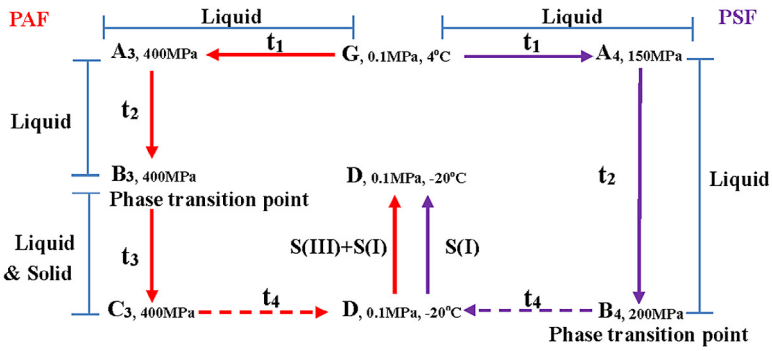


Fig. 1. Process chart of high pressure freezing of prawn natural actomyosin with temperature lowered to −20 °C and pressure elevated to 0.1, 100, 150, 200, 300, 400, 500 MPa, respectively. **G**: starting point at 4 °C, 0.1 MPa; $\mathbf{A_{n=2-7}B_{n=1-7}}$: **A**, elevated pressure to the designed value. **B**, phase transition point; C, released pressure after reaching the desired time; **D**: terminal point; S: solid; L: liquid; +: mixture; I, III, V were the types of ice crystals; IF, normal immersion freezing; PAF, pressure assisted freezing; PSF, pressure shift freezing. Solid line: elevating pressure process and maintaining pressure process; Dash line: releasing pressure instantly. t_1, elevating pressure time (3 MPa/s); t_2, surpercooling period; t_3, phase transiting to designed constant pressure time; t_4, releasing pressure time (1–2 s); t_5, immersion after pressure released (5 min); $t_2 + t_3 = 25$ min.

of the milk protein. However, there is no general pattern of protein denaturation under pressure (Silva et al., 2014), especially in cold conditions.

Cold-denaturation is caused by absorption to the growing ice crystals (leading to the dehydration of protein or the destruction of its network structure) and/or the concentration effects (Dumay, Picart, Regnault, & Thiebaud, 2006); and generally includes intermolecular cross-linking between adjacent protein molecules, reduction of Ca^{2+}-ATPase activity and of surface hydrophobicity (Paredi & Crupkin, 2007; Wang et al., 2014; Wu, Wang, Luo, Hong, & Shen, 2014). The mechanism of pressure-denaturation can be described by the Chatelier's principle (Baier et al., 2015), in which pressure decreases the volume of protein, resulting in the unfolding of globular proteins. Schade, Rudolph, Luedemann, and Jaenicke (1980) reported a large negative volume change (500 mL·mol^{-1}) resulting from the dissociation of oligomeric proteins, leading to a change of protein conformation. Changes in protein molecules can occur in covalent bonds (primary structure), hydrogen bonds (secondary structure), electrostatic interactions and hydrophobic interactions (tertiary structure). The change of electrostatic interactions and hydrophobic interactions would cause considerable volume increase (10–20 mL·mol^{-1}), while the volume variation caused by hydrogen bonds and covalent bonds is very small (+1 to −3 mL·mol^{-1}) (Baier et al., 2015). As such, the predominant effect of pressure on protein is on the higher conformation which is maintained by non-covalent bonds.

Protein denaturation caused by the combination of low temperature and high pressure during HPF is complicated and controversial (Bianco & Franzese, 2015; Marqués, Borreguero, Stanley, & Dokholyan, 2003) because it is affected by Pressure-Factors (pressure and time) and Freezing-Factors (temperature, phase transition, recrystallization, and ice crystal types). Furthermore, protein denaturation under the HPF process is more complex than that induced by the normal immersion freezing process at atmospheric pressure, requiring more research to elucidate the relevant mechanisms.

Actomyosin, consisting of myosin and actin, is the main protein component of seafood meat (Benjakul, Visessanguan, Ishizaki, & Tanaka, 2001) including prawn. Although the actomyosin denaturation has been considered by some previous studies (Hsu et al., 2007; Ko, Jao, & Hsu, 2003; Zhou et al., 2014), these