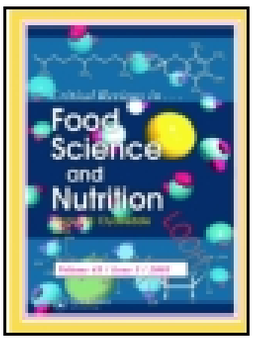

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Critical Reviews in Food Science and Nutrition

ISSN: 1040-8398 (Print) 1549-7852 (Online) Journal homepage: http://www.tandfonline.com/loi/bfsn20

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To cite this article: Xing Chen, Ronald Keith Tume, Youling Xiong, Xinglian Xu, Guanghong Zhou, Conggui Chen & Tadayuki Nishiumi (2017): Structural modification of myofibrillar proteins by high-pressure processing for functionally improved, value-added and healthy muscle gelled foods, Critical Reviews in Food Science and Nutrition, DOI: 10.1080/10408398.2017.1347557

To link to this article: http://dx.doi.org/10.1080/10408398.2017.1347557

Accepted author version posted online: 05 Jul 2017.

Structural modification of myofibrillar proteins by high-pressure processing for functionally improved, value-added and healthy muscle gelled foods

Xing Chen[a,c], Ronald Keith Tume[b], Youling Xiong[c], Xinglian Xu[a,*], Guanghong Zhou[a], Conggui Chen[d] and Tadayuki Nishiumi[e]

[a]Key Laboratory of Animal Products Processing, Ministry of Agriculture, Key Laboratory of Meat Processing and Quality Control, Ministry of Education, Jiangsu Synergetic Innovation Center of Meat Production and Processing, and College of Food Science and Technology, Nanjing Agricultural University, Nanjing, Jiangsu 210095, China

[b]Honorary Visiting Professor, Nanjing Agricultural University, Jiangsu 210095, China

[c]Department of Animal and Food Sciences, University of Kentucky, Lexington, Kentucky 40546, United States

[d]School of Food Science and Engineering, Hefei University of Technology, Hefei, Anhui 230009, China

[e]Graduate School of Science and Technology, Niigata University, Niigata 950-2181, Japan

[*]Corresponding author, Telephone: +86 25 843 95730. Fax: +86 25 843 95939. *E-mail address*: xlxus@njau.edu.cn (X. Xu)

Abstract: The texture, yield and organoleptic properties of comminuted meat products are closely related to the structure and functionality of myofibrillar proteins (MP). To enhance functional properties of MP, high hydrostatic pressure (HHP) has been widely utilized to modify the structure of MP through protein denaturation, solubilization, aggregation or gelation. This modification depends on the protein system (specie, type and formulation) and HHP condition (pressure intensity, pressurizing gradient, duration time, temperature, pressure/temperature and the sequence of application). However, there remains a lack of a systematic summary of structural changes and structure-function relationship of MP in response to various HHP conditions. Hence, this review first explored the profound knowledge on the structural and functional changes of MP induced by HHP based on previous works and recent progress. Second, to meet the growing demand for economical, nutritional and healthy meat products, recent applications of HHP on the manufacture of low salt, low phosphate and/or low fat gel-type meat products, as well as value-added and texture-modified meat products were highlighted. Finally, future considerations were presented to facilitate progress in this area and to enable HHP as an efficient strategy in tailoring the manufacture of functionally improved, value-added and healthy muscle gelled foods.

Keywords

Myofibrillar protein; High pressure; Structure; Functionality; Gel property; Healthy meat products.

■ Abbreviations

MP myofibrillar proteins

HP high pressure

HPP high pressure processing

HHP high hydrostatic pressure

HPH high-pressure homogenization

HDP hydrodynamic pressure processing

WHC water holding capacity

CL cooking loss

NaCl sodium chloride

KCl potassium chloride

S-1 subfragment-1

G' storage modulus

DSC differential scanning calorimetry

△Henthalpy

STPP sodium tri-polyphosphate

NMR nuclear magnetic resonance

SEM scanning electron microscopy

TEM transmission electron microscopy

DSC differential scanning calorimetry

FTIR fourier transform infrared

UV ultraviolet

P+Tcombined pressure/thermal

PATS pressure assisted thermal sterilization

TG transglutaminase

KC κ-carrageenan

SA sodium alginate

XG xanthan gum

CMC carboxymethyl cellulose

LBG locust bean gum

PG pressure gradient

PSEpale, soft, exudative

TMF texture-modified food

1 Introduction

The structural and physicochemical properties of myofibrillar proteins (MP) directly affect their functionality and are essential for the manufacture of high quality comminuted meat products (Xiong 1997). MP play an important role in determining the quality of muscle products because they impart textural attributes and provide functional properties such as gel-forming abilities, emulsifying properties, and water-holding abilities (Qiu et al., 2014; Sun and Holley 2011). The presence of high concentrations of salt (2%-3%) favors the extraction of MP, which enables the protein-sol matrix to emulsify and disperse any fat component present to form a 3-dimensional network structure that holds water and fat in a less mobile state (Sikes et al., 2009; Colmenero 2002). Heat treatment is currently the main method for inducing the gelation of MP. Factors including pH, ionic strength, rates of heating, protein oxidation, food additives influence the structural, functional and heat-induced gelation properties of MP, and they have been progressively summarized by Asghar et al., (1985), Xiong (1997) and Sun and Holley (2011). These factors will influence the yield, texture, cohesion and sensory acceptability of the final product. Attempts towards a better understanding of the basis for the conformational, physicochemical and functional changes of MP would be of benefit for the manufacture of more economical, nutritional and healthy processed meat products.

High pressure processing (HPP) is a technology by which a product is treated at or above 100 MPa by means of a compression fluid. This non-thermal technology can be used to create new

products without thermal degradation, or to obtain analogue products with minimal effects on flavor, color, and nutritional value (Simonin et al., 2012). HPP can be applied under three different forms: a static process in a vessel known as high hydrostatic pressure (HHP), a dynamic process in which a fluid is forced through a nozzle jet under HP known as high pressure homogenization (HPH) and hydrodynamic pressure processing (HDP) or shockwaves which instantaneously development pressure waves up to 1 GPa (Bolumar et al., 2013). For the last decade, HHP has been used for the production of a number of meat products for the inactivation of pathogenic microorganisms and thus for the extension of shelf-life (Speroni et al., 2014). However, high pressure (HP) is no longer seen just as a simple alternative to conventional pasteurization but as a technique to create innovative meat products. Pressure has been demonstrated to influence the processes of meat protein denaturation, solubilization, aggregation and gelation (Sun and Holley 2011; Sikes et al., 2009; Iwasaki et al., 2006; Colmenero 2002). This has provided great potential for modifying comminuted meat products by affecting structural and functional properties of MP, creating innovative products (Tintchev et al., 2013). During the last four decades, many studies have been carried out on the effects of HHP on MP and its gelation properties. A large number of interesting results were produced, leading to theories that have sometimes become controversial, often because of the different treatment conditions used. Currently, there are few summaries providing systematic and

insightful perspective, which elucidate the structural and functional changes occurring in MP in response to various conditions of HHP.

With the increasing changes in consumer lifestyle, food patterns, expanding knowledge of meat processing and a growing awareness of the relationship between diet and health, the past few years have seen many transitions and challenges within the processed meat industry. An increasing consumer demand for fresh-like, economical, nutritious, healthy and convenience products has triggered both scientific and industrial research towards the invention and application of new processing strategies. These technologies enable formulation of tailor-made meat products having reduced salt, phosphate and/or fat (Inguglia et al., 2017; Yang et al., 2015a; Xue et al., 2016), additional value with improved functional properties (Matak et al., 2015) or modified texture for specific populations (Aguilera and Park 2016). These growing markets constitute both a challenge and a unique opportunity for further R&D in the meat industry.

To overcome these challenges in processed meat products, many different strategies have been used. These include the replacement of sodium chloride (NaCl) with other chloride salts (Totosaus and Pérez-Chabela 2009; Tahergorabi and Jaczynski 2012), mineral salt mixtures (Ruusunen et al., 2005) and amino acids (Chen et al., 2016a), the replacement of fat by hydrocolloids (Totosaus and Pérez-Chabela 2009; Xiong et al., 1999; Jiménz-Colmenero et al., 2013), functionality improvement of pale, soft, exudative (PSE)-meat proteins by incorporating

adjunct like soy protein, carrageenan or pH shifting (Zhang and Barbut 2005; Daigle et al., 2005; Zhao et al., 2016), and modifying texture by enzymatic softening (Eom et al., 2015). However, the consumer demand for less processed and additive-free foods, together with an extended shelf life, has stimulated intensive research of alternative methods to meet this target. Apart from food additives, HHP has gained considerable interest in the meat industry for the processing and preservation of meat products (Sun and Holley 2010; Hygreeva and Pandey 2016). In part, this is because of its potential to manipulate the functionality and texture of comminuted meat products whilst fulfilling consumer requirements for minimally processed and free or low-additive products, yet maintain quality and nutritional properties (Buckow et al., 2013).

HHP can affect MP conformations which lead to protein denaturation, aggregation and/or gelation, and modify the textural properties, enhancing water binding and the stability of meat gels (Sun and Holley 2011; Colmenero 2002). These modifications can be controlled by the protein systems (e.g. species, type of protein, structural disintegration, pH, ionic strength and presence of otheringredients) and by the HHP conditions (e.g. pressure intensity, duration, pressure gradient, temperature and treatment sequence) (Fig. 1). The appropriate selection of the HHP parameters for treatment of MP can markedly modify meat quality and partially compensate for the reduction of NaCl, phosphate or fat contents thereby improving the value of

the meat products to meet consumers' and worldwide regulatory demands for healthy and nutritious meat products.

A number of excellent reviews have appeared in recent years dealing with the application of HP to meat and meat products. Colmenero (2002) examined the muscle gelation process, particularly that modulated by the sequence in which pressure/temperature combinations were applied. Sun and Holley (2010) summarized the influence of hydrostatic pressure based on meat texture and sensory characteristics while Buckow et al., (2013) discussed the knowledge of HP on the physicochemical characteristics of muscle tissue and its effect on lipid oxidation in muscle with some information on pressure-induced gelation of MP. Simonin et al., (2012) presented abroad treatise regarding the effects of HP treatment on quality and microbial inactivation of raw meat and meat-products. However, there is no comprehensive and insightful summary of the pressure-induced structural, physiochemical and functional changes of MP, and how these changes are being adopted for the fabrication of the comminuted muscle products currently in demand (low salt, low phosphate and/or low fat, value-added or texture-modified). Therefore, the present review only addresses the structure/structure-function relationship of MP and its comminuted/gelled muscle products. The aims of this review are (1) to explore the underlying details of structural and functional changes of MP modified by HHP, (2) to summarize recent applications of HHP for functionality improved, value-added and healthy

meat products, (3) to outline areas for future research that enable commercial utilization of HHP and the innovation of meat products.

2 MP structure and functionality

Muscle consists of three groups of proteins classified by their solubility and by their location within muscle tissue. They are represented by myofibrillar, sarcoplasmic and stromal fractions (Lee et al., 2010). MP or salt-soluble proteins, which are soluble only in solutions of relatively high ionic strength (> 0.3 M NaCl or KCl), comprise about 50 to 56% of the total skeletal muscle protein. Muscle myofibrils are comprised of thin and thick myofilaments (Pearce et al., 2011). The backbone of the thin filaments consists of the protein actin while the largest component of the thick filament is the protein myosin. Thus, MP extracted from muscle fibrils are mainly composed of myosin and actin. Myosin is the predominant protein in MP, consisting of two heavy chains (Mw 220 kDa) and two pairs of small subunits referred as light chains (Mw 130 kDa) (Bandman 1999). Under physiological conditions, myosin consists of a helical tail or rod region forming the backbone of the thick filament and a globular head region that extends from the thick filament and interacts with actin in the thin filament. The rigor complex (absence of ATP) formed by the interaction of myosin and actin is referred as actomyosin (Huff-Lonergan and Lonergan 2005). Actin usually exists as double helical filaments (known as F-actin) composed of polymerized globular monomers (G-actin), each with a molecular size of approximately 43 kDa (Xiong 1994).

Traditional comminuted meat products are produced by initial comminution of meat to form meat batters, which are complex systems consisting of solubilized muscle proteins and fibers, fragmented myofibrils, fat, water, salts, phosphates, and other ingredients. In this paste-like batter, myofibrils are fragmented into shorter pieces, and MP are extracted and solubilized by the salts in which water and fat are held and stabilized. Following thermal treatment, MP can form a cross-linked gel matrix that binds the water and fat, giving rise to typical water holding and texture associated with cooked comminuted products. In general, myosin in pre-rigor muscle, actomyosin, in post-rigor muscle, and actin are considered to contribute to the functional properties of MP and hence to the quality attributes of comminuted products (Smith 2001).

The functional properties of proteins are those physicochemical properties that allows protein molecules to interact among themselves and their environment to produce or improve the quality and stability of final products (Smith 2001; Xiong 1997). These functional properties of muscle proteins are broadly classified into three categories: (1) protein-water interactions, (2) protein-protein interactions, and (3) protein-fat interactions (Santhi et al., 2017b).

In general, all functional properties of proteins are influenced by the interaction of protein with water, including protein extraction/solubility and water holding, as well as viscosity. Sodium chloride (NaCl) and potassium chloride (KCl) affect muscle protein solubility and viscosity (Nayak et al., 1996; Smith 2001). Salt alters the electrostatic, hydration, and water structuring effects of MP, resulting in enhanced solubility (salting-in effect) or insolubility

(salting-out) (Chang 1997). Myosin, the major protein in MP, has been proposed to be responsible for the overall solubility of MP (Chen et al., 2017). Myosin filaments disaggregate into myosin monomers and are solubilized in high ionic strength buffers (> 0.3 M) and monomeric forms can subsequently re-aggregate back into filaments and become insoluble when the ionic strength is lowered (< 0.3 M) (Bandman et al., 1997; Sinard et al., 1989). During comminution, muscle fibers swell and absorb water, with even very low concentrations of extracted myosin increasing the solution viscosity, leading to improved batter stability prior to heating.

Meat batters, also considered as oil-in-water emulsions, are heterogeneous composite materials composed of protein-coated fat globules (oil droplets) dispersed in a MP gel matrix (Dickinson 2012). Therefore, the emulsifying property of MP, which describe the protein-fat interactions, is one of the functional properties responsible for batter stability and product texture in emulsion-based meat products (Santhi et al., 2017b). The stabilization of fat is accomplished in two steps: the high viscosity of the meat batter helps to prevent coalescence of the fat, and the fat droplets are surrounded by a MP film that reduces the interfacial tension between the dispersed and continuous phases (Smith 2001). Myosin is the major component of the interfacial film surrounding the fat droplets (Gordon and Barbut 1990). Factors such as protein solubility, structure and processing conditions play a key role in stabilizing the fat droplets (Santhi et al., 2017b; Xiong 1997).

The gel-forming ability of MP occurs during thermal processing and is probably the most important functional property in comminuted products and has a large influence on textural and sensory properties, as well as on cooking yields of the final products. Heat-induced MP gels are the result of protein denaturation which leads to the formation of a three dimensional network via inter-molecular covalent bonds such as disulfide bonds, as well as non-covalent hydrophobic interactions, with entrapment of water and/or fat (Zhang et al., 2015; Cao et al., 2012). Of the MP, myosin and actin contribute most to the development of desirable gel characteristics in processed meat products. Upon heating (60 to 70 °C), myosin alone can form excellent gels whereas actin does not show any signs of gelation (Samejima et al., 1981; Sano et al., 1989a). However, low contents of F-actin enhance the gelling ability of myosin while higher levels of F-actin lead to decreased elasticity of gels as a result of its thermally induced viscous and curdy-sol nature (Sano et al., 1989b). Filamentous myosin in 0.2 M KC1 at pH 6.0 forms more rigid gels than the monomeric form in 0.6 M KC1 at the same pH (Ishioroshi et al., 1979).Water holding capacity (WHC) and cook loss (CL) of MP gels are essential physical quality parameters of meat products. These attributes relate to the retention of the moisture in the comminuted product matrix when subjected for external forces such as heating, cutting and slicing. They determine the composition and palatability characteristics of the finished product (texture, appearance, juiciness and overall eating quality), and further, influence consumer acceptability and market value of the product (Hygreeva and Pandey 2016). Both WHC and CL of

comminuted meats and meat emulsions largely depend on protein solubilization, denaturation and gelation of MP during processing (Yang and Powers 2016).

Therefore, myosin, the major protein in MP, has been proposed to be responsible for the overall solubility, emulsification, gelation and water retention of MP. These functional properties are significant determining factors for yield, textural, and sensory attributes of comminuted meat products.

3 HHP-induced structural changes of MP for improved functionalities

HHP has been widely investigated and used to modify food proteins and produce textural and functional products different from those foods produced by conventional thermal processing methods. The technique is governed by Le Châtelier's principle, which indicates that high pressure enhances those conformational alterations or phase changes that are associated with a decrease in volume. In the case of these aqueous protein systems, they are accompanied with the penetration of water molecules into the proteins' interior by pressure (Buckow et al., 2013). HHP modifies protein by the rupture of non-covalent interactions (electrostatic and hydrophobic) in tertiary and secondary structures within protein molecules and the subsequent re-formation of intra/inter-molecular bonds (Sun and Holley 2010). Controlling the pressure-induced structural and functional changes of MP in meat products are important issues for meat-product manufacturers.

3.1 Previous studies on the effects of HHP treatment on MP components and their gelation

3.1.1. HHP induced conformational changes of MP

One of the major effects of HHP on MP is de-polymerization**.** Early studies showed that actin was sensitive to high pressure and underwent de-polymerization at 100 MPa (Ivanov et al., 1960), whereas myosin filaments from rabbit skeletal muscle in 0.15 M KCl were shortened as pressure was increased up to 35 MPa (Davis 1981). Actomyosin from ovine muscle in 0.6 M KC1 was disaggregated and F-actin was de-polymerization when pressure treated at up to 150 MPa (O'shea et al., 1976). It was summarized that de-polymerization of F-actin and myosin took place at pressure intensities between 100 and 300 MPa (Macfarlane 1985). More recently it was reported that actin undergoes a reversible F-G transformation under pressures less than 250 MPa (Ikeuchi et al., 2002). As a consequence of de-polymerization, it was anticipated that HHP increased the solubility of MP. This was further confirmed by Macfarlane (1974); Macfarlane and McKenzie (1976) and Lee et al., (2007) who observed a significant increase in the solubility of ovine and bovine MP after HHP treatment (< 300 MPa). Therefore, HHP has been shown to de-polymerize isolated myosin, F-actin and actomyosin and to promote the solubilization of MP.

Pressure-induced structural changes within MP have also been well described. Myosin begins to unfold at 50 MPa (Hsu and Ko 2001), and its tertiary structure changes above 200 MPa with the exposure of the hydrophobic and buried sulfhydryl groups; the secondary structure was

barely affected at pressures from 50 MPa to 600 MPa (Chapleau and de Lamballerie-Anton 2003; Chapleau et al., 2004). At pressures greater than 700 MPa, secondary structural changes occurred which led to irreversible denaturation (Sun and Holley 2010). The structural changes of the myosin head at pressures higher than 200 MPa were characterized by protein unfolding and the exposure of the hydrophobic cores to the surface of the molecule (Iwasaki and Yamamoto 2003). Denaturation of individual muscle proteins was also induced by HHP. Cod myosin was denatured at about 100 to 200 MPa (20 min, 30-50°C), whereas actin became denatured at 300 MPa (Angsupanich and Ledward 1998). In contrast to fish species, myosin from warm-blooded animals is presumed to be more stable to pressure. It was found that pork and bovine myosin and actin were both denatured at 200 to 400 MPa (10 min, 20-50°C) (Cheah and Ledward 1996; Ma and Ledward 2004; Chapleau and de Lamballerie-Anton 2003). HHP treatment can cause aggregation of myosin and actomyosin at pressures higher than 100 MPa. With 100 MPa HHP treatment, cod myosin in 0.6 KCl at pH 7.0 began to aggregate and become insoluble (Hsu and Ko 2001). The structure of tilapia actomyosin in 0.6 M KCl at pH 7.0 was aggregated mainly via hydrogen bonds at pressures above 100 MPa and the aggregation increased up to 250 MPa (Hsu et al., 2007). Ko et al., (2004) reported that the subfragment-1 (S-1) of myosin was the major portion contributing to the pressure-induced aggregation rather than the myosin rod. These results demonstrated that HHP can cause partial or complete unfolding, denaturation and reversible or irreversible aggregation of MP.

3.1.2. Effects of HHP on the gelation properties of MP

Heat-induced gelation of MP is fundamental for the manufacture of cooked meat products or fish meat pastes. Pressure affected/induced gelation of meat proteins depends upon the protein system (e.g., species, pH, ionic strength, ingredients) and upon the HHP conditions (e.g., pressure level, time, and pressurizing temperature).

The application of appropriate pressure treatment, at low and constant temperature, prior to heating enhances thermal gelation of MP. Studies by Macfarlane and team showed that pressure treatment (150 MPa/10 min/0°C) improved the heat-induced (70°C/10 min) gelation of myosin from ovine muscle at low ionic strength (Suzuki and Macfarlane 1984). They also showed that HHP pre-treatment (150 MPa/20 min/0-3°C) increased the tensile strength and water holding capacity of cooked (70°C/30 min) beef patties (1% salt at pH 5 to 6) (Macfarlane et al., 1984). In later studies HHP (300 MP) pre-treatment followed by heating at 90°C, was shown to reduce the gelling time and improve the rheological properties of washed sardine mince (2.5% NaCl) in comparison with heating alone (Montero et al., 1997; Pérez-Mateos and Montero 1997). Heat-induced (80°C/30 min) gels of pressurized (150 MPa/5 min/cold water) rabbit actomyosin displayed storage modulus (G') values higher than those of unpressurized actomyosin at KC1 concentrations of 0.2 to 0.6 M at pH 6.0 (Ikeuchi et al., 1992a). Ikeuchi's team proposed that the improved thermal gelation of actomyosin, induced by HHP pre-treatment, was attributable to the denaturation of actin in actomyosin and the increased sulfhydryl content and increased surface

hydrophobicity (Ikeuchi et al., 1992b). As depicted in Fig. 3, a high amount of F-actin exhibited a negative effect on the heat-induced gelation of actomyosin at low and high KC1 concentrations according to the theory proposed by Yasui et al., (1980). Pre-treatment at 100-150 MPa HHP would effectively diminish the negative effects of an excess amount of F-actin in actomyosin on the gel strength of myosin by de-polymerizing actin. As a result, HHP pre-treatment at 150 MPa increased the heat-induced gel strength of actomyosin (Ikeuchi et al., 1992b). Iwasaki et al., (2006) also suggested that de-polymerization of thin filaments was the cause of the high apparent elasticity of a heat-induced MP gel (0.2 M NaCl at pH 6.0) when pre-treated at 200 MPa for 15 min. They speculated that treatment at 300 MPa HHP induced shortening of myosin filaments, which might be responsible for the decrease in apparent elasticity of heat-induced MP gels (0.2 M NaCl at pH 6.0) when compared to that found at 200 MPa.

Combinations of high pressure with a range of thermal treatments have also been studied. The effects of pressure/temperature combinations on the muscle gelling properties have been thoroughly reviewed by Colmenero (2002). HHP of meat batters at 200 or 400 MPa and at 70 to 80°C led to improved fat and water retention, compared with cooked-only samples (Jiménz-Colmenero et al., 1998). However, it was summarized that HHP combined with heating (above 40°C) limited the gelation process of meat systems. The gel structure of pork and chicken meat batters treated with HP at 200-400 MPa/30 min at 60-80°C were weaker than those prepared by heating (non-pressurized) or pressurized prior to heating. This can be explained by

either an enhancement in the thermal stability of MP under HHP or an increase in protein hydrolysis under this severe condition (Colmenero 2002). HHP treatment after cooking can also affect the functional attributes of MP gel. HHP of cooked sausages at 500 MPa (65°C/15 min) induced less firm, more cohesive products having a higher yield compared to those subjected to conventional heat pasteurization (80-85°C/40 min) (Mor-Mur and Yuste 2003). Moreover, HHP (500 MPa) at mild temperatures (65°C) has been shown to be a valid technology for the pasteurization of cooked sausages (Yuste et al., 2000). Thus, HHP can be an efficient alternative to heat pasteurization for commercial enterprises in the secondary sterilization of cooked sausages, which also have improved functionalities (Mor-Mur and Yuste 2003).

Both the conformational state of myosin and the salt content can affect the gelation of MP. Without heat treatment, myosin filaments in 0.1 M KCI at pH 6 formed a gel by exposure to, and release of HHP at 210 MPa or above, whereas no gelation occurred in monomeric myosin dissolved in high salt solution (0.5 KCl at pH 6) upon HHP treatment at pressures up to 600 MPa. Under high ionic strength conditions, unpressurized myosin molecules display a typical two-headed shape in the monomeric state (Fig.2). Upon pressurization, either intra-molecular association of two heads in a single molecule or inter-molecular head to head association takes place. They then retain the ability to associate, so that daisywheel-shaped oligomers are finally formed. Due to pressure resistance of the myosin rod, and later, the lack of linkage between particles through entanglement of the myosin tail, the monomeric structure cannot form a gel

with HHP alone. However, myosin pretreated with HHP still retained the capability to form an aggregated structured gel following heating treatment (Yamamoto et al., 1993). In the case of myosin filaments at low ionic strength (0.1 M KCl at pH 6), a pressure of 210 MPa induced distortion of the myosin filament shaft and the projection of myosin head from filament surface, enabling inter-filamentous cross-linking involving side-by-side, end-to-end and random orientation of the filaments. Under these HHP conditions, a strand-type gel was produced (Yamamoto et al., 1990). A rheological and morphological comparative study between thermal and HHP-induced filamentous myosin gels showed that they both had similar fine-strand network structures, which were formed by side-by-side association of several filaments. The elasticity of the pressure-induced strand gel increased with increasing pressures up to 500 MPa (Iwasaki et al., 2005). When chicken myofibrils were subjected to HHP (up to 500 MPa/30 min/room temperature), they also induced gel formation at a protein concentration of 40 mg/mL in 0.1 or 0.2 M NaCl at pH 6 or 7, and the gel strength increased with pressure increasing at 0.1 M NaCl, but it remained almost the same at 0.2 M NaCl (Yamamoto et al., 2002).

Differing from mammalian and avian meat proteins, fish muscle protein gels were induced by HHP pre-treatment at low temperatures, presumably because of the low pressure and temperature resistance of fish proteins, as well as the presence of high transglutaminase activity (Buckow et al., 2013). Pressure treatment of fish proteins at 4°C prior to incubation at 25°C or 40°C (setting) increased the gel strength 2 to 3-fold in uncooked surimi gels, whereas minced turkey meat was

unable to form a proper gel upon 300 MPa HHP for 5 min, at ~10°C (Ashie and Lanier 1999). The gelation temperature of milkfish paste was reduced from 50 to 30°C by preliminary pressure treatment at 300 MPa for 60 min at 0°C (Ko 1996). Compared with the heat-set controls (90°C/15 min), HHP (170--240 MPa/60 min/28-35°C) was very effective in forming gels from Pacific whiting and Alaska Pollack surimi which resulted insignificantly higher shear stress and strain values (Chung et al., 1994).

The physical properties of meat emulsion gels formulated with various fat contents can also be influenced by HHP treatment. A study on the effects of HHP (100 or 300 MPa/5 or 20 min/about 8°C) on the texture of uncooked/cooked low fat (6%) and high fat (23%) meat batters indicated that pressure caused a significant increase in the mechanical resistance of uncooked batters, but HHP pre-treatment at 300 MPa decreased the elasticity of the meat matrix in high fat cooked batters (Carballo et al., 1996). In the case of beef patties, compared to high-fat formulation (20.3%), the low-fat (9.2%) product exhibited significantly higher Kramer shear force and Kramer energy values after HHP (100 or 300 MPa/5 or 20 min) pretreatment. It was also noted that HHP resulted in a higher fat release in comparison with untreated beef patties (Carballo et al., 1997). A similar observation of fat release was found with pork sausages containing low-fat (9% w/w) and high-fat(25% w/w) when treated with HHP (300 MPa/6-8°C/5-20 min) (Colmenero et al., 1997). It was proposed that HHP led to the rupture of adipocytes, causing a higher fat release (Carballo et al., 1997).

Overall, HHP induces various structural changes in MP, which affect their gelation properties.

3.2 Recent advances on the effects of HHP on the structural and functional properties of MP

Recent studies that have been conducted on the modification of muscle MP by pressure are summarized in Table 1 and are classified into several categories according to the meat system and conditions (raw muscle or extracted protein) and pressure/temperature combinations (Fig. 1).

3.2.1 Effects of HHP on denaturation, solubilization, aggregation and thermal gelation properties of MP

HHP pre-treatment modifies the conformation and functional properties of MP, which can subsequently affect the thermal gelation properties of MP in either a beneficial or a detrimental way.

The initial effect of HHP on MP is to dissociate the quaternary structure, induce unfolding and protein solubilization, and expose the functional groups to the protein surface. Studies by Zhang and colleagues investigated the effects of HHP pre-treatment (100-500 MPa/10 min/temperature not indicted) on conformational changes of chicken MP, including the primary, secondary, tertiary and quaternary structures (Zhang et al., 2017; Zhang et al., 2015). They found that surface hydrophobicity and reactive sulfhydryl contents increased as pressure increased. It was suggested that pressure treatment allowed entry of water into the interior of the protein, thus

protein molecules became stretched and unfolded, with the conformational structure becoming loose and destabilized, leading to unstable tertiary and quaternary structures. The hydrophobic groups that were previously buried in interior regions of the protein, and surrounded by a non-polar environment, then became exposed to an aqueous environment, with the structure becoming more open at higher pressures. As disruption of disulfide bonds requires 213.1 kJ/mol of energy (Pauling 1960) and 10,000 MPa of HHP only provides energy of 8.37 kJ/mol (Morild 1981), the disruption of the disulfide bonds by HHP could not be expected. Thus, the increase of reactive sulfhydryl content was speculated to be the result of the stretching and unfolding of protein molecules, with the interior SH groups becoming exposed with pressure. A similar trend was observed in rabbit myosin (0.6 M NaCl, pH 6.5) when subjected to various pressures (100--400 MPa/20°C/10 min) (Cao et al., 2012). The surface hydrophobicity and sulfhydryl group content of rabbit myosin changed little at 100 and 200 MPa, but showed significant increases at 300 and 400 MPa. For silver carp fish MP (0.6 M KCl), Qiu et al., (2014) indicated that HHP (200-500 MPa/20°C/10 min) caused protein unfolding and the exposure of hydrophobic groups. Analysis of the second derivatives of ultraviolet (UV) spectra and intrinsic fluorescence spectra suggested that, after HHP treatment, both the interior tyrosine and tryptophan residues moved to regions of higher polarity; namely, the surface of the protein, reflecting protein unfolding. A similar finding of increased surface hydrophobicity was also detected in natural actomyosin (0.6 M KCl at pH 7) from threadfin bream after HHP (200, 400,

600 MPa/room temperature/10, 30, 50 min) (Zhou et al., 2014).The surface sulfhydryl content of tilapia actomyosin (0.6 M KCl at pH 7) increased greatly at pressures of 100 MPa and higher (10-60 min/4°C) (Hsu et al., 2007). It is noted that the substantial changes leading to exposure of functional groups was essentially completed within the first 10 min of HHP treatment (Zhou et al., 2014; Hsu et al., 2007). In addition, HHP appears to induce the formation of disulfide bonds since the total sulfhydryl content of threadfin bream actomyosin gradually decreased as the pressure rose from 200 MPa to 500 MPa (Zhou et al., 2014). The total sulfhydryl contents of actomyosin from tilapia decreased substantially with increasing pressures above 200 MPa (Hsu et al., 2007) while actomyosin from chicken MP only declined significantly at pressures beyond 300 MPa (Zhang et al., 2017). These studies suggest that sulfhydryl groups reacted to form disulfide bonds at pressures above 200 MPa. However, this is not always the case as it has also been reported that HHP at 200 MPa or 500 MPa for 10 min at 10°C had no significant effects on the total sulfhydryl groups of hake MP (3% NaCl). Nevertheless, during subsequent heating (90°C/20 min), the production of disulfide bonds of the hake MP was promoted by HHP pre-treatment at 150 MPa to 500 MPa (Cando et al., 2014).

Protein unfolding was accompanied by the dissociation of MP, resulting in increased solubility, as aforementioned. Pressurization of MP at low pressures (100-200 MPa/10 min/ Temperature not indicated) caused a reduction in particle size, which was explained by the de-polymerization of actin and actomyosin (Zhang et al., 2017; Zhang et al., 2015; Hsu et al.,

2007). As a result of the dissociation of the quaternary structure, following moderate pressure treatments (100-200 MPa/10 min), the solubility of chicken breast MP increased significantly from 18.50 (0.1 MPa) to 47.86% (200 MPa) (Zhang et al., 2017). These results suggested that moderate HHP (≤ 200 MPa) improved the solubility of MP.

Inconsistent with those previous studies as discussed in section 3.1.1, the secondary structure of MP (0.6 M KCl) appears to be affected by HHP below 600 MPa. By analyzing the amide I Raman spectrum region, the ordered secondary structure proportions (α-helix and ß-sheet) of MP gradually decreased from 40.49% to 30.68% and from 22.11% to 9.79%, respectively, as pressures increased up to 500 MPa (10 min) (Zhang et al., 2017). Similarly, the α-helix fraction of silver carp MP, calculated from the far-UV CD spectra, gradually decreased as pressures increased to 500 MPa (20°C/10 min). Noticeably, these reductions only occurred at 400 MPa or higher (Qiu et al., 2014). Through estimation of amide I band content from Fourier transformed infrared (FTIR) spectra, HHP (150-500 MPa/10 min/10°C) reduced the α-helical structure of hake MP by about 50%. However, the content of ß-sheet structure increased after HHP (up to 500 MPa) treatment, which conflicts with that observed for MP from chicken breast (Cando et al., 2014). The α-helix structure of MP is mainly stabilized by hydrogen bonds between the carbonyloxygen (-CO) and the amino hydrogen (NH-) of the polypeptide chain (Cao and Xiong 2015; Liu et al., 2008). Zhang et al., (2017) deduced that HHP destabilized hydrogen bonds, leading to the loss of α-helix structure. However, there were no changes in the amino acid

profiles or rupture of covalent bonds; thus HHP treatment had no disruptive effect on protein primary structure.

Pressure-induced protein conformational changes and exposure of reactive groups are beneficial for inter-molecular bonding, which subsequently lead to protein denaturation, aggregation, or gelation (Zhou et al., 2014; Qiu et al., 2014).

The denaturation of proteins is very important in thermal gelation, which initiates various protein-protein interactions that affect the gelation properties of MP. Recent studies have indicated that pretreatment with HHP influences the thermal denaturation and stability of MP. Differential scanning calorimetry (DSC) is often used to investigate and identify the temperature range of denaturation transitions of individual proteins in MP after HHP treatment. Pressurization (100-500 MPa/10 min) caused protein denaturation in chicken MP (0.6 KCl) that shifted the thermal transition peaks to lower temperatures. As pressure increased, the enthalpy (ΔH) for each individual protein group in MP tended to decrease, indicating denaturation and loss of native structures (Zhang et al., 2017). Following treatment at 200 MPa, the degradation of muscle proteins and the de-polymerization of F-actin was initiated (Zhu et al., 2015). The ΔH of myosin and actin sharply decreased by 60.4% and 50.7%, respectively, indicating that more than half of the subunits had become severely denatured. Zhang and colleagues also reported that HHP at 300 MPa and above led to the disappearance of the myosin peak and the appearance of a new peak, suggesting the complete denaturation of myosin and the formation of an HHP-induced

aggregate (Zhang et al., 2017). The thermal denaturation pattern was similar to that of hake MP (3% NaCl) when subjected to 150-500 MPa/10°C/10 min (Cando et al., 2014) and to silver carpfish MP (0.6 M KCl) treated at 200-500 MPa/20°C/10 min HHP (Qiu et al., 2014), however, pressure did not induced an aggregate peak in these two works. A recent review by Buckow et al., (2013) also concluded that pressure-treated meat protein showed a loss of the DSC peaks for myosin and actin at treatments over 200 MPa. It was noted that actin appears to be the most pressure-sensitive fraction in MP, possibly as consequence of its F-G transition at relatively low pressures (Zhang et al., 2017; Buckow et al., 2013).

The presence of salts in MP systems can affect the response of individual proteins to pressure denaturation. For beef patties treated with HHP (200 MPa/5 min/5-10°C), the ΔH values of 200 MPa-treated patties containing 1% NaCl or 0.25% sodium tri-polyphosphate (STPP) + 1% NaCl tended to decrease in relation to that of those with no additions, suggesting that the presence of 1% NaCl may increase the sensitivity of meat proteins to HHP treatment. There was also an indication that the addition of 1% NaCl increased the denaturation of the myosin head region, whereas with the addition of 0.25% STPP, there was some protection from denaturation at 200 MPa. When HHP was applied at 300 MPa, the protective effect of 0.25% STPP was not evident. In contrast, the presence of 0.25% STPP favored denaturation of MP at this pressure level (Speroni et al., 2014). However, relatively high pressures at 350 MPa/6 min/20 ± 3°C totally denatured myosin and actin in pork batter, regardless of the salts and polyphosphates present,

and resulted in the formation of a new protein conformation. The addition of salts and polyphosphates destabilized this HHP-induced MP structure. This is thought to be the result of a pressure-induced structure having enhanced hydrogen bonding in the presence of salts and polyphosphates, which tended to be disassembled at low temperatures (Villamonte et al., 2013). In general, HHP above 200 MPa will denature MP and thereby significantly decrease the heat stability of both myosin and actin, causing them to denature at lower temperatures, thus requiring less energy input. This denaturation effect depends on the pressure intensity used and the presence or absence of salt and/or phosphate in MP preparations.

Protein denaturation induced by HHP can generate aggregations of MP. For chicken breast MP (0.6 M KCl), the particle size was enlarged by pressures higher than 200 MPa (10 min), which suggested that stronger HHP treatments (> 200 MPa) increased the formation of protein aggregates (Zhang et al., 2017). This was verified by transmission electron microscopy (TEM) observations in tilapia actomyosin (0.6 KCl at pH 7). At pressures up to 250 MPa (10 min/4°C), actomyosin formed aggregates and lost its arrowhead structure. With increasing pressure intensities and times, actomyosin became more aggregated with the solutions becoming turbid, causing an increase in light scattering as predicted (Hsu et al., 2007). Zhou et al., (2014) showed that the turbidity of natural actomyosin from threadfin bream increased sharply at pressures of 200 MPa or higher for 10-60 min. It is likely that this aggregation resulted mainly from the polymerization of myosin. The stained bands in the SDS-PAGE pattern corresponding to myosin

heavy chains became weak when the proteins were treated with HHP at intensities greater than 300 MPa (Zhang et al., 2015; Zhou et al., 2014; Cao et al., 2012) for chicken breast MP, for natural threadfin bream actomyosin and for rabbit myosin. When myosin polymerizes into large molecules, it is unable to pass through the pores of the polyacrylamide gel and thus remains in the stacking gel. Speroni et al., (2014) suggested that HHP (200 or 300 MPa/5-10°C/5 min) of MP in beef patties induced the formation of aggregates, and the presence of salts (0.25% STPP or 1% NaCl) decreased the aggregation induced by HHP. It was demonstrated that HHP-induced aggregates are stabilized through hydrogen bonding and hydrophobic interactions and this aggregation could likely be ascribed to the formation of intermolecular disulfide bonds at higher pressures (≥ 300 MPa) (Zhang et al., 2017; Speroni et al., 2014; Hsu et al., 2007; Zhang et al., 2015). It is because of the formation of large molecular size aggregates that a decrease in the solubility of MP was detected when proteins were subjected to high pressure at 200 MPa, or greater (Zhou et al., 2014). Therefore, HHP above 200 MPa can lead to aggregation via hydrogen bonds, hydrophobic interactions and disulfide bonding, hence reducing the solubility of MP.

HHP-induced conformational changes in MP can influence the behaviors of gelation and the properties of gels induced by heat. Several studies have recently been conducted on the thermal gelation process of pressurized MP, and their results have varied. The measurement of storage modulus (G′) changes (representing the elastic portion) during linear heating is commonly used

to investigate the heat-induced gelation process of MP. An increase in this modulus has a bearing on the structure formed (Cando et al., 2015). The total G′ of chicken breast MP (0.6 M KCl) during the heating ramp decreased in a dramatic manner as pressures increased up to 500 MPa (10 min). The peak corresponding to heat-induced myosin aggregation disappeared at pressures of 200 MPa and higher (Zhang et al., 2017). On the contrary, heat treatment of pressurized silver carp MP (0.6 M NaCl) resulted in higher gelation rates (between 50°C and 60°C) with increasing treatment pressures (200-500 MPa/20°C/10 min) (Qiu et al., 2014). Cando et al., (2014) found that heat-induced gels prepared from hake MP (3% NaCl) had similar elasticity whether they had been pressurized (0-500 MPa/10°C /10 min) or not. In the case of Alaskan Pollock surimi pastes (3% NaCl), those treated at 150 MPa (10 min/10°C) displayed a slight increase in gelation rigidity (G′) compared with the untreated sample, whereas at higher pressures of 300 MPa it was also decreased. It appears that 150 MPa may be optimal for production of the strongest thermal gel networks for surimi pastes (Cando et al., 2015). Similar results were also reported for rabbit myosin (0.3 M NaCl or 0.6 M KCl at pH 6.5) and for rabbit actomyosin (0.6 KCl at pH 6.0). The G′ value of pressurized myosin at 100 MPa (20-25°C/9-10 min) was higher than that of the non-treated and that treated at 200-400 MPa HHP following heating, indicating that the heated myosin gels modified by treatment at 100 MPa were the most rigid and elastic among the samples tested. With increasing pressures from 200 MPa to 400 MPa, the G′ values reduced sharply during the heating ramp (Wang et al., 2017; Cao et al., 2012). For actomyosin, there was

a linear increase in G' values at 80°C with increasing pressures in the range of 100-200 MPa, but further increases in pressure led to a decrease in gel rigidity (G') (Ikeuchi et al., 1992a). Basing on these results, combined with the theory of Ikeuchi et al., (1992b), the effects of HHP on the thermal gelation of actomyosin is depicted in Fig. 3. Pressure-induced enhancement (150 MPa) of subsequently heat-induced gelation of actomyosin was ascribed to the dissociation of F-actin in actomyosin and to the increased sulfhydryl content and surface hydrophobicity induced by HHP treatment (as discussed in section 3.1.2). However, HHP-induced protein unfolding occurred simultaneously with the protein denaturation and the formation of aggregates. When the HHP-induced myosin denaturation and aggregation overwhelmed the protein unfolding at higher pressures (≥ 300 MPa) (Ikeuchi et al., 1992b, a; Wang et al., 2017; Cao et al., 2012; Iwasaki et al., 2006), HHP would be detrimental for the thermal gelation of MP and cause a decrease in the elasticity of heat-induced MP gels. Notably, pressures higher than 300 MPa might induce denaturation and random aggregations of G-actin. However, the effects of randomly-aggregated actin, induced by HHP, on the thermal gelation process of actomyosin remain unclear (Fig. 3).

As a consequence of the various conformational and gelation changes induced by HHP, the resulting water binding and texture attributes of thermally processed MP gels can be regulated by HHP. The WHC of heat-induced chicken MP gel (20 to 65°C and maintained at 65°C for 20 min at 1°C/min) increased gradually from 0.1 MPa to 200 MPa (10 min), and then dropped sharply to an even lower value than that of untreated sample when the pressure was increased to 300 MPa

or higher (Zhang et al., 2015). Compared with the non-pressurized (0.1 MPa) rabbit myosin gel (0.6 M KCl at pH 6.5 heated from 20°C to 75°Cat 1°C/min and maintained at 75°C for 20 min), the WHC of the thermal gel that had been pre-treated at 100 MPa was higher, and increased significantly. Nevertheless, when increasing the pressure intensity from 200 to 400 MPa (9 min/25°C), the WHC decreased sharply (Wang et al., 2017). This is also consistent with a previous study in which application of 150 MPa (20 min) improved the WHC of cooked (70 ± 0.5°C/30 min) beef patties formulated with 1% or 3% salt (pH 5-6). However, such effects were not evident when lower pressures (50 MPa) were used (Macfarlane et al., 1984). It can be concluded that moderate HHP treatment (100-200 MPa) can increase the WHC of MP gel, whereas higher pressures (> 200 MPa) reduce its WHC. This dual function of HHP on the water binding properties of heat-induced MP gel is linked to the water-protein and protein-protein interactions within the gel microstructure. Using nuclear magnetic resonance (NMR), it was revealed that interactions between muscle proteins and water were enhanced following HHP treatment, where more free water was attracted by proteins, or trapped within the gel structure, which then became bound and immobilized (Zhang et al., 2015). Analysis of scanning electron microscopy (SEM) micrographs of chicken MP and rabbit myosin gels showed that application of 100 MPa or 200 MPa, led to a more regular homogeneous and filamentous network structure containing numerous smaller cavities. The pores embedded within the network were small while the strands appeared strong and fine, enabling water molecules to be strongly bound, preventing

them from removal by external forces (Zhang et al., 2017; Wang et al., 2017; Cao et al., 2012). When the pressure was increased above 200 MPa, myosin was highly aggregated and further heating caused a voluminous structures surrounding large and irregular-shaped pores (Wang et al., 2017). In other work at 300-400 MPa, the filamentous structure of the myosin gels diminished and large cavities were created as the gel structure began to form globular aggregates, suggesting a network of strengthened protein-protein interactions (Cao et al., 2012). It has been suggested that the formation of the three-dimensional network resulted from a balance between both protein-protein and protein-solvent interactions during heat-induced gelation (Boyer et al., 1996). As protein-protein interactions became stronger than those between protein and water at pressures higher than 200 MPa, the expulsion of the aqueous phase and shrinkage of the protein structure within the gel network occurred, resulting in low WHC (Wang et al., 2017). Regarding texture, with pressures increased from 0.1 to 200 MPa (10 min), thermal (20 to 65°Cat 1°C/min and then maintained at 65°C for 20 min) gel hardness of chicken MP increased from 20.3 g to 46.6 g, but then gradually decreased to 33.3 g as pressures further rose up to 500 MPa (Zhang et al., 2017). It was also found that moderate HHP (150 MPa/10 min/10°C) pretreated surimi (3% NaCl) registered a higher breaking-force and breaking-deformation values of thermal (90°C/30 min) gel than their counterparts of untreated and 300 MPa-treated samples (Cando et al., 2015). However, HHP treatment of surimi (2% salt) at pressures beyond 200 MPa (5-40°C/ 15 min) produced significant decreases in both fracture

stress and strain values of thermal (90°C/20 min) gels, although the effects at pressures below 200 MPa on gel texture was not shown (Zhu et al., 2015). These findings suggested that mild HHP pressures, in the range of 100 MPa to 200 MPa, can enhance the gel strength of MP. The physical property of gel texture is highly related to its microstructure. It is considered that the MP gels have a more dense and homogeneous gel microstructure following treatment at 100-200 MPa (10 min), contributing to higher hardness, whereas treatment at 300-500 MPa results in larger cavities with coarse microstructures, leading to lower hardness (Zhang et al., 2017). Treatment of surimi samples (3% NaCl) at 150 MPa (10 min/10°C) induced the formation of a very compact and homogenous structure of the thermally-induced gel compared to the non-treated and to other treated samples, which was in accordance with its higher gel strength (Cando et al., 2015).

Ingredient formulation can also greatly influence the texture and water retention of heated meat batters following HHP treatment. A significant triple interaction was observed between HHP (350 MPa/20°C/6 min), salt (1.5%--3%) and polyphosphate (0.25%--0.5%) on water binding properties of cooked (80°C/21 min) pork batters. Without salt, HHP had no positive effects on the WHC of comminuted meat products, but HHP synergized with salt can increase the WHC and decrease the cooking loss. HHP increased the cooking loss of the batters containing only polyphosphates in comparison with the non-HHP treated batters and HPP treated batters without polyphosphates (Villamonte et al., 2013). High pressure alone has an important

hardening effect on cooked pork batters, but the presence of salt and polyphosphates can counteract this hardening effect (Villamonte et al., 2013). Previous studies have also reported that HHP (100 to 400 MPa at 10 to 20°C) and salt (1 to 2%) can interact to reduce the cooking loss of meat batters (Sikes et al., 2009; Iwasaki et al., 2006).

In general, as proposed in Fig. 4, HHP treatment at moderate pressures (100--200 MPa) increased the solubility of MP by complex disassociation and de-polymerization of filament structures. The MP denatured and stretched moderately, causing the protein structures to become destabilized, exposing hydrophobic groups and sulfhydryl groups. During subsequent heating, sufficient unfolding of the helical tail portion of myosin, as well as abundant tail--tail cross-linking for water entrapment, would occur (Wang et al., 2017), forming a regular, homogeneous and filamentous three-dimensional network with enhanced water binding capacity and strength. On the other hand, HHP above 200 MPa caused strong protein-protein interactions through hydrophobic interactions, hydrogen bonding or even disulfide bonds, and generated large insoluble aggregates. Upon heating, less α-helix structure of myosin tail unfolded, thus the protein-water interactions during myosin tail associations might be inhibited, whereas protein-protein interactions would be strengthened (Wang et al., 2017), resulting in a globular aggregated network having large cavitations. This coarse and heterogeneous gel microstructure would be weak and conductive to water loss. To increase the water-retention ability of heat-induced MP gels, pressure should be applied at relatively low intensities. However, salt and

phosphate concentrations should be considered as vital factors influencing the role of HHP on meat gelation properties.

3.2.2 HHP pretreatment on whole muscle for structural modification of MP and functional improvement in processed meat products

Apart from its ability to reduce bacterial contamination, HHP of whole muscle was initially used for treatment of raw meat for tenderization and texture modification, and has been shown to effectively improve meat tenderness of both pre-rigor and post-rigor meat by breakage of the myofibril structures and activation of endogenous proteases during conditioning (Simonin et al., 2012; Sun and Holley 2010). Recent studies have demonstrated that HHP can be applied to whole muscle for the manufacture of comminuted meat products with improved functionality. HHP pre-treatment of muscle prior MP extraction can cause either protein de-polymerization or aggregation in MP. Xue and colleagues reported that HHP (100-300 MPa/25°C/15 s) pre-treatment of pre-rigor rabbit muscle caused the de-polymerization of F-actin into G-actin and the production of newly formed aggregates or peptide fragments with a molecular size between 55-70 kDa in the salt extracted MP (Xue et al., 2017). It was also found that pressures of 400-800 MPa/5 or 20°C/10 min modified extracted MP from the treated pork muscle in a manner that led to protein aggregation and the formation of a new polypeptide having molecular size of 50-75 kDa. The aggregation was mainly ascribed to myosin and actin interactions stabilized by hydrogen bonding, whereas the presence of the newly formed 50-75 kDa polypeptide was

attributed to either degradation of larger proteins into small sub-fragments or aggregation of low molecular size proteins (Grossi et al., 2016). As a consequence of protein aggregation, treating raw muscles (rabbit and pork) at pressures above 200 MPa led to reduced solubility of extracted MP (Xue et al., 2017; Grossi et al., 2016). Even with mild pressure treatment (100-200 MPa), no significant increase in MP solubility was observed (Xue et al., 2017; Grossi et al., 2016; Marcos and Mullen 2014), which is inconsistent with the conclusion from Section 3.2.1. It has been argued that any mechanical and/or chemical pre-treatment of the meat into MP fractions or meat batters prior to HP treatment may influence the pressure's effect on solubility (Grossi et al., 2016). Moderate pressure treatment of raw muscle altered the tertiary conformation of extracted MP, as was evidenced by HHP (100 MPa/180 s) of rabbit pre-rigor muscles, which led to the exposure of hydrophobic groups and reactive sufhydryl groups (Xue et al., 2017). Despite the lack of solubilization, the de-polymerization of F-actin to G-actin, and the exposure of functional sites with moderate pressures (100 MPa/180s, 200 MPa/15s, and 300 MPa/15s), benefited subsequent thermal protein-protein interactions, leading to improvements in the WHC and textural properties of heat-induced gels (Xue et al., 2017). Similar to previous theory, harsh pressures ($\geq$ 300 MPa/ $\geq$ 180 s) caused severe protein denaturation and aggregation in treated muscle, resulting in deteriorated functionality of extracted MP (Xue et al., 2017; Grossi et al., 2016).

3.2.3. Effects of combined pressure/thermal (P+T) treatment on structural and functional properties of MP

Traditionally, denaturation and gelation processes transforming batter to sausage, are induced by heating to a core temperature of approximate 72°C. This process can be also achieved by P+T, which acts differently from heat-induced changes. Previous studies have shown that P+T caused undesirable gels, having a lower quality compared with heat-induced gels, because of protein breakdown under this severe pressure/heat condition or preserving effects of HHP during thermal denaturation (As discussed in section 3.1.2) (Fernández-Martín et al., 1997; Jiménz-Colmenero et al., 1998). However, recent works have shown that P+T can be beneficial for textural and gel-forming properties of comminuted meat products, depending on the pressure/thermal combination used (pressure intensity, temperature, duration of treatments, pressurization/decompression rates) together with the specific conditions of the product system (initial temperature, size and geometry, composition, pH, ionic strength) (Zheng et al., 2015; Tintchev et al., 2013; Simonin et al., 2012).

The effects of P+T treatments (200 MPa/75°C/30 min) on chicken batters were investigated and compared with conventional heating (75°C/30 min). It was found that the P+T promoted the batter gelation process, forming a finely stranded gel having smooth appearance (Fig. 5), and importantly, improved texture and WHC. NMR results suggested that P+T can increase the proportion of immobilized water, which contributes to the low water release from the P+T

treated sausage. Distinct from the heat-induced sausage, which had a coarse gel network, SEM confirmed that the P+T treatment favored the formation of a fine gel network in MP sausage, inducing improved quality (Zheng et al., 2015). It was also suggested that the pressure/temperature sequence applied, e.g., heating before pressure treatment (H-P), pressure treatment before heating (P-H), and heating under pressure (P+H = P+T) (Fig. 5), can influence the gel properties of chicken batter. Briefly, P+H or P-H leads to improved gelation properties of chicken batter compared with those processed by H-P or heating alone (Zheng et al., 2015). The unfolding of native meat proteins (myosin) under P+T conditions leads to the formation of a gel having a more flexible structure that has enhanced moisture retention. The more severe processing conditions appear to have more effect on improving the WHC of the product as it was shown that the total released water of ostrich meat yor (Thai sausage) was gradually reduced with the increase of either pressure intensity from 200 to 400 MPa, with pressure duration from 40 to 60 min or with pressure temperature from 40 to 50°C (Chattong and Apichartsrangkoon 2009). To confirm this, the effects of P+T (100-600 MPa/10-40°C/240 s) treatments on frankfurters formulated with 0.5%-2% NaCl and 0.3%-0.15% phosphate were investigated. It was reported that batter firmness and WHC increased with increasing pressures and temperatures. Maximum batter firmness and WHC for all formulations and combinations were induced at 600 MPa and 40°C (Tintchev et al., 2013). A hypothetical mechanism of P+T induced denaturation, solubilization, aggregation and the gelation of myosin has also been proposed by

Tintchev et al., (2013) (Fig. 6A). According to the analysis of SDS-PAGE, myosin S-1 and S-2, N terminal, C-terminals, MLC, and actin are the main sub-units participating in the solubilization, aggregation, and gelation processes during P+T treatment (Tintchev et al., 2013). Using conditions of 150--250 MPa at 40°C, there was a maximum solubilization of the three S-1 myosin domains (N-terminal and C-terminal-50 kDa and 20 kDa) and the regulatory and essential light chains, as well as actin. As a result of the hydrophobic characteristic of the myosin-head domains, agglomeration via hydrophobic packing commenced (Iwasaki and Yamamoto 2003). Further cross-linking of different hydrophobic packs is speculated to produce the first stage of the secondary matrix network, although the specific configuration of these aggregates remains unclear. At higher pressures (350-600 MPa) combined with high temperature (40°C), the agglomerations developed themselves in a protein network, can form gel structure (Fig. 6A). The processes of protein solubilization, aggregation, denaturation, and gel formation with treatments longer than 240 s (The relevant preservation treatment time for industry) were also summarized in a pressure-temperature diagram by Tintchev et al., (2013) (Fig. 6B). It is suggested that P+T conditions ≥ 500 MPa/≥ 40°C/240 s may be commercially relevant for the single-step production of functionally improved frankfurter sausages, together with inactivation of microorganisms. Also, a low pressure gradient (PG) was recommended for industrial application as slow compression rate can develop sausages with improved functional attributes and lead to a reduction in salt content by 50% in the formulation (Tintchev et al., 2013). In

addition to the functional improvement, P+T can be applied as a pressure-assisted thermal sterilization (PATS) in the manufacture of meat products. Because of the shorter process time and lower maximum temperature, PATS has the advantages of achieving very safe foods of superior quality compared to those processed by conventional thermal treatments (Barbosa-Cánovas et al., 2014).

3.2.4. Effects of HHP on the structure and functionality of MP containing other ingredients

HHP can influence the susceptibility of the MP to other ingredients. Transglutaminase (TG), an enzyme that catalyzes the cross-linking of proteins (including beef and poultry actomyosin) via the formation of non-disulfide covalent bonds between glutamine and lysine of meat proteins, is capable of enhancing the binding ability of MP in comminuted meat products (Santhi et al., 2017a). Both endogenous (in fish meat) and exogenous TG has been shown to be remarkably stable under HHP conditions (up to 300 MPa at room temperature) (Menéndez et al., 2006; Zhu et al., 2014). Consequently, in addition to HHP-induced non-thermal denaturation and unfolding of MP, and promotion of cross-linking and gel strengthening, HHP also renders greater access of TG to the glutamine and lysine residues it targets, thereby enhancing intermolecular cross-link formation and improving gel strength (Simonin et al., 2012). During the production of surimi and turkey breast gels, previous studies have incorporated HHP prior to the TG gel-setting step to modify the MP structure, assisting TG (endogenous or added microbial enzyme) to improve the mechanical properties of heat-induced (90°C) gels previously set at 25°C or 40°C for 2 h (Uresti

et al., 2006; Ashie and Lanier 1999). Recent works have tested whether the setting step (cross-linking by endogenous or added TG) can be conducted simultaneously with the HHP step to effectively enhance strength of cooked gels. For this work, Tilapia pastes were subjected to pre-treatment using a combination of HPP (0.1-300 MPa/25°C/60 min) with the simultaneous addition of TG (0.044 unit/g of paste). This was followed by cooking at 90°C for 20 min. It was shown that HHP effectively modified the conformation of proteins and caused the amino acids in the protein to become more accessible to the acyl-binding sites of TG, leading to the formation of an enhanced gel network with improved strength and WHC (Hsieh et al., 2009). For Alaska Pollock surimi, setting with endogenous TG at 200 MPa/5°C/30 min HHP was found to induce high gel-fracture stress and strain, whereas increasing the temperature of the HHP treatment to 25°C, or extending the time of HHP to 60 min for setting with endogenous TG was detrimental to gel strength development. When exogenous TG was added for HHP setting (25°C), gel strength and deformability (fracture stress, strain) were higher than that of all other treatments tested (Zhu et al., 2014). Overall, a combination of HHP and the use of TG (either HHP prior setting or HHP setting) in comminuted meat products offer new potentials for the development of products having improved texture.

Gums or polysaccharides are commonly incorporated into formulations of comminuted meat products for manipulation of functional properties. When combined with HHP, various water binding and textural characteristics of MP gel have been obtained. The interactions between MP

and hydrocolloids in meat formulations are influenced by HHP, giving rise to gel having diverse properties. Without HHP, the addition of κ-carrageenan (KC) caused phase separation of the microstructure due to protein-polysaccharide repulsion. However, HHP at 300 MPa/10 min/29 ± 1°C reduced the extent of phase separation, and enhanced hydrogen bonding, thus improving the WHC of thermally-treated MP gel containing KC (Ma et al., 2013). HHP (100-400 MP/10 min/20°C) promoted the formation of an actomyosin-0.5% sodium alginate (SA) complex. Due to the strong interaction between actomyosin and SA, the pressure-treated actomyosin-SA displayed a low ability to denature and aggregate during subsequent heating, resulting in an increased CL from the actomyosin-SA gel (Chen et al., 2014b). On the other hand, the addition of specific hydrocolloids can modify the impact of HHP on the conformational and functional properties of MP. Pressure induced unfolding of MP, exposing sites that interact with xanthan gum (XG). The complex formation between MP and XG modified the impact of high pressure (600 MPa/20°C/6 min) on the secondary structures of MP by inhibiting the loss of α-helix structures and the increase of β-sheet structures. As a result, the HHP-induced protein-protein aggregation was inhibited, resulting in a higher solubility and surface hydrophobicity of MP than its counterpart without XG addition. These characteristics may improve emulsifying capacity of MP (Villamonte et al., 2015). The viscoelastic characteristics and WHC of P+T (600 MPa/50°C/40 min) induced ostrich-meat emulsion gels were investigated as influenced by the addition of various proportions of gum powder, provided as carboxymethyl cellulose (CMC),

locust bean gum (LBG) or XG (Chattong et al., 2015). The addition of CMC and XG had an antagonistic effect on the elasticity and WHC of P+T induced gel. This could possibly be explained by the fact that the ionic interaction between the basic ostrich-meat protein matrix and the anionic XG or XG plus CMC can prevent the protein matrix from interacting directly with water during P+T induced gelation. Contrary to CMC and XG, 1% LBG enabled the formation of a gel with increased elasticity and WHC (Chattong et al., 2015). Other works have also reported that addition of LBG and KC improved the thermal gelling properties, WHC, elasticity, cohesiveness and hardness of pressurized MP, which was dependent on the pressure intensities and formulations used (Ma et al., 2013; Ma et al., 2012; Trespalacios and Pla 2009).

In summary, HHP induces structural modifications (solubilization, denaturation, aggregation and gelation) of MP and results in varying effects on the final product texture and water retention. These largely depend on the product compositions (salt, phosphate, fat, and other ingredients), HHP parameters, and the pressure/temperature combinations or specific sequences. The information regarding the structural and functional changes of MP induced by HHP offers important theoretical foundations for technologies to develop MP gels having specific properties through application of defined combinations of HHP conditions and food ingredients.

4 Recent applications of HHP for functionally improved, value-added and healthy muscle gelled products

Recently, to meet the demands of consumers and manufacturers, HHP has been employed in various ways to modify the MP for the fabrication of new comminuted meat products, such as low salt, low phosphate and/or low fat, value-added and texture-modified meat products (Table 2).

4.1. Application of HHP for low salt, low phosphate and/or low fat meat products

Traditionally, NaCl, sodium phosphate and fat are essential components required for the manufacture of comminuted meat products. They play an important role in affecting their functional characteristics, such as cooking loss, water holding capacity, and textural properties, as well as sensorial properties of tastes and flavors(Yang et al., 2015b; Yang et al., 2015a; O'Flynn et al., 2014b). However, with a growing awareness of a link between diet and health, many processed meat products containing high levels of sodium salt and fat are now being criticized because of the health concerns. High dietary NaCl is associated with cardiovascular disease and strokes (Aburto et al., 2013; Strazzullo et al., 2009). High phosphate intake has potential risk, particularly in relation to bone metabolism, cardiovascular and renal functions (Ritz et al., 2012). Also, phosphates are currently perceived as being undesirable amongst consumers because of the clean-labeling issues (Speroni et al., 2014). High dietary fat induces the risk of obesity and chronic disorders (De Vries 2007; Chen et al., 2014a). Thus, there is growing demand for healthy meat products with low sodium salt, low phosphate and low fat, while still maintaining their palatability attributes. This poses a major challenge for the meat

industry. However, HHP has been found to be a useful method for successfully assisting in salt, phosphate and/or fat reduction in meat products.

HHP pre-treatment prior heating/cooking has been applied for the production of low salt, low phosphate and/or low fat meat products.

Studies on beef meat, pork meat and surimi used for the preparation of low sodium cooked sausages and gels, have demonstrated the viability of HHP for salt reduction (Sikes et al., 2009; O'Flynn et al., 2014b; Cando et al., 2015). Sikes and colleagues treated beef batters (containing 0, 0.5, 1 and 2% NaCl), with different pressure intensities (0.1-400 MPa) at 10°C for 2 min. They found that when cooked, the sausages treated at 200 MPa, containing just 1% NaCl, had similar CL and texture attributes to those containing 2% NaCl, demonstrating that application of high pressure to beef sausages with low-salt content resulted in improved yield and texture. It was suggested that the improved binding capacity in low-salt sausages resulted from enhancement of protein solubility and gelation through partial protein unfolding induced by HHP (Sikes et al., 2009). In the case of breakfast sausages, a reduction of salt levels from 2.5% to 0.5% without HHP led to increased CL, from 15.78% to 21.51%. However, HHP pre-treatment at 150 MPa/20°C/5 min, independent of salt level, significantly reduced the CL. Although contents of at least 2% NaCl are required to maintain an acceptable saltiness of the products, it has been recommended that HHP pretreatment (150 MPa) of low-salt cooked breakfast sausages (in the presence of 0.25% phosphate) allows a reduction in salt levels to 1.5%, and still retain

acceptable organoleptic and functional properties (O'Flynn et al., 2014b). It was reported that HHP-induced protein denaturation and/or unfolding in the reduced NaCl samples in a similar manner to that found with high levels of NaCl (Cando et al., 2015). Based on similar reasoning, HHP has also been used to achieve similar benefits for gelled products from fish muscle (Truong et al., 2017; Cando et al., 2015). Cando and colleagues found that HHP processing at 300 MPa/10°C/10 min improved the mechanical and sensory properties of reduced-NaCl (0.3%) surimi gels and stabilized the protein structures within surimi gels to a similar extent as determined for those with 3% NaCl content (Cando et al., 2015). The gelling process in fish muscle differs somewhat from that of meat from land animals in that fish muscle usually contains TG, which, under specific conditions, enables cross-linking of MP within a low temperature range (40-50°C for setting) (Hwang et al., 2007). A recent report by Truong et al., (2017) investigated differences in the gelation of low-salt barramundi products following the use of various processing methods (HHP and cooking, cooking alone or various sequence combinations of HHP and setting). Using 1 and 2% NaCl and 300 to 500 MPa at 4°C for 10 min, either followed by, or preceded by setting at 50°C for 2 h, they found that at 1% salt, with pressure followed by cooking, or pressure followed by setting, provided large improvements in both mechanical and functional properties compared to cooking alone. It was suggested to use pressure followed by cooking treatment to obtain high quality gels using only 1% added salt

(Truong et al., 2017). Thus, HHP treatment can be used to compensate for a reduction in sodium content in processed meats.

Moreover, HHP pre-treatment prior to heating/cooking can be successively adapted to develop low phosphate or reduced fat meat products. This is supported by the findings that pressure induces solubilization of MP without the need for additives, and by modification of protein structure, producing comminuted meat products having improved functionality (O'Flynn et al., 2014a; Yang et al., 2015a). Subjecting pork minced meat to HHP pre-treatment (150 or 300 MPa/20°C/5 min) before formulation with phosphate for manufacturing of cooked breakfast sausage, significantly improved the hardness, adhesiveness, cohesiveness and chewiness of sausages containing 0.25% phosphate, compared to those prepared with non-HHP meat containing 0.5% phosphate. It was concluded that a low phosphate (0.25%) breakfast sausage, with improved functionality and acceptability comparable to that with high phosphate (0.5%), can be achieved using 150 MPa HHP (O'Flynn et al., 2014a). In the manufacturing of bologna-type cooked sausage, pressure treatment at low pressure prior to heating was employed. In the absence of phosphates, HHP treatment (100 MPa/20°C/5 min) improved the functionality of meat proteins, allowing the formation of cooked sausage with the appropriate structure required for functionality (Bolumar et al., 2016). Using NMR, Yang and colleagues investigated the changes occurring in the various states of water and its distribution in pork batters formulated with 0 to 30% fat and 1% NaCl, as affected by pressure (Yang et al., 2015b; Yang et al., 2015a).

With HHP (0.1-400 MPa/10°C/2 min), it was found that at 200 MPa, there was greater water retention in the fast relaxation compartment, which was in accordance with the observations of cooking loss, texture and microstructure. The pork sausage containing 20% fat treated by 200 MPa exhibited similar yield, texture and sensory attributes to that of commercialized emulsion-type sausages, demonstrating that HHP at 200 MPa was capable of producing low fat (20%) pork sausages with acceptable technological and functional properties.

Low salt and/or low fat comminuted meat products can be attained through intelligent combination of HHP with other non-meat ingredients. This has been proved in studies in which HHP was used with TG treatment to successfully produce low salt/fat chicken meat gels (Trespalacios and Pla 2007a, b). Chicken meat gels formulated with 1.5% salt and 0.3% STPP, together with 0.3% TG, when treated with HHP at 500 MPa/30 min/40°C, followed by heating at 75°C for 5 min, exhibited improved binding and textural properties and microstructure, as compared to those pressurized without TG addition. Also, the gels had increased hardness, chewiness, and springiness, with a similar cohesiveness and cutting force to those obtained by heat alone (Trespalacios and Pla 2007a). In chicken gels containing 1.0% salt and no added phosphate, the addition of TG followed by HHP (700 or 900 MPa/40°C/30 min) markedly increased the hardness and chewiness compared to gels without added enzyme or those obtained by heat alone. It had been suggested that treatment with 700 MPa HHP (40°C/30 min), together with the TG addition, gave a synergistic effect, enabling the production of low-fat, low-sodium,

phosphate-free products with enhanced quality and sensory attributes, whilst assuring nutritive value and microbiological safety (Trespalacios and Pla 2007b). Recent studies have demonstrated that addition of amino acids (lysine or cystine) to surimi is beneficial for reducing the salt content when HHP is being used for surimi manufacturing (Cando et al., 2016b; Cando et al., 2016a). Breaking force, breaking deformation and WHC of the gels containing low NaCl (0% or 0.3%) were enhanced by the addition of 0.1% cystine and lysine and by HHP (300 MPa/10°C /10 min). This is ascribed to protein unfolding induced by both HHP treatment and the additives used, making it feasible to produce low salt (0.3% NaCl) surimu gels, having the appropriate mechanical and functional properties, being similar to those gels containing the regular amount of NaCl (3.0%) (Cando et al., 2016b). Further, incorporation of TG was shown to increase the WHC and strengthen the mechanical properties of low-salt surimi gels induced by HHP treatment in the presence of lysine (0.1%) or cystine (0.1%). It was proposed that HHP induced MP unfolding which promoted protein aggregation required for gelation, making a major contribution to the enhancement of physicochemical properties of low-salt surimi gels. Without HHP, but with the addition of TG and lysine and cystine, the gelation enhancement was less effective (Cando et al., 2016a).

Combined pressure/thermal (P+T) treatment has great potential for the production of low-temperature ready-to-eat (RTE) gel-type meat products with low salt, low phosphate and/or low fat in single-step process.

RTE chicken breast sausages containing reduced salt or phosphate have been developed with P+T (600 MPa/40°C/5 min) (Xue et al., 2016; Guo et al., 2017). Low salt contents (0.6%-1.4%) have been effective for gelation of meat proteins with application of P+T. With products having NaCl contents above 1.0%, there was a pronounced increase in water retention, and improved microstructure and texture. It was revealed that the NaCl content could be decreased to 1.2% when using 600 MPa/40°C/5 min for RTE gel products while maintaining suitable WHC, texture and palatability, being comparable to the higher salt (1.4%) counterpart (Guo et al., 2017). The influences of STPP contents (0.1, 0.2, 0.3 and 0.4%) on P+T induced gel in the presence of 1.2% salt were also investigated. It was demonstrated that the STPP contents commonly used for cooked sausages (0.3-0.5%) were excessive for P+T induced gels, causing a soft and tacky texture, whereas with 0.1% of STPP in meat batter, a juicy and more tender sausage with desirable WHC and palatability was obtained (Xue et al., 2016). These findings were related to the gelation process during P-T treatment. The decrease in α-helix and the increase in β-sheet in the protein secondary structure of the meat proteins during gelation are positively related to the WHC and textural properties of the pressure-induced gels (Guo et al., 2017). STPP content higher than 0.1% in the meat batter system hinders the gelling process of MP, which manifests as a higher α-helix content and lower ß-sheet content in the final gels. Besides, the enhancement of hydrogen bonding, as well as the reduced hydrophobic interactions, leads to inappropriate softness and tackiness of P+T induced meat gels when formulated with a higher content of STPP

(Xue et al., 2016). Generally, an optimal chicken breast gel product with low salt/phosphate (0.1% STPP and 1.2% NaCl), can be produced using P+T treatment at 600 MPa/40°C/5 min. Without doubt, specific pressure intensities and/or temperatures during P+T treatment are expected to affect the gel properties of low-salt meat gels (Omana et al., 2011). The gel hardness of chicken meat containing 1% or 2.5% NaCl was found to increase with increasing applied pressures (200-600 MPa/30 min) and temperatures (20-60°C). This significant increase in hardness was due to P+T induced denaturation of muscle proteins. It has been suggested that pressure and temperature have synergistic effects on gel hardness within the temperature range of 20--50°C of HHP, whereas the hardness is mostly affected by temperature at 60°C. With the addition of 0.3% ß-glucan, P+T induced chicken meat gels containing 1% NaCl and in the absence of STPP can attain similar properties to those with 2.5% NaCl addition, indicating that low salt and phosphate free chicken meat gels can be produced by combination of P+T treatment and the use of ß-glucan as salt partial substitute (Omana et al., 2011). Apart from the pressures or temperatures that affect the gel properties of P+T-induced low salt/phosphate meat products, the pressure gradient (PG) has a significant impact on the WHC of meat products. Pork frankfurter batters formulated with 0.5-2% NaCl and 0.15% and 0.3% phosphate were subjected to P+T (100-600 MPa/240 s/40°C) treatment under high (40 MPa/s) or low PG (2.5 MPa/s) for gel formation. It was shown that drip losses of the 0.5% NaCl batter processed with low PG were equivalent to the samples containing higher NaCl contents (2% and 1%), whereas drip losses in

the 0.5% NaCl batters treated at high PG were significantly higher than those in all other formulations. Additionally, an improvement of protein solubilization and gelation also occurred at low rates of PG. Thus proper adjustment of PG can lead to a reduction of salt content and improvement of WHC in P+T induced gel products (Tintchev et al., 2013). It has been proposed that P+T increases the content of free side chains of proteins to intensify the water-protein interactions, thereby improving their binding ability. Similar to the function of phosphate, P+T induces the dissociation of the actomyosin complex. In addition, water molecules are ionized by P+T treatment. Thus, the ionic strength is enhanced by P+T treatment, which can perform analogous effects of salt towards meat proteins. As a result, hydrogen and hydroxide ions are attracted to the protein charged groups and increase the solubility of meat proteins (Tintchev et al., 2013). The structural modification, water-protein interactions as well as the solubilization effects of P+T, contribute to the enhancement of such functional properties as WHC in low salt and/or low phosphate meat gel products.

With the use of fat substitutes, P+T can be adapted for the production of low fat meat products. Chicken meat products, with low fat and high protein, are obtained by a combination of P+T and the use of dried egg white as a fat replacement. The application of P+T together with the addition of dried egg white enabled the production of low-fat gel products having enhanced WHC, yield, hardness and cutting force. In order to shortening the treatment time, it was suggested that an increase in pressure above 500 MPa, whilst maintaining the temperature close

to 40°C, was beneficial for the production of reduced-fat chicken meat products having desirable textural properties (Trespalacios and Pla 2009).

4.2. Application of HHP to improve the functional properties of pale, soft, exudative (PSE)-like meat for value-added meat products

Along with the considerably growing consumption of poultry meat, and its expanding global market, an increasing occurrence of PSE-like meat has become a major concern for the entire poultry industry. Denaturation of MP in PSE meat, caused by a rapid postmortem pH decline and high temperature, results in weak gel formation, reduced WHC and gel strength of processed meat products, which leads to considerable economic losses (Li et al., 2014; Zhao et al., 2016; Chan et al., 2011). HHP has been shown to be effective for improving the functional attributes of MP and provides an alternative technology to enhance the value of PSE meat products. Low pH (PSE) and normal pH turkey meat batter formulated with 0.5% NaCl and 10% added water were subjected to 50, 100, 150 and 200 MPa treatment for 5 min at 4°C and then heated at 95°C for gel formation. The application of HHP significantly increased the solubility of meat proteins in both the low- and normal-pH batters, compared with untreated samples, and maximum protein solubility was found in samples treated at 50 and 100 MPa (Chan et al., 2011). This was suggested to result from the de-polymerization of MP, promoted by HHP. The higher surface hydrophobicity and greater exposure of sulfhydryl groups of proteins at 50 MPa and 100 MPa led to increased protein aggregation and an improved gelation ability, which contributed to

higher water retention in the low pH meat gel (Chan et al., 2011). Therefore, HHP treatment at 50 MPa or 100 MPa can be used for the production of functionally improved gel products prepared from PSE meat.

4.3. Application of HHP for texture-modified meat products

As the aging population is increasing rapidly, there are large increases in numbers of people with mastication and swallowing impairments due to their advanced age, neurological and muscular diseases, and the after-effects of stroke. Thus, the requirements for texture-modified food (TMF) to nourish the elderly, or specific populations, are demanding urgent attention (Aguilera and Park 2016). Meat is a nutrient-rich food, but comminuted meat products always have a firm texture, which makes it difficult to consume for those with mastication and swallowing impairments (dysphagia) (Chen et al., 2016b; Chen et al., 2016a; Chen et al., 2017; Tokifuji et al., 2013). Hence, there is a growing trend for the development of processed meat products having novel textures to meet the needs for this special population. Fortunately, HHP can be applied to meet this target. It has been reported that cooked pork meat gels made from meat batters formulated with 1:1 (w/w) water and 1.5% NaCl, and pre-treated at 400 MPa/17±2°C/20 min showed lower hardness and adhesiveness values, but scored higher elasticity, smoothness and ease of swallowing in sensory evaluations, compared to the untreated cooked gel. The pressure treated gel was easy-to-swallow and left little residue in the oropharynx (observed by video-fluoroscopic examination). Thus it has potential for use as a dysphagia meat

diet (Tokifuji et al., 2013). Similarly, a muscle product of minced fish meat produced using HHP was also suitable for dysphagia. Minced fish meats formulated with 1:1 (w/w) water and 1.5% NaCl were either subjected to 400 MPa/17±2°C/20 min HPP or 80˚C/10 min heating for gel formation. The pressure-induced gels were more lustrous in appearance, juicier, moderately elastic and smoother than that of those heat induced, and these gels achieved the criteria of dietary uses for elderly and dysphagic people (Yoshioka et al., 2016). The unique textural properties of the pressurized gel are attributed to the filamentous network structure filled with high amounts of water, formed by irregular lateral associations of myosin filaments at low ionic strength under pressure (Tokifuji et al., 2013; Yoshioka et al., 2016). When heated, α-actinin, tropomyosin and troponin can aggregate and attach to the former filamentous network, forming a comparatively coarser and more adhesive gel, as compared to the unheated-pressure induced gel (Tokifuji et al., 2013).

The above studies have clearly demonstrated HPP can be adopted as an efficient strategy to 1) reduce the salt, phosphate and/or fat levels in meat products while maintaining better quality characteristics compared to conventionally formulated meat products, 2) to improve the functional properties of PSE meat gel for increase in value and 3) to modify the texture attributes of meat gels for a dysphagia diet. Nevertheless, each specific product should be formulated individually by considered application of the pressure conditions used and the protein conditions for the expected functionality of gel products desired.

5. Summary and future considerations

It is known that functional and textural characteristics of comminuted meat and gel-type products depend mainly on the structure and functionality of MP. Many studies have indicated that HP-treatment of MP provides great potential for their structural modification, thereby creating innovative functional properties of MP. In order to clearly demonstrate the developmental progress of the fundamental theory and to thoroughly understand the relationship between structural changes of MP and its functional properties under pressure treatment, comprehensive discussions of both the previous studies and the recent advances achieved in structural modification of MP by HHP for improved functionality were presented. HHP can affect MP conformations and lead to protein denaturation, solubilization, aggregation or gelation, and thereby modify the textural properties, enhancing water binding and the stability of meat gels. This is dependent upon the protein systems (species, type and formulations) and pressure conditions (intensities, time, gradient, temperatures and its sequence). A moderate pressure (50-200 MPa) can affect the MP structure in a beneficial way resulting in enhanced water binding and texture of MP gelled product. A suitable combination of pressure and thermal (P+T) treatment can be used as single step process for the manufacture of RTE gel-type meat products with improved quality and safety. To meet the growing demand for economical, nutritional and healthy meat products, this review also summarized recent applications of HHP on the reduction of salt, phosphate and/or fat, value addition and textural modification of novel meat products.

The appropriate selection of the HHP parameters and product formulations can markedly modify their quality, and tailor-make novel comminuted meat products having functional improved, value-added and healthy attributes.

There is no doubt that once adopted, HHP will establish a clear competitive advantage in meat products processing. In addition to the considerable applications of HHP as non thermal preservation and decontamination/pasteurization/sterilization technology for extending the shelf life of commercial processed meat products (Hygreeva and Pandey 2016), relatively low pressures HHP are advantageous for altering functionality of muscle proteins in processed meat products for additive-reduced or free formulations. The advantages of low-pressure operations have a significant impact on the costs of HHP processing, where pressure cycle times are shortened, downtime for equipment maintenance is reduced and overall operational costs are significantly reduced.

However, there are some limitations before it can gain credibility. Future considerations are described as followings:

▪Due to lack of uniform standards and reliable and reproducible data for process validation, modifications of MP by HHP still remain elusive and the complex interrelationships between structural rearrangements occurring during HPP treatment and their resultant functionalities are not fully understood. The logging of standardized operational and processing parameters during

optimization of desired outcomes is required to facilitate the full potential of HHP in comminuted meat products.

▪ Modifications at higher pressures (> 400 MPa) in comminuted products can accelerate lipid oxidation during storage and lead to color and flavor changes, which may decrease sensory acceptability. More studies should be conducted to counteract this side effect through controlling the process parameters as well as developing the formulation of the product, or its packaging.

▪Although improved texture and WHC in reduced salt, phosphate and/or fat meat products can be achieved by HHP, a challenge to be faced is that the taste, color, flavor or aroma which may become unacceptable to some consumers. Since these perceptions are determined by a number of elements, and there is no single solution, a combination of multiple tools should be further assessed to develop the desired effect. Moreover, the effects of these low salt, low phosphate and/or fat meat products on peoples' health need to be considered.

▪The use of HHP as a personalized method for structure formation has promising perspectives of being accepted by consumers. Future studies can address the production of novel gel-type meat products having customized textures and enhanced nutritional profiles.

▪ Although the present range of HHP units available globally (size and throughput) provide meat companies with specific choices for their particular requirements in a cost-effective and safety manner (Balasubramaniam et al., 2016), product volumes fabricated through the use of

HHP are likely to remain low due to present market demand. However, even with market growth, it would appear that the development of new HHP equipment (e.g., horizontal continuous loading and unloading process) will be more than adequate to meet the requirements. New method of meat packaging in the vessel, automation solution and integration with the production line as well as knowledge of materials including stress analysis, fracture mechanics, non-destruction inspective, and high strength/high fracture toughness materials should be advanced by the equipment manufacturers and engineers in order to design and build units having higher pressure, larger volume, lager number of cycles, higher efficacy and safer pressure systems.

■ Conflicts of Interest

The authors declare no conflict of interests.

■ Acknowledgments

This work was supported by the National Natural Science Foundation of China under Grant 31471601 and Grant 31671875; by the Oversea Study Fellowship from the China Scholarship Council under Grant 201606850029 (to Xing Chen); and by the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD).

■ Author Contributions

Xing Chen collected data from the literature, drew all tables and figures, and drafted the manuscript. Ron Tume, Youling Xiong, Xinglian Xu, Guanghong Zhou, Conggui Chen and Tadayuki Nishiumi contributed to discussion of the content, preparation of the manuscript and checking of the language.

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Table 1 Recent studies on the effects of HHP on structural modifications and functional properties of MP of comminuted muscle products

Protein	HHP conditions	Structural modification	Functional properties	Reference
Effects of HHP pre-treatment on the isolated or formulated MP				
Chicken breast MP	0.1-500 MPa/10 min/ pressurizing gradient (PG) = 3.5 MPa·s^{-1} treatment after MP isolation	Actin and myosin denaturation; protein unfolding to expose surface hydrophobic and SH groups; α-helix and ß-sheet content decreased with increasing pressures. For thermal MP gels, 200 MPa pre-treatment was optimum for hydrophobic interactions while it was ≥ 300 MPa for hydrogen bonding.	Moderate HHP (200 MPa) improved MP solubility, gel hardness, WHC and microstructures of thermal MP gel; Stronger HHP treatment (≥ 300 MPa) weakened hardness while sharply decreasing WHC.	Zhang et al. (2017); Zhang et al. (2015)
Rabbit myosin	0.1-400 MPa/20 or 25°C/9 or 10 min/ PG = 20 MPa·s^{-1} after myosin isolation in 0.3 M NaCl or 0.6 M KCl at pH 6.5	Myosin showed increase in the content of sulfhydryl groups and surface hydrophobicity following HHP.100 MPa pre-treatment promoted unfolding of myosin tail during subsequent heating while HHP > 200 MPa pre-treatment facilitated aggregation of myosin head prior to heating and inhibited unfolding of myosin tail during heating.	100 MPa HHP improved the thermal gelation of myosin but was decreased at ≥ 300 MPa HHP. HHP ≤ 200 MPa induced filament structure with many small cavities in thermal gels; HHP ≥ 300 MPa induced globular aggregates with big cavities in thermal gels.100 MPa improved the WHC of myosin gels; HHP treatment (≥ 200 MPa) decreased the WHC of myosin gels.	Wang et al. (2017); Cao et al. (2012)
Silver carp MP	0.1, 200, 300, 400, 500 MPa/20°C/10 min after MP isolation in 0.6 M NaCl	HHP (≥ 200 MPa) reduced conformational stability, α-helix content gradually decreased and surface hydrophobicity increased with increasing pressures.	HHP resulted in high thermal gelation rates.	Qiu et al. (2014)
Threadfin bream natural actomyosin	200, 400, 600 MPa/room temperature/10, 30, 50 min after protein isolation in 0.6 M NaCl at pH 7.0	Pressures above 200 MPa induced denaturation and aggregation of actomyosin; exposed hydrophobic residues increased with HHP. HHP above 200 MPa induced intra- or extra-protein disulfide bonds.	Protein solubility decreased with increasing pressure above 200 MPa.	Zhou et al. (2014)
Hake MP	0, 150, 250, 500 MPa/10°C/10 min after protein isolation in 3% NaCl	HHP caused a reduction in α-helix and an increase in β-sheet content; HHP induced a reduction in myosin thermal denaturation temperature indicating protein unfolding; HHP had no significant effects on total sulfhydryl groups, but promote the	Heat-induced gel of HHP pre-treated MP has similar elasticity to that of untreated.	Cando et al. (2014)

		production of disulfide bonds during subsequent heating.		
MP in Beef patties	200, 300 MP /5-10°C/5 min/PG = 300 MPa·min^{-1} after MP formulated with 0, 1%, 2% NaCl and 0, 0.25%, 0.5% sodium tri-polyphosphate (STPP)	1% NaCl favored 200 MPa HHP-induced denaturation of myosin head and actin, whereas 0.25% STPP protected against that effect. At 300 MPa, STPP favored HHP-induced denaturation of myosin head, actin and other proteins. HHP-induced aggregates were stabilized by hydrogen bonds and hydrophobic interactions. The presence of salts in formulation can decrease the aggregation.	Soluble HHP-denatured beef proteins may provide interesting texture and technological properties to meat products with reduced salt content.	Speroni et al. (2014)
MP in pork batters	350 MPa/20±3°C/6 min/PG = 3 MPa·s^{-1} after MP formulated with 0%, 1.5%, 3% NaCl and 0%, 0.25%, 0.5% polyphosphate	350 MPa HHP denatured myosin and actin regardless of salts and polyphosphates; Addition of salts and phosphates destabilized the 350 MPa induced-protein structure.	HHP without salts had hardening effect on cooked pork meat batters; Salt and polyphosphates counteracted this hardening effect by improving the water-binding capacity; HHP enhanced water-binding capacity with less salt and without polyphosphate.	Villamonte et al. (2013)
Effects of HHP pretreatment on the raw muscle				
Rabbit MP	Muscle pre-treatment with 100, 200, 300 MPa/25°C/15s, 180s/PG = 20 MPa·s^{-1}	100 MPa depolymerized F-actin to G-actin and slightly exposed hydrophobic and sulfhydryl groups.	100 MPa/180s, 200 MPa/15s, and 300 MPa/15s improved the WHC and textural properties; 300 MPa/180 s caused severe protein denaturation.	Xue et al. (2017)
Pork MP	Muscle pre-treatment by 200, 400, 600, or 800 MPa/5 or 20°C/10 min	HHP (≥ 200 MPa) induced insoluble myosin and actin aggregates mainly formed by hydrogen bonds.	Low HHP pressure (200 MPa) facilitated protein hydration; Pressure at 400 MPa was found to be the threshold for loss of solubility.	Grossi et al. (2016)
Effects of pressure/thermal (P+T) combination on the isolated and formulated MP				
Pork MP	P+T treatment for 240 s: 100-600 MPa/10-40°C/PG = 40 MPa·s^{-1} or 2.5 MPa·s^{-1} formulated with 0.5%-2% NaCl and 0.15, 0.3% phosphate.	Maximum solubilization of S-1 domains with molecular weight of 50 kDa and at 27 kDa, as well as S-2, was found at 200--300 MPa; 100-200 MPa induced protein subunits below 6.4 kDa, which aggregated to form clusters via hydrophobic packing at > 300 MPa.	P+T application to frankfurter sausages with 240 s holding time improved firmness, WHC and CL and microbial inactivation above 500 MPa when initial temperatures are above 40°C. HHP adiabatic heating can uniformly modify the meat batters in a short time.	Tintchev et al. (2013)

Chicken breast MP	High pressure/thermal sequence treatment. H: 75°C/30min heating; H-P: 75°C/30min heating before 200 MPa/5 min/20°C HHP; P-H: 200 MPa/5 min/20°C HHP followed by 75°C/ 30 min heating; P+H (P+T): Heating (75°C) under 200 MPa/30 min pressure formulated with 2% NaCl and 0.3% STPP.	-	200 MPa/75°C/30 min P+H yielded the smoothest-textured sample having a high WHC and fine texture compared to H, H-P and P-H.	Zheng et al. (2015)
Effects of HHP on the isolated or formulated MP containing other ingredients				
MP in *Alaska pollock* (*Theragra chalcogramma*) surimi	High pressure/transglutaminase(TG) treatment: 200, 300, 400 MPa/5°C or 25°C/30 min/endogenous TG or 0.1% TG addition in the presence of 2% NaCl	HHP setting at 25°C with TG addition gave enhanced MP polymerization.	HHP setting at 25°C by endogenous TG was detrimental to cooked gel strength development while setting with endogenous TG under 300 MPa at 5°C gave high gel fracture stress and strain. When TG was added for setting under HHP at 25°C, gel strength and deformability (fracture stress, strain) were enhanced	Zhu et al. (2014)
Pork MP	High pressure with xanthan gum (XG) addition: 200, 400, or 600 MPa/20°C/6 min/ PG = 3 $MPa \cdot s^{-1}$. MP was formulated with 0.3 M NaCl at pH 6.0	XG inhibited the loss of α-helix structures and increased β-sheet structures induced by 600 MPa HHP; Unfolding of MP at high-pressure intensities exposed sites that interacted with the anionic polysaccharide, which prevented HHP induced protein aggregations; Xanthan increased the surface hydrophobicity of soluble MP after HHP treatment.	HHP above 200 MPa with xanthan addition showed higher solubility of MP than that in the absence of xanthan.	Villamonte et al. (2015)
MP in ostrich-meat	600 MPa/50°C/40 min/330 MPa/min PG HHP; Ostrich-meat formulated with carboxymethyl cellulose (CMC), locust bean gum (LBG) or xanthan gum (XG) in the presence of 2% NaCl, 5% STPP and 5% linseed oil	XG and/or CMC interacted to some degree with the MP matrix by HHP treatment. This prevented the protein matrix from interacting with the water or protein aggregates, leading to a reduced WHC.	1% LBG addition strengthened the elasticity and WHC of HHP-induced gel while the addition of CMC and XG had an antagonistic effect.	Chattong et al. (2015)

Note: HHP-high hydrostatic pressure, MP-myofibrillar proteins, PG-pressurizing gradient, STPP-sodium tri-polyphosphate, S-1-myosin subfragment 1, WHC-water holding capacity,

CL-cooking loss, P+T-pressure/thermal, TG-transglutaminase, XG-xanthan gum, CMC-carboxymethyl cellulose, LBG-locust bean gum.

Table 2 Recent applications of HHP for functionally improved, value-added and healthy muscle gelled products

Applications	HHP conditions	Achievements	Proposed mechanism	References
Application of HHP pretreatment prior heating or cooking for low salt, phosphate and/or low fat meat products				
Low-salt beef sausage	Formulated with 0.6%, 0.8% or 1% NaCl followed by 0.1-400 MPa/10°C/2 min/PG = 20 MPa·s^{-1} HHP	200 MPa HHP on meat batters containing 1% NaCl produced products having similar texture and cooked yield compared to the non-HHP samples with normal (2%) salt equivalents.	Increased solubilization of myosin and actin by HHP improved binding of muscle components.	Sikes et al. (2009)
Low-salt pork breakfast sausage with reduced-phosphate	Minced muscle pre-treatment by 0.1, 150 MPa/20°C/5 min prior to mixture with 0.5%-2.5% NaCl and 0.25% phosphate	150 MPa HHP can be adapted to reduce salt contents in the manufacture of low-salt breakfast sausages to 1.5% with acceptable organoleptic and functional properties.	-	O'Flynn et al. (2014b)
Low-salt surimi gel	Formulated with 0.3% or 3% NaCl followed by 0.1, 150 or 300 MPa/10°C/10 min HHP	Mechanical and sensory properties of reduced-NaCl (0.3%) gels were improved by the application of 300 MPa, obtaining similar values to gels made with 3% NaCl.	HHP induced protein denaturation and/or unfolding in reduced NaCl sample was similar to that with high content of NaCl.	Cando et al. (2015)
Low-phosphate pork breakfast sausages	Minced muscle pretreatment with 150 or 300 MPa/20°C/5 min prior to mixture with 0%, 0.25% or 0.5% phosphate	150 MPa has potential for reducing phosphate contents (0% or 0.25%) in sausages without significant changes in their functionality and improved their acceptability.	HP caused the solubilization and extraction of MP without the use of additives; HHP modified protein structure, promoting the cohesive properties of meat particles and improving the functionality of comminuted meat products.	O'Flynn et al. (2014a)
Reduced fat/salt pork sausage	Formulated with 0, 5, 10, 15, 20, 25 or 30% fat and 1% NaCl following 0.1-400 MPa/10°C/2 min/PG = 20 MPa·s^{-1} HHP	200 MPa treated sausages with 20% fat and 1% salt showed similar yield, texture and sensory properties to commercial emulsion-type sausages.	HHP at 200 MPa resulted in greater water retention in the fast relaxation compartment (strong water-protein interaction) and induced more protein denaturation.	Yang et al. (2015b); Yang et al. (2015a); Yang et al. (2016)
Application of HHP combined with food additives for low salt, low phosphate and/or low fat meat products				
Low-salt surimi gel	Surimi formulated with 0.3% NaCl, 0 or 0.1% lysine/cystine and 0 or	300 MPa HHP of surimi containing 0.5% TG and 0.1% cystine improved the physicochemical properties of low-salt	HHP induced primary protein denaturation or unfolding of MP, facilitating the further formation of	Cando et al. (2016a)

	0.5% transglutaminase (TG); 0, 300 MPa/10°C /10 min HHP	(0.3%) surimi gel.	different types of bonds assisted by additives.	
Low-salt surimi gel	Sumiri formulated with 0%, 0.3% or 3% NaCl, 0% or 0.1% lysine/cystine; 0, 300 MPa/10°C /10 min HHP	300 MPa or 0.1% lysine/cystine produced gels with low NaCl content (0.3% NaCl) having appropriate mechanical and functional properties, similar to those gels with 3.0% NaCl.	HHP treatment or the additives used can induce protein unfolding.	Cando et al. (2016b)
Application of high pressure/thermal (P+T) for low salt and/or low phosphate ready-to-eat (RTE) meat products				
Low-salt pork frankfurter	Formulated with 0.5%, 1% or 2% NaCl and 0.15% and 0.3% phosphate following P+T treatment for 240 s: 100-600 MPa/40°C/PG = 40 $MPa{\cdot}s^{-1}$ or PG = 2.5 $MPa{\cdot}s^{-1}$	2.5 $MPa{\cdot}s^{-1}$ PG rates at 600 MPa/40°C greatly improved WHC of reduced salt batters (0.5% NaCl).	HHP synergistically induced water-protein interaction; opened the protein molecule; dissociated actomyosin complex; disrupted myosin into subunits, increased the ionic strength through water dissociation induced by HHP.	Tintchev et al. (2013)
Pressure induced RTE chicken breast meat gel with low salt	Formulated with 0.6, 0.8, 1.0, 1.2 or 1.4% NaCl following 600 MPa/40°C/5 min/PG = 15 $MPa{\cdot}s^{-1}$ P+T treatment	NaCl content can be decreased to 1.2% in 600 MPa/40°C/5 min pressurized RTE gel products while maintaining suitable WHC, texture and palatability.	The high fraction of β-sheet of the meat proteins induced by HHP might lead to improvement in the WHC and textural properties of the pressure-induced gels.	Guo et al. (2015)
Low-salt/phosphate RTE chicken breast sausage	Formulated with 0.1, 0.2, 0.3 or 0.4% phosphate and 1.2% NaCl following P+T treatment (600 MPa/40°C/5 min/PG = 20 $MPa{\cdot}s^{-1}$)	600 MPa/40°C/5 min produced RTE sausages with reduced salt (1.2%)/phosphate (0.1%) while maintaining desirable functional properties and palatabilities.	Sausage with 0.1% phosphate treated with 600 MPa/40°C/5 min showed the highest ß-sheet contents and lowest α-helix contents, indicative of an enhanced gelling process.	Xue et al. (2016)
Low-salt chicken breast meat gel	Formulated with 1% or 2.5% NaCl, 0 or 0.3% sodium tri-polyphosphate (STPP) and 0 or 0.3% ß-glucan following P+T treatment (200, 400 or 600 MPa/20, 40 or 60°C/30 min)	Combination of 40°C and 400/600 MPa pressure were optimal for HHP processing of chicken breast gel, having adequate hardness and low nutritional loss; ß-glucan can be considered as a prospective partial substitute for NaCl in chicken breast gel produced by temperature-assisted HHP.	P+T induced hydrophobic interaction and disulfide bond formations are responsible for gel formation of chicken meat protein. Relative low temperature (40°C) together with high pressures (400/600 MPa) have synergistic effects on protein denaturation and gelation.	Omana et al. (2011)
Application of HHP to improve the functionality of pale, soft, and exudative (PSE) like meat for value added meat products				
PSE-like turkey meat value-added products	Low pH (PSE) and normal pH meat batters formulated with 10% water and 0.5% NaCl following 50 MPa,	50 and 100 MPa treatments gave the most improvement in WHC of low pH meat.	HHP at 50 or 100 MPa induced high protein surface hydrophobicity and great exposure of sulfhydryl groups, increased protein solubility and gelation	Chan et al. (2011)

	100 MPa, 150 MPa or 200 MPa/4°C/5 min HHP treatment		ability.	
Application of HHP for development of texture-modified meat products				
Pork meat gel as a dysphagia diet	Ground pork formulated with 1:0.5 or 1:1 (w/w) water, 1.5% NaCl and 0.05% rosemary followed by 400 MPa/17±2°C/20 min HHP	Gels with 1:1 (w/w) water treated with 400 MPa/17±2°C/20 min HHP were easy-to-swallow and left little residue in the oropharynx, it could be used as a dysphagia meat diet.	The network of myosin filaments gave superior textural properties to meat gels for dysphagic patients.	Tokifuji et al. (2013)
Minced fish meat gel as a dysphagia diet	Minced fish formulated with 1:0.5 (w/w), 1:1 (w/w) or 1:1.5 (w/w) water and 1.5% NaCl followed by 400 MPa/17±2°C/20 min HHP	Gels with 1:1 or 1:1.5 water treated with 400 MPa/17±2°C/20 min HHP were evaluated to be moderately elastic and smooth and to be useful for a dysphagia diet and for elderly individuals.	The HHP-induced fish gel was considered to be formed by irregular lateral associations of myosin filaments.	Yoshioka et al. (2016)

Note: HHP-high hydrostatic pressure, MP-myofibrillar proteins, PG-pressurizing gradient, RTE-ready-to-eat, PSE- pale, soft, and exudative, WHC-water holding capacity, P+T-pressure/thermal, TG-transglutaminase, STPP-sodium tri-polyphosphate.

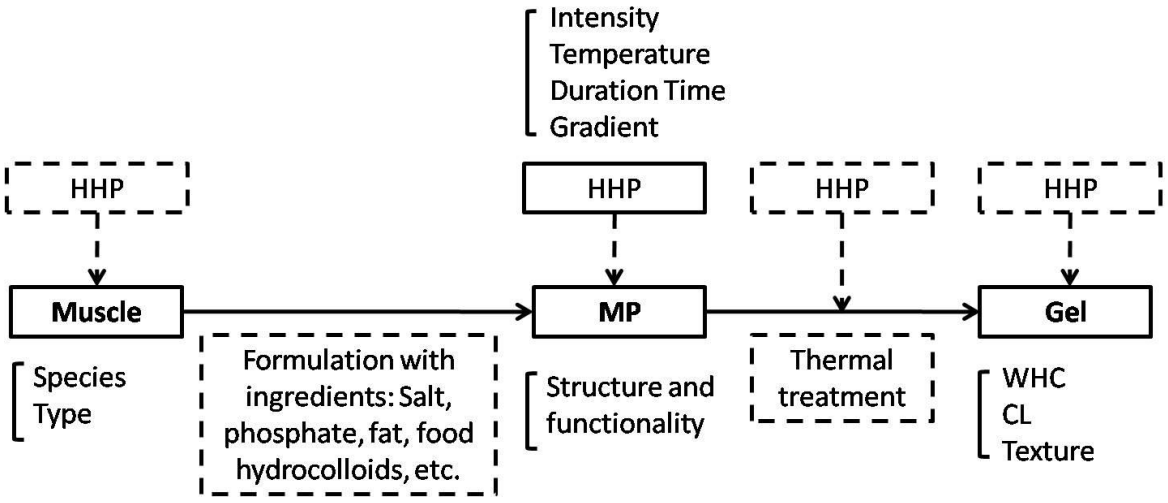


Fig. 1 Diagrammatic illustration of the formulation and gelation processes of MP and their resulting quality attributes as affected by HHP conditions during the manufacture of muscle gel-type products. HHP: high hydrostatic pressure, MP: myofibrillar proteins, WHC: water holding capacity, CL: cooking loss.

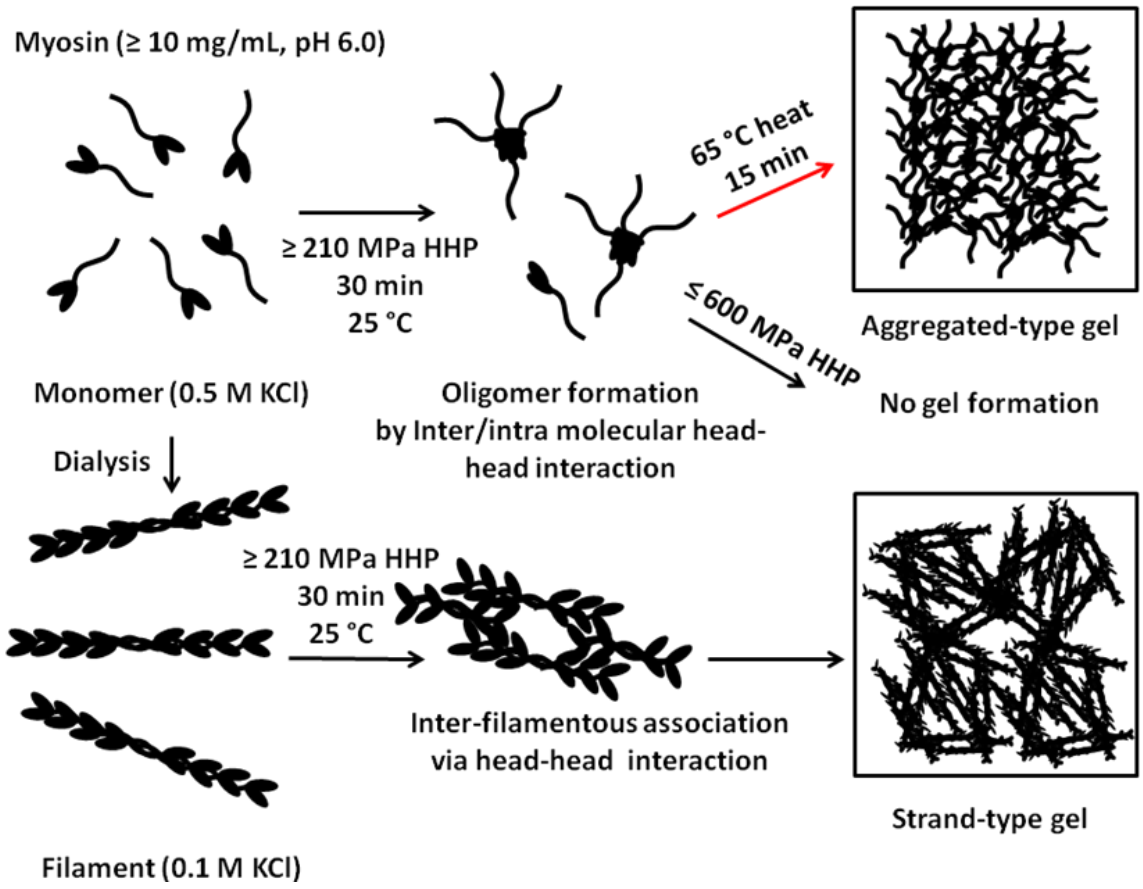


Fig. 2 Proposed mechanism of the HHP-induced aggregation and gelation of myosin in high ionic strength (0.5 M) or low ionic strength (0.1 M) solution at pH 6.0 based on the findings of Iwasaki et al. (2005); Yamamoto et al. (1990); Yamamoto et al. (2002) and Yamamoto et al. (1993). HHP: high hydrostatic pressure

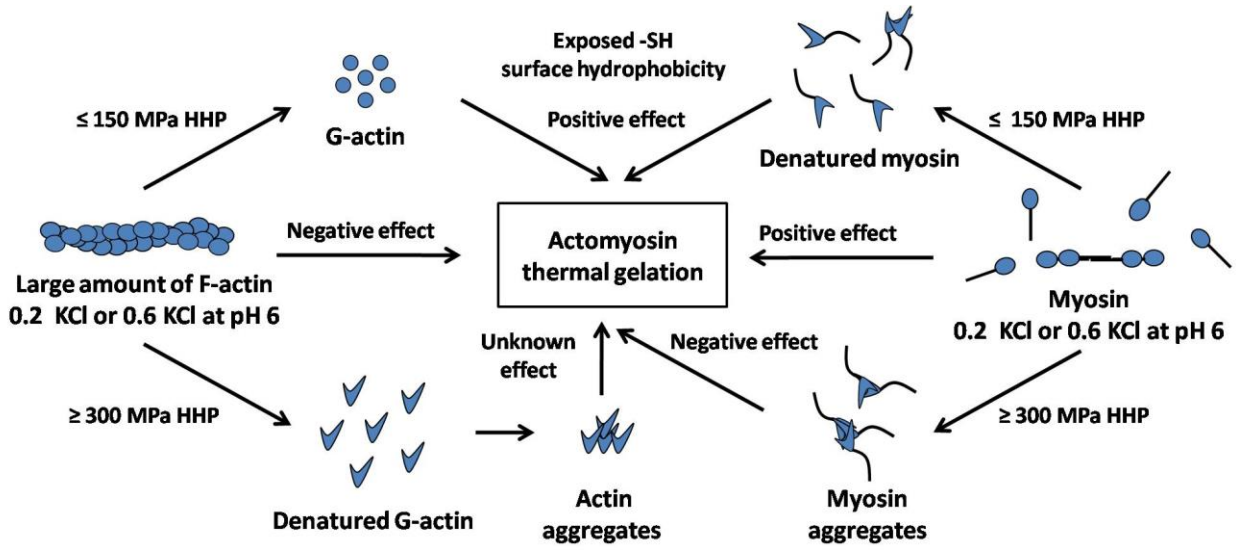


Fig. 3 Proposed mechanism on the effects of HHP on the thermal gelation of actomyosin in 0.6 M or 0.2 M NaCl/KCl at pH 6.0 based on the findings of Wang et al. (2017); Cao et al. (2012); Iwasaki et al. (2006); Ikeuchi et al. (1992b, 1992a) and Yasui et al. (1980). HHP: high hydrostatic pressure, -SH: sufhydryl groups.

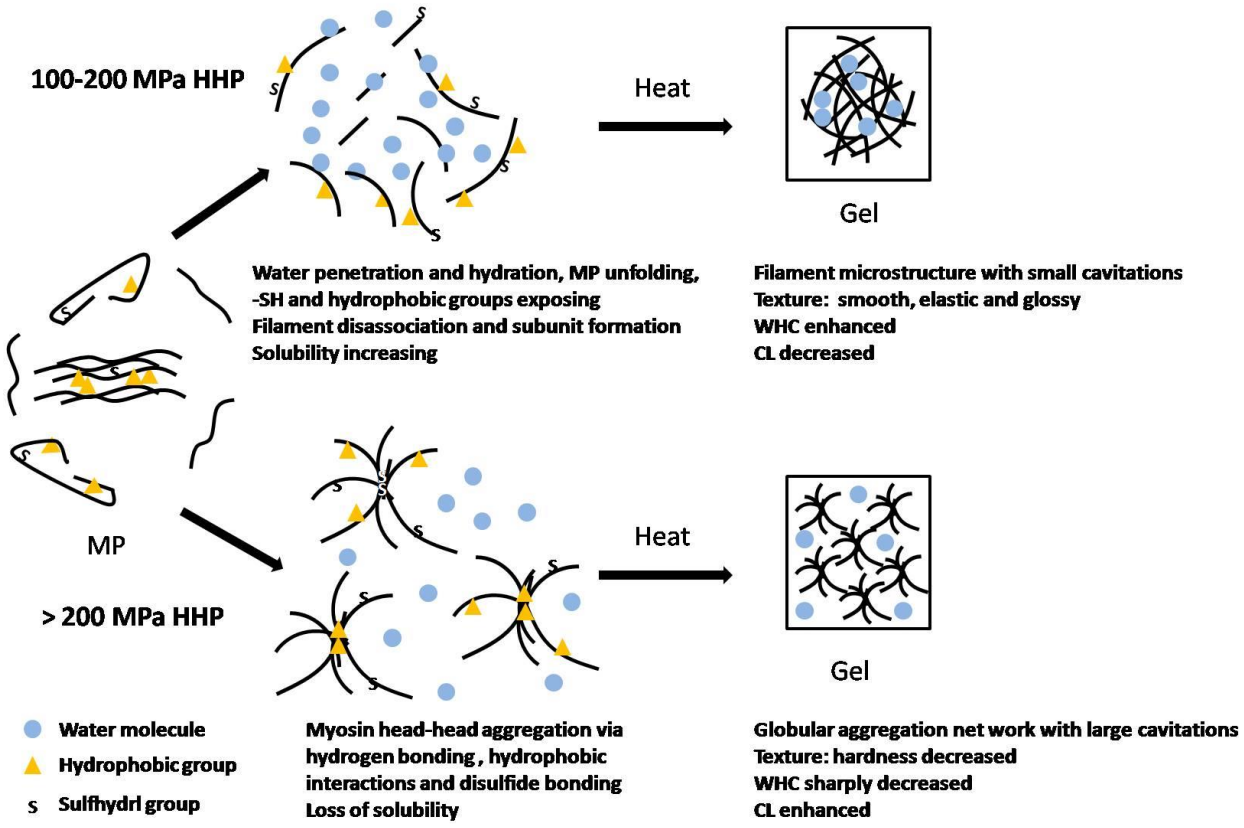


Fig. 4 Proposed mechanism on the effects of HHP on the denaturation, solubilization, aggregation and thermal gelation of MP in 0.6 M NaCl/KCl at pH 6-7. HHP: high hydrostatic pressure, -SH: sufhydryl groups, WHC: water holding capacity, CL: cooking loss.

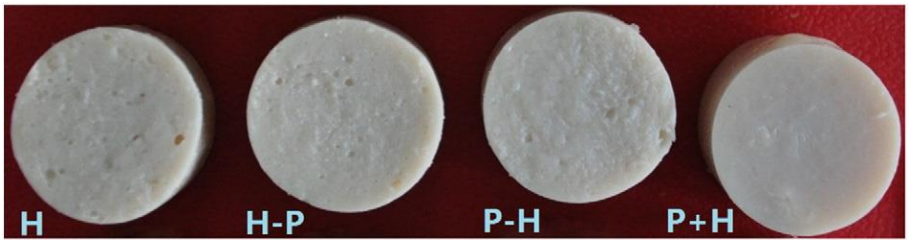
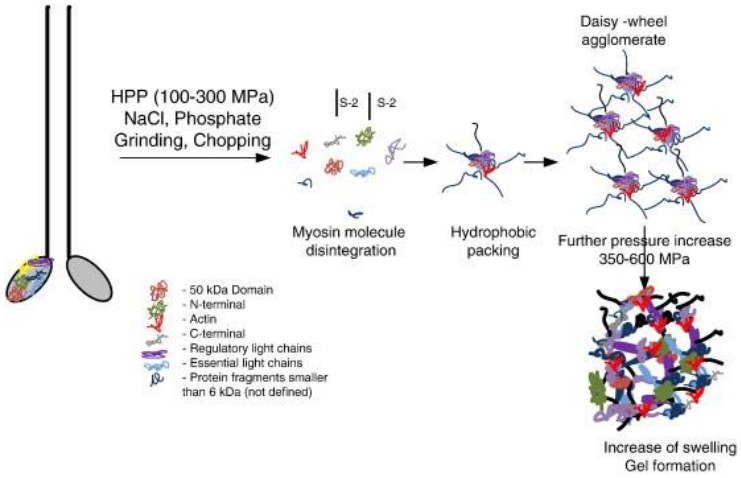


Fig. 5 Representative photographs of chicken sausages showing the appearance of transverse sections after pressure/thermal treatment. H: heating treatment (75°C, 30 min); H-P: heating treatment (75°C, 30min) followed by high-pressure treatment (200 MPa/20°C/5 min); P-H: high-pressure treatment (200 MPa/20°C/5 min) followed by heating treatment (75°C, 30min); P+H: heating under pressure (200 MPa/75°C/30 min). Source: adapted from Zheng et al. (2015).

(A)



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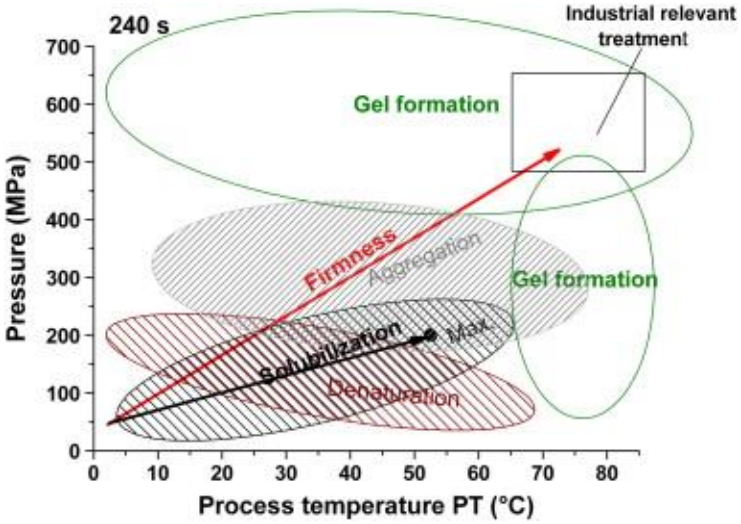

Fig. 6 (A) Theoretical mechanism of pressure/thermal (P+T) induced denaturation, solublization, aggregation and gelation of myosin. (B) Theoretical pressure-temperature diagram of myosin indicating commercially-relevant ranges for the manufacture of frankfurter sausage. Source: adapted from Tintchev et al. (2013).