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REVIEW

Structural and functional modification of food proteins by high power ultrasound and its application in meat processing

Dacheng Kang[a,b], Wangang Zhang[b], Jose M. Lorenzo[c], and Xing Chen[d,e]

[a]School of Life Sciences, Linyi University, Linyi, Shandong, China; [b]College of Food Science and Technology, Nanjing Agricultural University, Nanjing, Jiangsu, China; [c]Centro Tecnológico de la Carne de Galicia, Parque Tecnológico de Galicia, San Cibrao das Viñas, Spain Ourense; [d]State Key Laboratory of Food Science and Technology, Jiangnan University, Wuxi, Jiangsu, China; [e]School of Food Science and Technology, Jiangnan University, Wuxi, Jiangsu, China

ABSTRACT
In the field of agricultural and food processing, high power ultrasound (HPUS) is recognized as a green, physical and non-thermal technology in improving the safety and quality of foods. The functional properties of food proteins are responsible for texture, yield and organoleptic of food products which are the theoretical basis for food processing optimizing. HPUS treatment could provide the possibility for creating novel functional properties of new foods with desirable properties due to the modification of protein structure. In this article, an overview of the previous studies and recent progress of the relationship between structure modification and functional properties of food proteins using the HPUS technique were presented. The research results revealed that HPUS could significantly affect the conformation and structure of protein due to the cavitation effect resulting in the improvement of solubility, interfacial, viscosity, gelation and flavor binding properties of proteins. During meat processing, HPUS can modify the structure and thereby improve the functional properties of myofibrillar protein (MP), leading to the quality enhancement, low fat and/or salt products development and the shelf life extending. In view of this review, the recent findings of applications of HPUS in the production of meat products based on the modification of MP including curing, freezing/thawing and thermal processing have been summarized. Finally, the future considerations were presented in order to facilitate the progress of HPUS in meat industry and provided the suggestions based on the advanced protein modification by HPUS for the commercial utilization of HPUS in producing the innovative meat products.

KEYWORDS
Ultrasound; protein functional properties; myofibrillar protein; meat processing

Introduction

Proteins play a critical role in biological systems. By consuming protein-containing food products, the amino acids are provided for muscle protein synthesis and body growth, which encompasses important nutritional characteristics for humans. In addition, from the point of food system itself, the chemical and physical properties of proteins have vital influence on the protein performance in food systems during preparation, processing, consumption and storage (Klompong et al. 2007). These functionalities not only are related to their physicochemical and structural characteristics, but also provide the theoretical basis for optimizing food processing (Higuera-Barraza et al. 2016). On an empirical level, the various functional properties of proteins can be classified as three molecular aspects: (1) hydration properties including solubility, dispersibility, swelling and water absorption, (2) protein surface-related properties including emulsification, foaming and flavor binding, and (3) size- and shape-dependent hydrodynamic/rheological properties which are explained by gelation, elasticity, viscosity, dough formation and texturization (Speroni et al. 2009).

The understanding of the functional properties of proteins affected by physical and/or chemical modifications during processing and storage is essential for evaluating the quality changes of foods. During food processing, the functional properties of proteins can be changed due to the interaction with water, fat, sugar and other components in food (Amiri, Sharifian, and Soltanizadeh 2018). In addition, external processing conditions such as temperature, water activity, ionic strength and pH can modify the structure and the functional properties. In the last few decades, pH shifting (Abdollahi et al. 2016; Jiang et al. 2017), heat (Malik and Saini 2018), oxidation (Duan et al. 2018; Fu et al. 2019; Zhang, Xiao, and Ahn 2013; Zhou et al. 2014), S-nitrosylation (Liu et al. 2018) or dehydration (Dehnad, Jafari, and Afrasiabi 2016) treatment for the modification of proteins have been widely studied. However, it should be cautioned that although the conventional methods improve functional properties of proteins, they somehow impair the nutritional value, create some toxic side products and require high energy consumption. Thus, the minimum level of protein denaturation during processing is generally desired because this helps retain acceptable

CONTACT Wangang Zhang wangang.zhang@yahoo.com College of Food Science and Technology, Nanjing Agricultural University, Nanjing, Jiangsu 210095, China; Dacheng Kang jiangmeishanren68@163.com School of Life Sciences, Linyi University, Linyi, Shandong 276000, China.

protein solubility which is often a prerequisite functionality of these proteins in food products.

In recent years, the non-thermal processes, which refer to a group of techniques including supercritical fluid extraction (Panja 2018), extrusion (Nikmaram et al. 2017), pulse electromagnetic field (Han et al. 2018), high pressure (Chen et al. 2017) and high power ultrasound (Kang, et al. 2017; Wang, Zhang, and Zhou 2019) process or preserve foods without heating or drying and thereby have prominent advantages in improving product quality, energy efficiency and developing new product (Knorr et al. 2011).

High power ultrasound (HPUS) is a technology which is employed for food processing treated with the mechanical wave by the frequency ranging from 16-100 kHz (power intensity in the range of 10-1000 W/cm^2) by a cavitation effect (Awad et al. 2012; Chemat, Zill-e-Huma, and Khan, 2011). The extreme high temperatures and pressures from the area of cavitation generated by HPUS in liquid can lead to produce microstirring and microchannels on interfaces meanwhile the free radicals are appeared and produced (Kuijpers, Kemmere, and Keurentjes 2002; Tao and Sun 2015; Turantas, Kilic, and Kilic 2015). These phenomena are responsible for the modification of molecular reactions, characteristic and microstructure of food (Gulseren et al. 2007; Li et al. 2014; Vimini, Kemp, and Fox 1983). Studies have identified that this green technology could be used to improve or create new products with minimal effects on texture, nutritional, flavor and color value of foods, and even without thermal degradation for food during processing (Troy et al. 2016; Wang et al. 2018). Meanwhile, the mass transport as well as the textural or structural properties of food products could be influenced by these methods.

According to the energy or intensity of ultrasound, HPUS can usually be classified to two regions: low energy (low intensity) ultrasound with the intensities being below 1 W/cm^2 while the high power or high-intensity (>1 W/cm^2) has the frequencies between 20 and 500 kHz (Awad et al. 2012). Studies have identified that the chemical, physical and biochemical properties of food products could be influenced by HPUS cavitations in liquid. Thus, the HPUS has been used in research for more than eight decades which mainly focused on the food processing, the extraction of active ingredient, and the microorganism inactivation (Chemat, Zill-e-Huma, and Khan, 2011; Haughton et al. 2012; McClements 1995; Kang et al. 2017). The application of HPUS to proteins can also affect the structural and functional properties and then modify the textural and sensory qualities of products. Myofiber is the fundamental structural unit of the muscle, and the myofibril is the main organelle of the myofiber. Therefore, the quality and function of meat and meat products are mainly related to the structural and physicochemical properties of myofibrillar proteins (Huff-Lonergan, Zhang, and Lonergan 2010; Sun and Holley 2010). The purpose of this review was to illustrate the basic principle of functional and structural changes of proteins modified by HPUS, summarized the current developments of application of HPUS in the production of meat products based on the modification of myofibrillar proteins and provided the suggestions for the commercial utilization of HPUS in meat industry to produce innovative meat products.

Hpus-induced improvement of the functional properties of proteins

HPUS has been investigated to produce functional products through modifying food proteins which varies from that manufactured by conventional methods. In general, the modifications of proteins by HPUS are owned to the rupture and re-formation of covalent or non-covalent interactions with or within protein molecules (Tao and Sun 2015). In aqueous systems, the interaction of proteins with the water molecules can be improved by the effect of cavitation (Nazari et al. 2018). In this sense, the changes of functional properties of protein caused by HPUS and their effects on the solubility, interfacial property, viscosity, gelling and flavor combination or production were summarized.

Effects of HPUS treatment on the solubility of proteins

Many functional properties in food are affected by the solubility of proteins. As for meat processing, the solubility or extraction of myofibrillar proteins are significant in determining the textural, sensory and yield attributes of meat products (Higuera-Barraza et al. 2016). Therefore, exploring a technical method to improve the solubility by reducing the hydrophobic interaction between proteins and enhance the interaction between protein and water is meaningful.

Recently, HPUS has been identified as an alternative method to improve the solubility of proteins by modifying the structure, exposing more hydrophilic groups which buries in the internal molecular and decreasing the particle sizes (Jambrak et al. 2009; Morales et al. 2015). Arzeni et al. (2012) employed HPUS with 20 kHz at 4.27 W for 20 min on soy protein isolate, egg white protein and whey protein concentrate to investigate the solubility property changes of these proteins. The results showed that the solubility of soy protein isolate and egg white protein was improved after the HPUS treatment except the whey protein concentrate. The authors also found that after ultrasound treatment, the highest temperature of liquid was 43-45°Cwhich had minimal impact on the denaturation of proteins. However, the cavitation-induced high temperature in local area can promote the disintegration of protein polymers, reduce the macromolecular protein particles and improve the interaction between proteins and water, thus facilitating the solubility of proteins. However, Tang et al. (2009) and Hu et al. (2013) reported that the soybean protein isolate formed a soluble polymer during the HPUS processing, in which hydrophobic groups might be located in the center of the polymer while increased number of

Table 1. Recent studies on the effects of HPUS on structural modifications and functional properties of proteins.

Protein	HPUS conditions	Structural modification	Functional properties		Reference
Whey protein concentrate (WPC), soy protein isolate (500E) and egg white protein (EW), each solution prepared with 10% w/w at pH 6.5–7.1	20 kHz, 4.27 ± 0.71 W and 20% of amplitude for 20 min, Temperatures at the end of sonication was below 49 °C.	• Decreased the aggregates size of WPC and 500E but increased for EW. • No changed of sulfhydryl content • Increased surface hydrophobicity of all proteins	Solubility	500E and EW improved with the HPUS except the WPC	Arzeni, et al. (2012)
			Foaming	–	
			Emulsifying	–	
			Viscosity	Decreased in the consistency index of all protein	
			Gelation	Unchanged of the gelation performance of EW, WPC presented a higher elastic character, but no changed of 500E upon heating.	
Soybean protein isolate (SPI), 10.0%, w/v	20 kHz, 200, 400 and 600 W for 15 or 30 min, respectively	• Partial unfolding and reduction of intermolecular interactions • Increases in free sulfhydryl groups and surface hydrophobicity • Formed the larger aggregates during freeze drying	Solubility	Increased with HPUS power and treatment time	Hu, et al. (2013)
			Foaming	–	
			Emulsifying	–	
			Viscosity	• Decreased the consistency coefficients and increased the flow behavior index • Untreated SPI and SPI sonicated at 200 W for 15 min exhibited pseudoplastic behavior • The other sonicated dispersion was shear thinning with a Newtonian region	
			Gelation	–	
Beef myofibrillar proteins, 3%, w/v	20 kHz, 100 and 300 W for 10, 20 and 30 min, respectively	• Increased the pH value and reactive sulfhydryl content • Decreased particle size distribution	Solubility	Increased with HPUS power and treatment time	Amiri, et al. (2018)
			Foaming	Increased the foaming capacity and foaming stability	
			Emulsifying	Increased the emulsifying activity index and emulsion stability index	
			Viscosity	A reducing trend by increasing time and power of HPUS.	
			Gelation	Improved of WHC and gel strength	
Chicken actomyosin (CAM), 20 mg/mL	20 kHz, 1.15, 2.36 and 11.43 W/cm^2 for 20 min, respectively. Sample temperature was maintained below 8 °C	• 2.36 W/cm^2 resulted in the lowest particle size of CAM, and increase protein hydrophobicity and reactive SH groups. • Decreased in α-helix and increased thermally instable. • A small and homogeneous sub-bunch formed by 1.15-2.36 W/cm^2	Solubility	Increased significantly at 2.36 W/cm^2	Zou, et al. (2018)
			Foaming	–	
			Emulsifying	• Emulsifying activity index increased significantly at 2.36 W/cm^2 • Decreased the emulsion stability index	
			Viscosity	–	
			Gelation	–	
Egg white with pH 7.5	20 kHz, 90, 120, 240, 360 and 480 W for 10 min. Sample temperature was maintained below 25 °C	• Increased free sulfhydryl groups and surface hydrophobicity • Degradation of poorly water-soluble ovomucin. • A smaller aggregates and pore structure were formed by HPUS application	Solubility	Increased with HPUS power	Sheng, et al. (2018)
			Foaming	Highest foaming ability (260.00%) was obtained after 360 W HPUS treatment	
			Emulsifying	–	
			Viscosity	Decreased with HPUS power	
			Gelation	–	

(*continued*)

Table 1. Continued.

Protein	HPUS conditions	Structural modification	Functional properties		Reference
Ovalbumin (OVA), 5% (w/v)	20 kHz, 34-36 and 45-48 W/cm^2 for 20 and 40 min, respectively	• Unchanged of subunits and secondary structure of OVA • Increased free sulfhydryl groups and surface hydrophobicity • Decreased the surface net charge • Formation of protein aggregates after HPUS due to increased particle size.	Solubility	–	Xiong, et al. (2016)
			Foaming	• Increased the foaming ability • No changed of foaming stability	
			Emulsifying	• Increased the emulsifying activity • No changed of emulsifying stability	
			Viscosity	–	
			Gelation	Gelation temperatures of HPUS treated samples were higher than the untreated OVA	
Reconstituted milk protein concentrate (MPC) produced by ultrafiltered/diafiltered, final concentration 5% (w/v)	20 kHz, 12.50 ± 0.31 W for 0.5, 1, 2, and 5 min. Sample temperature was maintained below 50 °C.	• Particle size reduced from 28.45 μm to 0.13 μm after 0.5 min of sonication. • Increased the surface hydrophobicity with more hydrophobic groups exposed	Solubility	Solubility increased significantly from 35.78% to 88.30% after 5 min of HPUS pretreatment	Sun, et al. (2014)
			Foaming	–	
			Emulsifying	• Increased the emulsifying activity index • Emulsion stability index increased after HPUS treatment for 1 min. but decreased as the prolonged treatment time	
			Viscosity	Decreased after HPUS pretreatment	
			Gelation	Higher elasticity property than the control.	
Chicken myofibrillar protein (MP), 30 g/L	20 kHz, 88, 117, 150, 173 and 193 W/cm^2 for 15 min, respectively	• Increased the absolute value of the negative zeta potential and surface hydrophobicity • Decreased the particle size and total sulfhydryl (SH) group content • Decreased the G′ and G″ of MP during thermal gelation	Solubility	Increased with HPUS intensity	Zhang, et al. (2017)
			Foaming	–	
			Emulsifying	–	
			Viscosity	–	
			Gelation	• Denser and uniform gel microstructure when moderate HPUS (≦150 W/cm^2) treatments with improved water holding capacity (WHC) • Larger and irregular gel microstructures accompanied by decreased WHC with stronger HPUS (>W/cm^2) treatments	
Sodium caseinate and lactoferrin, 1.0 (wt %), pH 7.0	20 kHz, 300 W for 2, 4 and 6 min. Sample temperature was maintained below 30 °C.	• Decreased the size of sodium caseinate while the lactoferrin was increased. • Increased the surface hydrophobicity of all proteins • No differences for sodium caseinate structure but slight changes for lactoferrin.	Solubility	–	Figueiredo Furtado, et al. (2017)
			Foaming	–	
			Emulsifying	• Reduced droplet size • Improved emulsifying stability • A slightly higher stability of coarse emulsions	
			Viscosity	Increased intrinsic viscosity	
			Gelation	–	

(continued)

Table 1. Continued.

Protein	HPUS conditions	Structural modification	Functional properties		Reference
Animal proteins including bovine gelatin (BG), fish gelatin (FG) and egg white protein (EWP); vegetable proteins including pea protein isolate (PPI), soy protein isolate (SPI) and rice protein isolate (RPI), pH 7.08 ± 0.04, 0.1-10 wt.%	20 kHz, 34 W/cm^2 for 2 min. Before HPUS treatment, the temperature of protein solutions was within the range of 5-10 °C, whilst BG and FG solutions were within a range of 45-50 °C. After HPUS, the temperature of all protein solutions was below 45 °C.	• Decreased of the size of all proteins, except for RPI. • Unchanged of primary structure molecular weight of proteins. • No effect on the rate of FG or RPI surface denaturation, • No significant reduction in the equilibrium interfacial tension between untreated and HPUS irradiated FG or RPI	Solubility	Improved the solubility of PPI and SPI	O'Sullivan, et al. (2016)
			Foaming	–	
			Emulsifying	• HPUS- BG and FG had similar droplet sizes as their untreated counterparts. • HPUS- BG, PPI and EWP produced smaller emulsion droplets than control groups.	
			Viscosity	Reduction in the intrinsic viscosity of BG, FG, EWP, PPI and SPI except for RPI in solution	
			Gelation	–	
Whey protein (WP)	20 kHz, 150 W for 19.75 min. Sample temperature was kept at 29 °C.	• Exhibited smaller particle size, higher heat stability and storage modulus (G′) compared to raw WP. • Decreased the content of low molecular weight molecules. • Positive impact on the alteration in molecular and secondary structure of modified WP	Solubility	Increased with HPUS treatment	Khatkar, et al. (2018)
			Foaming	–	
			Emulsifying	–	
			Viscosity	No significant effect on the viscosity	
			Gelation	Formed a more elastic and stronger gel	
Fresh pasteurized homogenized skim milk (33 g/L protein)	20 kHz, 20 and 41W for 15, 30, 45 and 60 min. Sample temperature was maintained below 37 °C.	• An overall particle size reduction of fat globules • Soluble particles led to the minor changes of proteins • Denatured the whey proteins and formed soluble whey-whey/whey-casein aggregates during 30 min of sonication. • Partial disruption of whey proteins from aggregates with prolonged sonication time	Solubility	–	Shanmugam, et al. (2012)
			Foaming	–	
			Emulsifying	–	
			Viscosity	Unchanged of viscosity of milk	
			Gelation	–	
PSE-like chicken breast meat, 7.5 % with 2 % NaCl (w/w)	20 kHz, 28–32 W/cm^2 for 3 and 6 min.	• Reduced the content of salt solubility of myosin • Decreased the particle size of all batter samples. • Decreased the α-helical content and increased the formation β-sheet, β-turns, and unordered contents of all batter samples.	Solubility	–	Li, et al. (2014)
			Foaming	–	
			Emulsifying	–	
			Viscosity	Increased the final viscosity of PSE-like and normal batter samples.	
			Gelation	• Formed a compact and more dense gel network • Increased gel strength and water holding capacity of normal and PSE-like meat gels.	
Pre-heated whey protein isolate (WPI) (10% w/v, 85 °C for 30 min)	20 kHz, 107 W/cm^2 for 5, 10, 20 and 40 min. The pH modified to 7.0. Sample temperature was maintained below 45 °C.	• Reduced the particle size • Increased surface free sulfhydryl groups • Facilitated formation of more disulfide bonds during/after the gelation process	Solubility	–	Shen, et al. (2017)
			Foaming	–	
			Emulsifying	–	
			Viscosity	–	
			Gelation	Increased the water holding capacity, gel strength and gel firmness (G′) before GDL-induced gel (GIWG) formation	
Casein (CN) and whey protein (WP), CN:WP	20 kHz, 20.8 W for 1, 2	• Large aggregates were to be broken down to a	Solubility	–	Leong, et al. (2018)
			Foaming	–	

(*continued*)

Table 1. Continued.

Protein	HPUS conditions	Structural modification	Functional properties		Reference
ratios of 97:3 (high purity casein), 80:20 (similar to skim milk) and 50:50. The total protein concentration with 7.5% w/w	and 3 min. The pH ranged from 6.6 to 6.8. Sample temperature was maintained below 30 °C.	similar size when HPUS was applied post-heating • A considerable increase in aggregates size upon heating when HPUS was used prior to heating	Emulsifying	–	
			Viscosity	• HPUS alone reduced the viscosity • The use of HPUS post-heating produce a lower viscosity than those applied to pre-heating. • As for the 80:20 group, the application of HPUS prior to heating resulted in a higher viscosity than heating alone.	
			Gelation	• The sonicated casein stream produced a denser gel than the untreated casein sample. • The combined heat and HPUS treatments of high-purity casein produced gels with similar microstructures to the controls. • For the 50:50 system, the combined treatments resulted in gels that were less dense. • HPUS applied post-heating resulted in an improved gelation property.	

hydrophilic groups were exposed on its outer layer. These two opposed arguments may be due to the variation of protein source and concentration, pH, temperature and the frequency or power of the HPUS applied during food processing (Table 1).

Many researches have revealed that the protein solubility including soybean protein isolate and bovine myofibrillar protein could be enhanced with the increase of HPUS intensity and treatment time (HPUS intensity $> 2.39\,W/cm^2$, treatment time 20-90 min). Meanwhile the surface hydrophobicity of protein was positively correlated with the solubility (Amiri, Sharifian, and Soltanizadeh 2018; Hu et al. 2013; Kang et al. 2016b). In a recent study, Zou, Kang, et al. (2018) demonstrated that the solubility and surface hydrophobicity of actomyosin from chicken could be significantly improved by HPUS treatment within the range of $1.15 \sim 2.36\ W/cm^2$ while the protein polymers could be formed under the HPUS intensity of $11.43\,W/cm^2$, leading to decreased protein solubility and surface hydrophobicity (Table 1). Therefore, it is suggested that the hydrophobic groups embedded in the protein can be exposed by moderate HPUS treatment, which improved the hydrophobicity of the protein and the protein-water interaction (Arzeni et al. 2012). Notably, excessive HPUS treatment may cause denaturation and aggregation of proteins which can result in the loss of protein solubility.

Effects of HPUS treatment on the interfacial property of proteins

Foaming ability

Proteins are amphiphilic molecules and can migrate spontaneously to the oil-water or air-water interface to form a firm and thin film. Foaming properties, which refer to the ability of protein to incorporate and stabilize the gas bubbles, can be evaluated by foaming ability or foaming stability. Previous studies have demonstrated that the foaming stability of proteins could be improved by the effect of HPUS cavitation as a result of the partial denaturation and expanding of proteins. Accompanied by these modifications, the protein particle size can be reduced while the hydrophobic regions will be exposed to the surface so that proteins can rapidly distribute to air-water interface (Hayakawa and Nakai 2006; Jambrak et al. 2008; Shanmugam, Chandrapala, and Ashokkumar 2012; Tan et al. 2015).

Studies by Sheng et al. (2018) investigated the effects of HPUS pretreatments (20 kHz) at different power (90, 120, 240, 360 and 480 W) for 10 min on the foaming properties of egg white. The results revealed that, after 360 W HPUS treatment, the volume of bubbles was 4.9-fold higher than the control group with the highest foaming ability of 260.00%. The authors also found that the protein solubility was improved and the protein was easier to move to the gas-liquid interface after HPUS treatment. However, the foaming stability was reduced by HPUS application (Table 1). Xiong et al. (2016) compared the structure and interface properties of the untreated ovalbumin (OVA) with HPUS (20 kHz, 34-36 and 45-48 W/cm^2) treatment. The foaming ability and emulsifying activity were increased while the interface tension was reduced. This could be explained by the decreased surface net charge and increased surface hydrophobicity in OVA after HPUS treatment. However, the foaming stability and emulsifying had no remarkable differences (Table 1). Arzeni et al. (2012) found that the foaming ability was increased after applying the HPUS with the power of 4.27 ± 0.71 W (20 kHz). However, as for the beef myofibrillar proteins, Amiri, Sharifian, and Soltanizadeh (2018) showed the improvement of foaming capacity and stability because of the cavitation force of HPUS in both 300 W and 100 W (Table 1). The inconsistent results of those studies were possibly due to the HPUS equipment and option settings, and the molecular properties of proteins including solubility, hydrophobicity and molecular weight. For a protein with good foaming ability and stability, it should have an appropriate balance between rigidity and flexibility, which can support the abundant unfolding and cohesive interactions at the interface. In recent years, it has been found that covalent or non-covalent polymers could be formed in proteins (myofibrillar proteins and ovalbumin) after excessive HPUS treatment (Kang et al. 2016b; Xiong et al. 2016). The decreasing effect seems to be related to the increased protein particle size by HPUS treatment, which can not be quickly absorbed to the gas-liquid surface during the foaming process. In order to achieve an appropriate balance between protein unfolding and polymerization to obtain protein with well foaming ability and stability, it is necessary to take the properties of proteins and appropriate HPUS settings into consideration when applying HPUS.

Emulsifying properties

The proteins in natural and processed foods including milk, butter, sausage or cakes play an important role as an emulsifier. The proteins are needed to be unfolded and dispersed spontaneously and form protein adsorption layer at the oil-water interface to stabilize the emulsion. For the development of low-fat foods, the application of emulsifying technology will contribute to the improvement of food quality. In cooked sausage, the emulsifying ability of myofibrillar proteins have an important role on the water/fat retention and texture characteristics of the product since it is the theoretical basis for improving the gelatability and quality of final product. Therefore, it is meaningful for food enterprise to select food proteins with excellent emulsification ability or to adapt new technology for quality.

The emulsifying properties of food proteins can be evaluated by several methods such as emulsion stability and emulsion capacity. Application of HPUS could disorder the structures and decrease the droplet size of proteins resulting in enhanced emulsion activity. In addition, an increase in the number of exposed hydrophobic groups due to the decreased α-helix and increased β-sheet during HPUS treatment would improve the emulsifying property of proteins (Yanjun et al. 2014; Zhang, Pan, et al. 2018).

Figueiredo Furtado et al. (2017) investigated the effects of HPUS on the emulsifying properties of lactoferrin (1%, w/v) and sodium caseinate (1%, w/v), including the protein structure, conformation and hydrophobicity. They found that the protein diameter of sodium caseinate was decreased with the power of 300 W for 2-6 min treatment while the increased effect was obtained for lactoferrin (Table 1). This phenomenon was attributed to the various protein conformation that exhibited opposite behavior when exposed to the HPUS treatment. This could be explained by the production of non-covalent polymers, decreased β-sheet in protein secondary structure and increased protein surface hydrophobicity for the lactoferrin after HPUS treatment. HPUS treatment could further disperse the lactoferrin into the smaller droplet size in oil-water solution and stabilize the emulsion system. A recent report by O'Sullivan et al. (2016) investigated differences in the structural, physical and emulsifying properties of vegetable proteins including soy protein isolate, rice protein isolate and pea protein isolate, and animal proteins including egg white protein, bovine gelatin and fish gelatin with the treatment of HPUS. Each protein solution (0.1-10%, w/v) was sonicated at the HPUS intensity of 34 W cm^{-2} for 2 min. The result revealed that HPUS reduced the size of all proteins, except for rice protein. Comparing to the untreated proteins, the rice protein, soy protein and fish gelatin at the same concentrations had the similar droplet sizes after HPUS treatment, while the sonicated bovine gelatin, egg white protein and pea protein emulsions obtained a smaller droplet size when the concentrations were no more than 1% (w/v) (Table 1). This is ascribed to the increased surface hydrophobic and reduced interfacial tension between these untreated and HPUS treated groups. Therefore, in order to obtain a stable emulsion during HPUS processing, the sources and molecular structure of the protein should be taken into account comprehensively and the appropriate HPUS conditions should be selected.

The oil presented in protein solutions is essential for the texture and emulsifying stability in emulsion-based products (Santhi, Kalaikannan, and Sureshkumar 2017). The role of stabilization of fat droplets in aqueous is owe to the protein film surrounding the oil phase that reduces the interfacial tension between the continuous and dispersed phases. Taha et al. (2018) investigated the effects of HPUS (20 kHz at 50-55 W/cm^2; 2, 6, 12 or 18 min) on the emulsion properties of soybean protein isolate containing the long chain triglycerides and medium chain triglycerides. The results revealed that, after 18 min of HPUS treatment, emulsions composed with protein and medium chain triglycerides had the minimum droplet size ($d_{4,3}$) with 0.5 ± 0.0 µm due to the lowest particle size and highest adsorbed protein on the oil-water interface. The emulsion stability of oil-in-water emulsions were improved with the longer HPUS treatment time. In addition, the higher water solubility of medium chain triglycerides was also contributed to the movement and the adsorption of soybean protein isolate at the medium chain triglycerides oil/water interface. The ultrasonic treatment time and the type of oil had a great influence on the physical and chemical properties of the emulsion (Table 1). The author concluded that, by adding the soy protein isolate as the stabilizer, HPUS technology could be considered as a useful emulsification tool to produce emulsion-based products.

In other studies, combinations of HPUS with thermal or pH-shifting treatments for the emulsifying properties of proteins have also been studied. As for the study of HPUS/temperature for the emulsifying and physicochemical properties of heat-induced (85 °C for 30 min) aggregation of whey protein isolate by Shen, Fang, et al. (2017), who adopting an ultrasonic probe (20 kHz) for 20 min with pre- and post-thermal treatment. They found that whey protein solutions obtained the highest emulsion stability with the HPUS intensity 31 W/cm^2 for 20 min (Table 1). These changes could be due to the decreased droplets size and the increased surface hydrophobicity and free sulfhydryl group content. The researcher believed that the whey protein with the combination of post-thermal and HPUS treatment had improving effect on emulsifying and physiochemical properties which provided the potential for industrial applications. A similar research from Jiang et al. (2019) who investigated the effects of combination of pre-thermal treatment and HPUS treatment (20 kHz, 41-45 W/cm^2 for 0, 20, 40 and 60 min, respectively) on the functional and physicochemical properties of transglutaminase (TGase)-crosslinked whey protein isolate. A more (13%) emulsifying activity of the group of ultrasound-treated whey protein was found than that untreated whey protein, but the combination of pre-heated and HPUS treatment obtained a lower emulsifying activity than ultrasound-treated whey protein (Table 1). This is supported by the findings that after the combination of pre-heated and HPUS treatment, the TGase-crosslinked whey protein formed the largest size of protein molecular. The extended structure of high molar mass protein molecules could promote the aggregation and slow the adsorption of proteins on the emulsion interface. However, the higher emulsion stability was achieved by the method of HPUS-TGase and pre-heated-HPUS-TGase treatment which was owed to the poor flexibility of protein that reduced the oil/water interface adsorption capacity and prevented the reaggregation of oil droplets. On the other hand, it is beneficial to exert the effect of HPUS treatment on the emulsifying properties when protein was pre-heated. In other study, the HPUS was combined with pH-shifting to evaluate the effect on the structure and functional properties of pea protein isolate (Jiang et al. 2017). The pea protein dispersion (30 mg/mL, pH 7.0) was adjusted to pH 2, 4, 10, or 12 and then sonicated using a probe (13 mm diameter) with the acoustic power density at 68 W/100 mL for 5 min. The treated protein solution was adjusted to pH 7 after HPUS treatment. The best result was obtained for the combined of pH-shifting at pH 12 and HPUS treatment (pH12-US) which achieved the improved surface hydrophobicity and solubility of pea protein and meanwhile the soluble protein aggregates sizes were reduced below 100 nm (Table 1). The balance between hydrophobic and hydrophilic groups determined the emulsifying properties of proteins in solution. The alkaline condition of a solution could partially unfold and expose the hydrophobic cores to the surface of the molecule of pea protein. The cavitation effect of HPUS further broke the intermolecular interactions or disulfide bonds between proteins, thus producing smaller molar mass molecular proteins and resulting in emulsifying capacity and stability for emulsion systems.

Effects of HPUS treatment on the viscosity of proteins

During the transportation, mixing, heating/cooling or spray drying processing, the mass and heat transfer must be considered so that the viscosity of a protein is an important functional property for the fluidized food. The viscosity of protein solution is mainly affected by the inherent characteristics of protein molecules, protein-solvent and protein-protein interaction. The viscosity presented an exponential correlation between protein solubility or absorbency in most studies (Galush, Le, and Moore 2012).

As discussed previously, the HPUS treatment could affect the particle size and degree of aggregation of proteins, and thereby the rheological properties of protein solution might be changed by HPUS. However, there existed the distinct results on the effects of HPUS treatment on the type and viscosity of protein fluid. Arzeni et al. (2012) found that soy protein isolate, egg white protein and whey protein concentrate exhibited a shear thinning behavior as a consequence of HPUS treatment with the consistency index (m, mPa·s) $<$ 1. For soy protein solutions (10%, w/w), a more Newtonian behavior was observed as it was close to 1 and the m value was decreased from 2896 mPa·s to 46.60 mPa·s (Table 1). The authors further found that the flow behavior of protein suspensions tended to be a Newtonian one after HPUS application. Similar findings were obtained from the studies on myofibrillar proteins (Amiri, Sharifian, and Soltanizadeh 2018) and calcium caseinate (Yanjun et al. 2014). In another study carried out by Khatkar et al. (2018) the effects of HPUS with 150 W for 19.5 min treatment on the structure, solubility, particle size, rheology and morphology of whey protein (12.5%, w/v) were evaluated. They reported a decreased viscosity of protein after HPUS processing and classified the whey protein solution as the Newtonian behavior fluid (Table 1).

On the contrary, Krešić et al. (2008) demonstrated that the flow behavior of whey protein concentrate (10%) and whey protein isolate (10%) was observed to be shear-thickening behavior after HPUS treatments. The apparent viscosity of all proteins increased from 7.0 mPa·s to 8.0 mPa·s at the shear rate of 1240s^{-1} whereas no changes of fluid behavior were found (Table 1). Jambrak et al. (2009) discovered that soy protein isolates showed increased viscosity when subjected to HPUS (40 and 500 kHz) for 15 and 30 min (Table 1). The increased viscosity of protein after HPUS treatment could be ascribed to the promoted hydration of hydrophilic groups on the surface of protein with the water surrounding resulting in an increased water binding. However, the flow behavior indices indicated that soy protein isolates fluid was belonging to the pseudoplastic ($n < 1$) behavior before and after HPUS treatment. In addition, the increase in temperature during HPUS treatment could partially explain the enhanced viscosity (Hu et al. 2013).

Other studies conducted by Jambrak et al. (2010) evaluated the effects of HPUS (20 kHz, 40 kHz and 500 kHz for 15 min and 30 min, respectively) on the functional and physicochemical properties of the α-lactalbumin (α-LA). They reported that the apparent viscosity (6.0 mPa·s) of α-LA samples was not changed significantly by HPUS treatment ($p > 0.05$). The flow behavior was observed to be the shear-thickening after all treatments (Table 1). In another study, Shanmugam, Chandrapala, and Ashokkumar (2012) found that the viscosity of pasteurized skim milk was not altered after the HPUS (20 kHz, 20 and 41 W for 15, 30, 45 and 60 min) treatment (Table 1). These investigations tended to believe that the overall reduction of particle size of soluble particles and fat globules were responsible for the minimum changes to the flow behavior of proteins.

In addition, the viscosity of protein solution could also be influenced by sources and structure of proteins and the sequence of HPUS treatment. O'Sullivan et al. (2016) studied the effects of HPUS (20 kHz, 34 W/cm^2, 2 min) for the viscosity of proteins from animal and vegetable. The intrinsic viscosity of BG, FG, SPI, EWP and PPI was decreased significantly by HPUS treatment in solution ($p < 0.05$). However, the intrinsic viscosity of RPI was found unchanged due to the highly aggregated structure of the insoluble protein after HPUS treatment. The researchers believed that the restricted size reduction during the HPUS treatment was owned to the presence of carbohydrate interacted with the aggregation and the denaturation of protein (Mujoo, Chandrashekar, and Zakiuddin Ali 1998) (Table 1). In addition, to evaluate the effect of HPUS combined with heat treatment on the functional interactions between whey and casein in milk systems with different casein-whey protein ratios (97:3, 80:20 and 50:50), Leong et al. (2018) illustrated that the application of HPUS alone reduced the viscosity of all treatment groups, which may be ascribed to the decreased in the particle size. On the other hand, a lower viscosity of the solution could be obtained by the use of HPUS post-heating (Table 1). This could be explained by the destructive effect of HPUS for the heat-induced protein aggregations. However, the higher viscosity of 80:20 group was found when applying HPUS prior to heating than heating alone. According to the size distributions, it was proposed that the larger aggregates might be formed when HPUS was applied prior to heating.

Effects of HPUS treatment on the gelling properties of proteins

Gelation is the important functional properties for food proteins, which plays a major role in the preparation of foods such as tofu, emulsified sausage and cheese. The protein gel is the product of network structure which is formed by the cross-linked of polymers due to the covalent or noncovalent bonds. The cells in gel are capable of entrapping water and other small molecular substances. As a method to improve protein gelation, the effects of HPUS on the gelling property of proteins have been thoroughly reviewed by Higuera-Barraza et al. (2016). Most of researches have confirmed that HPUS treatment could improve the quality and water retention of the protein gels due to the changed molecular structure, and thereby increased the fluidity of proteins and the interaction between protein and protein/water. The modification of proteins by HPUS is conducive to the rapid formation of protein cross-linking when subjected to heating treatment (Amiri, Sharifian, and Soltanizadeh 2018; Leong et al. 2018; Li et al. 2014; Li et al. 2015; Wang, Yang, Tang, et al. 2017). The water holding capacity of MP gel, chemical forces and their relationship as influenced by HPUS (20 kHz, 240 W) was evaluated by Wang, Yang, Tang, et al. (2017). Both the water holding capacity and chemical forces of the MP gel were influenced significantly by HPUS treatment ($p < 0.05$). Hydrophobic force, electrostatic repulsion and hydrogen bonding were essential in holding water in MP gels while disulfide bonds were not. The 6 min treatment was the optimum condition for the water holding capacity of MP gel accompanied with the maximum of hydrophobic force, hydrogen bonding and electrostatic repulsion of the gel. The network structure with uniform density and the best retaining water properties of gels were obtained (Table 1).

The PSE-like (Pale, soft, exudative-like) meat is the major quality defect that affects the benefits of the poultry enterprises (Barbut 2009). In order to evaluate the effect of HPUS on the modification of PSE-like chicken meat, Li et al. (2014) employed the HPUS (20 kHz, 450 W) with 0, 3, or 6 min for meat batter suspension (7.5% meat proteins with 2% NaCl, w/w) to assess the change of gelling properties. The gel strength and water holding capacity of PSE-like group were improved significantly by HPUS treatment. The rheological behavior as well as the elastic (G′) and viscous (G″) modulus of PSE-like samples was improved by HPUS (Table 1). The decrease of myofibril particle size and the change of protein secondary structure caused by HPUS were the reasons for the improved gel properties of PSE-like meat. These results imply that the functionality of PSE-like meat might be modified by the applying of HPUS which provides the potential to increase economic benefits of poultry enterprises.

Another work conducted by Shen, Zhao, et al. (2017) sonicated (20 kHz) the pre-heated whey protein isolate solution (10% w/v, 85 °C for 30 min) at different time (5-40 min), and then analyzed the gelation properties of glucono-δ-lactone induced whey protein gel. Results showed that the gel strength, gel firmness (G′) and water holding capacity were significantly improved by HPUS treatment (Table 1). The cavitation generated by HUPS could destroy the non-covalent interactions between proteins, reduce the particle size of proteins and increase the free -SH content of the preheated WPI protein. These modifications above are beneficial to the formation of a uniform and dense cross-linking structure of the network gel when subjected to acid-induced gel process. The gelling properties of whey protein could be improved by HPUS to develop the new dairy products in the forms of gels. Nevertheless, as for the myofibrillar protein solution (30 g/L), Zhang et al. (2017) found that the stronger HPUS ($> 150\ W/cm^2$) treatments might induce the irregular and larger gel structures with the decreased water holding capacity. However, when the intensity of HPUS was less than $150\ W/cm^2$, a uniform and denser gel structure with the improved water holding capacity was observed (Table 1).

The HPUS can be used as an alternative method to improve the characteristics of protein gels. In terms of its application, not only protein sources and processing temperature should be considered, but the selection of appropriate HPUS condition is also essential for the formation of high-quality gels. In addition, the oxidation effect on proteins due to the free radicals (·OH) generated by HPUS cavitation can not be neglected (Kang et al. 2016b). Previous studies have confirmed that the moderate oxidation of proteins was beneficial for the formation of stable gels, but the increased degree of oxidation could reduce the gel quality (Zhou et al. 2014). Therefore, it is necessary to further evaluate the effect of oxidation of HPUS on the quality of gels and the content of essential amino acids.

Effects of HPUS treatment on the flavor binding and generation property of proteins

The flavor-binding property of proteins in food, especially in meat and meat products, are essential to consumer acceptance due to their functioned as the flavor carriers or flavor modifiers in fabricated foods. Though the modifications of protein structure and function by HPUS treatment have been fully reviewed, the effect of protein structure changes on the binding ability of flavor substances has not been reported. Recently, there had been some studies exploring the production, structure, solubility, emulsification, oxidation resistance and other features of the adducts which were produced from the reaction between protein and saccharides during the HPUS assisted processing. The researchers found that the adducts were obtained through the Maillard reaction as well as the HPUS assisted processing accelerated the rate of reaction. Meanwhile, the solubility and oxidation resistance in the product were improved significantly (Abdelhedi et al. 2017; Chen et al., 2019; Stanic-Vucinic et al. 2013). Furthermore, the alteration of protein functional characteristics due to Maillard reaction might impact the flavor binding ability, and thus affect the sensory properties of foods. To our knowledge, the surface hydrophobicity or hydrophobic interactions of proteins can affect the flavor binding ability due to the volatile flavors interact with proteins mainly through hydrophobic interactions. Therefore, the binding ability of proteins to flavor substances still needs to be further studied from the aspects of protein structure, conformation, interaction between proteins and flavor substances when protein is processed with HPUS. In addition, it is necessary to further evaluate whether the added products can produce novel flavor or harmful substances in subsequent processing process.

In cooked meat, the flavor compounds were created by the Maillard reaction process. In terms of the formation of flavor substances, Yu et al. (2016, 2017) employed amino acids (L-serine, L-lysine) and sugars (D-glucose, D-xylose) to build a model system to view the effect of HPUS treatment on the reaction rate of Maillard reaction and content or species of flavor substances. These studies found that the HPUS treatment could significantly lower the activation energy as which was needed to Millard reaction and improve the reaction rate (Yu et al. 2018). Ong et al. (2015) took the cysteine-xylose model system to evaluate the HPUS assisted processing on the composition of odor-active of flavor compounds by Maillard reaction. Compared to the conventional heat treatment, fewer sulfur-containing volatile flavor compounds were generated by HPUS-assisted Maillard reactions. It was proposed that the inefficient transmission of HPUS energy and the HPUS induced degassing were responsible for the expulsion of sulfuretted hydrogen resulting in the difference in flavor profile. However, as for a complex system, such as peptides or proteins with reducing sugars, the mechanism of forming the flavor substances still needs to be further studied when subject to the HPUS treatment. Furthermore, the influence of HPUS treatment on the formation and binding of flavor substances of proteins should be paid more attention in the following studies.

Recent applications of HPUS for meat processing based on MP modification

With the awareness of the relationship between diet and nutrition of consumers and the increasing changes in lifestyle, the scientific and industrial research is inclined to develop the rich flavor, healthy, convenience and economical meat products (Sitz et al. 2005). For the last decades, HPUS has been considered as a useful tool in the meat processing and responsible for the modification of molecules of MP that improve or provide more functional properties of meat such as water holding capacities and gel-forming abilities (Arzeni et al. 2012; Li et al. 2015; Stadnik, Dolatowski, and Baranowska 2008). In the industrial application, the HPUS technologies can provide a new processing strategy for formulation of reduced salt, phosphate and/or fat in emulsified meat products (Inguglia et al. 2017; Li et al. 2015), improving the functional properties of low value meat (Inguglia et al. 2017; Li et al. 2014; Li et al. 2015) or modifying texture of fresh meat (Alves et al. 2013; Sikes et al. 2014; Wang et al. 2018). Thus, in order to improve the meat quality and meet the consumer demands for the value-added meat products, controlling the HPUS-induced functional and structural changes of MP and optimization the parameters of HPUS are still important issues for meat science and meat industry. Therefore, it is necessary to understand the mechanism of functional, conformational and physicochemical changes of

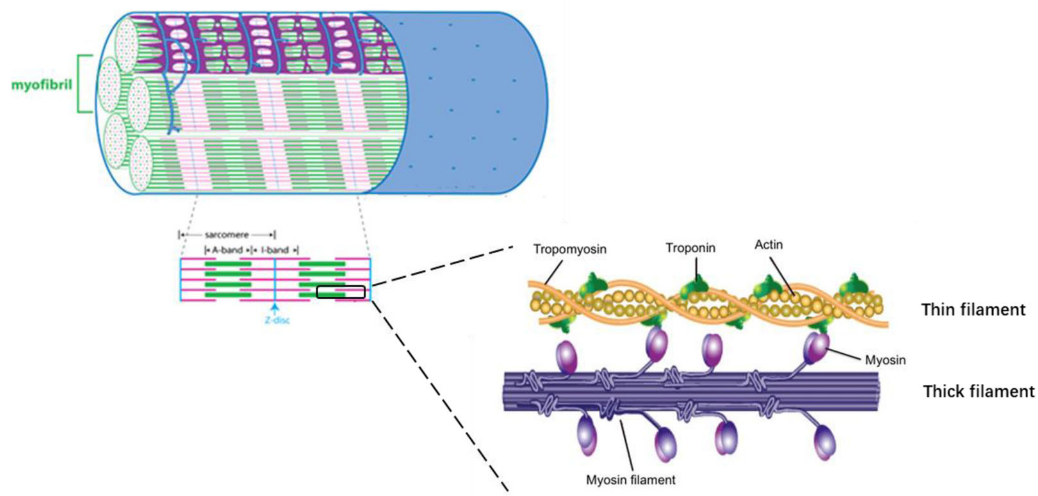

Figure 1. The structure and protein composition of myofibrils.

MP during the HPUS treatment to produce the healthy, nutritional and economical processed meat products.

Structure and functional properties of MP for meat quality

According to the solubility and their location in myofiber cells, the protein of muscle can be classified as three groups: myofibrillar, sarcoplasmic and stromal fractions (Lee, Joo, and Ryu 2010). The myofibrils are the long and cylindrical strands of contractile proteins which is almost the entire cross section of the muscle fiber (50 ~ 56% of the total skeletal muscle protein). The thick filaments and thin filaments are comprised the sarcomeres which is the basic component unit of myofibril. The thick filaments are consisting of an elongated protein called the myosin while the thin filaments are mainly of the actin protein (Fig. 1). Most studies have confirmed that the structures of cross bridge formed by the myosin and actin when Ca^{2+} binds to troponin are related to the meat quality (Chen et al. 2017, Wang et al. 2018). Under the postmortem conditions, the cross-linked structure is referred as the actomyosin in the absence of ATP (Barbut et al. 2008).

In general, the intact meat and meat batters are the common research subjects that the HPUS proccessed in order to accelarate the mass transfer and improve the tenderness and gel-forming porproties. Myosin, which is the major constituent in myofiber protein, has been suggested to be mainly responsible for the overall function of MP (Chen et al. 2017). Thus the interaction between myosin and salts, water, fat and other ingredients can significantly affect the quality attributes of meat and meat products. At present, the effects of HPUS assisted processing on the modification of physicochemical and quality of meat during curing, frozen/thawing and drying treatment have been detailly studied (Chemat, Zill-e-Huma, and Khan, 2011) (Fig. 2).

Curing

Curing is the method for enhancing the tenderness, juiciness, flavor and shelf-life of meat and meat products that is widely used in processing and preservation (Alarcon-Rojo et al. 2015). The conventional salt-brining processes are mainly encountered to three drawbacks: (1) the desalting process is needed due to the high NaCl content of products otherwise be harmful for human health; (2) the occurrence of natural microorganisms provides the potential risks in the contamination of products; and (3) the marinating process with extended time may leads to the textural damage including structural changes and bloating. For the last dacade, most studies have shown that the shorten brining time and improved meat quality and other characteristics could be achieved by applying the HPUS due to the mechanical and thermal effects of the cavitation. Meanwhile, the cured meat could be obtained under the reduced salt concentration due to the rapid material balance when adopt HPUS assisted curing technology (Alarcon-Rojo et al. 2015; Barretto et al. 2018; McDonnell, Allen, et al. 2014).

Kang and other colleagues reported that the intensity of HPUS, treatment time and salt concentration were the main factors influencing the rate of mass transfer and meat quality during HPUS assisted curing (Kang et al. 2016a; Leal-Ramos et al. 2011) (Table 2). The factor of salt concentration slightly affected the values of diffusion coefficients (D) while the intensity of HPUS was responsible for the changes of water or NaCl. Furthermore, the efficiency of NaCl uptake was also influenced by the geometric parameters of HPUS systems such as probe size, distances between the meat and transducer and the thickness of the sample. These factors above were crucial in determining the salt content of meat during HPUS processing (Elena S. Inguglia et al. 2018). The authers illustrated that the distance between the probe and the meat of 0.3 cm was the best efficient distance to ensure a higher D value of salt. However, a tradeoff distance of 0.5 cm could be considered if the balance between meat quality parameters and salt diffusion was achieved.

As for meat quality, studies implies that HPUS processing could improve the meat texture which lied in the synergistic action of mechanical of ultrasonic and salt that altered the functional, physicochemical and conformational of MP

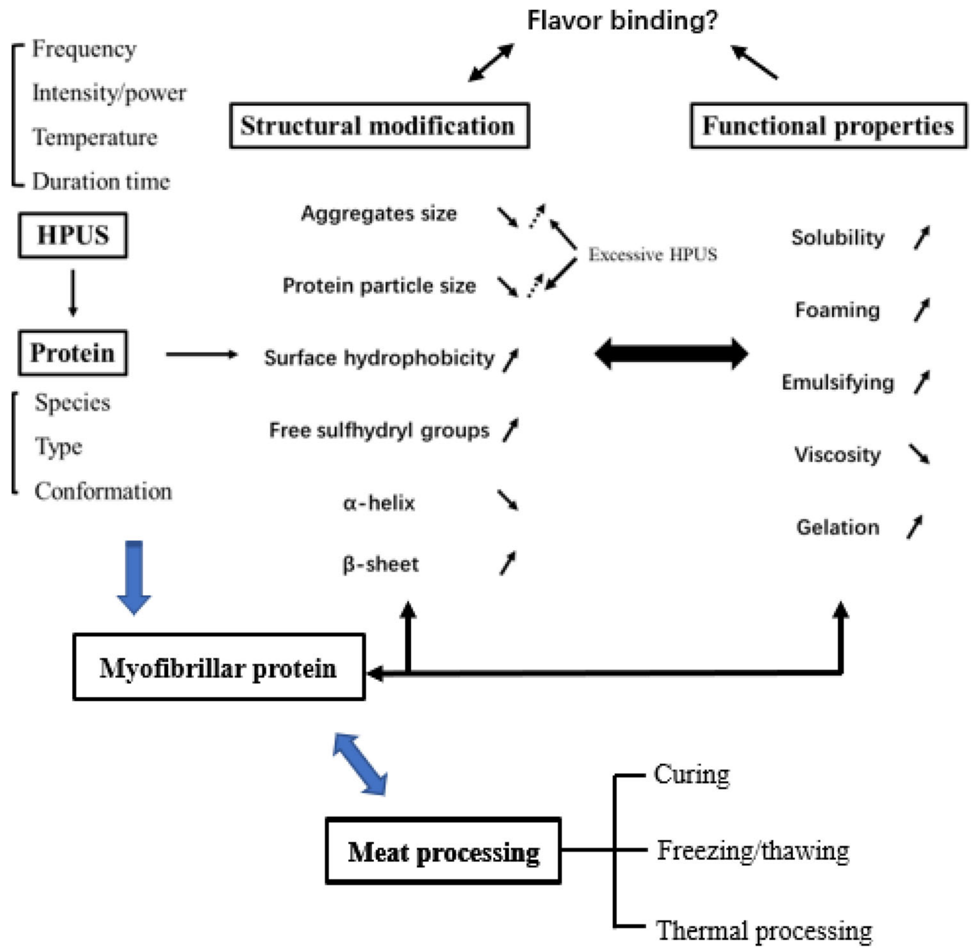

Figure 2. Diagrammatic illustration of the relationship between high power ultrasound (HPUS) on protein structure modification and functional characteristics, and its application in meat processing.

(Yang et al. 2015). McDonnell, Allen, et al. (2014) evaluated the water-protein interactions of pork during the HPUS-assisted curing (4.2, 11 or 19 W/cm^2 for 10, 25 or 40 min). Except to 4.2 W/cm^2 for 10 and 25 min, the protein extraction was increased than that of the control in all HPUS treatments (Table 2). The result could be confirmed by the evidence of unchanged of meat matrix determined by light microscopy. These findings imply that the meat proteins could be further extracted during the HPUS assisted curing. The myosin at the surface of the meat could be denatured with high power inputs. In contrast, Kang et al. (2017) demonstrated that HPUS (20 kHz, 150 and 300 W, 30 and 120 min) assisted curing could significantly increase the water holding capacity and the tenderness of beef compared to static brining due to the swelling and disruption of myofibrils. Beef with HPUS treatment could result in protein oxidation with decreased total sulfydryl groups leading to protein aggregation with covalent cross-linking. In addition, the increase of protein free sulfydryl residues, surface hydrophobicity and β-sheets while the decrease in α-helix indicate that the protein structure could be altered after HPUS treatment (Kang et al. 2016b) (Table 2). Thus HPUS treatment could improve the meat quality along with the MP oxidation and structure changed which could be ascribed to the salt and the mechanical effects of cavitations. Meanwhile, Krasulya et al. (2014) employing the acoustic activation of brine (pre-treated with HPUS 20 kHz, 200 W/L) investigated the effect of activated brine on the protein hydration of shredded raw meat. The mechanism of activation of brines based on HPUS cavitation energy was belonging to high-energy chemistry and epithermal phenomenon (Krasulya et al. 2019). The epithermal effect of HPUS could increase the solvency and capacity of water for dissociation due to the destruction of the intermolecular hydrogen bonds of cluster structure of water. As for the salt solution, the declusterizated water was beneficial for the Na^+ and Cl^- ions acquiring dense solvate shells, which prevented their association and created conditions for their getting into myofibrillar proteins. The brining pre-actived by HPUS could improve the process properties and functional of meat, and meanwhile the level of protein hydration and the formation of thermal resistant hydrocolloids were increased (Table 2). The experiments confirmed that acoustic activation of brine not only improved the rate of penetration of brine into interfibrillar spaces but also destructed the muscle fibers resulting in essential changes in meat structure and acceleration of biochemical processes. Further research could focus on the analysis of penetration kinetics of the pre-actived curing and the binding mechanism of hydrated ions and MP. In additon, McDonnell, Lyng, et al. (2014) firstly explored the application of HPUS (0, 10.7, 17.1 and 25.4 W/cm^2) in pilot-scale and evaluated the potential detrimental effects on meat

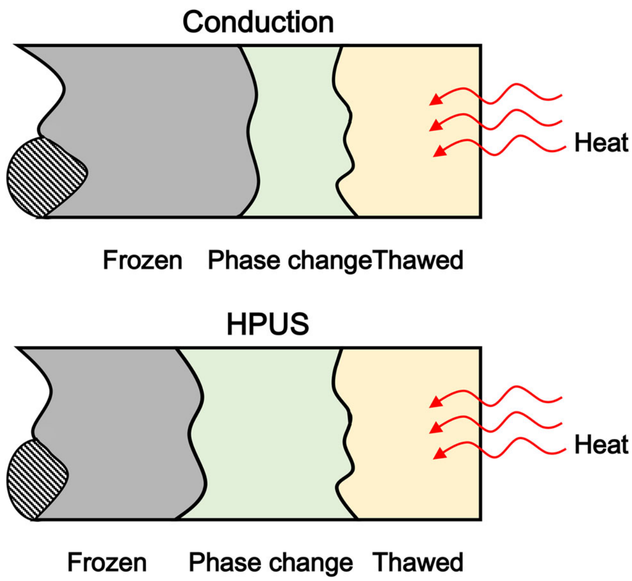

Figure 3. Effect of acoustic waves on the phase change region as compared to conduction heating.

quality. The results revealed that the quality of premium wet-cured cooked hams were minimumly influenced by the HPUS treament and the processing time achieved up to a 50% reduction (Table 2). It is suggested that further study should be conducted by cost analysis, condition optimization and comparison to conventional techniques to consummate and implement HPUS into industrial processes.

Freezing and thawing

Freezing is one of the most widespread industrial methods for food preservation which has strong relationship between temperature and the sensory and stability properties of foods (Kim et al. 2018). The lower temperature as well as the decreased water activity due to the converting of water into ice exhibit the preserving effect of freezing. During freezing processing, the chemical processes of dehydration and concentration of solutes which is induced by the formation and accretion of ice crystals in myofibers are associated with the physical damage and protein denaturation of meat. The protein denatured by freezing is especially notable during slow freezing treatment (Hou et al. 2020; Jiang, Jia, et al. 2019). Therefore, any methods which attempt to accelerate the rate of freezing are benefitial for the weakening of this damage (Gambuteanu, Patrascu, & Alexe, 2014).

Usually reducing the damage of cellular structure during freezing could be achieved by HPUS treatment. Studies have informed that the fine ice crystals were formed and the time between the crystallization and the complete of ice could be shortened by HPUS-assisted processing. The cavitation presented in liquid could also enhance both the rate of nucleation and crystal growth in a supercooled or saturated medium throughout the ultrasonic exposure (Chemat, Zill-e-Huma, and Khan, 2011).

As for meat freezing, in order to maintain the immobilized water and minimize the drip loss of meat, the integrity of cell structure and the extent of denaturation of myofibrillar protein are both important during freezing (Li, Zhu, and Sun 2018). Sun et al. (2019) evaluated the physicochemical properties and thermal stability of protein from common carp (*Cyprinus carpio*) muscle with the treatments of the HPUS-assisted immersion freezing (UIF), immersion freezing (IF) and air freezing (AF). The results showed that UIF was more efficient in maintaininging the muscle tissue integrity while reducing the thawing and cooking loss of samples. In addition, the fat oxidation and the protein denaturation of common carp muscle were inhibited by UIF treatment than that of AF and IF samples, which could be owned to the production of small ice crystals in meat tissue by the cavitation phenomenon of HPUS (Table 2). A similar research conducted by Zhang, Niu, et al. (2018) revealed that porcine *longissimus* muscles treated with the HPUS condition of 180 W and 30 kHz could achieve a significant reduction in thawing loss compared with other treatments. These results indicate that the freezing rate of muscle and meat quality could be improved at certain powers of HPUS (Table 2). Therefore, HPUS assisted freezing has great application prospect in maintaining meat quality and energy efficient.

Thawing is the reverse process of freezing. Major losses of sensory and nutritional qualities of frozen meat as well as the damage of muscle fiber usually take place during the faster thawing at room temperature. Meanwhile, the conventional thawing with low temperature takes a long time and is not suitable for the efficiency of food processing (Kalichevsky, Knorr, and Lillford 1995). Studies have shown that thawing rate had a great influence on meat quality. Fast thawing of food was desirable to assure quality of frozen vegetables, but slow thawing was better for fish and meat (Singha and Muthukumarappan 2016). Since HPUS could accelerate the rate of heat/mass transfer, HPUS assisted thawing might achieve rapid thawing at low temperature and improve the quality of meat when the appropriate frequency and power of HPUS is selected (Fig. 3). Nevertheless, the high energy requirement and localized heating hindered the application of this method (Li and Sun 2002). Miles, Morley, and Rendell (1999) found that, with accompanied by HPUS (500 kHz) with intensity of 0.5 W/cm^2, the cod, pork and beef samples could be thawed to a depth of 7.6 cm within 2.5 h. The application of HPUS had minimum impact on the protein denaturation of surface (Table 2). A similar research by Gambuteanu, Patrascu, & Alexe (2014) found that HPUS assisted thawing (0.6 W/cm^2) of pork *Longissimus dorsi* muscle had no significant difference from the method of water immersion thawing in terms of chemical and quality characteristics, and the thawing time could be shortened (Table 2). More importantly, improved elasticity by rheological determination of the samples suggested that HUPS assisted thawing could result in a better meat structure. Moreover, when the frequency of HPUS was applied near to the relaxation frequency range of ice crystals, the frozen foods could absorb the acoustic energy that accelerated the thawing rate (Kissam, et al. 1982). However, there is no uniform relaxation frequency to reference when applying HPUS to meat thawing due to the complex composition of meat staffs. Therefore, the HPUS-assisted thawing is still

Table 2 Recent applications of HPUS in meat processing.

Applications	Sample	HPUS conditions	Advantages	Ultrasound principle	Reference
Curing	Beef, *longissimus dorsi* (50 × 50 × 10mm)	20 kHz, HPUS (2.39, 6.23, 11.32 and 20.96 W/cm^2), NaCl concentration of brining (3, 4, 5 and 6%) and HPUS time (30, 60, 90 and 120 min). Temperature was maintained at 10 °C	• Diffusion coefficients (*D*) of NaCl or water could be significantly influenced by HPUS intensity while the salt concentration with ultrasound processing was slightly affected the values of *D*. • Reduced the NaCl supplemented in brining • Shortened the processing time	Increasing mass transfer	Kang, et al. (2016a)
	Beef, *longissimus dorsi* (50 × 50 × 10mm)	20 kHz, HPUS (2.39 and 20.96 W/cm^2), NaCl concentration 6%, and HPUS time (30 and 120 min). Temperature was maintained at 10 °C	• Increased the WHC of beef • Increased the tenderness of beef	• Cavitation phenomenon • Moderate oxidation of myosin induced polymerization, which contribute to increased water retention • Increased MFI values • Improved the proteolysis of desmin and troponin-T. • Swelling and disruption of myofibrils	Kang, et al. (2017)
	Pork, *m. longissimus thoracis et lumborum* (LTL), 300 ± 2 g (90 × 80 × 30 mm)	30-40 kHz bath and 20 kHz probe, total US intensities (0, 10.7, 17.1 and 25.4 W/cm ^{2}) and treatment times (2, 4 or 6 h). Temperature was maintained at 12 °C	• Decreased the brining time of meat by up to 50% in pilot-scale • No effect on the quality or sensory attributes of the product • Increased the hue angle, a* values and lipid oxidation over storage while no effect of HPUS treatment on quality attributes.	• Increasing mass transfer • A surface phenomenon during HPUS processing	McDonnell, et al. (2014)
	Pork	• HPUS activated brine: 20 kHz, 200 W/L for the saturated NaCl solution (salt to water ratio was 1:3). • Reference group with sodium chloride concentrations (3, 4 and 5%) • Temperature was maintained at 4 °C	• Increased the kinetics of brining • Decreased the aging time of whole-muscle raw meat in a brine by increasing the • Formed a uniform distribution of the brine through the entire muscle tissue	• Increasing mass transfer • Increase the hydration properties of pork proteins by activated brine • Speeds up the physico-chemical and biochemical processes with the HPUS activated brine • Activated brine improved the extraction of salt- and water- soluble proteins • Swelling of the sarcolemma and thickening of the fibers	Krasulya et al. (2018)
Freezing and thawing	Common carp (*Cyprinus carpio*), 5 cm-long chops perpendicular to the length, weight 210 ± 15 g	• 30 kHz, 175 W for 9 min • The frozen samples were stored at -18 ± 1 °C for 180 days.	• Smaller ice crystals throughout the storage period • Less damage of the muscle tissue	• Uniform heat transfer • Reduced the mobility and loss of	Sun, et al. (2019)

(*continued*)

Table 2 Continued.

Applications	Sample	HPUS conditions	Advantages	Ultrasound principle	Reference
			• Reduced the thawing and cooking losses than the IF(immersion freezing) and AF (air freezing) samples • Higher shear force than the AF and IF samples during the storage • Lower TBARS and TVB-N during storage	immobilized and free water • Higher protein thermal stability than AF and IF samples	
	Pork, *longissimus dorsi* 30-mm-thick chops perpendicular to the direction of the fiber. Weight 120 ± 2 g	• 30 kHz, 120, 180, 240 or 300 W until the center temperature reached -18 °C • The sample immersed at -20.0 ± 0.5 °C	• Accelerated the freezing rate obtained at 180 W • No significant differences in a* (redness), b* (yellowness), pH values or cooking loss • Reduced the thawing loss	• Uniform heat transfer • The shortest phase transition times obtained at 180 W • HPUIS with 180 W reduced the size of ice crystals and made their distribution more uniform • Reduce the mobility and loss of immobilized and free water accompanied by the reducing T_{21} and T_{22} relaxation times	Zhang, et al. (2018)
	Pork, *longissimus dorsi*, cut in cuboids of 100 g	• 0.6 W/cm^2 in a water bath at 15 °C. • Time needed to achieve the temperature range from -7 °C to -1 °C was 8 min	• No significant difference in terms of chemical and quality characteristics • Shortened thawing time • Improved elastic with a better structure of the samples	• Increasing heat transfer • Acoustic energy absorbed by frozen foods when a frequency in the relaxation frequency range of ice crystals in the food	Gambuteanu, et al. (2014)
Thermal processing	Topside ham, pork	20 kHz, 600 W/cm^2 for 10 min. 1.5, 1.12, 0.75 % NaCl and 0.75% NaCl + HPUS	• Reduced the sodium content of 32% with HPUS application. • Improved the physicochemical properties with reduced sodium • Improved the yield and the color without negatively affecting the oxidative stability. • Improved sensory acceptance for taste and texture parameters and for global acceptance	• Cavitation phenomenon • Increasing mass transfer	Barretto, et al. (2018)
	Beef, *longissimus dorsi*, 8 × 8×8 cm^3 with the weight of 500 g	20 kHz, 63.00, 74.76, 93.33 and 109.67 W/cm^2 cooked for 80, 100 and 120 min, respectively	• Increased the salt content • Reduced the pressure loss and free water content while improving the immobilized water content • Improved hardness • No effects on the springiness, chewiness and resilience	• Cavitation phenomenon • Uniform heat transfer • Myofibrils of beef were ruptured by HPUS treatment along with the Z-lines leading to the muscle swelling	Zou, et al. (2018)

(*continued*)

Table 2 Continued.

Applications	Sample	HPUS conditions	Advantages	Ultrasound principle	Reference
	Mortadellas, sausage	25 kHz, 301 W, cooked 900 s at 60 °C, 900 s at 65 °C, and 450 s at 76 °C	• Reduced the cooking time of mortadellas. • Provided a higher homogeneity in the internal temperature of the mortadellas. • Did not increase the lipid and protein oxidation. • Positive effect on gel formation and in the microbiological quality.	• Cavitation phenomenon • Uniform heat transfer	da Silva, et al. (2020)

a promising technology in meat industry. Future studies will engage in selecting the proper frequencies and power of HPUS to explore the mechanism for meat thawing.

Thermal processing

The sensory property of foods could be improved by thermal processing mostly due to the aggregation, unfolding, and formation of dimers and larger oligomers of proteins (Aryee and Boye 2017). Food proteins are denatured when exposed to moderate heat treatments (60-90 °C, 1 h or less). Extensive denaturation of proteins often results in insolubilization, which may impair those functional properties and product qualities. During meat processing, the most important functional properties of MP in reconstituted products is the gel-forming ability and the sensory and textural properties of the final products are mainly influenced by this functional character. In addition, the heat-induced gelation is prerequisite for the thermal denaturation of MP (Chen et al. 2017). Research demonstrated that MP treated with moderate HPUS ($\leq$150 W/cm^2) conditions could produce a uniform and denser gel microstructure as well as improved water holding capacity of the gels. This is supported by the findings with increased absolute value of the zeta potential, surface hydrophobicity and solubility of MP while decreased turbidity and total sulfhydryl group content (Zhang et al. 2017).

In conventional cooking, the outside of food may be overcooked while the interior was insufficiently cooked when exposed to elevated temperatures. These drawbacks could be partially solved by the use of HPUS due to the accelerated rate of heat transfer, and this have been utilized in thermal processing of meat (Chemat, Zill-e-Huma, and Khan, 2011). Pohlman et al. (1997) first confirmed that the HPUS assisted cooking (20 kHz, 1000 W) for *Longissimus* and *pectoralis* muscles of beef could result in an improved efficiency of energy consumption, moisture retention and cooking speed. Recently, Zou, Zhang, et al. (2018) and Zou, Kang, et al. (2018) further evaluated the effect of HPUS (0, 400, 600, 800 and 1000 W for 80, 100, 120 min, respectively; 20 kHz) assisted cooking on the quality trials of spiced beef. The hardness of beef was improved significantly by HPUS-assisted cooking when compared with the control, while the other texture index of the resilience, chewiness and springiness were less influenced by HPUS treatment (Table 2). These results could be confirmed by muscle swelling and the ruptured myofibrils along with the Z-lines during HPUS treatment. In addition, the type and relative content of volatile flavor substances were significantly increased by the HPUS treatment, especially for aldehydes, alcohols and ketones. The author concluded that the power of 800 W had a positive effect on textural and chemical profiles of spiced beef during cooking. Recently, da Silva, Voss, de Menezes, Barin, Wagner, Campagnol and Cichoski (2020) employed the HPUS (25 kHz) to evaluate the effect of HPUS on the cooking time and oxidation of mortadellas. The results revealed that HPUS could reduce the cooking time of the sausage. A positive effect on gel formation while no increase in the lipid and protein oxidation during HPUS assisted cooking was also reported (Table 2).

These above studies have clearly demonstrated that the phosphate or salt levels could be reduced in meat and the texture attributes of products could be modified by adopting an efficient strategy of HPUS assisted thermal processing. Nevertheless, in order to obtain the expected sensory quality of products, the considered HPUS and the protein conditions must be taken into consideration.

Summary and future considerations

The functionality and structure of proteins determines the textural and quality characteristics of food products. Most published studies indicate that HPUS treatment provide great potential for protein structural changes, thereby creating desirable functional properties. Recent advances of application of HPUS in structure and functional properties of proteins were presented in this paper to demonstrate the development of the fundamental theory. In summary, HPUS technology can significantly affect the conformation and structure of protein due to the cavitation effect resulting in the improvement of solubilization, interfacial, viscosity, gelation and flavor binding properties of proteins. Therefore, the textural properties of products, especially water holding capacity and the stability of protein gels, are ameliorated in short time and low temperature. The protein characters (formulations, type and species) and HPUS parameters (temperatures, time, intensities and applying sequence) are responsible for the effect of HPUS-assisted treatment. Secondly, in order to meet the growing demand for the high-quality meat products, this paper also presented the newly applications of HPUS on the curing, freezing-thawing and thermal processing of meat because the MP was responsible for the overall function and sensory quality of products. The appropriate selection of the HPUS parameters according to the product quality demand and meat formulations could markedly modify the quality and accelerate the rate of processing. In addition, HPUS assisted treatment could provide platform for the development of new products. However, the application of HPUS in meat is mainly in the laboratory stage at present, and it has not been widely used in meat industries. Therefore, further studies should be carried out from the following aspects:

1. The oxidation effect of HPUS (>100 W/cm^2) can not be ignored during processing. The current studies only pay attention to the process of HPUS on protein and lipid oxidation, whereas in the subsequent storage, the sensory acceptability of products might be influenced due to the effect of oxidation. More research should be conducted to manage the oxidation effects through optimizing product formula, adapting the new packaging or controlling the process parameters. Furthermore, studies have found that the brining after HPUS pretreatment could effectively accelerate the curing rate and improve the quality of meat. The novel application of HPUS could avoid the excessive oxidation of meat during the process of curing processing, but its mechanism still needs further investigation from molecular activation energy or intermolecular interaction.
2. Due to difference of equipment and processing conditions, the data obtained from researches sometimes are not comparable. The lack of uniform standards for process validation limits the full potential application of HPUS in meat products. Thus, it is necessary to take all the factors that influence the HPUS treatment (like mass diffusion rate, temperature, protein concentration, distance between probe and materials) into account, and the dimensional analysis in engineering is adopted to establish the mathematical model in determining the characteristics numbers for HPUS treatment. In addition, the standardized processing and operational parameters during application of HPUS are required in various facilitating occasions.
3. The combination of HPUS with heat, pressure or chemical solution can achieve better quality of production and the energy consumption and production efficiency can also be greatly improved. In addition, the production of innovative meat products with customized texture and nutritional enhancement profiles by HPUS with other method should be actively explored in the following studies.
4. Research and development of industrial HPUS equipment with stable power output, larger volume, higher efficacy and safer acoustics systems to meet technical demand for meat production. More studies should explore the industrial application of HPUS assisted processing so as to achieve technology-productivity transformation.

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ORCID

Jose M. Lorenzo http://orcid.org/0000-0002-7725-9294

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