High pressure treatment and the effects on meat proteins

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Abstract

High pressure (HP) has the prospect to engineer protein conformation, but the fundamentals of physicochemical HP-effects on biomolecules need to be addressed before HP can be fully exploited.

When understanding the mechanisms of HP-induced changes, HP can be used in targeted and unique biomolecule architecture. Thereby, HP offers opportunities to modify structure and interactions within or between biopolymers. This review introduces the principle of HP and its characteristic followed by a description of the pressure effects on meat proteins. Overall, meat protein systems and meat proteins as such are rather pressure-labile of nature. It is shown that HP results in significant changes in the sarcomere and is capable of modifying meat proteins by pressure-induced changes of the molecular structure. Pressurization (200-800 MPa) of meat affects the structure and functionality of myofibrillar proteins resulting in a considerably decreased solubility due to formation of insoluble aggregates. The underlying mechanisms of these pressure-induced molecular changes consist of two overall steps: 1) rupture of the noncovalent, intermolecular interactions resulting in protein denaturation and 2) formation of new intra- and/or intermolecular non-covalent bonds resulting in aggregation. How these findings may be useful for future research in medical and pharmaceutical applications is speculated upon.

Keywords: High pressure, meat proteins, myofibrillar proteins.

1. Introduction

High hydrostatic pressure is the isostatic pressure increase in a liquid (typically water) due to compression of the liquid. High pressure (HP) treatment used for biochemical application or food processing is in the range from 100 to 700 MPa (Megapascal equaling 1000-7000 atmosphere). Pressure-induced biochemical and physicochemical changes can be reversible or irreversible depending on the HP-conditions (pressure level, time, and duration) and milieu (system, substances). However, such changes will be completely different from those induced by temperature, because the energy developed by pressure is very small compared to that developed by temperature (Rivalain et al., 2010). Moreover, according to the fundamental thermodynamic relation (Gibbs free energy) pressure and volume are connected, meaning that a pressure-induced change is accompanied with a volume reduction (see next section). Accordingly, only the weak chemical bonds and interactions are affected by high pressure, making the effect essential different from heat-induced changes. Exactly this fact underpins HP as a powerful tool that opens new doors for both basic and applied research in bioscience. For example, high pressure has been studied in order to inactivate different microorganisms in relation to food development or biological applications. The main application of high pressure in the food industry is for elimination of microbial pathogens and spoilage microorganisms, and thus extension of shelf-life (Barba et al., 2017b). Pressure-induced inactivation has also been applied on antigens and antibodies and their influence on the immunogenicity, the role of high pressures on tumors, viruses in the development of vaccines (Murchie et al., 2005), and disinfection of human bone crafts (Gollwitzer et al., 2009; Steinhauser et al., 2006). The tuning of enzyme activity or inactivation of thermostable enzymes by HP treatment has also been established. Studies of the structure of pressure-populated folding intermediates (molten globule state) of proteins has been conducted in order to understand the protein folding regime (Silva et al., 2001). Especially, HP has been proved useful in the studies of the misfolding of proteins in relation to the conformational diseases such as Alzheimer's, Parkinsons's, and prion diseases (Lullien-Pellerin and Balny, 2002; Winter 2010). It has been shown that the use of HP can optimise the recovery of the native protein from inclusion bodies (biopurification under pressure) (Chura-Chambi et al., 2013). For more examples and details, see (Aertsen et al, 2009; Rivalain et al, 2010; Silva et al, 2001). Hence, pressure appears as an important tool for the investigation of biological systems as it can be used to discriminate between thermal and volumetric effects, alter weak bonds, keep liquid phase at subzero temperatures (Rivalain et al., 2010). This article will give an overview of HP modification of meat proteins.

2. High pressure fundamentals

The superior property of HP is the isostatic and instantaneous effect that ensures that pressure is transmitted uniformly throughout any treated sample. The three principles: Le Chatelier's principle, the isostatic principle and the principle of microscopic ordering applies to HP. Le Chatelier's principle means that when a force is applied to a system in equilibrium, the system will react to counteract the applied force. The isostatic principle means that a pressure change occurring anywhere in a confined incompressible fluid is transmitted throughout the fluid such that the same pressure change occurs everywhere. The principle of microscopic ordering means that an increase in pressure increases the degrees of ordering molecules of a given substance. Relating to HP, the Le Chatelier principle

dictates that any chemical reactions or physical transitions involving a decrease in volume are favored, while changes involving an increase of volume will not occur. According to the isostatic principle, any sample subjected to HP is compressed by uniform pressure from every direction, thereby no gradient is formed. Consequently, HP treatments are independent of sample size and geometry, and the effect is uniform and instantaneous, being mass/time independent, offering a great advantage to HP treatment over any heat treatment. It is not the intension of this review to provide a thorough description of the theoretical background or equipment technologies as detailed information has recently been published (Barba et al., 2017a; Barba et al., 2017b).

The thermodynamic description relates the change in energy with the change in reaction volume during the change from initial pressure to the final pressure at constant temperature:

$$\frac{\partial \Delta G}{\partial P} = \Delta V \qquad (1)$$

This shows that a change in pressure is associated with volume changes during reaction. This volume change is the difference between the initial molecular volumes ($V_A + V_B$) and the final molecular volume after reaction (V_{AB}), hence $\Delta V = V_{AB} - (V_A + V_B)$ for the reaction $A + B \rightarrow AB$. Moreover, the following relationship between change in free energy and the equilibrium constant for a given equilibrium is given:

$$\Delta G^{\theta} = -\mathrm{R}T \ln \mathrm{K} \qquad (2)$$

Together, equations (1) and (2), give the relationship between the equilibrium constant and the reaction volume:

$$\frac{\partial \ln \mathrm{K}}{\partial P} = -\frac{\Delta V^{\theta}}{\mathrm{R}T} \qquad (3)$$

Obviously, if the chemical reaction is increased in the forward direction (increasing the product AB) under pressure, i.e. K increases, it corresponds to a negative volume change and, thus, the final state takes up less space than the initial state, as $\Delta V^{\theta} < 0 \Rightarrow V_{AB} < V_A + V_B$ (Figure 1). Accordingly, the system will be shifted to the most compact state.

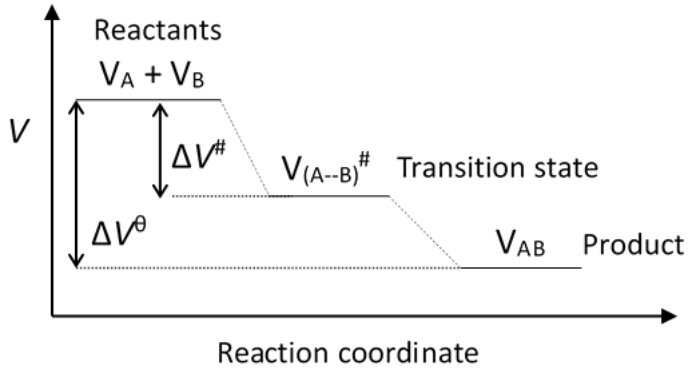

Figure 1. Volume diagram for the reaction $A + B \rightarrow AB$. Since $\Delta V^{\#}$ is less than 0, the reactants will be displaced under pressure towards the transition state A--B and finally to the product AB.

It is known, that the self-ionization of water is enhanced upon pressure, resulting in a significant increase in the ionic product of water, K_w, with increasing pressure (Lown et al., 1968; Marshall and Franck, 1981). This is due to the fact that under pressure, water ionization will reduce the volume by 22 mL/mole and the water ions will occupy less space:

$$H_2O + H_2O \Leftrightarrow H_3O^+ + OH^-$$

This is because the positive oxonium ion attracts the negative hydroxy ion, thus the two ions can be packed closer than two water molecules can. This also applies to weak acids used as buffer solutions in biochemical systems. Hence, the dissociation of acids increases upon pressurizing if the ion-pair formation is accompanied by a substantial volume reduction. Consequently, the buffering capacity of certain buffers decreases under HP-treatment with significant changes in pH (Orlien et al., 2007; Olsen et al., 2015).

Surely, native proteins are in a stable conformational state defined by the nature of the protein itself and the environmental conditions. However, this structural form of the protein is only stable in a narrow pressure and temperature region. The native structure is stabilized by a delicate balance of disulfide bonds and the relative weak non-covalent forces hydrogen bonds and hydrophobic, electrostatic, and van der Waals interactions. The primary structure and secondary structures of proteins are rarely affected by high pressure because of the very low compressibility and high energy of covalent bonds. Since the energy developed by pressure is very small, it will only affect weak forces; hence, the tertiary and quaternary structures of proteins are doomed to undergo changes due to high pressure (Boonyaratanakornkit et al., 2002). The dissolved protein's volume is made up by the volume of the atoms, of the cavities, and by the water solvation of peptide bonds and amino acid side chains. Accordingly, HP effect on the conformation of proteins is also influenced by its hydration patterns. Because of changes in the balance of the molecular forces, HP drives the stable protein system towards a smaller volume, thereby forcing changes in the structure of proteins. The conformational changes during pressurization occur due to the swelling of the folded polypeptide chain (by pressing water into the interior of the protein matrix) and the disruption of the non-covalent interactions. The resulting loss of contact between atoms/groups in the nonpolar domains causes unfolding of parts of the protein and subsequently denaturation. These pressure-induced changes are not all reversible, thus upon depressurization new molecular interactions may be formed, and consequently new protein structures can be formed. For example, some protein stability phase diagrams show elliptical shaped P-T relationship meaning that one or more of the fundamental thermodynamic material parameters (c_p, ΔH, and ΔS) for the native and denatured state have a complicated dependency on pressure and temperature. Indeed, the pressure-induced changes in protein structure and function will vary considerably depending on the type and concentration of protein and on the intensity and duration of the pressure treatment.

3. High pressure and meat proteins

In the very early high-pressure years, the technology was used to examine the effect on microorganisms (Hite, 1899). HP has since been documented to improve the microbiological safety of the final meat product and is currently used widely in the meat industry for preservation of especially ready-to-eat products like sausages, ham, salami. During various investigations, it was observed that HP affects protein structures leading to different changes in the meat

proteins. The pressure-induced structural changes of the protein molecules due to different states of unfolding and denaturation result in changed reactivity of the meat proteins. Therefore, HP became of increasing scientific interest and investigation of the pressure-induced modification of meat proteins intensified and numerous reviews of the HP effect on meat exist (Bajovic et al., 2012; Buckow et al., 2013; Colmenero, 2002; Ma and Ledward, 2013; Olsen and Orlien, 2016; Simonin et al., 2012; Sun and Holley, 2009). Meat or muscles are composed of collagen, sarcoplasmic proteins, and myofibrillar contractile proteins. However, collagen, the connective tissue, is highly structured and is not affected by high pressure as such. The sarcoplasmic protein fraction consists mainly of heme pigments and enzymes and constitute around one-third of the total muscle proteins. The basis of the meat structure, the myofibrillar protein fraction, consists mainly of myosin, actin, actinin, tropomyosin, troponin, and constitutes around two-thirds of total muscle proteins. Since the myofibrillar proteins are the structural proteins and, as such, more stable than the sarcoplasmic proteins, they contribute mostly to the functionality in meat, and have been investigated in more detail regarding pressure effects. Such proteins are obviously closely related to the human body and various applications can be reflected on and tied to a medical perspective: for scaffolding in tissue engineering, protecting agent, and as drug delivery and release agents.

The myofibrillar proteins are located differently in the thin and thick filaments in the filamentous structure and are involved in the process of muscle contraction. Pressurization of meat above 200 MPa leads to modification of the ultrastructure. The extensive changes in the sarcomere were attributed to the disruption of I-band and loss of M-line, but no effect on Z-line (Iwasaki et al., 2006; Jung et al., 2000; Rusman et al., 2007; Suzuki et al., 1990). Such pressure-induced rupture of the filamentous structure upon increasing pressure results in dissociation of the thin and thick filaments, thereby affecting solubilization of the myofibrillar proteins. Thus, the effect of HP-treatment on the solubility of myofibrillar proteins has been investigated for beef (Chapleau and Lamballerie-Anton, 2003; Lee et al., 2007; Speroni et al., 2014), pork (Grossi et al., 2012a, b; Tintchev et al., 2013), turkey (Chan et al., 2011), and chicken (Iwasaki et al., 2006). In general, the solubility was found to decrease upon increasing pressure level above 200 MPa. However, the solubility was also observed to increase at low or moderate pressure (Iwasaki et al., 2006; Lee et al., 2007; Tintchev et al., 2013; Speroni et al., 2014) or not affecting myofibrillar protein solubility at all (Marcos and Mullen, 2014). It is noted, that in the latter studies protein suspensions or meat batters were studied, and it is likely that the protein extraction or mincing prior to HP-treatment had already disrupted molecular interactions, thereby inducing greater solubility. Pressure-modification of proteins is initiated by the destabilization and/or disruption of the hydrophobic and electrostatic interactions of the quaternary and tertiary structure resulting in a partial or full protein unfolding or denaturation, thereby exposing various reactive domains. Once the domains are accessible for other constituents, new hydrophobic interactions, hydrogen bonding, or disulfide bonds are possible and responsible for protein aggregation. Such changes in the protein conformation will affect molecular sizes and thereby protein solubility due to denaturation following covalent linking (decreased solubility) or degradation into lower molecular-weight compounds (increased solubility). Hence, the mechanism consists of two overall steps: 1) the protein denatures due to the rupture of noncovalent

interactions within the protein molecules and 2) new exposed areas of the denatured protein facilitate formation of new intra- and/or intermolecular bonds resulting in large aggregates. The loss of solubility was examined in details by different reagents targeting the disruption of specific molecular interactions in order to evaluate the type and degree of the protein-protein interactions responsible for the aggregation mechanism (Grossi et al., 2016). It was found that formation of hydrogen bonds had a major role in protein aggregation during pressurization, while disulfide crosslinks and hydrophobic interactions may not be responsible for the loss of protein solubility in HP-treated meat. Molecular chaperones have a remarkable ability to stabilize proteins, i.e., to protect proteins against aberrant aggregation and inhibit precipitation. In order for a molecular chaperone to be effective it must be able to bind aggregation-prone targets. The hydrogen bonding ability of pressurized myofibrillar proteins may provide chaperone activity by binding, shielding and stabilizing sensitive proteins of biomedical relevance e.g. inclusion bodies.

Indeed, the decreased solubility of the myofibrillar proteins is a result of the pressure effect on the individual proteins. In this context, analyzing the solubilized proteins by electrophoresis will reveal which and to what extent proteins have been affected by the HP treatment. In this way, the disappearing of bands or newly formed bands is attributed to either degradation of larger proteins into small subfragments or aggregation of low molecular-weight proteins. Such electrophoretic profiling showed that the myofibrillar proteins were modified by pressure-induced denaturation, and immediately followed by either degradation or aggregation (Angsupanich et al., 1999; Grossi et al., 2016; Speroni et al., 2014; Tinchev et al., 2013). The aggregation can maybe be controlled in the direction of assembly into highly ordered nanostructures such as spheres, tubes, fibrils or disks, which can act as carrier systems for therapeutic purpose. The protein (not being foreign to the body) assemblies may be able to incorporate a given medicament or drug, then, upon injection into the muscle can act as a depot and release the medicament slowly and controlled.

Myosin is the most abundant protein in the myofibrils and it has two identical globular head regions, which are composed of various fragments that connect with the light chains and the heavy chains as tails. It was suggested that monomer myosin initially associated by head-to-head intra- or intermolecular interactions followed by aggregation into a so-called daisywheel (the heads are connected together in the middle and the tails are pointing outwards) oligomer, with the tails remaining intact (Yamamoto et al., 1993). Tintchev et al. (2013) expanded the aggregation mechanism to: the initial (up to 300 MPa) disruption of the myosin molecules is followed by a hydrophobic packing into daisy-wheels, which further (350-600 MPa) form larger aggregates with other unidentified proteins into a protein network. Later, Speroni et al. (2014) found that pressure resulted in a decrease in the myosin-HC content, while the myosin-LC content did not change and proposed another mechanism: the pressure-induced aggregation involved the dissociation of myosin heavy and light chains followed by aggregation of the heavy chains. These suggestions emphasize that the exact configuration of the aggregates formed and the underlying protein-protein interactions are still unknown. However, a Western blotting analysis, which targets selected myofibrillar proteins, thereby monitoring their individual and specific behavior at different HP levels, showed that myosin and actin lose their native solubility at HP-treatment above 400 MPa, while α-actinin and troponin-T are less affected by

pressure (Grossi et al., 2016). This supports the findings of other authors that myosin, especially the globular head (which is composed of several low-molecular-weight fragments) (Iwasaki and Yamamoto, 2003), is the most pressure-sensitive, and it dissociates under pressure, followed by the formation of non-covalent protein aggregates (Iwasaki et al., 2006; Tintchev et al., 2013; Yamamoto et al., 1993). Considering a great similarity of mammalian meat proteins and human tissues, it can be speculated if pressurized myofibrillar proteins could contribute to the development of a new family of bioactive scaffolds for tissue engineering applications. The goal of scaffolds in tissue engineering is to create a temporary solution until new tissue is generated. Hence, the question arises: can pressure-induced aggregates of myofibrillar proteins be used as scaffolds with the goal of treating damaged and degenerated tissues?

4. Conclusion

Pressure is a fundamental physical parameter that affects biomolecules differently than temperature. HP induces severe changes in meat morphology and consequently meat proteins. In general, proteins denature at pressure above 200 MPa due to disruption of the weak molecular forces responsible for maintaining the native conformation of the molecule in effect affecting protein solubility. The soluble proteins are exposed to remarkable structural changes and lose their native functionality. The pressure-induced changes of the molecular structure increases the accessible surface area and exposes new binding sites facilitating new intra- and intermolecular interactions. Thus, HP modifies the structure of the myofibrillar proteins in a manner that leads to protein aggregation and reduced solubility. However, the complexity of the HP-effects is high, and the exact action of pressure on the various molecular forces affecting the protein structure is still unknown and not described. This review highlights that using pressure for structure modification of meat proteins is a delicate balance between destroying and building intra- and intermolecular forces in order to affect the structure of the proteins in the expected way e.g. in relation to aggregation, gelation and textural properties. Even though the emphasis is on mammalian meat proteins, the knowledge and application of HP to provoke or inhibit aggregation may be transferable and useful in a medical or biomedical perspective.

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