

<https://doi.org/10.17221/87/2023-CJFS>

Ultra-high-pressure technology for preservation of fresh aquatic foods: A review

JIAKUN PENG^{1,3}, CHUNE LIU¹, KAIKAI BAI^{1,3}, SHAOHUA XING², FENG LIU^{1*}

¹*Yantai Research Institute of China Agricultural University, China Agricultural University, Yantai, China*

²*School of Food Engineering, Ludong University, Yantai, China*

³*College of Engineering, China Agricultural University, Beijing, China*

*Corresponding author: liufeng2008@cau.edu.cn

Citation: Peng J., Liu C., Bai K., Xing S., Liu F. (2023): Ultra-high-pressure technology for preservation of fresh aquatic foods: A review. *Czech J. Food Sci.*, 41: 313–322.

Abstract: Aquatic foods such as fish, shrimp, and shellfish are important human nutrient sources. However, aquatic foods spoil quickly during processing and storage due to spoilage bacteria and endogenous enzymes. Ultra-high-pressure (UHP) technology, as an advanced non-thermal treatment method, is an effective preservation technique for aquatic foods. The mechanism of UHP technology is to destroy non-covalent bonds by UHP, which leads to the change of cell membrane permeability and the destruction of high-level structure of proteins, making apoptosis and enzyme inactivation. The technology can effectively sterilise and preserve food's colour, taste and nutritional value. The paper provides an introduction to the working principles, types, sources and equipment and describes the progress of the research and application of UHP technology in pascalisation, enzyme inactivation, parasite inactivation and quality modification of aquatic products. Potential limitations and prospects of the technology are also outlined. We hope to lay the theoretical groundwork for using this technique in aquatic product processing and provide guidance for its application in industrial production.

Keywords: emerging technologies; microbial inactivation; non-thermal preservation technology; quality control; shelf-life

Aquatic products are the general term for aquatic animal and plant products produced by marine and freshwater fisheries and their processed products. Consumers widely favoured them for their flavour and high nutritional value. However, due to spoilage bacteria and endogenous enzymes, aquatic foods spoil quickly during processing and storage, causing the

rapid deterioration of their quality and limiting their shelf-life (Arshad et al. 2021). To ensure the food safety and freshness of aquatic foods, it is necessary to maintain their quality and increase their economic value through appropriate preservation methods. Currently, commonly available preservation methods for aquatic foods include physical, chemical, and biological pres-

Supported by the National Key Research and Development Plan (Project No. 2019YFD0901705).

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Table 1. Characteristics of aquatic product preservation by different preservation techniques

Fresh way	Preservation technology	Characteristics
Physical preservation	electrostatic field	pros: the effect is obvious, the treatment time is short,
	UHP	the preservation time is long, retains the original flavour
Chemical preservation	modified atmosphere	cons: the equipment is complex
	salt preservation	pros: the operation is simple, and the effect is good
	smoked preservation	cons: some deficiencies, such as antimicrobial residue
	immersion preservation	and bacterial resistance
Biological preservation	chitosan	pros: tasteless, non-toxic, safe,
	tea polyphenols	and will not cause secondary pollution
		cons: making complex

UHP – ultra-high pressure

ervation (Boziaris et al. 2021). Compared to the other two technologies, the physical technique has low energy consumption, high efficiency, and no secondary pollution. So, physical preservation will be extensively used in food storage. The methods and characteristics commonly used for preserving aquatic products are shown in Table 1.

Ultra-high-pressure (UHP) treatment is a non-thermal food processing technique that relies on liquid as the pressure transmission medium. In UHP treatment, food is subjected to 100–1 000 MPa for effective pascalisation and enzyme inactivation (Hygreeva and Pandey 2016). The method reduces the loss of thermal-sensitive components in food and preserves the food's actual colour, flavour, and nutrients maximally (Truong et al. 2015). This technology possesses the advantages of low energy consumption and high efficiency while not causing secondary pollution and is simple to operate (Sukmanov et al. 2019). In addition, European Food Safety Authority (EFSA) concluded that UHP of food does not present any additional microbiological or chemical food safety concerns compared to other conventional application treatments, such as pasteurisation. Pathogen reductions in milk/colostrum caused by the current UHP conditions applied by the industry are lower than those achieved by the legal requirements for thermal pasteurisation (Gomez-Estaca et al. 2018).

In aquatic product processing, UHP treatment is regarded as the critical technology for pascalisation, preservation, enzyme inactivation, and quality modification (Wang et al. 2016). It features uniform pressure transmission, effective pascalisation, and thorough enzyme inactivation. This paper provides an overview of the principles, types, sources and equipment used in UHP preservation, focusing on research on the UHP inactivation of microorganisms and en-

zymes in aquatic foods, to provide a reference for the wide application of UHP technology in aquatic product preservation.

METHODOLOGY

Web of Science and Scopus database were used to conduct a literature search. The search was performed only on papers published in English from January 2012 to March 2022. Phrases related to ultra-high pressure (including 'UHP', 'HHP', and 'HPP') combined with phrases related to food preservation (including 'pascalisation', 'enzymatic inactivation', and 'quality modification') were used in the query. The references cited in the obtained articles were also reviewed to locate further relevant studies.

The following information was collected from the retrieved articles: first author, author affiliation, publication year, food-safety-related storage and preservation technology, microbial control in food, UHP technology in the food industry, the microbial inactivation mechanism of UHP, the impact of UHP on enzymes in aquatic foods, and the use of UHP in aquatic product processing and the results achieved.

PRINCIPLES, TYPES, SOURCES, AND EQUIPMENT OF UHP

UHP processing technology, also known as ultra-high-pressure technology (UHP), high hydrostatic pressure technology (HHP), or high-pressure processing (HPP). UHP technology refers to the technology of pressurising liquid or gas to more than 100 MPa. The early applications of UHP technology were not in the food industry but in ceramics, steel and alloys. UHP food processing technology began at the end of the 19th century (Ledward et al. 1995). In 1899, American

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mechanics scientist Hite found that milk treated under 450 MPa could prolong the fresh life. The possibility that UHP can be used in food processing methods was first proposed (Hendrickx et al. 2001).

The generation of UHP is largely based on Pascal's principle. Food UHP technology refers to the soft packaging or bulk food into a sealed UHP container, with water or other liquids as the medium of pressure transfer, applying (100 to 1 000 MPa) pressure at normal temperature or low temperature after a certain period of maintenance. To achieve pascalisation, material modification, produce new organisational structure, change the quality of food and change the speed of some physical and chemical reactions of food a processing method (Raghubeer et al. 2020). The working principle of UHP preservation is that because the liquid (water) is compressed under the action of UHP, and the proteins, starch, enzymes, etc., in the pressurised food medium are compressed by pressure denaturation, the non-covalent bonding part (hydrogen bonding, ionic bonding and hydrophobic bonding and other interactions) of the polymeric stereo structure of biological substances changes, i.e. the structure of the substance changes (Houska et al. 2018). For cell morphology and structure, UHP can destroy the functionality of microbial cell membranes, increasing cell membrane permeability and affecting nutrient intake and metabolic waste discharge by cells (Barba et al. 2015). For protein, UHP makes protein solidify and denatures. As most enzymes are essentially proteins, the disruption of protein conformation inevitably affects the activity and function of enzymes (Abid et al. 2014). When both the cell membrane and enzymes of microorganisms are damaged, DNA replication is hindered, gene functions are lost, and the normal physiological functioning of microorganisms is impaired. This can be lethal to microorganisms.

UHP equipment can be divided into four types: intermittent, semi-continuous, continuous and pulse according to the processing and operation mode. Studies have reported better pascalisation results with staged pressure changes than continuous static pressure processing. During UHP treatment, aquatic foods are packaged into appropriate forms or sizes and are placed in a closed pressure vessel. The pressure in the vessel is increased with a pump. 100–1 000 MPa is always applied to aquatic foods through the pressure transmission medium (Pottier et al. 2017). The entire process happens in three stages: pressurisation, pressure holding, and pressure unloading (Figure 1). The opening of a UHP vessel is equipped with a piston, which generates the required pressure when driven by the stressor. Water or other liquids is usually used as the transmission medium to apply pressure to the food. The food is then kept at the required pressure for a specified period and removed after rapid pressure unloading.

UHP equipment can be classified into two types according to the pressure-generation mode: internal and external (Aganovic et al. 2021). Internal pressurisation mainly consists of a UHP container (high-pressure chamber) and pressurising cylinder (low-pressure chamber). The UHP container and pressurising cylinder work together. In the upward stroke of the piston movement in the pressurising cylinder, the piston compresses the medium in the container, generating UHP so that the material is subjected to ultra-high static pressure; in the downward stroke of the piston movement, the decompression unloads the material. External pressurisation consists of a UHP container and pressurising device. UHP vessel and pressurising device are separated, and available pressurising pump and booster to generate a pressure medium, and send the pressure medium to the UHP vessel through

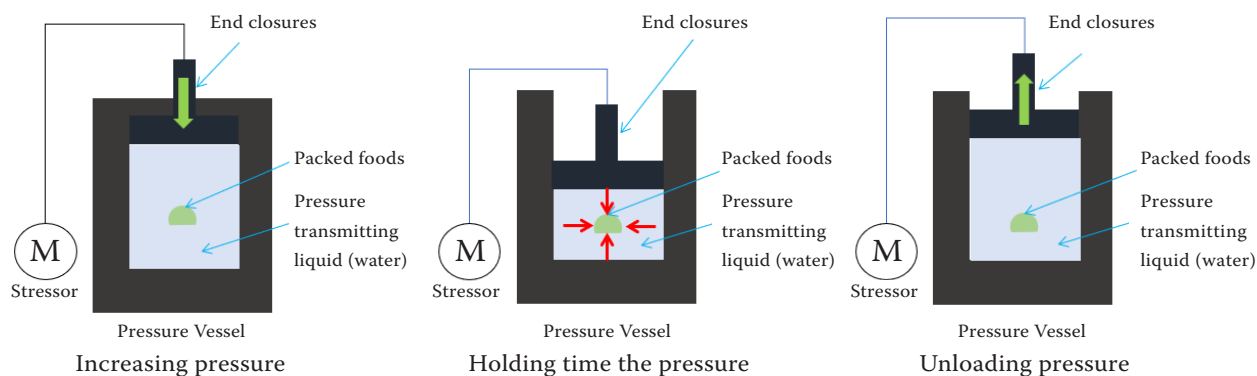


Figure 1. Schematic representation of ultra-high pressure (UHP) procedure

M – machine

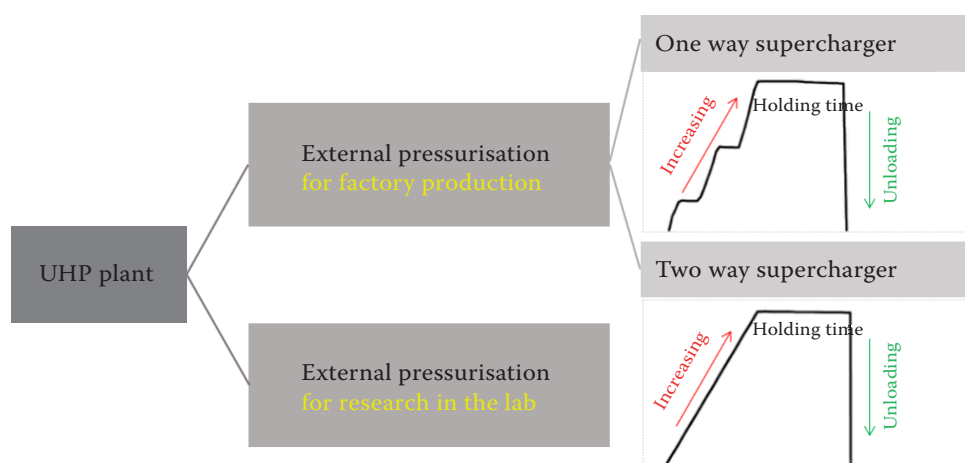


Figure 2. Characteristics of different UHP plant

UHP – ultra-high pressure

high-pressure piping (Huang et al. 2017). The pressure of UHP equipment relying on external pressure generation can be obtained by unidirectional or bidirectional vessel. A unidirectional vessel produces a fixed amount of pressure in one loading cycle. The compression direction needs to be reversed at the end of the cycle (at this time, there will be a stagnation period in pressure generation) before subsequent pressurisation processes can be carried out. For this reason, the pressure curve shows multiple plateaus and adopts a stair-like shape (Figure 2). A bidirectional vessel resembles two unidirectional vessels operating together in opposite horizontal directions. When one piston is moving, the pressure-transmission medium on the other side of the vessel is replenished, allowing pressure generation to continue in the other direction when one side completes its loading cycle. As a result, there is no stagnation in pressure generation, and the pressure curve is a straight line (Figure 2) (Fan et al. 2020).

UHP equipment can be divided into liquid and solid materials according to the material processing status. According to the different ways of UHP pascalisation of liquid materials, the corresponding equipment can be summarised into two categories: *i*) similar to the treatment of solid food; *ii*) by liquid materials instead of pressure media directly with UHP treatment. However, when liquid materials are used instead of pressure media for processing, the requirements for UHP containers are higher, and the containers must be cleaned and disinfected after each use. Solid materials generally need to be processed after packaging because the hydraulic pressure inside the UHP container has isotropic pressure characteristics; the pressure treatment will not affect the shape of solid materials, but

whether the material itself has pressure resistance may affect the volume of the material after treatment. The key aspect of the solid-state UHP food pascalisation equipment is the design of the UHP container in the UHP processing chamber, which is also the core of the whole device.

UHP equipment can be divided into vertical and horizontal types according to how the UHP vessel is placed.

CONTRIBUTIONS OF UHP TO AQUATIC FOODS

UHP treatment can significantly extend the shelf-life of fresh foods. This is achieved mainly through inactivating spoilage microorganisms, enzymes, and parasites, as well as improving the quality of aquatic foods (Figure 3). Generally speaking, a slightly lower pressure is required to inactivate parasites and viruses than bacteria (Aganovic et al. 2021).

Microbial inactivation. UHP as a method of microbial inactivation was first studied in Japan in 1987 under the direction of Professor Hayashi Rikimaru of the University of Tokyo (Ohara et al. 2015). UHP treatment is mainly through the structure of the cell wall and cell membrane and cell space destruction of microorganisms, the protein composition of degeneration, and the enzyme activity decreased to achieve the purpose of pascalisation. UHP compacted the otherwise fluid phospholipid bilayer of the bacterial cell membrane into a dense gel state, causing irreversible damage to the cell membrane and eventually leading to cell death and induced mechanical breakage in the bacterial cell wall (Yuan et al. 2017).

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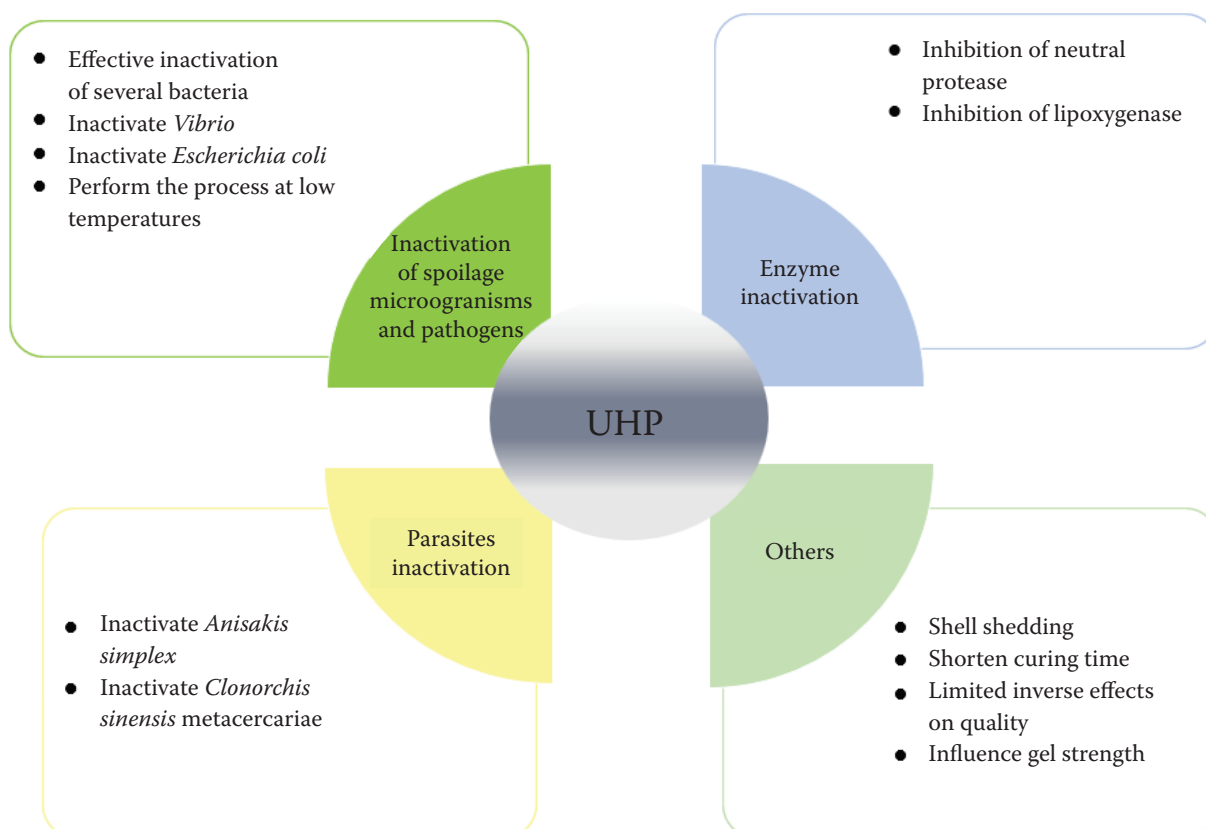


Figure 3. Main contributions of UHP in aquatic food preservations based on recently published research papers

UHP – ultra-high pressure

UHP inactivates microbes by destroying the biological membranes of microorganisms (Figure 4). This is accomplished in two ways: *i*) UHP causes the vacuoles in microbial cells to rupture, which changes the cell morphology irreversibly and inactivates the cells (Yamamoto et al. 2021); *ii*) UHP can instead alter cell membrane permeability and indirectly inhibit enzyme activity and genetic material replication in microorganisms, thus, realising microbial inactivation (Xu et al. 2021).

UHP treatment can inactivate common bacteria in a variety of aquatic foods. Examples of these bacteria include *Vibrio parahaemolyticus*, *Vibrio vulnificus*, mesophiles, psychrophiles, proteolytic bacteria, Enterobacteriaceae, lactic acid bacteria, *Pseudomonas* and H_2S -producing bacteria (Table 2).

The factors influencing the effectiveness of UHP pascalisation include operation temperature, pressure, pressure holding time, and the type of organism. Com-

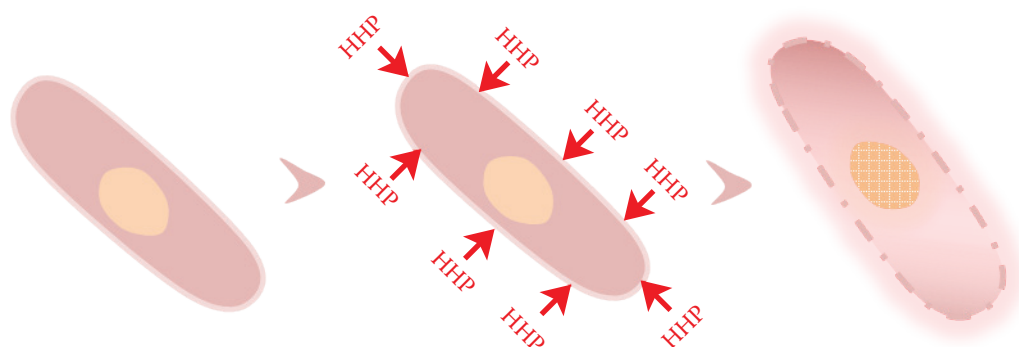


Figure 4. Schematic representation of UHP destruction of the microbial cell membrane

UHP – ultra-high pressure; HHP – high hydrostatic pressure

Table 2. Different UHP's remarkable effects on inactivating microorganisms in various aquatic foods

Microorganism	Treated food	UHP source	Results	References
<i>Vibrio parahaemolyticus</i> <i>Vibrio vulnificus</i>	<i>Crassostrea virginica</i>	300 MPa 2 min 0/25 °C	reduced more than 5 log CFU·g ⁻¹ completely inactivated	Ye et al. 2015
Mesophiles Psychrophiles Proteolytic bacteria Enterobacteriaceae <i>Pseudomonas</i> H ₂ S producing bacteria Lactic acid bacteria	<i>Fenneropenaeus indicus</i>	250 MPa 6 min 25 °C	decreased by 0.7 log CFU·g ⁻¹ reduced by 1.2 log CFU·g ⁻¹ reduced by 1.2 log CFU·g ⁻¹ reduced by 1.65 log CFU·g ⁻¹ reduced by 2.2 log 10 CFU·g ⁻¹ reduced by 1.4 log CFU·g ⁻¹ reduced by 0.91 log 10 CFU·g ⁻¹	Ginson et al. 2015
<i>Vibrio parahaemolyticus</i>	eastern oyster	250 MPa 5 min 5 °C	decreased by 6.2 log 10 CFU·g ⁻¹	Phuvasate and Su 2015
Psychrophiles Mesophiles <i>Shewanella putrefaciens</i>	<i>Trachurus murphyi</i>	400 MPa 20 min 4 °C	reduced by 4.5 log CFU·g ⁻¹ reduced by 4.4 log CFU·g ⁻¹ reduced by 3.2 log CFU·g ⁻¹	Reyes et al. 2015

UHP – ultra-high pressure; CFU – colony forming unit

pared to treatment at room temperature (15–30 °C), UHP treatment at elevated temperature (50–60 °C) achieves significantly higher pascalisation efficiency of *Escherichia coli* by two- to six-fold (Meng et al. 2016). The effect of UHP treatment is directly proportional to pressure and pressure holding time. The concentration of *V. parahaemolyticus* in oysters dropped from 10⁹ CFU·mL⁻¹ to 10 CFU·mL⁻¹ (CFU – colony forming unit) at 350 MPa was applied for 14.5 min, while at 500 MPa was only 30 s (Calik et al. 2002). Different microorganisms have different levels of pressure tolerance, which directly influences the effectiveness of UHP treatment (Simonin et al. 2012). Bacterial spores can withstand 1 200 MPa pressure at room temperature. In addition, the rate of pressure rise/release and the mode of pressurisation can also affect the pascalisation effect of UHP treatment (Ferreira et al. 2016).

Enzyme inactivation. Endogenous enzymes are biocatalysts closely related to biological metabolism that directly affect organisms' material metabolism, nutrient and energy conversion. The activity of endogenous enzymes is the key factor affecting the quality of aquatic foods and contributes primarily to the protein hydrolysis and softening of aquatic foods during refrigeration (Ge et al. 2015). Most enzymes are proteins or RNA and possess primary, secondary, tertiary, and even quaternary structures. An enzyme's primary structure is largely comprised of peptide bonds, a type of covalent bond that remains stable under UHP. Non-

covalent bonds, however, are present in the secondary, tertiary, and quaternary structures of enzymes (Cheng et al. 2021). UHP (< 700 MPa) does not affect the primary structure of proteins and favours the stabilisation of the secondary structure but destroys their tertiary and quaternary structures. UHP forces the original structure of proteins to stretch, and the molecules change from ordered and compact structure to disordered and loose structure, or deformation occurs, and the active site is damaged, and the biological activity is lost. In addition, protein denaturation was reversible at 100 MPa and irreversible above 200 MPa (Qi et al. 2015). Their results established a reference for determining appropriate parameters in inhibiting enzyme activity by UHP.

UHP also inhibits enzyme activity and results in the loss of activity in spoilage-related enzymes, thus preserving food's inherent quality and flavour. Such treatment alters the molecular structure of protein by applying pressure, thereby affecting enzyme activity in vivo (Dos Santos Aguilar et al. 2018). When the enzyme is subjected to UHP, the non-covalent bonds that maintain its spatial structure (salt bond, hydrogen bond, hydrophobic bond) are broken thus the peptide bond molecule stretches into irregular linear polypeptides so that the active site no longer exists (Figure 5). The enzyme is inactivated due to the disruption of its spatial structure, thereby delaying the deterioration of aquatic products during storage (Balakrishna et al. 2020). Currently, acidic protease, alkaline protease, lipase, chitinase, and

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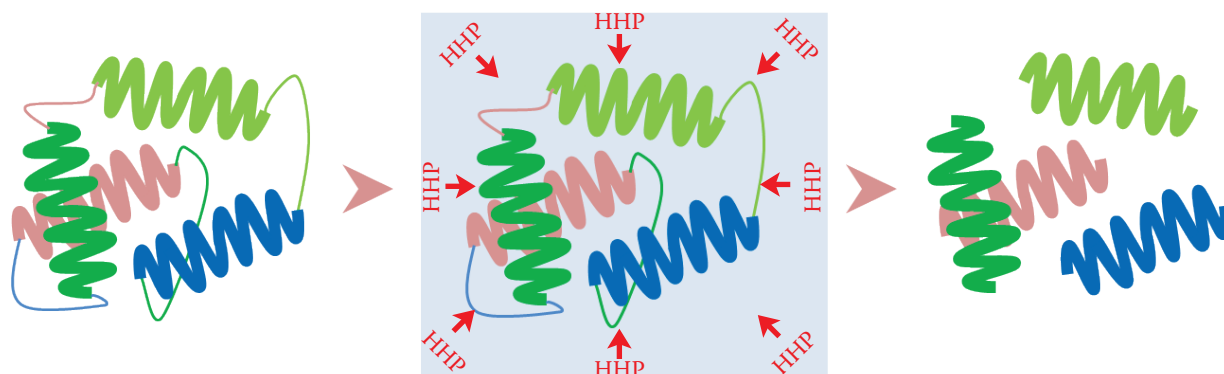


Figure 5. Schematic representation of enzymatic inactivation by UHP

UHP – ultra-high pressure; HHP – high hydrostatic pressure

polyphenol oxidase are research focuses on aquatic product preservation (Table 3).

Parasite inactivation. Fish, shrimp, and shellfish are usually the intermediate hosts of parasites. However, many people follow the dietary trend of consuming uncooked or semi-cooked aquatic foods. Such eating habits increase the infection risk of foodborne parasitic diseases (Kultur et al. 2017). While the gastric acid secreted by the human stomach kills some parasites, it cannot destroy certain zoonotic parasites. Humans can be infected with parasitic diseases if they ingest raw food containing the larvae or metacercariae of these parasites (Rux et al. 2020). The parasites are mostly proteins, UHP treatment is highly effective at killing common foodborne parasites in aquatic

foods, such as heterophyid trematodes, Digenea, *Clonorchis sinensis*, *Echinochasmus*, *Centrocestus*, and *Gnathostoma*, substantially improving the food safety of aquatic foods while preserving their colour, flavour, and nutritional value.

Others. The combination state of flavour substances, vitamins, pigments and various small molecules in food is in the form of a covalent bond, so the UHP treatment process has almost no effect. Food viscosity, uniformity and structure are sensitive to high pressure, but these changes are often beneficial. UHP technology is also an effective means to improve the quality of aquatic food. For example, the whiteness, gelation, water retention, hardness, and elasticity of Australian lungfish protein jelly after UHP treatment were enhanced

Table 3. Application of UHP in enzymatic inactivation of aquatic foods

Enzyme	Treated food	UHP source	Results	References
Calpain MBSP Collagenase Cathepsin B Cathepsin D Cathepsin L	grass carp	200/300/400/500/600 MPa 15 min 4 °C	sharply reduced by UHP (≤ 300 MPa) sharply reduced by UHP (≤ 300 MPa) inhibited significantly when ≥ 400 MPa only inhibited under ≥ 400 MPa pressures only inhibited under ≥ 400 MPa pressures activated by UHP treatments	Yu et al. 2018
Polyphenoloxidase	black tiger shrimp	300/400/500/600 MPa 3–15 min 30/40/50/60 °C	the inactivation rate increased with an increase in pressure, temperature and time, from 2% in 300 MPa/30 °C/3 min to 87% in 600 MPa/60 °C/15 min	Kaur and Rao 2017
Lactoperoxidase	smoked salmon	250/450 MPa 10 min 5 °C	UHP at 450 MPa in combination with Lactoperoxidase was the most effective treatment avoiding biogenic amine formation	Montiel et al. 2012

MBSP – myofibril-bound serine proteinases; UHP – ultra-high pressure

Table 4. Application of UHP in the quality improvement and protection of aquatic foods

Application	Treated food	UHP source	Results	References
Shucking	<i>Procambarus clarkia</i>	100/200/300/400/500 MPa 5 min 25 °C	treatment at 200 MPa for 5 min is the optimum for crayfish shucking and maintaining the meat qualities	Shao et al. 2018
Texture improving	<i>Crassostrea gigas</i>	100/200/300/400/500 MPa 5 min 25 °C	at 400 MPa, UHP significantly changed the texture of oysters by increasing hardness, springiness, chewiness, and cohesiveness	Ma et al. 2021
Taste improving	<i>Lates calcarifer</i>	300/400/500 MPa 10 min 4 °C	whiteness, gel-forming ability, water-holding capacity, hardness, and springiness of the barramundi gels increased as applied pressure and salt concentration increased	Truong et al. 2017
Fishy smell inhibition	mackerel	200/300/400 MPa 3 min 19 °C	at 500 MPa, UHP treatment can inhibit urease activity, thereby reducing the fishy smells from fish and shellfish	Kim et al. 2021

UHP – ultra-high pressure

when cooking at 90 °C for 30 min (Herranz et al. 2013). High-pressure treatment also gives tuna protein jelly higher gel strength, better mechanical properties, and a smoother texture (Truong et al. 2017). Other applications of UHP technology in improving the quality of aquatic foods are shown in Table 4.

UHP SAFETY AND LIMITATIONS

UHP treatment can pascalisation, not all micro-organisms (and bacterial spores) and enzymes are inactivated by commonly applicable doses. When UHP treatment exceeds a certain limit, the pressure will cause protein denaturation and gelation, and the quality of aquatic food will also change. For example, aquatic foods will appear cooked, although no obvious cooked flavour can be detected (≥ 650 MPa) (Marangoni Júnior et al. 2019). The texture of rainbow trout, grass carp, and Chinese pipe whip shrimp all become harder after pressure treatment (Yagiz et al. 2007). Further, the study of the UHP pascalisation mechanism is not deep enough to provide necessary guidance for the experiment, and the technical parameters and data of the UHP fresh-keeping processing of aquatic foods are not perfect, which needs a lot of research and development. The UHP equipment is large, and the cost is high, so the complete set of industrial production equipment and the collaborative measures to reduce the pressure of UHP pascalisation need in-depth and systematic research.

CONCLUSIONS AND FUTURE PROSPECTS

UHP has been shown to have an enormous potential for fresh aquatic food processing. UHP sea cucumber, oyster, abalone, and application and crab are successful Japanese UHP food application cases. As a non-thermal preservation technique, UHP destroys non-covalent bonds of aquatic foods to achieve pascalisation, blunt enzymes and improve the functional properties of aquatic foods. The colour, aroma, taste and nutritional integrity and safety of UHP food meet the psychological needs of consumers and are in line with the current requirements of green food. However, the basic theoretical research of UHP technology is not comprehensive enough, and the applicable conditions and main limiting factors of UHP technology need to be further studied. In addition, UHP technology can also be used in combination with other sterilisation methods such as thermal sterilisation, radiation, ultrasonic, bacteriostatic agent and so on to achieve good synergies.

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Received: June 7, 2023

Accepted: September 18, 2023

Published online: September 22, 2023