



# A review on high hydrostatic pressure for bivalve mollusk processing: relevant aspects concerning safety and quality

Rosiane Costa BONFIM<sup>1\*</sup>, Fabiano Alves de OLIVEIRA<sup>2</sup>, Ronoel Luiz de Oliveira GODOY<sup>3</sup>,  
Amauri ROSENTHAL<sup>3</sup>

## Abstract

Mollusks are considered a nutritious source of food and their consumption has increased worldwide. However, their consumption, mainly of bivalves, has been considered responsible for numerous cases of foodborne diseases. This is related to their food intake, as they are filter-feeders and, consequently, bioaccumulate toxic compounds. High hydrostatic pressure (HHP) is recognized as an efficient technology to control pathogenic and deteriorating microorganisms, with low damage to the sensorial and nutritional properties of foodstuffs. This review addresses the use of HHP on bivalve mollusks, based on recent relevant studies in this field.

**Keywords:** shellfish; bivalves; quality; isostatic.

**Practical Application:** Information generated from this study provide insights into HHP application and effects on bivalve mollusks, with information on process conditions, its effects on muscle proteins and microorganisms and its impact on extending product shelf-life. These data are extremely important for the development of further industrial applications of this novel, nonthermal, fresh seafood processing technology.

## 1 Introduction

Mollusks, particularly bivalves, are often associated with food safety issues, due to recurrent episodes of gastrointestinal infections and food poisoning (Murchie et al., 2005). This is due to the physiological characteristics related to their nutrition, as they are filter-feeding animals with the capacity to bioaccumulate toxic chemicals and waterborne pathogens, including human intestinal viruses, certain sewage and wastewater bacteria, and bacteria naturally present in estuarine waters. Furthermore, toxins derived from plankton and dinoflagellates present in marine environments may also bioaccumulate in mollusks, leading to serious neurological consequences for seafood consumers (Kingsley, 2014).

Most mollusks are consumed whole, including their gastrointestinal tract, either raw or only lightly cooked (Lees, 2000), since more severe heat treatments cause detrimental effects to the taste and appearance of these marine animals, causing consumer rejection (Murchie et al., 2005).

The use of high hydrostatic pressure (HHP) on bivalve mollusks is currently under study more and of interest, due to its minimal effects on the sensorial characteristics and nutritional qualities of these organisms. This technology is used for open shucking of oysters and other mollusks, and has proven efficient in reducing microorganism loads, including of certain pathogens, such as *Vibrio parahaemolyticus*.

In contrast to traditional heat treatments, high pressure processing is able to reduce microbial loads without altering

product physicochemical properties, since pressure is transmitted uniformly and instantaneously (isostatic process) and temperature variations in the process are low, of about 3 °C per 100MPa (adiabatic), depending on the food composition. These characteristics prevent food deforming or heating and any relevant organoleptic property alterations (Rendueles et al., 2011).

HHP is able to inactivate microorganisms and enzymes due to protein modifications and/or denaturation, while valuable lower molecular weight components, such as vitamins and volatile compounds, responsible for food nutritional and organoleptic quality, remain unchanged (Heinz & Buckow, 2010). Thus, the process makes it possible to extend the shelf life of food products with minimal effect on their nutritional properties and freshness (Truong et al., 2014).

This article presents a review of the HHP process applied to bivalve mollusks, pointing out effects on microbial load, shelf life, physical structure, chemical components and the advantages of this preservation industrial process.

## 2 High Hydrostatic Pressure (HHP)

HHP technology has been widely applied in the production of meat products, dairy products, aquatic products and vegetable and fruit products, as well as various beverage products. The global market for HPP foods reached approximately \$9.8 billion in 2015 and is expected to culminate in a market value of \$ 54.77 billion in 2025 (Huang et al., 2017).

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<sup>1</sup>Departamento de Tecnologia de Alimentos, Universidade Federal Rural do Rio de Janeiro – UFRRJ, Seropédica, RJ, Brasil

<sup>2</sup>Centro Federal de Educação Tecnológica – CEFET, Valença, RJ, Brasil

<sup>3</sup>Embrapa Agroindústria de Alimentos, Rio de Janeiro, RJ, Brasil

\*Corresponding author: [rcostabonfim@yahoo.com.br](mailto:rcostabonfim@yahoo.com.br)

HHP processing applied to food consists in subjecting the hermetically packaged food to pressures ranging from 100 to 700 MPa, for a certain time, according to the purpose and/or equipment capacity (Cheftel, 1995; Farkas & Hoover, 2000). Food packed in flexible packages is placed in a compression chamber to undergo the pressurization process. The chamber, which is hermetically sealed, is then filled with the pressure transmitting fluid (usually water), thus expelling all the air inside the chamber. A predefined pressure is then initiated and maintained for the set time and, at the end of this cycle, the chamber is depressurized (Farkas & Hoover, 2000; Hoover et al., 1989). As the packed food is pressurized inside the pressure chamber, this processing presents little risk of cross-recontamination and even contamination in case of operational failures (Pereira & Vicente, 2010).

HHP is based on the *Pascal* (or isostatic) and *Le Chatelier* principles. The former states that pressure is transmitted uniformly and almost instantaneously throughout the food, regardless of its mass, size or composition (with a certain minimum moisture content required for pressure transmission), while the latter states that any phenomenon (phase transition, molecular conformation change or chemical reaction) accompanied by a reduction in volume is favored by increased pressure (and vice versa) (Barbosa-Cánovas & Rodríguez, 2002; Cheftel, 1995).

The adiabatic condition of the process causes only a slight temperature variation with increasing pressure, regardless of the size and shape of the food, (the temperature increases approximately 3 °C per 100 MPa, depending on the food constitution), which prevents the food from being effectively deformed or heated (Chawla et al., 2011; Smelt, 1998). The pressurizing process is, therefore, independent of the volume and shape of the sample, unlike a thermal process.

However, although the food exhibits reduced compressibility, it shows a certain reduction in volume. According to Farkas & Hoover (2000), this reduction may reach up to 15% during the process, but reverts during depressurization, and is due to changes promoted, mainly, in proteins and water molecules (Cheftel & Culioli, 1997).

### 3 HHP effect on proteins

HHP processing is capable of altering the functional properties of food constituents. Protein conformation effects may lead to disruption, aggregation or gelation, depending on the protein system, applied pressure, temperature and treatment duration (Messens et al., 1997).

Under pressure, protein molecules behave according to "*Le Chatelier*" the law, where they suffer volume reduction due to the presence of internal spaces and the better packaging of water molecules (Cheftel & Culioli, 1997; Truong et al., 2014). These changes may promote the reduction of up to 1.0% of the protein volume, through changes in quaternary, tertiary and secondary structures (Silva et al., 2001).

Ionic bonds and hydrophobic interactions, responsible for maintaining protein tertiary and quaternary structures, are disrupted and more easily broken at pressure levels of around 150 and 200MPa, while the secondary structure requires higher

pressures, between 300 and 700MPa (Considine et al., 2008; Heremans & Smeller, 1998; Lullien-Pellerin & Balny, 2002; Oliveira et al., 2017). However, the primary structure of a protein, in other words, its native structure, is not influenced by HHP, since covalent bonds display reduced compressibility (Cheftel & Culioli, 1997).

As a consequence, complex organization structures that contain proteins, such as membranes, are altered due to the breakdown of hydrophobic and electrostatic interactions, as well as the disruption of some hydrogen bonds (Considine et al., 2008; Farkas & Hoover, 2000). Changes in protein structure can be reflect on numerous parameters such as texture, water content and color. Table 1 provides a compilation of the main effects caused by the HHP protein structure modifications in bivalve molluscs.

In bivalve mollusks, the main function of HHP application is shucking. Thus, this process can cause the disruption of non-covalent interactions in tertiary protein structures, leading to denaturation of muscle proteins and connective tissues and, ultimately, causing the release of the adductor muscle (Rong et al., 2018; Hsu et al., 2010; Cruz-Romero et al., 2004).

The effect of HHP on shucking has always been dominated by treatment pressure and time (He et al., 2002). However several shellfish species (including bay scallops, oysters, mussels and clams) display different sensitivities to pressure or time. For example, Yi et al. (2013) observed that bay scallops were fully released at 350 MPa/0 min (i.e. immediate decompression), while only 18% were released at 300 MPa/0 min, and that they were more affected by critical pressure thresholds than treatment time.

Texture is a quality parameter affected by HHP, although the measurement of texture parameters in seafood in general is controversial. Few studies concerning bivalve mollusks, in particular, are available, with no consensus. For example, some authors report increased shear strength and hardness of the adductor muscle in these animals and suggest that this may be due to aggregation and water loss induced by denaturation in the myofibrillar fraction (Hsu et al., 2010; Yi et al., 2013; Cruz-Romero et al., 2008b, c; Lopez-Caballero et al., 2000). However, others authors report contradictory data, where sample hardness decreased after pressure application (Pérez-Won et al., 2005).

Mootian et al. (2013) observed an increase in the hardness of pressurized clams at 276MPa and 552MPa and observed that 552MPa/3min disrupted the ultra-structure of the adductor muscle and mantle from continuous tightly packed muscle fibers, to open, broken, or twisted fibers, by Scanning electron images (SEM). However, these data do not agree with the report by Pérez-Won et al. (2005), who reported that the alveolar structure of the scallop adductor muscle was destroyed after exposure to 400 MPa for 10 min, with reduction in the size of interfiber spaces, resulting in a more compact structure. The loss of the honeycomb structure was accompanied by a decrease in shear values, indicating firmness reduction. This data variability is due to the great diversity of species included when using the term shellfish, even between species of the same bivalve mollusk

**Table 1.** Main effects caused by protein structure modifications when applying HHP.

Reference	Product	Treatment/Conditions	Application/ Objective	Main Conclusions
Hsu et al. (2010)	Oyster ( <i>Crassostrea gigas</i> )	150 to 300 MPa for 0, 1 and 2 min + fry cooking at 160 °C for 90 sec	Shucking	Shucking 250 MPa/1min - 92%, 250 MPa/2 min and 300 MPa/0 min - 100% shucking
			Colour	L*↑; a*↓; b*↑; ΔE ↑
			Texture	300MPa/0min increased cutting force
Yi et al. (2013)	Scallop ( <i>Aequipecten irradians</i> )	150 to 400 MPa for 0, 2 and 3 min.	Shucking	200 MPa/3min, 300 MPa/3min, 350 MPa / 2min and 400 MPa 0 min - 100% shucking
			Colour	L*↑; a*↓; b*↑; ΔE ↑
			Texture	Increased hardness by 300MPa / 0min
Rong et al. (2018)	Oyster ( <i>C. gigas</i> )	275, 300, 350 MPa/1min; 100, 200, 250, 275, 300 MPa/3min; 275 and 300MPa/2min.	Shucking	275 MPa for 3 min or 300 MPa for 2 min – 100% shucking
Briones- Labarca et al. (2012)	Red abalone ( <i>Haliotis rufescens</i> )	500 MPa/8 min, 550 MPa/3 min, 550 MPa/5 min, Storage at 4 °C for 60 days	Texture	More compact structure due to protein gelling.
Pérez-Won et al. (2005)	Scallops ( <i>A. irradians</i> )	400MPa+ one 10 min pulse; 400MPa+two 5 min pulses; 200MPa+ one 10 min pulse; 200MPa+two 5 min pulses;	Microstructure/ Texture	Hardness reduction at 400MPa + one 10 min pulse and 200MPa + one 10 min pulse. Compression of the muscle fibers with rearrangement of the perimysium and reduction of the endomysium.
Lopez- Caballero et al. (2000)	Oysters ( <i>Ostrea edulis</i> )	400 MPa at 7 °C for 10 min or 400 MPa at 7 °C for 5 min in two consecutive steps.	Texture	400 MPa por 5 and 10 min - Increase in the shear strength in storage
Cruz-Romero et al. (2004)	Oyster ( <i>C. gigas</i> )	100, 300, 500 or 800 MPa for 10 min at 20 °C	Colour	L*↑; a* ↓- 500 and 800MPa similar to cooked; b* ↑
			Protein profile (SDS-PAGE)	protein denaturation at a pressure level ≥ 300 MPa
Cruz-Romero et al. (2008a)	Oyster ( <i>C. gigas</i> )	100, 300, 500 or 800 MPa for 10 min at 20 °C	Colour	L*↑; a*↓; b*- unaffected
Cruz-Romero et al. (2008b)	Oyster ( <i>C. gigas</i> )	260, 400 or 600 MPa for 5 min at 20 °C+ storage	Texture	Increased cutting force
			Colour	L*↑; a*↓; b*↑ at 260 MPa
Bindu et al. (2015)	Mussels ( <i>Perna viridis</i> )	100, 200, 300 and 400 MPa for 5min at 30 ± 3 °C	Shucking	300 and 400 MPa –easily detached from the shell
			Texture	increased proportionately with pressure levels
			Colour	L*↑; a*↓; b* ↑
Mootian et al. (2013)	Clams ( <i>Mercanaria mercanaria</i> )	Pressure levels 250 to 552 MPa for hold times ranging between 2 and 6 min	Texture/ Microstructure	Increased hardness in 276Mpa and 552 MPa. 552 MPa for 3 min disrupted the ultra- structure of the adductor muscle and mantle.
			Colour	L*↑ and a* ↓- values stabilized at 276 MPa.
He et al. (2002)	Oyster ( <i>C. gigas</i> )	241, 275 and 310 MPa/ 0min; 241and 275MPa/ 1min; 207, 241 and 275MPa/2 min	Shucking	241 MPa for 2 min -88% detachment; 310MPa, 0 min -100% release.

\*↑ and ↓: increase or decrease in color index as a function of HP processing parameters.

genus, and also due to the variety of methodologies for gauging mollusk texture.

Another very important quality parameter concerning HPP-processed bivalve mollusks is color. According to Cruz-Romero et al. (2004, 2007, 2008a, b, c) regarding oysters and Briones-Labarca et al. (2012) for abalones, the L\* value increases with increasing pressure, indicating that HHP treatment could lead a to the brighter and less transparent adductor tissue. After high pressure treatment, seafood showed an opaque appearance

similar to that obtained by very light cooking (Murchie et al., 2005). Muscle paleness after HHP treatment resulted in brightness increases, and it was not only accounted for loss of active pigment, but also for protein coagulation, altering sample surface properties, reflecting reflected light and creating the whitish color (Kruk et al., 2011).

Although some differences are noted between different studies, most have reported decreased a\* (loss of red) and increased b\* (yellow), which varies according to species and pressurization

parameters (Table 1). The parameters that make up color in scallops, for example, can display great variability, as migration of carotenoids from the gonads to the adductor muscle occurs due to not yet fully elucidated genetic mechanisms (Li et al., 2010; Du et al., 2017). According to Rodriguez-Amaya (1993), lipid oxidation is another cause of colour loss in fish products, due to the degradation of highly unsaturated carotenoids such as astaxanthin, one of the major pigments in shellfish and fish products.

#### 4 HHP effect on microorganisms

High pressures cause morphological, biochemical and genetic changes, especially in membranes, leading to changes in microorganism functioning and reproduction (Cheftel & Culioli, 1997), including gaseous vacuole compression, cell stretching, cell wall membrane separation, cell wall contraction with pore formation, cytoskeletal modifications and nucleus and intracellular organelles changes (Campos et al., 2003). In addition, HHP increases cell permeability, inhibits energetic reactions and denatures enzymes essential for microorganism growth and reproduction (Calderón-Miranda et al., 1998).

Due to its special characteristics, the cell membrane is the main target of HHP treatment (Smelt, 1998), mainly resulting in permeability and functionality modifications (Pagán & Mackey, 2000). One hypothesis for microbial inactivation by HHP is linked to the decrease of sodium and potassium-dependent ATPase activity, located in the phospholipid layer of the cell membrane and involved in active membrane transport. In this way, ATPase becomes unable to maintain proton transport

through the membrane causing internal pH decreases and cell death (Cheftel & Culioli, 1997).

However, it seems that no single damage to a cellular structure or function is responsible for microorganism inactivation; cell death is due to a multiplicity of accumulated damages in different parts of the cell (Hoover et al., 1989). Thus, when accumulated damages exceed the ability of a cell to repair itself, cell death occurs (Rendueles et al., 2011).

Recently Rong et al. (2018) published a study on the use of high throughput sequencing (HTS) to investigate control microbiota and oysters treated with HHP during refrigerated storage. Fresh oysters (hand-shucked) were compared to a 300 MPa treatment for 2 min, due to its presented shucking efficiency. Shelf life was evaluated and fresh oyster samples became sensorially unacceptably on the eighth day of storage and microbiologically unfit for consumption on the sixth day of storage. Oysters treated with HHP, on the other hand, were valid for 12 days, as HHP promoted a reduction of 1.27 logs cycles ( $P < 0.01$ ). A principal component analysis (PCA) concerning odor analysis by electronic nose demonstrated discrepant positions for fresh, damaged and pressurized samples, confirming the hypothesis that HHP altered the oyster deterioration process during storage, influencing their microbiota. The dominant bacteria present in fresh oysters were *Vibrio*, *Shewanella* and *Pseudoalteromonas*, with *Pseudoalteromonas* and *Shewanella* dominant in spoiled oysters. The HHP treatment altered oyster deterioration microbiota dramatically, with *Psychrobacter* dominant in HHP treated spoiled oysters. Table 2 displays the main research on HHP applications in bivalve mollusks.

**Table 2.** Compiled on the effect of HHP on the microbiota of bivalve molluscs.

Seafood	Microorganisms Group/ Method	Pressure treatments	Main effects	Reference
Oyster ( <i>C. Gigas</i> )	TVC, APC and H2S-producing bacteria	100, 300, 500 or 800 MPa for 10 min at 20 °C	Bacterial load was initially reduced at all pressures to levels below the detection limit.	Cruz-Romero et al. (2008a)
Oyster ( <i>C. Gigas</i> )	TVC, APC and H2S-producing bacteria	260, 500 or 800 MPa for 3, 5 or 5 min, respectively, at 20 °C and stored at 2 °C	TVC, APC and counts of H2S-producing bacteria increased during storage, independently of pressure treatment	Cruz-Romero et al. (2008c)
Oyster ( <i>C. Gigas</i> )	Inoculated with titer of the MNV-1 stock ( $2 \times 10^{11}$ PFUs/ml). Plaque Assays and RT-PCR	200, 300 or 400 MPa for 5 min.	5-min 400-MPa treatment at 0 °C inactivated MNV-1 within oysters to undetectable levels; HPP might subtly alter the viral capsid proteins but that the RNA remains protected	Li et al. (2009)
Oyster ( <i>C. Gigas</i> )	Inoculation of 4-5 log CFU/ml of <i>V. parahaemolyticus</i> . APC and PPC by the pour-plate method; Total and fecal coliforms; <i>V. parahaemolyticus</i> for MNP method and PCR	293 MPa for 90,120,150,180 or 210 seg	293 MPa por 120 seg was capable of 3.52-log reductions of <i>V. parahaemolyticus</i> .	Ma & Su (2011)
Oyster ( <i>C. Gigas</i> )	APC and ANPC	207 to 310 MPa at 0, 1, and 2 min and stored at, 4 °C and evaluated over 27 d.	Reduction of 2-3 logs with APC and ANPC at reduced level during storage	He et al. (2002)

APC = Aerobic plate counts; PPC = Psychrotrophic plate counts; ANPC = Anaerobic plate counts; CFU = colony-forming units; MPN = most probable number; PFU = plaque-forming units; TVC = Total viable counts; PCR = polymerase chain reaction; RT-PCR = reverse transcription- polymerase chain reaction; TABC = numbers of total aerobic bacterial counts.

Table 2. Continued...

Seafood	Microorganisms Group/ Method	Pressure treatments	Main effects	Reference
Oyster (commercial)	Numbers of total aerobic bacterial counts (TABC), presumptive <i>Vibrio spp.</i> counts (PV), and presumptive <i>V. vulnificus</i> counts (PVv); 16S rDNA sequencing.	- HP -treated (250 at 400MPa for 1 at 3 min); - QF (quick frozen) - raw oysters; Stored for 21 days; Three sampling were carried: winter, summer and fall.	Numbers of bacterial flora in HP oysters were reduced in comparison to the controls (raw oysters), however increased in TABC over time (7,14,21 days) at levels higher than raw oysters in two out of the three samplings (fall and winter).	Prapaiwong et al. (2009)
Oyster ( <i>C. virginica</i> )	Counts of <i>V. vulnificus</i>	150 MPa/4 min and 200 MPa/1min at -2, 1, 5, 10, 20, 30, 40 and 45 °C.	Conditions for a 5-log reduction of <i>Vibrio vulnificus</i> : $\geq 250$ MPa; $\leq 4$ min at -2 or 1 °C.	Kural et al. (2008)
Oyster ( <i>C. virginica</i> )	Inoculation of the Hepatitis A virus (HAV); Plaque Assays and RT-PCR	300, 325, 350, 375, and 400 MPa for 1 min at approximately 9 °C	Reductions of $> 1$ , $> 2$ and $> 3 \log^{10}$ /PFU for 1 min treatments at 350, 375 and 400 MPa at 8, 7 and 10.3 °C, respectively.	Calci et al. (2005)
Oyster ( <i>C. virginica</i> )	Inoculation of the Hepatitis A virus (HAV); Plaque Assays.	350, 375 and 400 Mpa for 5 min at 17-22 °C; whole-in-shell oysters and shucked oysters.	2.56 and 2.96 log <sub>10</sub> inactivation of HAV, for whole-in-shell oysters and shucked, oysters respectively, after a 400-MPa treatment.	Kingsley et al. (2009)
Oyster ( <i>C. virginica</i> )	total Vibrionaceae (MPN), <i>Vibrio parahaemolyticus</i> (MPN), total coliform, faecal coliform and total aerobic bacteria.	Raw oysters at 600 MPa, in the presence or absence of hot sauce flavouring.	initially reduced aerobic plate counts by 2 log <sup>10</sup> when compared to raw untreated oysters and bacterial counts remained low over the 8 days of refrigerated storage	Kingsley et al. (2015)
Oyster ( <i>C. virginica</i> )	Inoculation of 7-8 log MPN/g of <i>V. parahaemolyticus</i> and <i>V. vulnificus</i>	225 MPa, 250 Mpa, 275 MPa and 300 MPa for 2 min. Stored at: 21 °C/5h; 35 °C/5h; 4 °C/1day; 4 °C/2 days; 10 °C/1day and -18 °C/2 weeks	HHP at 300 MPa/2 min achieved a $> 5$ -log MPN/g reduction of <i>V. parahaemolyticus</i> , completely inactivating <i>V. vulnificus</i> ; HHP at 200 MPa/2 min/-18 °C for 7days- completely inactivating <i>V. parahaemolyticus</i>	Ye et al. (2013)
Scallop ( <i>Argopecten irradians</i> )	Total coliforms for MPN and APC methods	150 to 400 MPa for 0, 2 and 3 min.	Level of 200MPa for 3 min produced reductions in the ACP and coliform to undetectable levels.	Yi et al. (2013)
Scallop ( <i>A. irradians</i> )	APC method	400MPa+1 pulse of 10 min; 400MPa+2 pulse of 5 min; 200MPa+1 pulse of 10 min; 200MPa+2 pulse of 5 min;	All UHP treatments reduced the initial load in total plate count of microorganisms to $< 10$ cfu/g.	Pérez-Won et al. (2005)
Clams ( <i>M. mercanaria</i> )	Inoculation of 7 log CFU/g of a cocktail of <i>V. parahaemolyticus</i>	Pressure levels 250 to 552 MPa for hold times ranging between 2 and 6 min.	450 MPa for 4 min and 350 MPa for 6 min reduced the initial concentration of <i>V. parahaemolyticus</i> to a nondetectable level ( $< 101$ CFU/g), achieving $> 5$ log reductions.	Mootian et al. (2013)
Abalone ( <i>Haliotis rufescens</i> )	APC method	100 or 300 MPa for 5 or 10 min and control	The pressure level of 300MPa per 5 or 1m min extended the shelf life from 14 days to 35 days. However it was not enough to reach the stationary phase.	Hughes et al. (2016)

APC = Aerobic plate counts; PPC = Psychrotrophic plate counts; ANPC = Anaerobic plate counts; CFU = colony-forming units; MPN = most probable number; PFU = plaque-forming units; TVC = Total viable counts; PCR = polymerase chain reaction; RT-PCR = reverse transcription- polymerase chain reaction; TABC = numbers of total aerobic bacterial counts.

Viruses are of great concern in foods, as they are obligatory intracellular parasites and can only replicate inside suitable living host cells. As a result, viruses cannot multiply in the environment or in foods, so traditional factors used to control bacterial levels in food systems (e.g., acidified pH, reduced temperature, or reduced water activity) are ineffective as barriers to viral hazards (Jaykus, 2000). In the case of viruses, experiments suggest that HHP inactivates viruses through the denaturation of their capsid proteins, which renders them unable to bind to their receptor on the surface of their host cell (Kingsley, 2014).

Investigations concerning the potential of HPP in inactivating human norovirus and hepatitis A virus, currently considered the two most significant foodborne virus threats in raw bivalve shellfish, have demonstrated that pressures  $\geq 400$  MPa will inactivate these viruses in shellfish tissues (Kingsley et al., 2002, 2005, 2007, 2009; Calci et al., 2005; Terio et al., 2010; Leon et al., 2011; Ye et al., 2014).

The efficiency of HHP technology in inactivating microorganisms depends, mainly, on the magnitude of the

applied pressure, pressurizing time, process temperature and type of microorganism, as well as cell growth phase, type of food material and the presence of microbial agents, among others (Farkas & Hoover, 2000).

HHP processing, alone or alongside other methods, has been investigated as a way to reduce pathogenic microorganism contamination in bivalve mollusks, mainly concerning *Vibrio parahaemolyticus* and *Vibrio vulnificus*. In addition, it is used to reduce spoilage burdens and, thus, extend seafood shelf life (He et al., 2002; Hughes et al., 2016; Mootian et al., 2013; Phuvasate & Su, 2015; Ye et al., 2012, 2013). In addition, reports of the potential use of HHP against hepatitis A virus and calicivirus are also found in the literature (Calci et al., 2005).

## 5 HHP effect on nutritional and sensory aspects

Food conservation by HHP originates from HHP ability to conserve original color, flavor, aroma, quality and nutritional content attributes. Pressurization is able to alter the structure of high molecular weight molecules, such as proteins and carbohydrates, while smaller molecules, such as volatile compounds, pigments, vitamins and other compounds related to sensory, nutritional and health characteristics, are less affected. Thus, this method is able to provide products displaying sensorial characteristics very close to those of the fresh food without the addition of preservatives (additives), while also displaying a favorable effect on texture characteristics and other desirable attributes, such as digestibility (Chawla et al., 2011; Ginson et al., 2015).

Some authors have reported that HHP may increase the total amount of carotenoids available in vegetable matrices (Patras et al., 2009a, b; Plaza et al., 2006; Sánchez-Moreno et al., 2005), and theorized that pressure modifies the permeability of the cell membrane and denatures carotenoid-bound proteins, at the same time making proteins more available (Barba et al., 2015). However, no studies along these lines are available for mollusks and fishes.

Kingsley et al. (2015) evaluated consumer acceptance of HHP (whole shell) treated oysters at 300, 400 and 500 MPa at 22 °C and 400, 500 and 600 MPa at 6 °C. All HHP-treated samples received the highest scores applying a hedonic scale for attributes such as appearance and texture in relation to control samples, indicating the possibility of the use of this technology for oyster processing.

However, marine foods are characterized by high levels of polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are highly susceptible to oxidation and oxidative lipid degradation during storage, directly affecting the quality of these products, impacting taste, color, texture and nutritional value. Lipid systems are the biological components most sensitive to pressure, mainly due to the predominance of hydrophobic bonds, which are very susceptible to the process (Medina-Meza et al., 2014).

A pressure level of 300 MPa leads to a small effect on lipid oxidation, which increases linearly at higher pressures. In the case of shellfish and marine animals in general, the presence of PUFAs promotes radical production, leading to accelerated

oxidation in subsequent storage periods. However, HHP can be combined with other methods that perform post-pressurizing antioxidant functions (Medina-Meza et al., 2014).

## 6 Advantages, challenges and perspectives

HHP technology displays many advantages over conventional methods, including uniform pressure distribution throughout the food with minimal increases in temperature, possibility of safely extending product shelf life with only minimal nutritional and sensorial losses, and only requiring a small amount of energy for the compression of a solid or a liquid, compared to heating the product at 100 °C. In addition, the technology is applicable to packaged foods, thus preventing unnecessary and obsolete recontamination or the need for aseptic packaging processes (Pereira & Vicente, 2010).

HHP consumes relatively low energy and requires low amounts of potable water, thus reducing its carbon footprint and decreasing effluent production, since the pressure transmission liquid (usually water) can be recycled. Consequently HHP can be considered an environmentally sustainable process (Truong et al., 2014).

Bermúdez-Aguirre & Barbosa-Cánovas (2011) pointed out that the number of HHP devices worldwide has developed at an annual exponential rate in several countries over the last 20 years, and that, currently, the use of HHP by food industries and the sale of pressurized products is a reality, ranging from fruits and vegetables to seafood and eggs, with wide consumer acceptance. In addition, Huang and colleagues (Huang et al., 2017) recently published a report on HHP growth and relevance in the food sector, and highlighted that this technology is the most commonly applied non-thermal processing technique in the world.

The Food and Drug Administration (FDA) and US Department of Agriculture (USDA) have approved the technology as a food preservation method, and the US National Advisory Committee on Microbiological Criteria for Foods regards HHP as a Non-thermal pasteurization process that can replace conventional pasteurization (Wang et al., 2013).

However, the method still presents obstacles to large-scale applications in the food industry, mainly in relation to the high initial capital to be invested. Besides the cost of the equipment, the use of HHP for shellfish and marine animals can be hampered due to seasonality. Although fish and shellfish can be consumed throughout the year, there may be peaks in production and consumption during certain periods. Thus, in order to meet product demand during harvesting periods, the company may be required to install more than one HHP unit (Sousa & Gonçalves, 2013), besides utilizing alternative products considering seasonality.

Although HHP is able to preserve the nutritional and sensory characteristics of foods, the full effects of treatment require individual study, due to the complexity of each food composition and the possibilities for changes and intrinsic reactions that may occur during pressurization. Therefore, several studies have been carried out to investigate microorganism and enzyme inactivation

kinetics, biopolymer structures (proteins, polysaccharides), as well as the effect on specific constituents of food products (juices, dairy products, meats, fish, fruits and vegetables). This aspect is related to the fact that HHP is associated with a promising tool not only for food preservation, but also because of its potential to promote positive effects on technological properties and to preserve the functional and nutritional characteristics of the food constituents. However, these effects, properties and characteristics should be studied both in the integrated system and concerning individual components.

## 7 Conclusions

The international fishing industry currently applies HHP in commercial oyster processing. However, further studies are required concerning its effects on biochemical and microflora characteristics in order to overcome the health risks associated with bivalve mollusk consumption. HHP displays many advantages, as it is a non-thermal technology compared to conventional treatments in relation to the preservation of food nutritional composition and sensory quality. Moreover, it is a clean technology, since it presents a significantly lower carbon footprint than thermal methods.

The limiting factor to its implementation remains the high capital cost of the technology. However considering its benefits, the resulting potential value aggregation and prospects of technological development, it is expected that implementation costs will become more accessible, expanding HHP use for the processing of bivalve mollusks and other shellfish in general.

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