HPP Seafood Applications

Seafood is highly regarded among a healthy eating lifestyle as it is an important source of lean protein, unsaturated fatty acid, lipid soluble vitamins A and D, and essential trace minerals. Approximately 75% of seafood consumed in the United States is fresh or frozen (Seafood Health Facts, 2018). Fish and seafood have less connective tissue than meat, which makes it more susceptible to protein degrading enzymes and spoilage microorganisms. Unsaturated fatty acids are also susceptible to oxidation, resulting in the formation of rancid off-flavors during storage. In addition to non-desirable sensory changes, foodborne pathogens may survive frozen and cold storage due to the availability of nutrients and neutral pH in seafood.

In this regard, the high pressure processing (HPP) technology may be of use to preserve the freshness and high quality attributes of seafood while achieving food safety objectives. HPP does not alter covalent bonds which are the backbone of chemical compounds associated with the nutritional profile and sensory characteristics of foods. Overall, the HPP technology provides:

Pathogen Reduction

Foodborne pathogens

Vibrio parahaemolyticus is the most recurrent shellfish pathogen leading to acute gastroenteritis (Su and Liu, 2007). In the United States, **the Interstate Shellfish Sanitation Conference (ISSC) established that ≥3.52 $\log_{10}$ of *Vibrio* spp. is the shellfish processing standard,** which is likely to be achieved by HPP at relatively mild pressure levels (150-350 MPa; Table 1).

Table 1. HPP inactivation of *Vibrio parahaemolyticus* in seafood.

Seafood	*P* (MPa)	*t* (min)	*T* (°C)	Log reduction (cfu ml^{-1} or cfu g^{-1})	Reference
Clam juice	172	10	23	6.00	(Styles et al., 1991)
Oyster	200	10	25	6.00	(Berlin et al., 1999)
	293	8	8	3.52	(Ma and Su, 2011)
	300	3	28	5.00	(Cook, 2003)
	350	2	20	5.30	(Kural and Chen, 2008)

Vibrio vulnificus is an opportunistic pathogen that is also found in bivalves and may induce septicemia (~50% mortality rate) when ingested by high risk consumers like individuals with immunocomprised systems or underlying chronic diseases (World Health Organization and Food and Agriculture Organization of the United Nations, 2005). *V. vulnificus* is less pressure resistant than *Vibrio parahaemolyticus*, and a microbial risk assessment suggests that processing oysters at 250 MPa achieves ≥3.52 $\log_{10}$ reductions of *V. vulnificus*, and reduced the probability of septicemia from ~4,900 to less than 4 cases for every 100 million consumption events (Serment-Moreno et al., 2015).

Other foodborne pathogens exhibit higher pressure resistance. For instance, processing at 500 MPa for 3-9 min holding time yielded 1.9-4.7 $\log_{10}$ reductions of *Escherichia coli* in raw black tiger shrimp, requiring 600 MPa and 6-9 min to achieve 4.0-6.0 $\log_{10}$ reductions (Kaur et al., 2016).

Foodborne viruses

Human norovirus (HuNov) is another major foodborne virus that may be potentially found in seafood. At present, there is no laboratory media or animal model to quantify HuNov concentration accurately. As for the current FDA thinking on HuNov, following good manufacturing practices (GMP's) is the most effective way to minimize the risk according this fact sheet. Recommendations are based on a risk assessment (RA) publication for retail establishments, and on the following website FDA announces an upcoming RA peer reviewed reference specific for shellfish. Additionally, shellfish processors may consult sanitation guidelines provided by the most recent version of the "Guide for the Control of Molluscan Shellfish" (Interstate Shellfish Sanitation Conference, 2015) and state guidelines available in the ISSC website.

According to the National Outbreak Reporting System (NORS) data consulted on April 2018 for HuNov, the 9-year period (1998-2006) preceding the shellfish sanitation guidelines reported 784 illnesses and this total dropped to 562 cases over the next 10-year period (Table 2; Center for Disease Control, 2018). On the other hand, the hospitalization risks slightly increased but could be related to better detection methodologies, more awareness by consumers and health organizations, etc. Still, the hospitalization rate is relatively low (<1%) and fortunately no fatalities had been reported over these 19 years. It should be noted that the current dashboard version does not distinguish between confirmed or suspected HuNov cases, thus the number of incidences could actually be lower.

Table 2. Foodborne illness incidences associated with Human norovirus (HuNov) exposure in oyster consumption in the United States (Center for Disease Control, 2018).

Period	Year	Outbreaks	Illnesses	Hospitalizations	Deaths	Food/Ingredient Search Crtieria
Before ISSC Guidelines	1998-2006	59	784	3	0	• Oyster shooters • Oysters • Oysters, raw • Raw oysters • Oysters, steamed • Oysters, baked • Oysters, fried • Oysters, uncategorized
5-year period with ISSC Guidelines	2007-2011	16	190	4	0	
Most recent 5-year period	2012-2016	20	372	4	0	

The available scientific evidence also suggests that HPP will help to reduce the risk associated with HuNov exposure during raw oyster consumption. Studies with surrogates (i. e. murine norovirus-1, Tulane virus) suggest that 400-600 MPa deliver >4 log_{10} PFU (Li et al., 2009; Li et al., 2013). Among the few studies available for HuNov, Imamura et al. (2017) observed 1.9-2.0 log_{10} reductions of the virus genomic equivalent copies (GEC) after HPP (400 MPa, 5 min, 25 °C) in naturally contaminated oysters (bioaccumulation by filter feeding) or by inoculating the adductor muscle with the virus. A study with human volunteers reported no infection cases for HuNov oysters treated at 600 MPa, 5 min, 6 °C (Leon et al., 2011). Processing at lower pressure levels (400 MPa, 5 min, 6 °C) infected 23% of subjects, whereas the consumption of raw HuNov-contaminated oysters resulted in a 50% infection rate. It should be noted that the volunteers in this clinical study were given sodium bicarbonate to lower the stomach pH to increase the likelihood of an active virus. Furthermore, the HuNov exposure dose (4 log_{10} GEC) was also higher than the 1-3 log_{10} GEC HuNov concentration range reported in other outbreaks (Imamura et al., 2017).

Relatively low pressure levels (250-400 MPa) may also prevent transmission of other foodborne viruses like Hepatitis A (Calci et al., 2005; Terio et al., 2010). Nonetheless, processing at 600 MPa is more likely to help inactivate other commonly found foodborne pathogens such as ubiquitous *Listeria monocytogenes*.

Shelf Life Extension

In addition to ensuring food safety, HPP also extends shelf life by reducing the initial load of spoilage bacteria of fresh seafood and slowing down non-desirable enzymatic and chemical reactions. Overall, pressure levels between 250-600 MPa may keep spoilage microorganisms below 10^5-10^6 cfu/g for 16-28 days when seafood is refrigerated at 2-4 °C (Table 3).

Table 3. Microbial quality parameters of fresh non-treated and HPP seafood products during refrigerated storage.

Microorganism	Seafood	HPP		Storage				Reference
		P (MPa)	*t* (min)	*T* (°C)	Day	$\log_{10} N_{Untreated}$ (cfu g^{-1})	$\log_{10} N_{HPP}$ (cfu g^{-1})	
Psychotropic bacteria	Octopus	450-600	6	4	0 16	5.2 7.7	<1.0 5.0-6.5	(Hsu et al., 2014)
	Oysters	300-500	2	4	0 15	4.0 8.0	2.1-2.2 7.0-7.5	(Lingham et al., 2016)
	Prawn	250	6	2	0 20	4.9 6.0	4.1 5.5	(Ginson et al., 2015)
	Shrimp	270-435	5	2	0 20	6.0* 8.0	4.5-5.0 6.0-6.1	(Kaur et al., 2015)
Total plate count	Mussels	300-400	5	2	0 28	2.0 ND	<1 4.3-5.1	(Bindu et al., 2015)
	Oysters	293	2	5	0 18	3.06 ND	2.5 5.2	(Ma and Su, 2011)
	Scallops	500-600	2	2	0 28	2.5 6.5	2.5 2.5-3.5	(Linton et al., 2003)
	Squid	400-600	10	4	0 10	6.0 7.5	<1.0 2.9-4.4	(Zhang et al., 2015)

During refrigerated and frozen storage, seafood is highly susceptible to amine compound formation by enzymatic and microbial activity, which results in off-flavors and may lead to intoxication (Biji et al., 2016; Cheng et al., 2014). Non-treated, hand-shucked oysters showed a concentration of total volatile basis nitrogen (TVB-N) of 4.32 mg N/100 g at day 0 of refrigerated storage (4 °C), which increased to 7.63 mg N/100 g after only 2 days (Rong et al., 2018). HPP-shucked oyster meat (300 MPa, 2 min) showed a 4 day lag phase before TVB-N increased from 4.19 mg N/100 g (day 0) to 5.28 mg N/100 g. During storage, the 4 day lag phase of the TVB-N concentration remained as consumers rejected non-treated oysters by day 8 with 20.7 mg N/100 g), whereas HPP oysters yielded 18.29 mg N/100 g (Rong et al., 2018).

Processing raw squid between 200-400 MPa reduced the concentration of diethylamine (DMA) and trimethylamine (TMA) by 20-51% after 20 days of refrigerated storage (Gou et al., 2010a; Gou et al., 2010b). Paarup et al. (2002) observed 40 TMA mg/100g concentration in non-treated squid mantle stored for 7 days, whereas HPP (300-400 MPa) significantly slowed down TMA formation, yielding 20-35 mg/100 g after 28 days. In black tiger shrimp, HPP at 300-600 MPa/3-9 min reduced TMA concentration by 20-63% (Kaur et al., 2015).

Packaging selection is another important attribute to extend the shelf life of seafood. HPP delivered 1.87-1.91 and 1.47-1.54 $\log_{10}$ counts of total plate count and psychrotrophic bacteria, respectively (Kaur and Rao, 2017). Shrimp packaged in multilayer polyester (MMP) and ethylene vinyl alcohol (EVOH) pouches extended shelf life over 30 days (<4.70 log_{10} counts) at 4 °C. Conversely, low density polyethylene (LDPE) pouches are more permeable to oxygen and spoilage microorganism growth (6.00 log_{10} counts) resulted in shrimp spoilage within 24 days (Kaur and Rao, 2017).

Mollusk Shucking and Crustacean Meat Extraction

Oysters are a cuisine delicacy and a healthy protein source. For most of us, opening up shellfish or crustaceans is a tricky, time-consuming and intensive task, as it requires skills, a sharp knife, and most importantly, lots of patience!

Eating raw oysters is highly regarded among consumers but this practice greatly increases the risk of foodborne illness. Oysters and other bivalves (i. e. clams, mussels) feed by filtering water through their body, leading to the accumulation of potentially hazardous microorganisms like Vibrio species. Raw oyster consumption during summer season is of particular concern since warm seawater temperatures provide ideal conditions for microorganisms to thrive. Cooking eliminates hazardous bacteria but is not appealing for those preferring to eat raw oysters since heat takes away the natural flavor, texture and large part of its nutrients.

Mollusk meat extraction is one of the best-known HPP seafood applications. HPP denatures the adductor muscles of shellfish like oysters, clams, etc., as well as the muscular attaching the muscle to the exoskeleton of crustaceans. The adductor muscle denaturation of shellfish by pressure becomes more evident around 250 MPa. HPP yielded over 80% of partial/full adductor muscle release of oysters and scallops around 250-300 MPa within 1-3 min (Table 4). Processing at higher pressure levels and extending holding time increases the probability of achieving 100% full release. However, it is more likely that harsher processing conditions break the bivalve shell and contaminate the meat with the debris (Rong et al., 2018). Several factors such as age and specie influence the adductor release rate. Thus, optimization of processing conditions for meat recovery is recommended.

Table 4. Bivalve adductor muscle release yields by HPP.

Seafood	*P* (MPa)	*t* (min)	*T* (°C)	% Release None	% Release Partial	% Release Full	Reference
Mussels	300	5.0	30	0	0	100	(Bindu et al., 2015)
Oysters	250	3.0	20	0	20	80	(Rong et al., 2018)
	275	1.0		0	20	80	
		2.0		0	10	90	
	300	1.0		0	15	85	
		2.0		0	0	100	
	242	1.0	NR	4	27	69	(He et al., 2002)
		2.0		6	6	88	
	276	1.0		0	4	96	
	275	1.0	NR	10	83	7	(Flick et al., 2001)
		1.5		0	95	5	
	310	1.0		0	87	13	
Scallops	200	3.0	NR	0	0	100	(Yi et al., 2013)
	300	2.0		0	8	92	

Crustacean meat extraction is another important HPP application for seafood, since the technology allows the recovery of nearly the complete edible meat portion while significantly simplifying this laborious task. Prior to HPP, seafood processors heated crustaceans with steam or boiling water to facilitate shell removal. However, heating reduces the meat moisture content and delivers lower meat extractions yields.

Lobster may be the best-documented HPP crustacean meat extraction, nearly recovering the entire edible portion by processing at 300-400 MPa (Fig. 1a). Other less exploited shucking applications include HPP crab (Fig. 1b) and shrimp/prawn. Martínez et al. (2017) recovered between 24-27% (w/w) of blue crab meat consisting of jumbo lump, backfin, special, and claw cuts with HPP (400-600 MPa). Contrastingly, hand extraction of cooked crabs resulted in 15% (w/w) meat recovery, whereas the extraction from raw, non-treated crabs yielded 18% (w/w) recovery. Moreover, Elamin et al. (2015) highlighted the easiness of shell removal for whole mud crabs processed at 345 MPa when compared to unprocessed samples, but observed no differences for shrimp peeling.

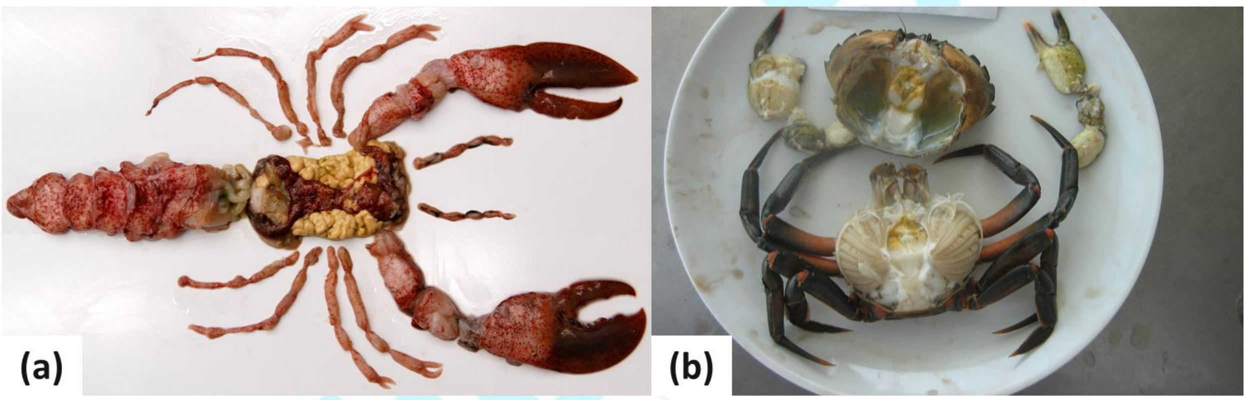

Figure 1. HPP shucking for crustacean meat extraction: (a) lobster; (b) Chinese crab. Hiperbaric 2018©.

References

Berlin, D.L., Herson, D.S., Hicks, D.T., Hoover, D.G., (1999). Response of pathogenic *Vibrio* species to high hydrostatic pressure. Applied and Environmental Microbiology 65(6), 2776-2780.

Biji, K.B., Ravishankar, C.N., Venkateswarlu, R., Mohan, C.O., Gopal, T.K.S., (2016). Biogenic amines in seafood: a review. Journal of Food Science and Technology 53(5), 2210-2218.

Bindu, J., Ginson, J., Kamalakanth, C.K., Gopal, T.K.S., (2015). High pressure treatment of green mussel *Perna viridis* Linnaeus, 1758: effect on shucking and quality changes in meat during chill storage. Indian Journal of Fisheries 62(2).

Calci, K.R., Meade, G.K., Tezloff, R.C., Kingsley, D.H., (2005). High-pressure inactivation of hepatitis A virus within oysters. Applied and Environmental Microbiology 71(1), 339-343.

Center for Disease Control, (2018). National Outbreak Reporting System (NORS).

Cheng, J.-H., Sun, D.-W., Zeng, X.-A., Pu, H.-B., (2014). Non-destructive and rapid determination of TVB-N content for freshness evaluation of grass carp (Ctenopharyngodon idella) by hyperspectral imaging. Innovative Food Science & Emerging Technologies 21, 179-187.

Cook, D.W., (2003). Sensitivity of *Vibrio* species in phosphate-buffered saline and in oysters to high-pressure processing. Journal of Food Protection 66(12), 2276-2282.

Elamin, W.M., Endan, J., Yusof, Y.-A., Shamsudin, R., Ahmedov, A., (2015). Effect of high pressure processing on sensory and physical attributes of malaysian shrimps and mud crabs. American Scientific Research Journal for Engineering, Technology, and Sciences 14(2), 91-96.

Flick, G.J., Penn, S., Oey, M., Meadows, B., (2001). Application of high pressure processing to Virginia's oyster industry. Virginia Sea Grant Marine Advisory Service Program, Blacksburb, VA, p. 16.

Ginson, J., Panda, S.K., Bindu, J., Kamalakanth, C.K., Srinivasa Gopal, T.K., (2015). Effect of high pressure treatment on microbiological quality of Indian white prawn (*Fenneropenaeus indicus*) during chilled storage. Food Microbiology 46, 596-603.

Gou, J., Lee, H.-Y., Ahn, J., (2010a). Effect of high pressure processing on the quality of squid (*Todarodes pacificus*) during refrigerated storage. Food Chemistry 119(2), 471-476.

Gou, J., Xu, H., Choi, G.-P., Lee, H.-Y., Ahn, J., (2010b). Application of high pressure processing for extending the shelf-life of sliced raw squid. Food Science and Biotechnology 19(4), 923-927.

He, H., Adams, R.M., Farkas, D., Morrissey, T.R., (2002). Use of high-pressure processing for oyster shucking and shelf life extension. Journal of Food Science 67(2), 640-645.

Hsu, C.-P., Huang, H.-W., Wang, C.-Y., (2014). Effects of high-pressure processing on the quality of chopped raw octopus. LWT - Food Science and Technology 56(2), 303-308.

Imamura, S., Kanezashi, H., Goshima, T., Suto, A., Ueki, Y., Sugawara, N., Ito, H., Zou, B., Uema, M., Noda, M., Akimoto, K., (2017). Effect of high-pressure processing on human noroviruses in laboratory-contaminated oysters by bio-accumulation. Foodborne Pathogens and Disease 14(9), 518-523.

Interstate Shellfish Sanitation Conference, (2015). National shellfish sanitation program (NSSP): Guide for the control of molluscan shellfish 2015 revision, in: Administration, U.S.F.a.D. (Ed.).

Kaur, B., Pal, Rao, P.S., (2017). Effect of storage temperature and packaging on quality and shelf-life of high pressure processed black tiger shrimp (*Penaeus monodon*). Journal of Food Processing and Preservation 42(1), e13366.

Kaur, B.P., Kaushik, N., Rao, P.S., Mishra, H.N., (2015). Chilled storage of high pressure processed black tiger shrimp (*Penaeus monodon*). Journal of Aquatic Food Product Technology 24(3), 283-299.

Kaur, B.P., Rao, P.S., Nema, P.K., (2016). Effect of hydrostatic pressure and holding time on physicochemical quality and microbial inactivation kinetics of black tiger shrimp (*Penaeus monodon*). Innovative Food Science & Emerging Technologies 33, 47-55.

Kural, A.G., Chen, H., (2008). Conditions for a 5-log reduction of *Vibrio vulnificus* in oysters through high hydrostatic pressure treatment. International Journal of Food Microbiology 122(1-2), 180-187.

Leon, J.S., Kingsley, D.H., Montes, J.S., Richards, G.P., Lyon, G.M., Abdulhafid, G.M., Seitz, S.R., Fernandez, M.L., Teunis, P.F., Flick, G.J., Moe, C.L., (2011). Randomized, double-blinded clinical trial for human norovirus inactivation in oysters by high hydrostatic pressure processing. Applied and Environmental Microbiology 77(15), 5476-5482.

Li, D., Tang, Q., Wang, J., Wang, Y., Zhao, Q., Xue, C., (2009). Effects of high-pressure processing on murine norovirus-1 in oysters (*Crassostrea gigas*) in situ. Food Control 20(11), 992-996.

Li, X., Ye, M., Neetoo, H., Golovan, S., Chen, H., (2013). Pressure inactivation of Tulane virus, a candidate surrogate for human norovirus and its potential application in food industry. International Journal of Food Microbiology 162(1), 37-42.

Lingham, T., Ye, M., Chen, H., Chintapenta, L.K., Handy, E., Zhao, J., Wu, C., Ozbay, G., (2016). Effects of high hydrostatic pressure on the physical, microbial, and chemical attributes of oysters (*Crassostrea virginica*). Journal of Food Science 81(5), M1158-M1166.

Linton, M., Mc Clements, J.M.J., Patterson, M.F., (2003). Changes in the microbiological quality of shellfish, brought about by treatment with high hydrostatic pressure. International Journal of Food Science & Technology 38(6), 713-727.

Ma, L., Su, Y.C., (2011). Validation of high pressure processing for inactivating *Vibrio parahaemolyticus* in Pacific oysters (*Crassostrea gigas*). International Journal of Food Microbiology 144(3), 469-474.

Martínez, M.A., Velazquez, G., Cando, D., Núñez-Flores, R., Borderías, A.J., Moreno, H.M., (2017). Effects of high pressure processing on protein fractions of blue crab (*Callinectes sapidus*) meat. Innovative Food Science & Emerging Technologies 41, 323-329.

Paarup, T., Sanchez, J.A., Peláez, C., Moral, A., (2002). Sensory, chemical and bacteriological changes in vacuum-packed pressurised squid mantle (*Todaropsis eblanae*) stored at 4 °C. International Journal of Food Microbiology 74(1), 1-12.

Rong, C., Ling, Z., Huihui, S., Qi, L., (2018). Characterization of microbial community in high-pressure treated oysters by high-throughput sequencing technology. Innovative Food Science & Emerging Technologies 45, 241-248.

Serment-Moreno, V., Deng, K., Wu, X., Su, Y.-C., Fuentes, C., Torres, J.A., Welti-Chanes, J., (2015). Monte Carlo analysis of the product handling and high-pressure treatment effects on the *Vibrio vulnificus* risk to raw oysters consumers. Journal of Food Engineering 144(1), 86-92.

Styles, M.F., Hoover, D.G., Farkas, D.F., (1991). Response of *Listeria monocytogenes* and *Vibrio parahaemolyticus* to high hydrostatic pressure. Journal of Food Science 56(5), 1404-1407.

Su, Y.C., Liu, C., (2007). *Vibrio parahaemolyticus*: a concern of seafood safety. Food Microbiology 24(6), 549-558.

Terio, V., Tantillo, G., Martella, V., Pinto, P.D., Buonavoglia, C., Kingsley, D.H., (2010). High pressure inactivation of HAV within mussels. Food and Environmental Virology 2(2), 83-88.

World Health Organization, Food and Agriculture Organization of the United Nations, (2005). Risk assessment of *Vibrio vulnificus* in raw oysters: interpretative summary and technical report, *Microbiological Risk Assessment*.

Yi, J., Xu, Q., Hu, X., Dong, P., Liao, X., Zhang, Y., (2013). Shucking of bay scallop (*Argopecten irradians*) using high hydrostatic pressure and its effect on microbiological and physical quality of adductor muscle. Innovative Food Science & Emerging Technologies 18, 57-64.

Zhang, Y., Jiao, S., Lian, Z., Deng, Y., Zhao, Y., (2015). Effect of single- and two-cycle high hydrostatic pressure treatments on water properties, physicochemical and microbial qualities of minimally processed squids (*Todarodes pacificus*). Journal of Food Science 80(5), E1012-E1020.