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## High-pressure processing offers varied applications



1 June 2003 George J. Flick, Jr., Ph.D.



## Expensive process has potential for value-added products



The new 10-ton HHP unit at Virginia Tech's High-Pressure Processing Laboratory is now available to food processors and distributors to evaluate the technology.

High-pressure processing, also called high hydrostatic pressure (HHP) processing, has various applications with fish and shellfish products. The technology's high pressures destroy or inactivate microbial cells in seafood to improve shelf life, quality, and food safety.

### Fish protein gels

HHP can be used to produce new surimi products. In surimi processing, sarcoplasmic proteins are removed by thorough washing, since they do not form gels when heated. However, if sarcoplasmic proteins are pressurized at over 300 MPa, they coagulate and can be incorporated into surimi and related products.

Pressure-induced gels have a springy texture similar to that of prepared sausage. Studies using hydrocolloids such as gums, alginates, and carrageenans have showed differences due to the pressures, temperatures, and times under which the gels were produced.

When gels were produced under high pressure with moderate heating, they were very elastic and light, with high water-holding capacities. Gels produced under high pressure at cold temperatures possessed high puncture resistance, cohesiveness, and water holding capacities. In contrast, gels produced by heating at atmospheric pressure exhibited low cohesiveness, yellowness, lightness and high adhesiveness and hardness. The combination of pressure treatments and ingredients presents a new potential for surimi type products.

### Muscle appearance, texture

When fish with red and white muscles are pressurized, the muscle tissue becomes opaque. The effect is similar to that of boiling or grilling with increasing pressures and holding times. Therefore, the fish obtains the characteristics of a cooked, rather than fresh, product.

This color change may not be perceived by consumers as desirable. Both red and white muscle tissues become lighter with increasing hydrostatic pressures. The redness in red-muscle fish decreases with increasing hydrostatic pressures. However, yellow color is not affected by pressure treatments.

Similar color effects have been observed when Alaska pollock surimi was pressurized to 500 MPa. There is a general hardening of the muscle tissue at 101 MPa. The upper limit for maintaining or enhancing tissue hardness is the application of 203 MPa for 10 minutes. Beyond this point, the tissue becomes softer. The hardening of fish muscle is not generally considered undesirable, but softening could be a problem for fish with soft muscle tissues.

Enzyme activity



Oyster processing is one of the leading uses of HHP technology. HHP provides a safer, preshucked product.

When a fish dies, rigor mortis produces a variety of changes in muscle tissue. The degradation of adenosine triphosphate (ATP) is important, since it affects fish flavor. When carp muscle was treated at 200, 350, and 500 MPa and stored at 5 degrees-C, the concentration of inosine monophosphate (IMP), an ATP degradation product, was suppressed in the samples treated at 350 and 500 MPa. Since increased concentrations of IMP are responsible for a loss in fish sensory quality, increased high-pressure processing should improve fish sensory characteristics.

Another change occurring during low-temperature storage of fish is the accumulation of free fatty acids. These acids are particularly reactive and rapidly oxidize in fish. When cod muscle was treated at 202 MPa for 15 minutes and stored at minus-2 degrees-C, the free fatty acid content was similar to non-pressurized samples. However, when the fish muscle was subjected to pressures greater than 405 MPa for 15 minutes, the free fatty acid level did not increase.

Table 1 illustrates the effects of pressure levels and holding times on the free fatty acid values and thiobarbituric (TBA) numbers for untreated and pressurized turbot (*Scophthalmus maximus*) fillets. TBA values are indicative of lipid oxidative rancidity. The greater the number, the higher the degree of undesirable oxidation.

Flick, Effects of pressure and pressurization time, Table 1

Treatment (MPa)	15-minute	15-minute	30-minute	30-minute
	FFA Value	TBA Number	FFA Value	TBA Number
.1	3.20	0.42	3.20	0.42
100	3.23	0.60	3.22	0.62
140	3.43	0.58	3.08	0.70
180	3.60	0.76	3.93	0.78
200	4.39	0.78	3.88	1.22

Table 1. Effects of pressure and pressurization time on FFA and TBA numbers on turbot muscle.

The TBA numbers of the samples pressurized at 100 MPa for 15 and 30 minutes and at 140 MPa for 15 minutes showed only a slight change when compared to the fresh sample. But the higher the pressure applied, the higher the TBA number. At 200 MPa, the pressure holding time was 60 percent higher for a 30-minute treatment than a 15-minute treatment.

The softening of fish post-mortem is caused by several protein digestive enzymes: cathepsins, collagenases, Ca2+-dependent proteases, alkaline proteases, trypsins, and chymotrypsins. While bacteria receive the most attention for their relation to fish spoilage, many of the deteriorative changes which reduce shelf life can also be due to biochemical activities.

The results of 303-MPa pressure applied for 30 minutes to enzymes obtained from sheephead and bluefish (*Pomatomus saltatrix*) are shown in Table 2. While the initial enzyme activities were substantially reduced, the enzyme activity underwent a reactivation when the fish was stored at 4 to 7 degrees-C (Table 3).

Flick, Effects of high pressure on selected enzyme activities, Table 2

Fish Species	Enzyme	% Activity Lost
Sheephead	cathepsin C	80
Bluefish		91

Fish Species	Enzyme	% Activity Lost
Sheephead	trypsin	64
Bluefish		74
Sheephead	chymotrypsin	75
Bluefish		65

Table 2. Effects of high pressure on selected enzyme activities from sheephead and bluefish.

Flick, Enzyme activities during refrigerated storage, Table 3

Fish Species	Enzyme	% Activity Lost Day 7	% Activity Lost Day 14	% Activity Lost Day 21
Sheephead	cathepsin C	50	28	32
Bluefish		70	10	15
Sheephead	trypsin	45	30	20
Bluefish		50	35	22
Sheephead	chymotrypsin	45	23	10
Bluefish		35	10	15

Table 3. Enzyme activities during refrigerated storage at 4-7° C.

There is an inconsistency in the effects of high pressure on fish enzymes. For example, when 202 MPa was applied to the enzymes from sheephead for 30 minutes, the activities lost for cathepsin C, chymotrypsin, and trypsin like enzymes were 56, 60 and 57 percent, respectively. However, when subjected to 101 MPa for 30 minutes, the three enzymes lost 42, 25 and 15 percent of their respective original activities. Therefore, no clear order of enzyme susceptibility to pressure treatments can be deduced.

A study of selected protease enzymes in cod (*Gadus morhua*) showed that some proteases survived pressures of 800 MPa. However, a marked decrease occurred in the activity of neutral proteases above 200 MPa.

Oxidation of tissue lipids

The oxidation of lipids in muscle tissue is accelerated by high-pressure treatment. The peroxide value levels of oils extracted from pressure-treated and refrigerated cod muscle were significantly higher than that of nonpressurized and refrigerated cod muscle. Similar increases in peroxide values have been reported in mackerel muscle that received high-pressure treatments.

These results indicated that fish muscle contains factors that accelerate lipid oxidation during high-pressure treatment. One of the factors reported to affect lipid oxidation is the concentration of heme and nonheme iron.

After treatment at pressures above 400 MPa, the oxidative stability of cod muscle lipids was markedly decreased as measured by the TBA number. This was thought to be due to the release of metal catalysts from complexes, since the addition of ethylenediaminetetraacetic acid inhibited the increased rates of oxidation.

Applications to fish, shellfish

Thawing

High-pressure thawing is effective in foods having high water content because the melting temperature of water is depressed under pressure from 0 degrees-C at atmospheric pressure to 22 degrees-C at 220 MPa. The latter pressure is the maximum of interest in thawing because the phase-change transfer increases with pressures below 220 MPa and decreases above this point.

Pressures exceeding 220 MPa modify the ice crystal structure and increase the phase-change temperature. Neither is desirable, since the first can cause texture changes and the second can affect the overall duration of the process.

In a test, tuna muscle thawed at 200 MPa saw a decrease in thawing time and reduction in thawing drip losses. The thawing drip was roughly 4 percent (2 to 6 percent drip volume on a wet-weight basis, depending on thaw temperature) at atmospheric pressure and less than 1 percent at high pressure, irrespective of the pressure level (50 to 150 MPa). The microbial content of the tuna was either maintained at a constant level or reduced.

The influences of pressure level, pressurization rate, and pressure holding time at a constant freezing rate were studied in comparison with a thawing process at atmospheric pressure in whiting, *Gadus merlangus*. A high freezing rate (0.77 vs. 0.14 degrees C/minute) and high pressurization rate (100 vs. 42 MPa per minute) reduced thawing drip loss at a given pressure. A decrease in the drip volume for high-pressure thawing compared to atmospheric thawing was obtained only by prolonging the pressure holding time. Table 4 contains the thawing times of whiting fillets as a function of pressure.

Flick, Thawing times of whiting fillets, Table 4

**Pressure (MPa) Thawing Time (min)**

.1	60
50	35
100	25
150	20
200	15

Table 4. Thawing times of whiting fillets as a function of pressure.

High-pressure thawing of spiny dogfish, *Squalus acanthias*; and scallops, *Pecten irradians*, was compared to thawing under atmospheric pressure. Results showed that for both products, the thawing drip loss was significantly reduced – 70 percent for dogfish and 31 percent for scallops. Optimal results were obtained at 150 MPa. The savings of only a few percent on a large volume of product, especially a high-value product, should be an important economic factor in including high-pressure technology as a unit operation.

**Parasites**

A common parasite in many marine finfish and squids is *Anisakis simplex*. The parasite presents a major health risk when fish is consumed raw (as with sushi, sashimi, and ceviche), improperly cooked, or subjected to a process employing minimal heat (carpaccios and cold-smoked fish). Treatment of the parasite at 200 MPa for 10 minutes at a temperature of 0 to 15 degrees-C kills all *Anisakis* larvae.

Lower pressures can be successfully employed down to 140 MPa, but the pressurization time must be increased up to one hour. Most larvae are destroyed at pressurization greater than 120 MPa with times exceeding 10 minutes.

**Vacuum, modified atmosphere packaging**

In testing, vacuum-packaged muscle from hake, *Merluccius capensis*, was subjected to 400 MPa in three five minute cycles at 7 degrees-C. The processed samples were more stable at chilled temperatures of 2 to 3 degrees-C and remained sensorially acceptable until 43 days of storage, in comparison with nine days for nonpressurized hake. When a pressure of 400 MPa was applied, the fish attained a cooked appearance, as previously discussed.

Fish pressurized at 400 MPa had a shelf life of 15 days, while samples treated at 200 MPa had a one-week shelf life. The 400-MPa-processed fish had a 99 percent reduction in microbial count, while the 200-MPa samples had a 90 percent microbial reduction. The samples processed at 400 MPa had very low trimethylamine nitrogen (TMA-N) values and a slower increase in drip loss from day 15 of storage. High TMA values have been associated with undesirable flavor and textural changes in foods during storage.

In further research, high-pressure processing at low temperature combined with modified-atmosphere packaging was used for the preservation of Atlantic salmon. A shelf life extension of two days was obtained after a treatment of 150 MPa for 10 minutes at 5 degrees-C compared to nonpressurized, vacuum-packaged salmon. Modified atmosphere storage (50 percent O<sub>2</sub> + 50 percent CO<sub>2</sub>) alone extended the shelf life for four days at 5 degrees-C.

When salmon were subjected to high pressure in the presence of 50 percent O<sub>2</sub> + 50 percent CO<sub>2</sub>, the threshold value for microbial spoilage as judged by a 7.0 to 7.2 log CCU/gram was not reached until at least 18 days at 5 degrees-C. Spoilage and the pathogenic microorganisms *Salmonella*, *Listeriamonocytogenes*, and *Shewanella putrefaciens* were more susceptible to high-pressure processing in the presence of the gas mixture.

**Application to squids, octopus**

Sensory, chemical, and microbial changes were reported in vacuum-packed *Todaropsis eblanae* squid mantles that were pressurized at 150, 200, 300, and 400 MPa for 15 minutes at ambient temperature and stored at 4 degrees-C. Sensory analyses showed that the higher the pressurization, the longer the shelf life. The samples pressurized at 400 MPa were rejected after 28 days of storage compared to seven days for the untreated samples.

Octopus, *Octopus vulgaris*, was stored at 2.5-3 degrees C after treatment at 400 MPa at 40 degrees-C. The shelf life was extended 43 days longer than the untreated samples. The pressure-treated samples contained a lower level of nitrogenous compounds, had reduced autolytic activity, and less drip loss. The shear strength values remained stable throughout storage.

**Conclusion**

High-pressure processing is an emerging technology that has applications in primary and further processed fish and shellfish. Although the process is expensive, economic potential exists for the production of value-added products and products degraded by conventional thermal treatments. Also, HHP has special application in ready to- eat products that do not have a specified pathogen-reduction process, such as cold-smoked fish, due to the United States Food and Drug Administration's zero-defect action policy for the presence of *Listeria monocytogenes*.

*Note: This is the third in a series of articles on high hydrostatic pressure processing. In the February Global Aquaculture Advocate, the first article briefly outlined the technology, economics, and some general applications of HHP. In April, the second article discussed the effects of high pressure processing on the destruction of spoilage and pathogenic microorganisms.*

*(Editor's Note: This article was originally published in the June 2003 print edition of the Global Aquaculture Advocate.)*

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