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Virginia Cooperative Extension
Virginia Tech
Commercial Fish & Shellfish Technology
fact sheet

Novel Applications of High Pressure Processing

Originally appearing in Global Aquaculture Advocate, Volume 6, Issue 3 (June 2003)

Fish Protein Gels

HPP can be used to produce new surimi products. In surimi processing, the 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 will coagulate and can be incorporated into surimi and related products. Pressure-induced gels have been reported to have a springy texture similar to that of prepared sausages. Studies using various hydrocolloids (for example, various gums, alginates, and carrageenans) showed that the main difference was due to the process (pressure, temperature, time) under which the gels were produced. When gels were produced under high pressure with moderate heating, the resulting gels were very elastic and light, with high water holding capacities. Gels produced under high pressure at cold temperatures possessed high puncture test properties (breaking deformation, breaking force) and high cohesiveness and water holding capacity. In contrast, gels produced by heating at atmospheric pressure exhibit low cohesiveness, high lightness and yellowness, high adhesiveness and hardness. The combined systems (pressure treatments and ingredients) present a new potential for surimi type products.

Muscle Appearance and Texture

When red and white muscle fish are pressurized, the muscle tissue becomes opaque similar to boiling or grilling with increasing pressures and holding times. Therefore the fish obtains the characteristics of a cooked, rather than a 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, the yellow color is not affected by various pressure treatments. Similar color effects have been observed when Alaska pollock surimi was pressurized up to 500 MPa. There is a general toughening/hardening of the muscle tissue when 101 MPa of pressure is applied. 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 an undesirable effect, however, softening could be a problem with fish having soft muscle tissues.

HPP can be used in the development of new seafood products for consumers.

Table 1. Effect of pressure and pressurization time on FFA and TBA number on turbot muscle.

Treatment (MPa)	15 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 2. The effect of high pressure on selected enzyme activities from sheephead and bluefish.

Fish Species	Enzyme	% Activity Lost
sheephead	cathepsin C	80
bluefish		91
sheephead	trypsin	64
bluefish		74
sheephead	chymotrypsin	75
bluefish		65

Table 3. Increase in enzyme activities during refrigerated storage at 4 - 7° C.

Fish Species	**Enzyme**	**% Activity Lost**		
		Day 7	Day 14	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

Enzyme Activity

When a fish dies, rigor mortis develops producing a variety of changes in muscle tissues. The degradation of ATP (adenosine triphosphate) is important since it affects fish flavor. When carp muscle was treated at 200, 350, and 500 MPA and stored at 5° C, the concentration of one of the ATP degradation products, IMP (inosine monophosphate) 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. Free fatty acids are particularly reactive and rapidly oxidized in fish. When cod muscles were treated at 202 MPa for 15 minutes and stored at -2° 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 effect of pressure level and pressure holding time on the free fatty acid values and TBA (the TBA number is indicative of lipid oxidative rancidity and the greater the number, the higher the degree of undesirable oxidation) numbers on untreated and pressurized turbot (*Scophthalmus maximus*) filets. 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 level applied, the higher the TBA number. At 200 MPa, the pressure holding time was 60% higher for a 30-minute treatment than for a 15-minute treatment.

The softening of fish *post-mortem* is caused by several protein digestive enzymes (cathepsins, collagenases, Ca^{2+}-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 may also be due to biochemical activities. When pressures of 303 MPa were applied for 30 minutes to enzymes obtained from sheephead (scientific name not provided) and bluefish (*Pomatomus saltatrix*), the following results were obtained (Table 2)

While the initial enzyme activities are substantially reduced, the enzyme activity undergoes a reactivation when the fish is stored at refrigerated temperatures (4 - 7° C). The increase in enzyme activities during storage is provided in Table 3.

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% respectively. However, when subjected to 101 MPa for 30 minutes, the three enzymes lost 42, 25, and 15% of their respective original activities. Therefore no clear order of enzyme susceptibility to pressure treatments can be deduced.

A study on 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 (POV) levels of oils extracted from pressure treated and refrigerated cod muscle were significantly higher than that of non-pressurized and refrigerated cod muscle. Similar increases in peroxide values have been reported in mackerel muscle receiving high pressure treatments. There results indicate that fish muscle contains certain factors that accelerate lipid oxidation during high pressure treatment. One of the factors reported to affect lipid oxidation is the concentration of heme and non-heme iron.

After treatment at pressures above 400 MPa, the oxidative stability of cod (*Gadus morhua*) muscle lipids were markedly decreased as measured by the thiobarbituric (TBA) number. The TBA number provides the relative oxidation of fat. The higher the number, the greater the

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

Pressure (MPa)	Thawing Time (Minutes)
.1	60
50	35
100	25
150	20
200	15

oxidation. This was thought to be due to the release of metal catalysts from complexes since the addition of EDTA (ethylenediaminetetraacetic acid) inhibited the increased rates of oxidation.

Applications to Fish and Shellfish

THAWING - High pressure thawing is effective in foods having a high water content. This is because the melting temperature of water is depressed under pressure from 0° C at atmospheric pressure down to -22° C at 220 MPa. A pressure of 220 MPa is the maximum of interest in thawing from the heat transfer process. This is because the phase change transfer increases with pressures below 220 MPa and decreases above this point. Pressures exceeding 220 MPA will cause a modification of the ice crystal structure and an increase in the phase change temperature. Neither of these phenomena are desirable since the first may cause an undesirable texture change and the second may affect the overall duration of the process. Tuna muscles were thawed using a pressure of 200 MPa. A decrease in thawing time was obtained together with a reduction in the thawing drip losses. It was observed that thawing drip was roughly 4% at the mean at atmospheric pressure (between 2 and 6% drip volume on a wet weight basis depending on the thaw temperature) and less than 1% at high pressure, irrespective of the pressure level (between 50 and 150 MPa). The microbial content of the tuna was either maintained at a constant level or reduced. The influence of the pressure level (between atmospheric pressure and 200 MPa), the pressurization rate, and the pressure holding time, at a constant freezing rate, was studied in comparison with a thawing process at atmospheric pressure in whiting (*Gadus merlangus*). A high freezing rate (0.77 vs. 0.14° C/minute), high pressurization rate 100 vs. 42 MPa/minute) reduced the 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 filets 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% for dogfish and 31% for scallops) and optimal results were obtained at 150 MPa. The savings of only a few percent on a large volume of product, especially a high-valued product, should be considered as an important economic factor in including high pressure technology as an unit operation.

High pressure processing has been shown to slow changes that typically occur post-mortem in the flesh of most species of fish.

PARASITES - A common parasite in many marine finfish and squid is *Anisakis simplex*. The parasite presents a major health risk when fish is consumed raw (sushi, sashimi, and ceviche), improperly cooked, or subjected to a process employing only minimal heat (carappacios and cold smoked fish). Treatment of the parasite at a pressure of 200 MPa for 10 minutes at a temperature between 0 and 15° C killed all *Anisakis* larvae. Lower pressures can be successfully employed down to 140 MPa, but with the lower pressures, the pressurization time must be increased up to 1 hour. Most larvae have been observed to be destroyed at pressurization greater than 120 MPa with times exceeding 10 minutes.

VACUUM AND MODIFIED ATMOSPHERE PACKAGING - Vacuum packaged hake (*Merluccius capensis*) muscles were subjected to 400 MPa (three 5 minute cycles) at 7° C. The processed samples were more stable at chilled temperatures (2 - 3° C) and remained sensorially acceptable until 43 days of storage, in comparison with 9 days for the non-pressurized 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 two log (99%) reduction in microbial count while the 200 MPa samples had a one-log

HPP can dramatically increase the shelf life of products such as octopus or squid rings.

(90%) microbial reduction. The samples processed at 400 MPa had very low trimethlyamine 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.

High pressure processing at low temperature combined with modified atmosphere packaging (MAP) was used for the preservation of Atlantic salmon. A shelf life extension of 2 days was obtained after a treatment of 150 MPa for 10 minutes at 5° compared to non-pressurized, vacuum packaged salmon. Modified atmosphere storage (50% O_2 + 50% CO_2) alone extended the shelf life for 4 days t 5° C. When salmon were subjected to high pressure in the presence of 50% O_2 + 50% CO_2, the threshold value for microbial spoilage as judged by a 7.0 - 7.2 log CCU/g was not reached until at least 18 days at 5° C. Spoilage and pathogenic microorganisms (*Salmonella, Listeria monocytogenes*, and *Shewanella putrefaciens*) were more susceptible to high pressure processing in the presence of the gas mixture.

APPLICATION TO SQUID AND OCTOPUS - Sensory, chemical, and microbial changes were reported in vacuum-packed squid (*Todaropsis eblanae*) mantles that were pressurized at 150, 200, 300, and 400 MPa for 15 minutes at ambient temperature and stored at 4° 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 with 7 days for the untreated samples. Octopus (*Octopus vulgaris*) was stored a t 2.5 - 3° C after treatment at 400 MPa at 40° 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 application to primary and further processed fish and shellfish. Although the process is still expensive, an economic potential exists for the production of value-added products and products that are degraded by conventional thermal treatments. Also, there is special application to ready-to-eat products that do not have a specified pathogen reduction process, such as cold smoked fish, due to the FDAs a zero defect action policy for the presence of *Listeria monocytogenes.* As research continues on the new process, many applications for the technology will emerge that are specially suited for use with fish and shellfish products.

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This ***fact sheet*** was published by: Virginia Tech, the Virginia Sea Grant College Program, and Virginia Cooperative Extension

This publication is the result of research supported by the NOAA Office of Sea Grant, the U.S. Department of Commerce, and the Virginia Sea Grant College Program.