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Effect of High Hydrostatic Pressure Processing on *Listeria spp.* Microorganisms

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High hydrostatic pressure can have a disruptive effect on living organisms. High pressures either destroy or inactivate microbial cells through a combination of physiological and biochemical effects on the microorganism. Scanning electron microscopy studies have revealed that cell morphology is not significantly affected at the lower pressures, although membrane integrity is damaged. At elevated pressures, (500 - 700 MPa) progressive morphology changes become evident. Cell lysis occurs with the highest pressure treatments. The article on high hydrostatic pressure in the last issue of *Advocate* magazine described the potential of high hydrostatic pressure (HHP) processing for improving the shelf-life, quality, and safety of fish and fishery products. This article discusses the variables that should be considered when developing a HHP process to kill or inactivate the pathogenic microorganism, *Listeria monocytogenes* in fish and fishery products.

Microorganisms sharing the same scientific name may have significantly different biochemical and physical properties. Unlike many other forms of life, microorganisms only have to share a few biochemical and morphological properties to be classified as identical. Consequently, many microorganisms with the same scientific name can have disparate responses when exposed to similar environmental effects. Therefore, any processes or procedures designed to control a single microorganism must consider the variation in properties that may occur within that species.

Effect of Pressure on *Listeria monocytogenes* Strains

Substantial differences between various *L. monocytogenes* isolates have been reported in the scientific literature. Table 1 provides an example of the magnitude of microbial inactivation that has been reported to occur within two isolates, one obtained from a microbial collection and the other isolated from a food product.

With 5 minutes of processing, one strain was reduced by a factor of 10 while the other was reduced by a factor of 1,000. The magnitude after 20 minutes of pressurization illustrated an even greater difference in pressure resistance between the strains. One strain was reduced by a factor of 100 while the other was reduced by a factor of ten million. A processor developing a process to reduce *L. monocytogenes* by 5 $\log_{10}$ could have a process as short as 5 min or over 30 depending on the pressure tolerance of the specific strain of microorganism encountered.

A second study (Table 2) utilizing various pressures and three strains of *L. monocytogenes* clearly demonstrated the lethal effect of increasing pressure on cells. However, a significant difference in pressure resistance was again observed between the three strains.

The time required for microorganism inactivation or death was significantly reduced by increasing the hydrostatic pressures applied. A processor producing a ready-to-eat product and employing reasonable sanitary practices should not have a *L. monocytogenes* population greater than 1,000/g in the final product. Therefore a listericidal HHP process could be less than 10 min at 450 MPa while at 300 MPa the process would exceed 30 min.

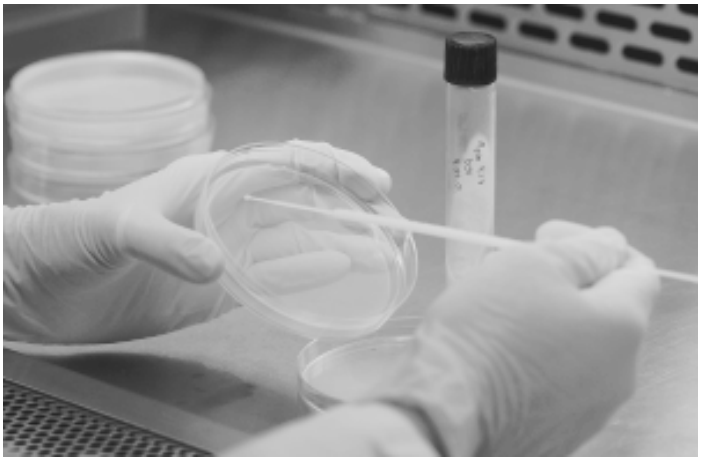

Table 1. Destruction of Two Strains of *L. monocytogenes* at 375 MPa.

Treatment Time (Min)	($\log_{10}$ Number of Organisms Destroyed) Strain 1	Strain 2
5	10^1	10^3
10	10^1	10^5
15	10^2	10^6
20	10^2	10^7
25	10^3	10^7
30	10^3	10^7

Table 2. Destruction of Three Strains of *L. monocytogenes* at 300, 375, and 450 MPa.

Treatment Time (Min)	Pressure 300 MPa 1	300 MPa 2	300 MPa 3	375MPa 1	375MPa 2	375MPa 3	450MPa 1	450MPa 2	450MPa 3
	(Log $_{10}$ Number of Organisms Destroyed)								
5	0	0	0	0	0	10^3	$10^{1.5}$	$10^{2.5}$	10^7
10	0	0	0	0	$10^{2.5}$	10^5	10^4	10^5	ND
15	0	0	10^1	$0^{1.5}$	10^4	10^6	$10^{5.5}$	10^5	
20	0	0	$10^{1.5}$	$10^{1.5}$	$10^{4.5}$	10^7	10^7	10^5	
25	0	0	10^2	$10^{2.5}$	$10^{4.5}$	10^7	10^7	10^5	
30	0	0	10^3	10^3	$10^{4.5}$	10^7	ND	10^5	

ND = not detected

Table 3. Destruction of One Strain of *L. monocytogenes* at 375 MPa in Two Different Food Products

Treatment Time (Min)	Product 1 (Log $_{10}$ Number of Organisms Destroyed)	Product 2
5	10^1	10^3
10	$10^{1.5}$	10^5
15	10^2	$10^{5.5}$
20	$10^{2.5}$	10^6
25	10^3	$10^{7.5}$
30	$10^{3.5}$	$10^{7.5}$

Effect of the Pressure on *Listeria monocytogenes* in Different Food Products

In additional to differences between stains and pressures, the environment in which the microorganism is contained during pressurization is important. Some products provide a sparing effect on microorganisms while others increase their vulnerability. Table 3 demonstrates the survival of one strain of *L. monocytogenes* in two different products at 375 MPa.

These results clearly show that if complete, or partial, destruction of *L. monocytogenes* in a food product is desired, research needs to be performed to determine if more than one strain is present and how readily the various strains succumb to or resist pressurization in that food product. Unfortunately, the information obtained on the destruction or inactivation of a microorganism in one food product cannot be readily transferred to another product.

Effect of the Pressurization Ramp on the Inactivation of *Listeria innocua*

L. innocua was used as a surrogate microorganism in a study to determine whether there was a resultant difference when slow pressurization with rapid depressurization was tested against a process with rapid pressurization and slow depressurization. The test was conducted over a range of 400 to 600 MPa with holding timers ranging from 0.5 min to 5 min. Results of the study indicated there was no difference in inactivation of the microorganism between the two techniques. It is interesting to note that only one strain of *L. innocua* was employed in the study.

Conclusion

Validation of a process requires some thought and at times, substantial laboratory research. When developing a process, the services of a food microbiologist and statistician could prove to be most useful. Since the U. S. Food and Drug Administration has established a zero defect action level for *L. monocytogenes* in cooked ready-to-eat products, a processor cannot afford to establish a process that does not perform as intended. Variables to be considered in developing a listericidal process based on HHP technology should include: the variation that may exist in the various *L. monocytogenes* serovars; the magnitude of the reduction required (e.g., 3 or 5 $\log_{10}$); the food product being processed; and the intended use of the product. Finally, the sanitary operation of the processing facility is most important. A facility employing acceptable sanitary practices may require a three $\log_{10}$ reduction while one with marginal sanitary practices may need a 5 $\log_{10}$ reduction. The process time required to achieve the two log difference could be significant. The longer process time could result in major increases in capital investments and production costs. As always, there is no substitute for establishing and maintaining high sanitary standards in the production of fish and fishery products.

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