

Research Note

High Hydrostatic Pressure Treatment of Finfish To Inactivate *Anisakis simplex*

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ABSTRACT

High hydrostatic pressure has been demonstrated to be a useful technique for treating food to reduce the number of pathogenic organisms and to extend shelf life. Most research in this area has focused on bacteria. However, a concern in the sashimi (raw fish) industry is that nematode worms such as *Anisakis simplex* occur naturally in cold-water marine fish. The objectives of this research were to perform a pilot study to determine the effect of high hydrostatic pressure on the viability of *Anisakis simplex* larvae, commonly found in king salmon and arrowtooth flounder, and to evaluate the effects of high hydrostatic pressure on the color and texture of the fish fillets. Pieces of fish (ca. 100 g per bag) containing 13 to 118 larvae were exposed to pressures of up to 80,000 lb/in² (552 MPa) for up to 180 s. The times and pressures required to kill 100% of the larvae were as follows: 30 to 60 s at 60,000 lb/in² (414 MPa), 90 to 180 s at 40,000 lb/in² (276 MPa), and 180 s at 30,000 lb/in² (207 MPa). For all salmon treatments that killed 100% of the larvae, a significant increase in the whiteness of the flesh was observed. Although high hydrostatic pressure was effective in killing *A. simplex* larvae in raw fish fillets, its significant effect on the color and overall appearance of the fillet may limit its application to the processing of fish for raw-fish markets.

The increasing popularity of restaurants that serve sashimi and sushi containing raw finfish has heightened concern about the safety of these products. The parasite *Anisakis simplex*, which is a human pathogen, is commonly present in the larval stage in the intestines of cold-water marine fish. Marine mammals, shellfish, and finfish serve as hosts to these nematodes. The most common method of treating finfish destined for the raw-fish market is to properly freeze it prior to serving. The U.S. Food and Drug Administration (7) specifies that to ensure that all nematodes are killed, finfish must be frozen and stored at -20°C (-4°F) or below for 168 h (7 days) in a freezer or frozen at -35°C (-31°F) or below until solid and then stored at -35°C (-31°F) for 15 h.

High hydrostatic pressure (HHP) processing has been suggested as a treatment for use in reducing pathogenic microorganisms associated with food and in extending shelf life. HHP has been applied to a variety of foods, including fruit juices, sauces, meats, and seafoods (2, 3, 5, 6). In a recent publication, Molina-Garcia and Sanz (4) described the effects of HHP on *A. simplex* larvae that were suspended in distilled water and in isotonic saline solutions. They found that treatment at 200 MPa for 10 min killed all of the larvae. At lower pressures, treatment was required for up to 1 h to kill all of the larvae. Such long treatment

times would be impractical for a commercial operation. In our investigation, we determined the effectiveness of HHP in reducing populations of *Anisakis simplex* in finfish. The purposes of this pilot project were to determine the time and pressure parameters that were effective in killing *Anisakis simplex* and to determine whether there were other effects that might affect the consumer acceptability of the raw product destined for the sashimi and sushi markets.

MATERIALS AND METHODS

To maximize the number of worms in the sample, the belly flap (muscular tissue surrounding the viscera) regions were obtained and processed when possible. Belly flaps from Pacific halibut (*Hippoglossus stenolepis*) and belly flaps and viscera from arrowtooth flounder (*Atheresthes stomias*) were obtained from local retailers and wholesalers and used to determine the survival of worms after HHP processing. Belly flaps of Alaska king salmon (*Oncorhynchus tshawytscha*) and fillets of Columbia River king salmon were packaged for color analysis after HHP processing. All fish samples were refrigerated (4°C) for 48 h and were then transported in a cooler to Avure Technologies Inc. (a subsidiary of Flow International Inc., Kent, Wash.), cut, and assembled into $\sim 100\text{-g}$ portions, which were placed into low-density polyethylene meat bags and then vacuum packed (Multivac Model A300/16, Multivac Inc., Kansas City, Mo.) at 60 Mbar (6,000 GPa).

For nematode analyses, several time and pressure parameters were tested. For halibut, one to four samples were treated at 2, 10, 20, 30, 60, and 80 lb/in² (14 to 552 MPa) for 30 s, and one or two samples were treated at 1, 20, 30, 60, and 80 lb/in² (6.9 to 552 MPa) for 60 s. For arrowtooth flounder, one to three samples were treated at 20, 30, 40, 50, and 60 lb/in² (138 to 414

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TABLE 1. *Survival of Anisakis simplex in finfish tissue subjected to high hydrostatic pressure processing^a*

Species	Treatment pressure		Treatment time (s)	n	Avg no. of total nematodes	Avg no. of live nematodes	Avg % of killed nematodes
	lb/in ² (×1,000)	MPa					
Halibut	0	0	0	1	5	5	0
	2	14	30	2	70	56	20
	10	69	30	4	47	28	35
	20	138	30	1	45	30	33
	30	207	30	2	22	4	84
	60	414	30	2	36	0	100
	80	552	30	1	88	0	100
	1	6.9	60	2	49	36	26
	20	138	60	2	26	19	25
	30	207	60	1	41	9	78
	60	414	60	1	13	0	100
	80	552	60	2	14	0	100
Arrowtooth flounder	0	0	0	3	138	116	16
	20	138	90	1	33	29	12
	30	207	90	3	96	6	92
	40	276	90	3	48	0	100
	50	345	90	3	56	0	100
	60	414	90	2	118	0	100
	20	138	180	1	18	5	72
	30	207	180	1	38	0	100
	40	276	180	1	53	0	100
	50	345	180	1	39	0	100

^a n, number of bags processed. Each bag contained ca. 100 g of fish tissue. The average numbers of total and live nematodes are expressed per ca. 100 g of tissue.

MPa) for 90 s, and one sample was treated at 20, 30, 40, and 50 lb/in² (138 to 345 MPa) for 180 s. For color analyses, two samples were prepared for each treatment of Alaska king salmon belly flaps (30, 40, and 50 lb/in² for 90 s, and 30, 40, and 50 lb/in² for

180 s) and of Columbia River king salmon fillets (30, 40, 50, and 60 lb/in² for 90 s).

Samples were loaded into a 2-liter HHP machine (The Food Unit Assembly, part no. 7XS-6000, Flow International), cooled to

TABLE 2. *Tristimulus color values for salmon processed by high hydrostatic pressure*

Sample	Treatment pressure		Treatment time (s)	L*	a*	b*
	lb/in ² (×1,000)	MPa				
Alaska king salmon belly flaps	0	0	0	56.9 ± 1.5 A	11.5 ± 2.6	17.4 ± 1.1
	30	207	90	57.7 ± 4.6 A	10.6 ± 4.0	17.2 ± 6.6
	40	276	90	61.7 ± 1.1 AB	12.8 ± 1.3	18.4 ± 2.3
	50	345	90	66.8 ± 1.1 B	12.9 ± 1.6	18.1 ± 1.4
	0	0	0	56.9 ± 1.5 A	11.5 ± 2.6	17.4 ± 1.1
	30	207	180	64.0 ± 3.7 B	13.3 ± 2.4	20.6 ± 0.4
	40	276	180	65.5 ± 0.2 B	13.7 ± 1.4	20.2 ± 2.2
	50	345	180	70.4 ± 0.6 B	12.2 ± 0.5	18.3 ± 0.0
Columbia River king salmon fillets	0 ^b	0	0	43.7 ± 2.6 A	8.8 ± 1.4	13.1 ± 3.5
	30	207	90	57.3 ± 4.9 B	11.4 ± 1.3	18.7 ± 2.8
	40	276	90	63.6 ± 2.9 BC	10.8 ± 0.7	14.9 ± 1.2
	50	345	90	69.5 ± 2.1 C	10.5 ± 0.8	15.2 ± 2.0
	60	414	90	66.7 ± 3.0 BC	10.3 ± 1.1	14.1 ± 1.6

^a L*, 0 = black, +100 = white; a*, -80 = green; +100 = red; b*, -80 = blue; +70 = yellow. Values presented are averages of three readings for each of two tissue samples. Data for the untreated samples are repeated for the 180-s data set. Values with different letters for the same processing time are significantly different by one-way analysis of variance and Tukey's HSD post hoc test ($P < 0.05$).

^b This set was stored for 2 days at 4°C.

10°C with a circulating ice bath, and processed at the specified pressures and times. Average temperatures (measured with a Raytek Mini Temp FS) going in and coming out of the HHP machine were $7.3 \pm 1.2^\circ\text{C}$ and $7.6 \pm 1.1^\circ\text{C}$, respectively, for packed fish samples ($n = 19$); $10.1 \pm 3.8^\circ\text{C}$ and $5.6 \pm 1.4^\circ\text{C}$, respectively, for water ($n = 5$); and $9.9 \pm 2.8^\circ\text{C}$ and $7.7 \pm 1.0^\circ\text{C}$, respectively, for the vessel ($n = 5$). After HHP processing, the samples were placed in a cooler with ice packs and transported to the University of Washington. Samples for worm analysis were processed for digestion within 48 h after high-pressure processing. Nematode larvae were isolated by the pepsin digestion method of Van Velzen (8) and tested for viability as described in Adams et al. (1). Tristimulus color was measured with a colorimeter (Minolta Chroma Meter CR-200, Minolta, Ramsey, N.J.) for two samples per treatment; three readings taken from random areas of each fillet were averaged ($n = 2$). The colorimeter was calibrated with a standard white reference tile to $L^* = 116$, $a^* = 0$, and $b^* = 0$. Where appropriate, data were analyzed by linear regression or univariate analysis of variance (Bonferroni multiple comparisons) with Statistical Packages for the Social Sciences (SPSS).

RESULTS AND DISCUSSION

We examined the effect of HHP processing for up to 180 s on the viability of *Anisakis simplex*. Relatively short processing times were tested in this pilot study, since we wanted to examine treatment times that would be of interest to the food industry. The minimum processing times necessary to kill the nematodes were found to be 180 s at 30,000 lb/in² (207 MPa), 90 s at 40,000 lb/in² (276 MPa), and 30 to 60 s at 60,000 lb/in² (414 MPa) (Table 1). Processing at higher pressures required proportionately less time to kill the larvae than processing at lower pressures did.

HHP affected the color of the fish. The tristimulus color L^* values for Alaska king salmon and Columbia River king salmon were most affected by HHP processing; as the pressure increased and as the time of pressure treatment increased (90 versus 180 s), the product became more white (as indicated by a higher L^* value; Table 2). When data for salmon were pooled according to either increasing pressure or increasing time, the trend was for the L^* value to increase (Table 3). The a^* and b^* values did not show similar trends. At the processing times and pressures that resulted in 100% mortality of the larvae (180 s at 30,000 lb/in² [207 MPa] and 90 s at 40,000 lb/in² [276 MPa]), the HHP-treated fillets or belly flaps were significantly more white than the untreated controls. The change in color was confirmed by visual observation. Storage of the control and the HHP-treated fillets in the refrigerator for 7 days did not result in any consistent changes in the L^* , a^* , or b^* values (data not shown).

In summary, nematode larvae of *Anisakis simplex* found in fish can be killed by HHP treatment. Our results for raw salmon and arrowtooth flounder agree with the results of Molina-Garcia and Sanz (4). However, the treat-

TABLE 3. Effects of pressure and processing time on L^* values for king salmon treated with high hydrostatic pressure

Treatment time (s)	Treatment pressure		Avg L^* value ^a
	lb/in ² ($\times 1,000$)	MPa	
	0	0	51.6 A
	30	207	59.7 B
	40	276	63.6 BC
	50	345	68.9 D
	60	414	66.7 CD
0			51.6 A
90			63.4 B
180			66.7 B

^a 0 = black, +100 = white. Means with different letters in the same data set (pressure or time) are significantly different by one-way analysis of variance and Tukey's HSD post hoc test ($P < 0.05$).

ments that proved to be effective in killing 100% of the worms also resulted in significant whitening of the salmon. Future studies should consider these effects for processed products destined for sashimi, sushi, or other raw-seafood markets.

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