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Research Article

Impact of Salt Concentrations and Storage Temperatures on Microbial Safety of Paddlefish Roe

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Abstract

In this study, authors have investigated the impacts of salt concentrations and storage temperatures on the microbial safety of paddlefish roe. Fresh caught fish raw samples were obtained from the Paddlefish Research Center in Miami, Oklahoma. Fish raw samples with the final salt concentrations 3, 2.5, and 2% (W/W) were prepared and inoculated with *L. monocytogenes* to achieve the final bacterial concentration of approximately 4 log CFU/g. Each inoculated sample was stored at -0.5oC, 3.3oC, and 10oC for 15 days. Each samples was analyzed for pathogen presence at 0, 5, 10, and 15 days of storage. Results indicated that salt concentrations have a significant impact on microbial growth, especially in product stored at an elevated temperature. For the samples stored at 10oC, no salt sample had the most *L. monocytogenes* growth of 4.92 log CFU/g compared to the 3% salt sample, which had a 2.52 log CFU/g increase over 15 days. The salt concentrations did not have a significant impact on the bacterial growth for the samples stored at–0.5oC. The research indicated that precise storage temperature control is the key to safety produce low salt-containing fish roe products.

Keywords: Caviar; Egg; L. monocytogenes; Salt Paddlefish

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Introduction

Caviar and Fish Roe products are aquatic animals' salt-cured and preserved eggs [1]. The word "caviar" originates from the Persian expression "Mahi Khaviari," which translates to "egg generating fish" [2]. Sturgeon and other fish roe-producing fish have been around for millions of years. The eggs of fish have been preserved and eaten in many ancient cultures [3]. The first references to the products most like Caviar today are found in the works of Homer and Aristotle. In those times, Caviar was served on platters garnished with flowers. Today, Caviar is served on lightly buttered or dry toast. For the past 200-300 years, caviar has widely been regarded as a 'luxury product' and has been coveted by elites for many years. Russian tsars implemented a caviar tax, adding to rarity of the product (Van Uhm & Siegel, 2016). Because of this, fish roe products have historically had a large black market [4].

Fish Roe and Caviar products are becoming increasingly popular in markets such as America, Russia, and Iran [1]. The market is projected to increase in the years 2020-2024 by 8%. Caviar and fish roe products are salt-cured unfertilized fish eggs. Caviar only comes from sturgeon fish; meanwhile, fish roe refers to all fish eggs. The terms are often used interchangeably by consumers. Caviar and fish roe products are ready-to-eat products that do not require further cooking but can be heat treated.

In the last decade, it was observed that there was a significant surge in the production of fish roe as the demand for the product increased steeply. In 2014, the United States exported two metric tons of fish roe prized at three hundred euros per kilogram. The U.S. export of fish roe increased from two matric tons in 2014 to almost ten metric tons in 2018 [5]. The increase in the fish roe production in the U.S. matches the worldwide trend of increased production, which observed over almost 300% increase in fish roe production between 2014 to 2018 [5].

Since 2020, COVID-19 has severely impacted food production worldwide. But, the pandemic has not slowed down the growth of the caviar market. Caviar and fish roe products are projected to have a high growth rate compared to the global GDP growth [6]. The compound annual growth rate of the caviar and fish roe markets is estimated to be between 7%-9% in the next five years, and the growth for the fiscal year 2021 was at 6.26% [6].

In the U.S., Paddlefish (*Polyodon spathula*) has been identified as one of the significant sources of fish roe. Paddlefish are found throughout the U.S., including Oklahoma. Paddlefish produce a fish roe that is sensitive to overexploitation because it is an expensive source of caviar [7]. The overexploitation of Paddlefish roe comes from overfishing. Paddlefish meat is highly regarded; additionally, the roe is held to a very high standard for a caviar product [8]. Overfishing was occurring because Caviar and Fish roe products are increasing in demand. Fish roe products are seen as luxurious and have seen a sharp increase in consumption. To curb this, other states across America have implemented measures such as bag limits, harvest caps or quotas, and restrictive licensure. To address the issue of Paddlefish overfishing in Oklahoma, the Oklahoma Department of Wildlife Conservation banned the commercial fishing of Paddlefish [7]. The Paddlefish conservation efforts in Oklahoma were further

strengthened by establishing the Paddlefish Research Center (PRC) by the Oklahoma Department of Wildlife Conservation near Miami, Oklahoma (Scarnecchia et al., 2013). The PRC engages in research related to Paddlefish and promotes conservation efforts for the fish. The center supports the anglers by cleaning and processing their Paddlefish for a donation of fish roe. The PRC further processes and salts the fish roe and sells the product worldwide.

L. monocytogenes infections can cause serious foodborne illnesses. The foodborne illness caused by *L. monocytogenes* is known as Listeriosis. The food contaminated with *L. monocytogenes* is the primary source of Listeriosis (Madjunkov et al., 2017). The mortality rate of *L. monocytogenes* infection is estimated to be around 20% to 30% [9]. This pathogenic agent is estimated to be the third-largest cause of death among infections caused by 31 known foodborne pathogens [10]. In the United States, the Centers for Disease Control and Prevention estimates that *L. monocytogenes* is responsible for approximately 260 deaths and 1500 illnesses each year [11]. Ready-to-eat food products including meat and seafood along with unpasteurized milk products are major sources of Listeria infection. Table 1 summarizes *L. monocytogenes* related foodborne illnesses and implicated foods worldwide.

Country	Survey period	Food product	*L. monocytogenes* contamination	Reference
EU	2010 to 2011	Fishery food	10.4%	EFSA (2013)
		RTE meat food	2.07%	
		Cheese	0.47%	
Ireland	2013 to 2014	Dairy products	3.2%	LEONG et al. (2014)
		Meat products	4.2%	
Sweden	2010	Fish	12%	LAMBERTZ et al. (2012)
		Meat products	1.2%	
		Cheese	0.4%	
Estonian	2008 to 2010	Raw meat and raw meat products	18.7%	KRAMARENKO et al. (2013)
		RTE meat product	2%	
		RTE milk product	0.3%	
Denmark	2014	RTE milk product	2.32%	ANONYMOUS, (2015)
		Fish and fishery products, RTE	8.45%	
		Cheese, milk and dairy products, RTE	0	
United States	2008	RTE meat products	0.52%	MAMBER, (2010)
Australia	1997 to 2003	Sliced RTE meat products	4.7%	ROSS et al., (2009)
		Pates	1.2%	
		Frankfurters	2.7%	
Chile	2008 to 2012	Sausages and cured meat	5%	SALUDES et al., (2015)
		Cheese	3%	
		Seafood	3%	

Table 1: *L. Monocytogenes* contamination by product type.

Caviar and fish roe products could be potential reservoirs of *L. monocytogenes* contamination. Table 2 shows *L. monocytogenes* contamination by fish species. Recognizing that there are *L. monocytogenes* in fish, there is also a potential for contamination in fish roe and caviar [12]. Some caviar producers utilize a heat process to pasteurize fish roe, but the processed fish roe is less attractive to the consumer and experiences a significant reduction in monetary value. Therefore, many fish roe producers rely on salt (at least 3%) and low-temperature storage to control pathogen growth (Huss et al., 2000; Farber, 1991).

Consumers are shifting toward products with less salt, and Caviar is no exception. Hypertension is one of the leading causes of death in America, and consuming less salt reduces the risk of high blood pressure [13]. But, there is a complete lack of research on the impact of varying levels of salt concentrations and storage temperatures on *L. monocytogenes* growth in Paddlefish roe. Therefore, this study measures the microbial risks associated with the low salt Paddlefish roe stored at varying temperatures.

Fish species	Country/year	Sampling place	No. of tested samples/ No. of positive samples (%)	Reference
Whiting fish (*Merlangius merlangus*)	France/n.s.*	Commercial outlets	26/0 (0)	Davies et al. 2001
European plaice (*Pleuronectes platessa*)	Great Britain/n.s.		5/0 (0)	
Atlantic salmon (*Salmo salar*)	Great Britain/n.s.		5/0 (0)	
	Denmark/1998–1999	Norway and the Faroe Islands	185/16 (8.6)	Fonnesbech Vogel et al. 2001
Salmonidae	USA/1998	Smoked fish processors	102/8 (7.8)	Norton et al. 2001
Rainbow trout (*Oncorhynchus mykiss*)	Great Britain/n.s.	Commercial outlets	20/2 (10)	Davies et al. 2001
	Greece/n.s.	Retail outlets	71/0 (0)	Papadopoulos et al. 2010
	Portugal/n.s.	Commercial outlets	10/0 (0)	Davies et al. 2001
	Finland/2000	Fish farms in lakes and sea areas	103/15 (14.6)	Miettinen and Wirtanen 2005
European pilchard (*Sardina pilchardus*)	Portugal/n.s.	Commercial outlets	10/0 (0)	Davies et al. 2001
Catfish (*Siluriformes*)	USA/n.s.	n.s.	30/0 (0)	Chen et al. 2010
Gibel carp (*Carassius gibelio*)	Greece/n.s.	Retail outlets	65/0 (0)	Papadopoulos et al. 2010
Silver Carp (*Hypophthalmichthys molitrix*)	Iran/n.s.	Warm-water fish ponds in Guilan province	42/2 (4.76)	Razavilar et al. 2012
		Fish farm, freshly caught	39/1 (2.6)	Basti et al. 2006
Sardine (*Sardina pilchardus*)	India/1997–2001	Retail outlets	15/0 (0)	Dhanashree et al. 2003
Croakers (*Sciaenidae*)			11/0 (0)	

Table 2: *L. Monocytogenes* contamination by fish species.

Materials and Methods

Bacterial cultures

For this set of experiments, four different isolates of *L. monocytogenes* were used, V7-2, 39-2, ScottA-2, and 383-2. All strains were generously given by Dr. Peter Muriana, Oklahoma State University. To improve recovery from inoculated fish roe, all *L. monocytogenes* strains were pre-screened for Rifamycin S/V (10 ug/ml) and Streptomycin sulfate (100 ug/ml). The strains were grown individually in 6 mL of tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, MD) supplemented with Rifamycin S/V (10 ug/ml) and Streptomycin sulfate (100 ug/ml). The strains were allowed to grow for 20 hours at 32°C. Following incubation, 1 mL was taken from each strain and combined in a 15ml sterile centrifuge tube to form a four-strain pathogen mixture for inoculation. The pathogen cocktail was washed three times by centrifugation at 4000Xg and suspended in phosphate-buffered saline (PBS) solution. The strain cocktail was serially diluted in PBS to achieve approximately 4 log CFU/g final concentration *L. monocytogenes* in fish roe once mixed.

Fish roe sample preparation

The fish roe samples were obtained from the Paddlefish Research Center(PRC) in Miami, Oklahoma. Briefly, fresh-caught paddlefishes were processed to harvest fish roe at PRC, Miami, Oklahoma. The harvested fish roe (unsalted) were carefully packed in sterile Whirl-PAK bags (Model no S-22730- 69 Oz.) and transported to the food microbiology laboratory in a pre-cooled ice chest. The fish roe was stored at -20°C until used in the experiments in the laboratory. Before the experiment, the fish roe samples were thawed by holding at 4±1°C overnight. The next day, the fish roe was mixed with non-iodized salt (Sigma-Aldrich, MO) to obtain a finished product with the final salt concentrations of 3, 2.5, and 2% (W/W). No salt added fish roe samples were used as controls. After curing, four samples weighing 25g each were prepared from each corresponding fish roe sample with different salt levels. Each 25 g sample was placed in sterile Whirl-PAK (model S-19792- 4 Oz.) and inoculated with 1 ml of *L. monocytogenes* to achieve the final bacterial concentration of approximately 4 log CFU/g. The samples were stored at three different temperatures -0.5°C, 3.3°C, and 10°C and samples were analyzed for pathogen presence at 0, 5, 10, and 15 days of storage. The storage temperature and salt concentrations were selected based on PRC's request. Salt intensity was measured using salt concentration and not water phase salt. Water Phase Salt is the amount of salt compared to the amount of moisture in the flesh. Moist fish products will require more salt than drier fish products and will taste saltier even with Water Phase Salt being the same. For this reason, measuring salt concentration is more

accurate for consumer preference; salt concentration provides a more precise measure of how salty a product is [14].

Microbial enumeration

At the end of each storage period, each sample was mixed with 50 ml PBS and blended at a medium speed for 1 min (Seward Stomacher®, 80 Biomaster, Worthington, U.K.). The resulting slurry samples were then serially diluted, and appropriate concentrations were plated on tryptic soy agar supplemented with Rifamycin S/V (10 ug/ml) and Streptomycin sulfate (100 ug/ml). Plates were incubated at 32°C overnight, and bacterial colonies were counted the next day.

Statistical analysis

All experiments were repeated at least three times. The data are presented as the mean and standard deviation of multiple replications. The statistical analysis was performed using repeated measures, one-way analysis of variance, and the Holm-sidak test for pairwise multiple comparisons to determine significant differences ($p < 0.05$) among treatments.

Results

The growth of *L. monocytogenes* in the paddlefish roe containing salt concentrations ranging from 0 to 3% and stored at -0.5°C, 3.3°C, and 10°C for 15 days are presented in figures 3.1, 3.2, and 3.3 respectively. It was observed that *L. monocytogenes* was not impacted by the presence of salt when stored at -0.5°C (Figure 1). The minimum growth temperature for *L. monocytogenes* is -0.1°C to -0.4°C; therefore, the storage temperature alone was sufficient to prevent the growth of the target pathogen [15].

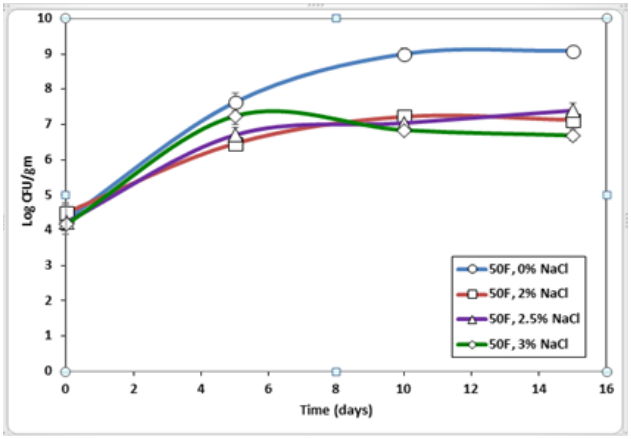

Figure 1: Survival of *Listeria monocytogenes* in Paddlefish roe with salt concentration ranging from 0 to 3% and stored at 10°C for 15 days.

When fish roe samples were stored at 3.3°C the impact of salt was a little more noticeable. For the sample with no salt, over the 15 days of storage *L. monocytogenes* number increased by 4.22 log CFU/g, while the bacterial growth was significantly less (2.2 log CFU/g) in the sample with 3% salt. All salt-containing fish roe samples were equally effective in preventing *L. monocytogenes* growth from the fish roe stored at 3.3 °C during the first 10 days of storage. But, the 3% salt-containing sample was significantly better at slowing the target pathogen growth than 2 and 2.5% salt-containing samples at the end of the storage period (Figure 2). A study by Hwang (2007) observed that the salt concentrations above 2% can effectively reduce *L. monocytogenes* growth from smoked salmon when stored at 5 °C. The direct comparison of our research findings is not possible due to the difference in the food source used in the study by [16]. [17] noticed that the salt concentrations over 2% effectively prevent *L. monocytogenes* from chum salmon roe and caviar when stored at 3°C. In our study, we have observed the growth of the pathogen during the storage temperature. The difference in storage temperature (3°C Vs 3.3°C in our experiment) could be the reason for the microbial growth. In addition, no pathogen growth in Shin and Rasco's study could be the function of selective media, modified oxford agar, to recover *L. monocytogenes*, which could /reduce the recovery of stressed microbes. [18] reported that modified oxford agar could reduce the recovery of heat-stressed *L. monocytogenes* by almost 3 log CFU/ml compared to tryptic soy agar.

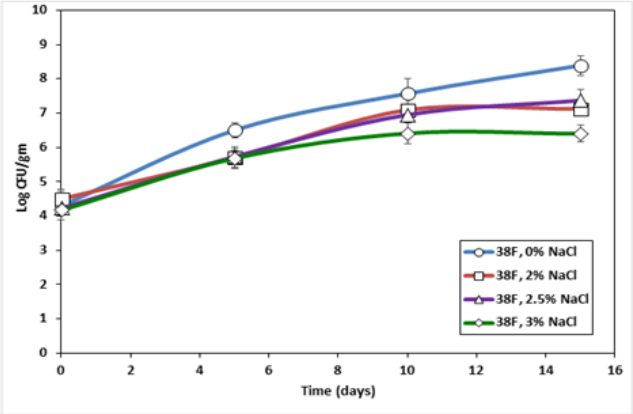

Figure 2: Survival of *Listeria monocytogenes* in Paddlefish roe with salt concentration ranging from 0 to 3% and stored at 3.3°C for 15 days.

The Figure 3 lists *L. monocytogenes* recoveries from fish roe containing varying amounts of salt and stored at 10 °C. Significant growth of pathogens was observed among all samples, but the no salt sample had the most *L. monocytogenes* growth of 4.92 log CFU/g compared to the 3% salt sample, which had a 2.52 log CFU/g increase over 15 days. Our results are in agreement with the findings reported by [17]. In their study of the impact of salt concentration on *L. monocytogenes* survival in chum salmon roe and caviar, Shin and Rasco reported a significant growth of the target pathogen in caviar with more than 4.36% salt concentration when the product was stored at 7°C.

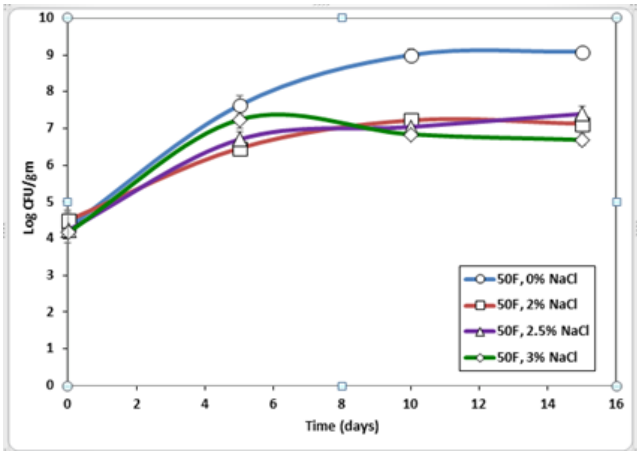

Figure 3: Survival of *Listeria monocytogenes* in Paddlefish roe with salt concentration ranging from 0 to 3% and stored at 10°C for 15 days.

Conclusion

In conclusion, it was observed that all salt concentrations used in the study were significantly more effective in controlling *L. monocytogenes* than the no-salt control samples. The study results indicate that precise low temperature control provides a more critical hurdle for preventing *L. monocytogenes* than salt alone.

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