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Tempering Large Tuna Prior to Thawing to Minimize Histamine Formation

ABSTRACT

The time and temperature controls for processing canned tuna to control histamine formation were first published in the 1998 edition of the *Fish & Fishery Products Hazards & Controls Guide* from the U.S. Food and Drug Administration (FDA). The controls have been refined since then with validation studies and FDA warning letters. To control histamine formation, the latest precooking validation study allows a 12-h thawing and butchering time limit, a critical limit of a minimum precooking temperature of 60°C at the backbone of the fish, and a 12-h critical limit from the end of precooking until the inhibitory temperatures is reached in the cold spot in the can in the retort. The largest tuna cannot be thawed within the initial 12-h limit prior to precooking. We propose a tempering phase in ambient air of –3 to –4°C to increase the enthalpy of the still frozen tuna so the thawing time in water can be shortened and the critical limits can be met. The potential for growth of bacteria that form histamine and the formation of histamine at temperatures near 0°C was evaluated based on published data. At the proposed ambient tempering temperatures of –3 to –4°C, there is minimal risk of the growth of histamine-forming bacteria and the formation of histamine.

INTRODUCTION

Canned tuna processing is a global business that is regulated from catch *(35)* to can *(69)*, and it provides a safe, sustainable high-protein food product for the world. Most tuna for canning is harvested and then frozen rapidly at sea *(10)*. The raw frozen whole fish are delivered to canning factories either directly from the harvesting vessels or indirectly via container ships or bulk carriers following transshipment *(17, 61)*. This frozen fish requires thawing before further processing; however, the larger tuna can take much longer to thaw than the smaller fish, and how much longer depends on the ambient temperature (water or air). The process of preparing whole tuna for canning has been described by Bell et al. *(7)* and DeBeer et al. *(15)*. Process flow diagrams are also available *(18, 74)*.

All seafood processed for the U.S. market must comply with the U.S. Food and Drug Administration (FDA) hazard

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analysis critical control point (HACCP) food safety system established in 1997 *(27)*. The fourth edition of the *Fish and Fishery Products Hazards and Controls Guidance* (HACCP Guidance) *(69)* was published in 2011. This document "is intended to assist processors … in the development of … HACCP plans."*(69)* Occasional updates have been issued since then, and the latest version of the fourth edition was published in April 2020 *(73)*. The FDA has issued clarifying points for critical control points (CCPs) and critical limits (CLs) by using warning letters for such details as monitoring times and temperatures or times to reach inhibitory processing temperatures *(66–68, 71, 72)*. These warning letters are sent to individual processors and are publicly available on-line. Thus, they have been used to refine the CLs for the impacted CCPs.

In the past 25 years (1994 through 2020), tuna processing from the steps of thawing through retorting or refreezing of cleaned loin meat has gone through four time and temperature adjustments, and we are proposing an additional scenario with adjusted CLs.

Scenario 1. The first edition of the HACCP Guidance in 1994 *(63)* had no restrictions for times and temperatures for tuna processing prior to the 12-D (12 decimal reduction) retort process. Thus, there were no time or temperature limits to thawing, butchering, precooking, cooling, cleaning, canning, or sealing except for an advisory time limit from the time the can is sealed until retorting *(11, 13)*.

Scenario 2. The second and third editions of the HACCP Guidance *(64, 65)* recommend a 12-h time limit for thawing, butchering, and precooker staging, with no guidance for time limits after the precooker. The purpose of this first time limit was to suppress the growth of histamine-forming bacteria and thus prevent histamine formation.

Scenario 3. The 2011 HACCP Guidance (fourth edition) and subsequent warning letters recommended 12 h from the start of thawing until inhibitory temperatures (high or low) were reached at the center of the retorted can or the cleaned and bagged loins while freezing *(66–69, 72)*. This 12-h time frame was not enough time to process the tuna from thawing through 60°C in the center of the can in the retort or 4°C in the center of the loin bag in the freezing chamber. This recommendation really applies to processing of only small fish with shorter process times. Larger fish require more time to process during the various stages of thawing, precooking, and cooling, so at times tuna with frozen backbones (or cores) were being precooked.

Scenario 4. Adams et al. *(2)* provided evidence that a 12-h thaw and butcher time limit, a precooking CCP with a final core temperature CL of 60°C, and an additional 12-h CL for the cleaning and processing time is safe *(73)*. Larger fish can be processed under this scenario; however, the 12-h time limit for thawing and butchering still is not effective for the largest fish. Thus, we are proposing a fifth scenario.

Scenario 5. This scenario adds a tempering step for the largest fish before the thawing step so these fish can be properly thawed. To increase the enthalpy in these fish, the temperature would be raised just before thawing from −20 to −3°C by putting them in a temperature-controlled room. After the core of the fish reaches −3°C but is still frozen, the fish would be moved to the water thawing area, and the thawing and butchering portion of scenario 4 would be completed.

The purpose of this article is to provide the background and develop a rationale for tempering large tuna in air to an equilibrium backbone temperature of −4 to −3°C before completely thawing them in water to the desired core temperature needed for precooking. This air tempering process may extend over more than one full day. Some factories temper frozen tuna by surrounding them with ice, but that procedure is not addressed here.

Histamine

Histamine poisoning from fish is caused by the ingestion of high levels of histamine from temperature-abused fish *(33)*. Histamine formation from bacterial action in tuna meat is a food safety concern during the handling and processing of nonfrozen raw tuna *(69)*. Hungerford *(33)* and Lehane and Olley *(48)* have provided reviews of histamine poisoning.

In 1982, the FDA established a defect action level of 20 mg% (200 ppm) for histamine in tuna as indicative of decomposition and an action level of 50 mg% (500 ppm) to signify a hazard to public health *(25)*. In 1995, the FDA lowered the defect action level to 5 mg% (50 ppm) but kept the action level the same *(26)* and included more species of fish in the compliance document.

Histamine-forming bacteria produce an enzyme, histidine decarboxylase, that converts free histidine into histamine. The conversion of histidine to histamine helps to regulate the pH of their environment *(28, 52)*. Histidine decarboxylase is the only known enzyme having a pathway to produce histamine from free histidine *(58)*. When the histamine-forming bacteria detect the presence of free histidine molecules, histidine decarboxylase is formed by the bacteria and conversion of the histidine to histamine begins. The reaction rates of different bacterial histidine decarboxylase enzymes are temperature and pH dependent *(37)*. These enzymes produce a proton transfer reaction: a histidine molecule is transferred into the bacterial cell and converted to histamine. Then the histamine molecule is passed out of the bacterial cell, and a proton is consumed to provide energy to the bacteria *(41)* during this electromotive reaction. Landete et al. *(46)* provided an explanation and schematic of the proton motive force generated by this enzymatic reaction.

Bacteria can be divided into three groups on the basis of their optimal growth temperature ranges: thermophiles, which grow best at high temperatures, mesophiles, which grow best at moderate temperatures, and psychrophiles, that grow best at cooler temperatures *(34)*. However, Ingraham

(34) found overlap in the optimal growth temperatures among these bacterial groups. Many species of bacteria found on tuna during the catching and freezing processes are associated with the fish, and many are associated with humans. Almost all humanborne pathogens are mesophilic bacteria *(50)*, but the psychrophiles are of the most concern at the proposed tuna tempering temperatures of −4 to −3°C. Histamine-forming bacteria represent <0.2% of the bacterial population on freshly caught tuna *(42, 43, 49)*. The histamine formed by these bacteria become a hazard when tuna die and are not rapidly chilled or frozen *(33)*. Taylor and Speckhard *(62)* suggested that "histamine-producing bacteria are not common components of the natural microflora of tuna" and that "the possibility remains that histamine-producing bacteria may not be part of the normal microflora of scombroid fish but may instead represent post-catching contaminants."

Histidine Decarboxylase Activity and Histamine Formation by Psychrotolerant and Mesophilic Bacteria

When Adams et al. *(2)* conducted a large-scale tuna decomposition study, they had a difficult time getting histamine formation started when they used previously frozen fish, so their experimental preparation had to be repeated with fresh (never frozen) fish *(75)*. The freezing process seems to have reduced the bacterial population significantly. Over the years, certain bacterial species have been reported to form histamine, but some were misidentified or have been replaced by newly reported bacterial species *(38)*. Some bacteria have been reported to be able to form histamine under certain conditions, but this production was not replicated in other studies under the same conditions. The key factor to consider from this variety of processes is whether temperature and the process of histamine formation have a material effect on the tempering of frozen tuna. This study was conducted to evaluate the possible risk of bacterial histamine formation at −4 to −3°C.

The many species of bacteria that can form histamine in tuna are both mesophilic and psychrotolerant, but only a small number of species are active and can produce histamine at temperatures <2°C. Examples in the literature are species of *Photobacterium* that are significant histamine formers and inhabit seawater, sea sediments, saline lake waters, and many marine organisms *(8, 45)*. Bjornsdottir-Butler et al. *(8)* tested 23 species of *Photobacterium* for histamine forming ability in LSW-70 broth cultures. Five *Photobacterium* species produced significant histamine (>200 ppm) at 20°C after 48 h of incubation: *P. angustum, P. aquimaris, P. kishitanii, P. damselae,* and *P. phosphoreum*. Bjornsdottir-Butler et al. *(9)* also tested *P. angustum, P. aquimaris, P. kishitanii*, and *P. phosphoreum* in an inoculum of 10^6 CFU/mL at 4°C. *P. kishitanii* and *P. phosphoreum* slowly formed >50 ppm of histamine in 3 days. For *P. kishitanii* inoculum at 10^2 CFU/mL, 6 to 7 days was needed to form >50 ppm of histamine.

Morganella morganii, a mesophilic bacterium, is the most troublesome bacterial species at higher temperatures because it is the most heat resistant and histaminogenic of the histamine-forming bacteria *(24)*. *Morganella psychrotolerans,* identified in 2007, is psychrotolerant *(19)* and can form histamine at temperatures <2.5°C *(21)*; however, its histamine formation time is measured in days rather than hours. The Seafood Spoilage and Safety Predictor estimates no histamine formation at 0°C for either *M. morganii* or *M. psychrotolerans (29)*.

Psychrotolerant Histamine-Forming Bacteria. *Alteromonas putrefaciens* is primarily a low-temperature spoilage organism *(56)* for which Frank et al. *(30)* reported <1 mg/100 mL histamine at 0°C after 14 days. *M. psychrotolerans* has been studied extensively by Emborg *(19)* and Emborg et al. *(21, 22)*, who reported growth at 0 to 2°C and production of histamine but no histamine formation for 10 days. Dalgaard et al. *(14)* found no histamine formation by *M. psychrotolerans* after 10 days of incubation at 2°C. *P. phosphoreum* can be isolated from fresh fish but is inactivated after being submitted to temperatures below freezing *(14, 23)*, so this species is not a factor in the tempering process for frozen tuna.

Mesophilic Bacteria. Behling and Taylor *(6)* tested *Citrobacter freundii* at 0 and −3°C in tuna infusion broth, and no histamine was formed at those temperatures. *Enterobacter aerogenes, M. morganii,* and *Proteus vulgaris* were isolated from Indian anchovy *(Stolephorus indicus)* by Rodtong et al. *(57)*. These bacterial species in homogenized fish formed 20 ppm of histamine after 15 days on ice. However, when Rodtong et al. isolated these three species and tested them individually on histamine evaluation broth, histamine was not detected at 0°C, although the cell counts were 10^8 CFU/mL. Behling and Taylor *(6)* found that *Klebsiella pneumoniae* produced histamine at 715 nmoles/mL (~143 ppm) after 158 h (6.5 days) at 0°C with a starting inoculum of 10^7 CFU/mL. Baranowski et al. *(4)* found no growth of *K. pnuemoniae* at 2°C but found very minor amounts of histamine that they suggested was formed by existing histidine decarboxylase.

Some low-temperature data come from studies of histamine formation by unknown bacteria in whole or minced fish. Frank and Yoshinaga *(31)* estimated increasing levels of histamine formation at 0°C for bacteria in skipjack *(Katsuwonus pelamis)* in days. Emborg et al. *(20)* also estimated histamine formation by *M. psychrotolerans* in sterile tuna meat and broth at 0°C in days. Yamanaka and Matsumoto *(76)* reported trace amounts of histamine from bacteria in saury pike *(Cololabis saira)* after 4 days at 0°C and 20 ppm after 7 days. At −1°C, no histamine formation was found after 6 days and only 12 ppm was found after 9 days. Kim et al. *(40)* tested for bacterial histamine formation at 0°C in whole and dressed albacore *(Thunnus alalunga)* and found no histamine formation after 18 days. Middlebrooks

et al. *(51)* tested for bacterial histamine formation at 0°C in mackerel sections *(Scomberomorus maculatus)* and found 6 ppm after 10 days. Kerr et al. *(39)* tested for bacterial histamine formation at 0°C in yellowfin tuna *(Thunnus albacares)* and found 1 ppm after 4 days and 25 ppm after 8 days. To date, these are the lowest temperatures at which histamine formation has been found, regardless of species of fish or bacteria.

The list of histamine-forming bacteria that have been reported to form histamine at ≤2°C and the relevant periods of time is given in *Table 1*. All other reported histamine-forming bacteria found need higher temperatures for histamine formation. *Table 1* includes some high histamine parts per million for various conditions. Behling and Taylor *(6)* reported ~143 ppm of histamine had formed after 6.5 days in tuna infusion broth at 0°C after starting with a *Klebsiella* inoculum of 10^7 CFU/mL, which was several orders of magnitude higher than the level expected on wild fish. Emborg and Dalgaard *(21)* started with an inoculum of *M. psychrotolerans* at 10^3 CFU/mL and obtained 100 ppm of histamine in broth after 9 days at 1.7°C.

Although raw fish rapidly chilled and held on ice or in refrigerated sea water at −1°C may allow some production of histamine at very low concentrations over long periods of time *(40)*, this short-term control of histamine formation at low temperatures is why boats using ice or refrigerated sea water have been successful. At these temperatures, decomposition by odor-causing bacteria is the primary cause for rejection of spoiled fish, not histamine concentrations. The HACCP Guidance of 2011 *(69)* recommends organoleptic evaluation of 118 fish per lot of tuna (max 25 mt) at receiving for all deliveries. This evaluation identifies decomposition in the fish from odor-causing bacteria. Every tuna packed under U.S. federal inspection must be organoleptically evaluated by trained factory workers, and federal inspectors must audit the process continuously *(59)*.

In summary, the decarboxylation of histidine to form histamine is a very slow process at ≤ 0°C. The use of ambient tempering room temperatures of −3 or −4°C (i.e., below the temperature of the phase change from ice to water; latent heat of fusion) will control the risk of histamine formation. At the end of the tempering phase, the fish core should be −3 to −4°C, and this process will decrease the ensuing thawing times in water to prepare the tuna for butchering, organoleptic inspection, and precooking.

Tuna Processing

A critical first phase in tuna processing is thawing the frozen fish because tuna usually is delivered and stored frozen. Previously frozen tuna and never frozen tuna require very different processing to control histamine formation *(69)*; therefore, here we will assume that the tuna arrives at the factory frozen or is frozen on arrival and that the factory uses a precooking CCP as described in scenario 4.

Tuna are usually stored at −18 to −20°C; thus, thawing starts with very cold fish. Extra time is required to thaw large fish because may be needed to thaw them properly depending on size, water temperature, and water circulation speed (JDB, personal communication). Results from thawing studies *(3, 36)* indicate that an increase in the water or air temperature has more impact on thawing rates than does increasing the speed of water or air circulation. If the initial temperatures of large fish were increased prior to water thawing, they could be thawed in less time. These considerations support increasing the body temperature of the large tuna through tempering before thawing.

During thawing, as the core temperature increases, the consistency of the fish flesh changes from frozen very hard to something less hard. When a fish starts with a very cold initial temperature, more time is required for thawing than when the fish starts with a higher core temperature (JDB, personal communication).

In this document we define the following terms for increasing the temperature of frozen tuna: (i) thawing happens in water or air at >4.4°C; (ii) tempering happens in air at ambient temperatures of less than −3°C.

Tempering for frozen fish that will be precooked does not involve holding at >4.4°C ambient air temperature for some amount of time before turning on the thaw water because at >4.4°C the initial 12-h CL for processing tuna starts. The process we propose does not affect that initial 12-h limit.

Tempering times for large tuna may be ≥ 48 h. The important aspect of using tempering as a CCP and CL is maintaining the air temperature of the environment throughout the tempering phase at below −3°C to control histamine formation as the increase in fish core temperature is occurring. In recent warning letters *(66, 72)*, the FDA suggested using a continuously recording thermometer for monitoring thawing temperatures (air or water) at temperatures <4.4°C (40°F) when thawing at that temperature is a CL and/or CCP.

One of our coauthors (F.N.) used Time to Temper software (Global Cold Chain Alliance, Arlington, VA) to estimate tempering time for fish of different sizes. The parameters used were an initial temperature of −18°C, an end point of −3°C, and a moving air temperature of −3°C. The estimated number of tempering hours by fish size are listed in *Table 2*. Each factory will have to verify tempering times and temperatures with their own equipment and operating conditions.

When the tuna precooking target CL of 60°C at the core is not met and the batch must be recooked, the entire initial time of the precooking cycle must be added to the 12-h thaw and butcher time limit. If the 12-h CL is exceeded, then necessary corrective actions are required *(70)*.

In practice, U.S. tuna boats were tempering the fish in the 1950s, 1960s, and 1970s before unloading directly to the canneries. At that time, the fish were packed very tightly in the wells, and the fish had to be tempered so they could be

TABLE 1. Studies on low-temperature histamine-forming bacteria (HFB)

Bacteria	Fish	Temp (°C)	Item tested	Medium	As reported	ppm	Period	Comments	Reference(s)
Alteromonas putrefaciens	Mahi-mahi (*Coryphaena hippurus*)	0	Isolates	Trypticase soy broth	<1 mg/100 mL	<10	14 days	Table IV, p. 337	*30*
Citrobacter freundii	Tuna broth	0, –3	Isolates, 10^7 cells/mL	TFIB	0 nmoles/ mL	0	158 h, 6.5 days		*6*
Enterobacter aerogenes, Morganella morganii, Proteus vulgaris	Anchovy (*Stolephorus indicus*)	0	Isolates	Histamine evaluation broth	0 mg/100 mL	0	18 h		57
		Ice	Homogenized fish	Homogenized fish	1.9 mg/100 mL	19	15 days		
Klebsiella pneumoniae UH-2	Skipjack (*Katsuwonus pelamis*)	2	Isolates	TSB	0.14 µmoles/ mL	28	6 days	No growth at 2°C	*4*
K. pneumoniae T2	Tuna broth	0	Isolates, 10^7 CFU/mL	TFIB	715 nmoles/ mL	143	158 h, 6.5 days	p. 1313	6
		–3	Isolates, 10^7 CFU/mL	TFIB	0 nmoles/ mL	0	158 h, 6.5 days		
Morganella psychrotolerans	FSSP model	2	Model		0 ppm	0	10 days		*14*
	Canned tuna, tuna broth, garfish (*Belone belone*)	1.7	Isolates, 10^3 CFU/mL	Broth	100 ppm	100	222 h, 9 days		*21*
Photobacterium leiognathi	Mackerel (*Scomber japonicus*)	Ice	Isolates	Moller's basal medium			16 days	Decreased on ice	*53*
P. phosphoreum	Mackerel	Ice	Histidine decarboxylase	SWYP broth	3.9 mg/100 g	39	24 days	Seemed to be the result of a single analysis	*54*
	Mackerel (*S. japonicus*)	Ice	Isolates	Muscle meat	13 µg/ gm	13	16 days		*53*
		Ice	Isolates	Moller's basal medium	146 mg/100 mL/	1,460	16 days	Table 7	
P. phosphoreum or N-group bacteria	Pacific saury (*Cololabis saira*)	2.5	Isolates	Mackerel infusion broth	144 mg%	1,440	12 days	Maximum on Pacific saury	*32, 55*
HFB	Albacore (*Thunnus alalunga*)	Ice	Whole fish		0 ppm	0	18 days	Whole and dresed fish	*40*
HFB (many)	Mackerel (*Scomberomorus maculatus*)	0	Fish sections		0.6 mg%	6	10 days		*51*
HFB	Yellowfin tuna (*Thunnus albacares*)	0	Minced flesh		1 mg/kg	1	4 days	Table 3	*39*
		0	Minced flesh		25 mg/ kg	25	8 days	Table 3	

continued on next page.

TABLE 1. Studies on low-temperature histamine-forming bacteria (HFB) (cont.)

Bacteria	Fish	Temp (°C)	Item tested	Medium	As reported	ppm	Period	Comments	Reference(s)
HFB	Saury pike, (*C. saira*)	0	Saury pike muscle		1.65 mg/100 mL	16.5	6 days	In Japanese	*76*
HFB	Saury pike, (*C. saira*)	-1	Saury pike muscle		1.22 mg/100 mL	12.2	9 days		
HFB	Skipjack (*K. pelamis*)	20						<0.1% HFB	*43*
HFB	Tonggol tuna (*Thunnus tonggol*)	20						<0.13% HFB	*42*
HFB	Sardines (*Sardina pilchardus*)	35					24 h	~1% HFB	*1*
HFB	Scombroid fish	37						<0.1% HFB	*49*

TABLE 2. Estimated tempering time with a fan by tuna species and size

Tuna species	Wt (kg)	Length (cm)	Thickness (cm)	Temp (°C)		Time (h)
				Starting	Ambient	
Albacore	10	77	17.9	–18	–3	16.7
Albacore	12	83	17.5	–18	–3	16.3
Albacore	17	93	18.6	–18	–3	17.8
Albacore	19	97	20.7	–18	–3	20.6
Albacore	20	100	21.4	–18	–3	21.6
Albacore	26	109	22.7	–18	–3	23.5
Yellowfin	26	120	26.2	–18	–3	29.3
Yellowfin	55	140	32.6	–18	–3	40.3
Yellowfin	64	146	34.8	–18	–3	44.5

loose enough (not frozen together) to unload, as described by DeBeer et al. *(17)* and Lassen and Rawlings *(47)*. The target temperature of the larger yellowfin tuna for unloading was –5 to –2°C (22 to 28°F).

The tuna processing guidelines or regulations in some countries specify a maximum allowable backbone (core) temperature at the start of precooking *(60)*. Both the Thailand's Dept of Fisheries (DOF) and the Canadian Food Inspection Agency (CFIA) stipulate that the core temperature of tuna must be ≤5°C at the start of precooking *(60)*.

Enthalpy and thawing tuna

Enthalpy (measured in kilojoules) is the amount of heat content used or released to change the temperature of a mass from one temperature to another at a constant pressure and volume *(12)*. In this case, heat is required to change the mass phase from frozen to thawed. Enthalpy is measured from one temperature to another and will be calculated for the temperature range of –20 to 4°C needed for thawing tuna. *Table 3* shows the amount of enthalpy required for this temperature and phase change. For ease of calculation, the fish is considered thawed at 0°C. During actual tuna thawing, the phase change occurs over a wide temperature range *(Fig. 1)*, but the enthalpy calculations are essentially the same. Depending on actual temperatures at the start and end of thawing, >80% of the energy needed to thaw the fish and increase the temperature by 24°C (from –20 to 4°C) is used for the latent heat of fusion during the phase change over the temperature range.

TABLE 3. Enthalpy to thaw tuna from –20 to 4°C

Temp (°C)	Delta (°C)	Specific heat (kJ/kg/°C)	Enthalpy (kJ/kg)	% change in enthalpy	Reference
0 to 4	4	3.3075	13.23	5	*15*, Appendix A
0	0	234.9	234.9	83	*15*, Appendix D
–20 to 0	20	1.7165	34.33	12	*15*, Appendix A
Total	24		282.46	100	

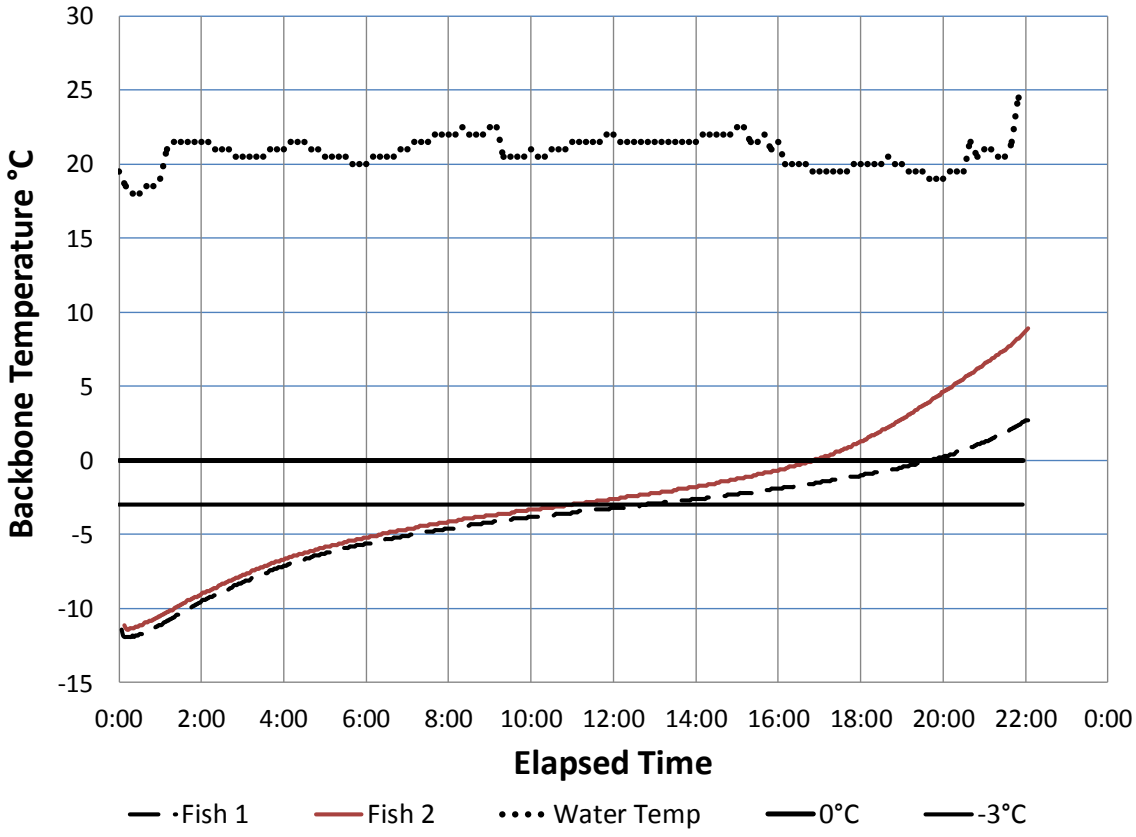

FIGURE 1. A thawing curve for 30- to 32-kg albacore.

The second law of thermodynamics states that heat transfers from hot to cold *(44)*, but there are time restrictions for this heat transfer from the ambient environment into a frozen fish and thus raising the temperature, especially at the core. These time restrictions are a function of the thermal conductivity, the temperature difference, the medium (air or water) giving up the heat energy to the colder item, and the heat transfer coefficient *(16)*. All of these factors affect the tempering phase time depending on the air speed and temperature difference.

A two-stage tempering and thawing system is suggested to reduce the total thawing time of large tuna in the critical temperature zone of >4.4°C. The first stage involves use of ambient air temperatures below –3°C, and the second stage involves use of air or water with temperatures >4.4°C.

The backbone or core thawing profile for tuna from −20 to −1°C is generally a smooth curve with a long horizontal lag at about −5 to −2°C where the phase change occurs *(Fig. 1)*. Every curve of a core temperature of a tuna being thawed will have a similar shape, but the slopes and inflection points will differ. The rate of temperature increase is faster from −20 to −5°C but then slows down or pauses as the phase change occurs. *Figure 1* also shows that thawing a 30- to 32-kg albacore may take ≥20 h depending on the initial temperature and the temperature of the thaw water.

Baranowski et al. *(5)* found that the populations of histamine-forming bacteria and their ability to form histamine are reduced when the tuna is frozen for some time. Vogl et al. *(75)* had difficulty starting a histamine decomposition experiment with frozen fish and had to use fresh, never frozen fish. The processing limits and CLs in the 2011 HACCP Guidance *(69)* are different for fresh fish and previously frozen fish because of the impact of freezing on the tuna and bacteria.

Tempering and maintaining frozen tuna at or below −3°C will improve control of the thawing process and of histamine formation. The reported temperatures required for histamine formation are too high for histamine to be formed during tempering of tuna. Holding the fish at or below −3°C can also serve as a short-term buffering time for scheduling the fish into the process.

Suggestions for tempering are as follows.

(i) Because tempering requires extra work, use this technique for fish that cannot be thawed in 12 h.

(ii) Use a tempering room at a constant temperature of −3 to −4°C, with good air flow *(Table 3)*. For better air contact with the fish, wire cage totes can be used (see cover photo).

(iii) Limit the tempering time to 72 h, which should be enough time for the largest fish to warm from −20 to −3°C and allows the operations team to start the fish on a Friday and temper it over a weekend with time to spare. When this operating limit is exceeded, the odor of every fish on the butcher line should be assessed.

(iv) Always use a temperature measuring system that keeps a continuous record of the ambient temperature per the warning letters from the FDA *(66, 72)*.

When the tempering room is attached to the cold storage area, it should be insulated. The ambient temperature must be continuously maintained below −3°C and continuously recorded. The time for determining the CL for thawing will not start until the tuna leaves both the tempering room and the cold storage and insulated transportation trucks. The time for determining the CL for thawing will not start until the fish are moved into a space with ambient temperatures >4°C.

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REFERENCES

1. Ababouch, L., M. E. Afilal, S. Rhafiri, and F. F. Busta. 1991. Identification of histamine-producing bacteria isolated from sardine *(Sardina pilchardus)* stored in ice and at ambient temperature (25°C). *Food Microbiol.* 8:127–136. https://doi.org/10.1016/0740-0020(91)90005-M.
2. Adams, F., F. Nolte, J. Colton, J. DeBeer, and L. Weddig. 2018. Precooking as a control for histamine formation during the processing of tuna: an industrial process validation. *J. Food Prot.* 81:444-455. https://doi.org/10.4315/0362-028X.JFP-17-276.
3. Bailey, C., S. J. James, A. G. Kitchell, and W. R. Hudson. 1974. Air-, water-, and vacuum-thawing of frozen pork legs. *J. Sci. Food Agric.* 25:81–97. https://doi.org/10.1002/jsfa.2740250110.
4. Baranowski, J. D., P. A. Brust, and H. A. Frank. 1985. Growth of *Klebsiella pneumoniae* UH-2 and properties of its histidine decarboxylase system in resting cells. *J. Food Biochem.* 9:349–360. https://doi.org/10.1111/j.1745-4514.1985.tb00357.x.
5. Baranowski, J. D., H. A. Frank, P. A. Brust, M. Chongsiriwatana, and R. J. Premaratne. 1990. Decomposition and histamine content in mahi-mahi *(Coryphaena hippurus)*. *J. Food. Prot.* 53:217–222. https://doi.org/10.4315/0362-028X-53.3.217.
6. Behling, A. R., and S. L. Taylor. 1982. Bacterial histamine production as a function of temperature and time of incubation. *J. Food Sci.* 47:1311–1314. https://doi.org/10.1111/j.1365-2621.1982.tb07675.x.
7. Bell, J. W., B. E. Farkas, S. A. Hale, and T. C. Lanier. 2001. Effect of thermal treatment on moisture transport during steam cooking of skipjack tuna *(Katsuwonus pelamis)*. *J. Food Sci.* 66:1–7. https://doi.org/10.1111/j.1365-2621.2001.tb11337.
8. Bjornsdottir-Butler, K., A. Abraham, A. Harper, P. V. Dunlap, and R. A. Benner, Jr. 2018. Biogenic amine production by and phylogenetic analysis of 23 *Photobacterium* species. *J. Food Prot.* 81:1264–1274. https://doi.org/10.4315/0362-028X.JFP-18-022.
9. Bjornsdottir-Butler, K., K. L. Baltzer, J. Nash, and R. A. Benner, Jr. 2018. Growth and histamine production of *Photobacterium* species at refrigeration temperatures. Presented at the IAFP 2018 Annual Meeting. International Association for Food Protection, Des Moines, IA.
10. Burns, F. D. 1985. Tuna handling and refrigeration on purse seiners. NOAA technical memorandum, National Marine Fisheries Service. Available at: ftp://ftp.library.noaa.gov/noaa_documents.lib/NMFS/SWFSC/TM_NMFS_SWR/NOAA-TM-NMFS-SWR-011.pdf. Accessed 2 December 2019.
11. California Department of Public Health. 1984. Cannery inspection regulations. California Code of Regulations, title 17, §12979. Available at: https://www.cdph.ca.gov/Programs/CEH/DFDCS/CDPH%20Document%20Library/FDB/FoodSafetyProgram/Cannery/CACanneryRegulations.pdf. Accessed 27 January 2020.
12. Charm, S. E. 1963. The fundamentals of food engineering. AVI Publishing, Westport, CT.
13. Cole, W. R. 2020. In-plant sampling plans to address potential incipient or thermophilic spoilage. Technical bulletin. TechniCAL, Metairie, LA.
14. Dalgaard, P., J. Emborg, A. Kjølby, N. D. Sørensen, and N. Z. Ballin. 2008. Histamine and biogenic amines—formation and importance in seafood, p. 292–324. *In* T. Børresen (ed.), Improving seafood products for the consumer. Woodhead Publishing, Cambridge, England. https://doi.org/10.1533/9781845694586.3.292.
15. DeBeer, J., F. Nolte, and C. W. Lord. 2015. Precooking tuna: a study of the factors impacting the time required for precooking. *Food Prot. Trends* 35:448–460.
16. DeBeer, J., F. Nolte, C. W. Lord, and J. Colley. 2019. Precooking tuna: a heat of summation analysis of different heating profiles. *Food Prot. Trends* 39:127–136.

17. DeBeer, J., F. Nolte, C. W. Lord, and J. Colley. 2019. Salt penetration in whole raw tuna frozen onboard vessel by brine immersion: an industrial study. *Mar. Fish. Rev.* 81:40–52. https://doi.org/10.7755/MFR.81.1.2.

18. DeBeer, J., F. Nolte, C. W. Lord, J. Colton, and J. Colley. 2017. Sampling plans to determine the minimum core temperature reached during the precooking of tuna. *Food Prot. Trends* 37:269–288.

19. Emborg, J. 2007. *Morganella psychrotolerans*—identification, histamine formation and importance for histamine fish poisoning. Ph.D. thesis. Danish Institute for Fisheries Research, Charlottenlund. Available at: https://orbit.dtu.dk/files/4687217/afhandling%20til%20orbit.pdf. Accessed 4 November 2019.

20. Emborg, J., and P. Dalgaard. 2008. Modelling the effect of temperature, carbon dioxide, water activity and pH on growth and histamine formation by *Morganella psychrotolerans*. *Int. J. Food Microbiol.* 128:226–233. https://doi.org/10.1016/j.ijfoodmicro.2008.08.016.

21. Emborg, J., and P. Dalgaard. 2008. Growth, inactivation and histamine formation of *Morganella psychrotolerans* and *Morganella morganii*—development and evaluation of predictive models. *Int. J. Food Microbiol.* 128:234–243. https://doi.org/10.1016/j.ijfoodmicro.2008.08.015.

22. Emborg, J., P. Dalgaard, and P. Ahrens. 2006. *Morganella psychrotolerans* sp. nov., a histamine-producing bacterium isolated from various seafoods. *Int. J. Syst. Evol. Microbiol.* 56:2473–2479. https://doi.org/10.1099/ijs.0.64357-0.

23. Emborg, J., B. G. Laursen, T. Rathjen, and P. Dalgaard. 2002. Microbial spoilage and formation of biogenic amines in fresh and thawed modified atmosphere–packed salmon *(Salmo salar)* at 2°C. *J. Appl. Microbiol.* 92:790–799. https://doi.org/10.1046/j.1365-2672.2002.01588.x.

24. Enache, E., A. Kataoka, D. G. Black, M. Hayman, L. Weddig, and K. Bjornsdottir-Butler. 2013. Heat resistance of histamine producing bacteria in irradiated tuna loins. *J. Food Prot.* 76:1608–1614. https://doi.org/10.4315/0362-028X.JFP-12-467.

25. Federal Register. 1982. Defect action levels for histamine in tuna. *Fed. Regist.* 470:40487–40488.

26. Federal Register. 1995. Decomposition and histamine—raw, frozen tuna and mahi-mahi; canned tuna; and related species; revised compliance policy guide. *Fed. Regist.* 60(49):39754–39756.

27. Federal Register. 1995. Rules and regulations. Procedures for the safe and sanitary processing and importing of fish and fishery products. *Fed. Regist.* 60(242):65096–65202. http://www.gpo.gov/fdsys/pkg/FR-1995-12-18/pdf/95-30332.pdf. Accessed 25 May 2018.

28. Ferrario, C., F. Borgo, B. De Las Rivas, R. Muñoz, G. Ricci, and M. G. Fortina. 2014. Sequencing, characterization, and gene expression analysis of the histidine decarboxylase gene cluster of *Morganella morganii*. *Curr. Microbiol.* 68:404–411. https://doi.org/10.1007/s00284-013-0490-7.

29. Food Spoilage and Safety Predictor. 2014. FSSP for Windows, v. 4.0. Available at: http://fssp.food.dtu.dk/. Accessed 7 December 2019.

30. Frank, H. A., J. D. Baranowski, M. Chongsiriwatana, P. A. Brust, and R. J. Premaratne. 1985. Identification and decarboxylase activities of bacteria isolated from decomposed mahi mahi (*Coryphaena hippurus)* after incubation at 0 and 32°C. *Int. J. Food Microbiol.* 2:331–340. https://doi.org/10.1016/0168-1605(85)90023-6.

31. Frank, H. A., and D. H. Yoshinaga. 1987. Table for estimating histamine formation in skipjack tuna, *Katsuwonus pelamis*, at low nonfreezing temperatures. *Mar. Fish. Rev.* 49:67–70.

32. Fujii, T., A. Hiraishi, T. Kobayashi, R. Yoguchi, and M. Okuzumi. 1997. Identification of the psychrophilic histamine-producing marine bacteria previously referred to as the N-group bacteria. *Fish. Sci.* 63:807–810. https://doi.org/10.2331/fishsci.63.807.

33. Hungerford, J. M. 2010. Scombroid poisoning: a review. *Toxicon* 56:231–243. https://doi.org/10.1016/j.toxicon.2010.02.006.

34. Ingraham, J. L. 1958. Growth of psychrophilic bacteria. *J. Bacteriol.* 76:75–80. https://doi.org/10.1128/JB.76.1.75-80.1958.

35. International Seafood Sustainability Foundation. 2018. Management (RFMOS): an overview. Available at: https://iss-foundation.org/knowledge-tools/databases/rfmo-management-database/. Accessed 25 May 2018.

36. James, S. J., and P. G. Creed. 1980. Predicting thawing time of frozen beef fore and hindquarters. *Int. J. Refrig.* 3:237–240. https://doi.org/10.1016/0140-7007(80)90054-7.

37. Kanki, M., T. Yoda, T. Tsukamoto, and E. Baba. 2007. Histidine decarboxylases and their role in accumulation of histamine in tuna and dried saury. *Appl. Environ. Microbiol.* 73:1467–1473. https://doi.org/10.1128/AEM.01907-06.

38. Kanki, M., T. Yoda, T. Tsukamoto, and T. Shibata. 2002. *Klebsiella pneumoniae* produces no histamine: *Raoultella planticola* and *Raoultella ornithinolytica* strains are histamine producers. *Appl. Environ. Microbiol.* 68:3462–3466. https://doi.org/10.1128/AEM.68.7.3462-3466.2002.

39. Kerr, M., P. Lawicki, S. Aguirre, and C. Rayner. 2002. Effect of storage conditions on histamine formation in fresh and canned tuna. Department of Human Services, State Chemistry Laboratory, Werribee, Victoria, Australia.

40. Kim, S. H., H. An, and R. J. Price. 1999. Histamine formation and bacterial spoilage of albacore harvested off the U.S. Northwest coast. *J. Food Sci.* 64:340–343. https://doi.org/10.1111/j.1365-2621.1999.tb15896.x.

41. Konings, W. N., J. S. Lolkema, and B. Poolman. 1995. The generation of metabolic energy by solute transport. *Arch. Microbiol.* 164:235–242. https://doi.org/10.1007/BF02529957.

42. Koohdar, V. A., V. Razavilar, K. Abolhassan, and S. Alireza. 2012. Histamine-producing bacteria isolated from frozen longtail tuna *(Thunnus tonggol)*. *Afr. J. Microbiol. Res.* 6:751–756. https://doi.org/10.5897/AJMR11.882.

43. Koohdar, V. A., V. Razavilar, A. A. Motalebi, F. Mosakhani, and T. Valinassab. 2011. Isolation and identification of histamine-forming bacteria in frozen skipjack tuna *(Katsuwonus pelamis)*. *Iran J. Fish. Sci.* 10:678–688.

44. Kreith, F., and M. S. Bohn. 2001. Principles of heat transfer, 6th ed. Brooks/Cole, Pacific Grove, CA.

45. Labella, A. M., D. R. Arahal, D. Castro, M. L. Lemos, and J. J. Borrego. 2017. Revisiting the genus *Photobacterium:* taxonomy, ecology and pathogenesis. *Int. Microbiol.* 20:1–10. https://doi.org/10.2436/20.1501.01.280.

46. Landete, J. M., B. De Las Rivas, A. Marcobal, and R. Muñoz. 2008. Updated molecular knowledge about histamine biosynthesis by bacteria. *Crit. Rev. Food Sci. Nutr.* 48:697–714. https://doi.org/10.1080/10408390701639041.

47. Lassen, S., and J. Rawlings. 1959. A manual for refrigeration practice for tuna clippers. California Fish Canners Association, Terminal Island, CA. Available from John DeBeers (jdebeer2005@gmail.com).

48. Lehane, L., and J. Olley. 2000. Histamine fish poisoning revisited. *Int. J. Food Microbiol.* 58:1–37. https://doi.org/10.1016/S0168-1605(00)00296-8.

49. López-Sabater, E. I., J. Rodríguez-Jerez, M. Hernández-Herrero, and M. T. Mora-Ventura. 1996. Incidence of histamine-forming bacteria and histamine content in scombroid fish species from retail markets in the Barcelona area. *Int. J. Food Microbiol.* 28:411–418. https://doi.org/10.1016/0168-1605(94)00007-7.

50. Mackowiak, P. A. 1981. Direct effects of hyperthermia on pathogenic microorganisms: teleologic implications with regard to fever. *Rev. Infect. Dis.* 3:508–520. https://doi.org/10.1093/clinids/3.3.508.

51. Middlebrooks, B. L., P. M. Toom, W. L. Douglas, R. E. Harrison, and S. McDowell. 1988. Effects of storage time and temperature on the microflora and amine-development in Spanish mackerel *(Scomberomorus maculatus)*. *J. Food Sci.* 53:1024–1029. https://doi.org/10.1111/j.1365-2621.1988.tb13522.x.

52. Molenaar, D., J. S. Bosscher, B. ten Brink, A. J. Driessen, and W. N. Konings. 1993. Generation of a proton motive force by histidine decarboxylation and electrogenic histidine/histamine antiport in *Lactobacillus buchneri*. *J. Bacteriol.* 175:2864–2870. https://doi.org/10.1128/jb.175.10.2864-2870.1993.

53. Morii, H., D. C. Cann, and L. Y. Taylor. 1988. Histamine formation by luminous bacteria in mackerel stored at low temperatures. *Nippon Suisan Gakkai Shi* 54:299–305. https://doi.org/10.2331/suisan.54.299.

54. Morii, H., and K. Kasama. 2004. Activity of two histidine decarboxylases from *Photobacterium phosphoreum* at different temperatures, pHs, and NaCl concentrations. *J. Food Prot.* 67:1736–1742. https://doi.org/10.4315/0362-028X-67.8.1736.

55. Okuzumi, M., S. Okuda, and M. Awano. 1982. Occurrence of psychrophilic and halophilic histamine-forming bacteria (N-group bacteria) on/in red meat fish. *Bull. Jap. Soc. Sci. Fish.* 48:799–804. https://doi.org/10.2331/suisan.48.799.

56. Parker, L. L., and R. E. Levin. 1983. Relative incidence of *Alteromonas putrefaciens* and *Pseudomonas putrefaciens* in ground beef. *Appl. Environ. Microbiol.* 45:796–799. https://doi.org/10.1128/AEM.45.3.796-799.1983.

57. Rodtong, S., S. Nawong, and J. Yongsawatdigul. 2005. Histamine accumulation and histamine-forming bacteria in Indian anchovy *(Stolephorus indicus). Food Microbiol.* 22:475–482. https://doi.org/10.1016/j.fm.2004.08.009.

58. Shahid, M., T. Tripathi, F. Sobia, S. Moin, M. Siddiqui, and R. A. Khan. 2009. Histamine, histamine receptors, and their role in immunomodulation: an updated systematic review. *Open Immunol. J.* 2:9–41. https://doi.org/10.2174/1874226200902010009.

59. Shenks, L. 2020. Personal communication. [lenanah1@aol.com].

60. Suwanrangsi, S., S. Keerathiviriyaporn, K. Sophonphong, K., S. Brillantes and L. Limpus. 1995. Canned tuna quality management manual. ASEAN, Canada fisheries post-harvest technology project, phase II. Available from: http://aquaticcommons.org/26792/1/9810076088.pdf. Accessed Dec 4, 2020.

61. Sylvester, J. 2018. Shared excerpts from "The first forty years." Marine Chartering Co., San Francisco, CA. [john@chartering.com].

62. Taylor, S. L., and M. W. Speckhard. 1983. Isolation of histamine-producing bacteria from frozen tuna. *Mar. Fish. Rev.* 45:35–39.

63. U.S. Food and Drug Administration. 1994. Fish & fishery products hazards & controls guide, 1st ed. Available at: https://catalog.hathitrust.org/Record/002810535r. Accessed 16 April 2020.

64. U.S. Food and Drug Administration. 1998. Fish & fisheries products hazards & controls guide, 2nd ed. Available at: https://catalog.hathitrust.org/ Record/009176166. Accessed 16 April 2020.

65. U.S. Food and Drug Administration. 2001. Fish & fisheries products hazards & controls guidance, 3rd ed. Available at: https://eos.ucs.uri.edu/seagrant_Linked_Documents/flsgp/flsgph01002.pdf. Accessed 16 April 2020.

66. U.S. Food and Drug Administration. 2008. Warning letter to Hai Soon Leong Sdn Bhd. Available at: https://www.fdalabelcompliance.com/letters/ucm161312. Accessed 23 October 2019.

67. U.S. Food and Drug Administration. 2008. Warning letter to Pacific Fishing Company. Available at: https://wayback.archive-it.org/7993/20170112194257/http://www.fda.gov/ICECI/EnforcementActions/WarningLetters/2010/ucm262239.htm. Accessed 4 November 2019.

68. U.S. Food and Drug Administration. 2010. Warning letter to Songkla Canning. Available at: https://www.fdalabelcompliance.com/letters/ucm214853. Accessed 23 October 2019.

69. U.S. Food and Drug Administration. 2011. Fish and fishery products hazards and controls guidance, 4th ed. Available at: http://www.fda.gov/downloads/food/guidancecomplianceregulatoryinformation/guidancedocuments/seafood/ucm251970.pdf. Accessed 24 December 2016.

70. U.S. Food and Drug Administration. 2014. Warning letter to Southeast Asian Packaging and Canning Limited. Available at: https://www.fda.gov/inspections-compliance-enforcement-and-criminal-investigations/warning-letters/southeast-asian-packaging-and-canning-limited-423596-05152014. Accessed 8 January 2020.

71. U.S. Food and Drug Administration. 2015. Warning letter to Procesamiento Especializado De Alimentos. Available at: https://www.fda.gov/inspections-compliance-enforcement-and-criminal-investigations/warning-letters/procesamiento-especializado-de-alimentos-sapi-de-cv-08122015. Accessed 6 January 2020.

72. U.S. Food and Drug Administration. 2016. Warning letter to Jin Tzer Marine Products. Available at: https://www.fda.gov/inspections-compliance-enforcement-and-criminal-investigations/warning-letters/jin-tzer-marine-products-co-ltd-500281-07282016. Accessed 23 October 2019.

73. U.S. Food and Drug Administration. 2020. Fish and fishery products hazards and controls guidance, 4th ed., 2020 version. Available at: https://www.fda.gov/media/80637/download. Accessed 16 April 2020.

74. Visvanathan, C., S. Duangpaseuth, Q. Das, N. Chotchamlong, J. Ariunbaatar, A. Khunchornyakong, V. Prashanthini, and W. Jutidamrongphan. 2007. Seafood processing. Industrial waste abatement and management. Available at: https://www.scribd.com/document/95857041/Industrial-Waste-Abatement-Seafood. Accessed 4 November 2019.

75. Vogl, F., R. Salazar, F. Nolte, G. Kontoh, and G. Ybanez. 2012. Validation for precooking as a control for potential histamine production in tuna loins for subsequent canning. Presented at TAFT 2012. Proceedings of the 4th Trans-Atlantic Fisheries Technology Conference, Clearwater Beach, FL, 30 October to 2 November 2012.

76. Yamanaka, H., and M. Matsumoto. 1989. Simultaneous determination of polyamines in red meat fishes by high performance liquid chromatography and evaluation of freshness. *Food Hyg. Safe. Sci. (Shokuhin Eiseigaku Zasshi)* 30:396–400. https://doi.org/10.3358/shokueishi.30.396. (In Japanese.)