

Time-Temperature Indicators

For some seafood products, particularly raw fish, refrigeration is the only barrier to growth and toxin production by *Clostridium botulinum*. Unless the seafood is in packaging that's permeable to oxygen, FDA recommends processors place a time-temperature indicator or "TTI" on the package to show consumers that proper temperature controls were in place from the time the product was packaged to the time the consumer or end user opens the package.

This video discusses how you can use TTIs with raw seafood products, focusing on TTIs that are considered to be full history indicators. Full history TTIs respond to time-temperature exposures continuously across a broad range of temperatures. FDA doesn't recommend reliance on TTIs that are considered partial history indicators because they only respond when a specified temperature is reached or exceeded. Partial history indicators don't account for all the time the product spends outside the target storage temperature and are of limited value for controlling *Clostridium botulinum*.

Let's first go over why we need to control the *Clostridium botulinum* hazard in seafood products.

Clostridium botulinum or *C. bot.* is a naturally occurring spore-forming bacterium that grows best in the absence of oxygen. There are various types found in most soils and in fresh or salt water and their sediments. It's also commonly found in fish, especially in the gut and on the gills.

C. bot. forms botulinum toxin which is the most powerful natural toxin known to man and causes botulism. The toxin inhibits muscle contraction; and if left untreated, may cause respiratory paralysis and even death. Botulism occurs when the food containing the toxin is ingested.

Initial symptoms may include fatigue, blurred vision, and difficulty swallowing. While the incidence of botulism is low, the disease is severe with 5 to 10 percent mortality rates in cases that go untreated.

In its vegetative state *C. bot.* is killed easily by heat, but its spores are heat-resistant and can withstand long periods of exposure to high temperatures. *Clostridium botulinum* produces a highly toxic neurotoxin. The FDA Fish & Fishery Products Hazards & Controls Guidance categorizes strains of *Clostridium botulinum* into two different groups.

The first group is proteolytic and includes strains of type A and proteolytic types B and F. These produce spores that are extremely heat resistant but they only grow at temperatures above 50 degrees Fahrenheit. Proteolytic means they degrade proteins when they grow, spoiling the product and making it look and smell bad. This group is most commonly associated with botulism outbreaks in canned foods due to improper preservation in both commercial and home-made foods.

The other group is non-proteolytic and is comprised of strains like type E and non-proteolytic types B and F. This group produces spores that are less heat resistant but they can grow at temperatures as low as 38 degrees F. Growth below 38 degrees hasn't been documented. The non-proteolytic forms don't degrade proteins so a product may look and smell normal even if it's toxic. The non-proteolytic types are associated with smoked, salted, unviscerated, and dried fish.

The bottom line is - refrigeration works well to control strains like type A, but not without additional precautions for strains like type E. Because temperature strongly affects the growth and toxin production in both of these groups, two primary strategies based upon temperature can be used to

control its formation. These involve either freezing the product or maintaining product temperatures below 38 degrees F. We'll discuss control of the hazard later but first let's turn our attention to how the hazard is created during packaging.

It's important to remember that *C. bot.* grows and produces toxin best when there is little to no oxygen present, so if products are packaged so that oxygen surrounds or permeates the food, you won't have to control for it. Oxygen permeable packaging allows spoilage organisms to grow and spoil the food before toxin can be formed. Packaging materials with oxygen transmission rates of 10,000 cubic centimeters per square meter per day or more at 24 degrees Celsius or 75 degrees Fahrenheit are considered oxygen permeable. These transmission rates allow enough oxygen to reach the product so aerobic spoilage bacteria will spoil it before botulinum toxin forms. As long as oxygen-permeable packaging material is used and there isn't some other condition of the food limiting oxygen penetration throughout the food, botulinum toxin isn't considered a likely hazard. However, if a refrigerated product is packaged in a way that reduces the amount of oxygen in contact with it, then growth of aerobic spoilage bacteria will be slowed and botulinum toxin may form before the product spoils. The product may not show any outward signs of spoilage but could contain deadly toxin. Let's take a look at some packaging methods which may limit the amount of oxygen in contact with the food.

Packaging that limits or excludes oxygen transmission is called Reduced Oxygen Packaging, or ROP. Examples include vacuum-packaging; packaging in hermetically sealed containers such as double-seamed cans, glass jars with sealed lids, and heat sealed plastic containers, modified or controlled atmosphere packaging, where the normal concentration of oxygen has been changed and is artificially low; packing in oil, or packing in deep containers where air may be prevented from reaching the product at the center.

Reduced oxygen packaging is becoming increasingly popular, because by limiting aerobic spoilage bacteria, it extends the shelf life of the food and allows processors to offer fresh seafood products that stay fresh longer with minimal processing. Of the packaging strategies listed, vacuum-packaging is one of the most popular methods used. Vacuum packaging offers the advantage of longer shelf life by not only preventing growth of spoilage bacteria but also by limiting oxygen-dependent chemical processes that spoil, age, or discolor the food. Unfortunately, raw, ROP, refrigerated products may allow growth of *C. bot.* If the product is exposed to moderate temperature abuse, it may look very appealing and fresh, but can potentially contain deadly toxin.

We mentioned earlier that *C. bot.* growth and toxin production are strongly affected by temperature. So, processors can choose between two strategies when it comes to controlling the hazard with temperature. These are either freeze the product or maintain product temperature below 38 degrees. Understand that in both cases, temperature control serves as a sole barrier to growth and toxin production by *Clostridium botulinum*.

Let's take a look at freezing first. If you intend to market a frozen ROP fishery product, it's recommended that you freeze the product immediately after processing and store the product frozen at the processing facility and during distribution. Also, label the product so that it clearly states that it needs to be held frozen and thawed under refrigeration immediately before use. As long as the product remains frozen, the hazard is controlled.

If refrigeration temperatures are used to control the hazard then FDA recommends the product be stored and shipped below 38 degrees Fahrenheit. Remember that *C. bot.* growth hasn't been

documented below 38 degrees. If the storage and distribution temperatures for ROP products is below 38 degrees, then we recommend placing a TTI on the package to alert the consumer when the product has been exposed to an unsafe combination of time and temperature.

A suitable TTI should indicate whether proper temperature control was maintained from the time the product is packaged to the time the consumer or end user opens the package. If refrigeration is the only defense against the growth of non-proteolytic strains of *C. bot.*, then a TTI is essential, because studies have shown that refrigerators in consumers' homes and in retail establishments are rarely at or below 38 degrees Fahrenheit. Then the TTI will let consumers know when the product has been exposed to enough time above 38 degrees to challenge its safety.

Examples of fishery products that should include a TTI are raw, vacuum-packaged fish fillets, loins or steaks, and unpasteurized, cooked fishery products, such as vacuum-packaged lobster meat.

Scientific studies show that *C. bot.* produces toxin very slowly at lower temperatures and more quickly at higher temperatures. This graph illustrates the relationship. The horizontal axis is temperature and increases to the right. The vertical axis is the time it takes for toxin to appear in days, increasing toward the top. The line on the graph is called the Skinner-Larkin curve and represents the minimum time for toxin to be formed at any given temperature. You can see the boundary line is not straight. Toxin is formed very quickly at higher temperatures but more slowly at lower temperatures. With only a small increase of temperature above 38 degrees, *C. bot.* can quickly turn an otherwise safe product into one which can make a consumer ill.

For example, the graph shows that at 38 degrees Fahrenheit, it takes over 20 days for toxin to appear, but at 50 degrees it takes only 2 ½ days. Even worse, temperature abuse near 95 degrees can result in the appearance of toxin in less than 2 hours. Remember, toxin may be present in the product without any signs of spoilage, including in appearance, smell or taste.

While they don't prevent toxin production, properly designed TTIs respond like the Skinner-Larkin curve and can provide a warning if a product has been exposed to temperature abuse or conditions which could result in toxin formation. And, consumers can check them to determine if the safety of the product may have been compromised.

There are many types of TTIs on the market that function in a variety of ways, but all show some type of visual change with temperature and time exposure. They are often manufactured as a kind of tag that can be affixed to the product. Color change is the most commonly used indication of temperature exposure, but other methods may work as new devices become available. It's important that the visual change be irreversible. Once exposed to dangerous cumulative time and temperature conditions, a properly designed TTI will always indicate that history, even if the product is later cooled to the correct temperature.

Like the rate of toxin production, the rate at which TTIs change is dependent upon temperature. If your food package is exposed to time and temperature that exceeds the TTI's design parameters, then the TTI will indicate that it's reached its end point. Also, the TTI should clearly instruct the consumer how to read the TTI and know it's reached its end point.

Now, let's review how to ensure the TTI functions correctly with refrigerated ROP seafood products.

Different TTIs have different factors that influence their function. For example, some are activated by the processor before use, while others are shipped from the manufacturer pre-activated or ready to run. Some have strict shipping and storage requirements, such as requiring refrigeration, while others may be stored at room temperature before being placed on the product.

Just as you identify controls for hazards in seafood products, you should identify whether TTIs require critical control points and appropriate critical limits to ensure that they function properly and are reliable. Processors should incorporate any necessary critical control points and critical limits into their existing HACCP plan and use appropriate monitoring, corrective actions, record keeping and verification procedures to ensure the TTIs operate correctly.

For example, if a TTI manufacturer recommends maintaining specific temperatures during shipping, then processors should list TTI receiving as a critical control point in their HACCP plan and designate appropriate monitoring procedures to ensure transit temperatures don't exceed the temperature critical limit. The processor can request that the TTI manufacturer ship every lot with a temperature data-logger and complete shipping records that can be kept on file.

Similarly, if they have strict requirements for proper storage, processors should include TTI storage as a critical control point in the HACCP plan, with storage temperature as a critical limit, and monitoring procedures to safeguard against temperature abuse. Processors should also determine if there's an expiration date and have proper procedures in place for enforcing it.

Whether protecting the TTIs during shipping and storage or protecting against other hazards that may cause them to fail, proper monitoring, record keeping, corrective action steps, and verification steps are essential to ensure that they will operate correctly. Not all TTIs have these or similar limitations. In those cases, processors may determine that critical control points aren't required and that no further action is necessary to ensure accurate performance.

In all cases, processors should make sure they operate correctly by using a challenge test to check the function of each lot upon receipt. A challenge test involves activating a small number of TTIs, exposing them to room temperature, and comparing their responses against those predicted by the manufacturer. Processors may also perform a challenge test at other temperatures besides room temperature.

In addition, processors should determine if their TTIs have restrictions or limitations that would make them unsuitable in their processing environment. For example, some won't function correctly if they are exposed to excessive heat, dry ice, chemicals, or light. Processors should be aware of any such limitations and safeguard against accidental exposure to any agent or condition that may impair proper function.

The best time to affix a TTI to the product is immediately after creating the reduced oxygen conditions that allow growth and toxin production to occur. For example, when vacuum-packaging fish fillets the best time to apply the TTI would be immediately after sealing the package. Make sure each one is properly activated and securely attached to the package. Upon application, processors should comply with all product storage recommendations and abide by all precautions prescribed by the manufacturer to ensure the TTIs perform as designed.

If activation is required, processors should institute steps to ensure proper activation and include these in their HACCP plan. And, as with shipping and storage, if specific activation procedures are required, we recommend processors establish monitoring, record keeping, corrective actions and verification procedures to ensure the consumer or end user gets a fully functional TTI. Processor records, which may include shipping records, storage records, and records of expiration dates, should show that these conditions have been met.

It may be difficult to determine if the right TTIs have been selected for the products. If processors have questions about whether it's the right one for their products, they should contact the manufacturer. Work closely with the manufacturer to ensure their TTIs adequately predict toxin formation and that they're using the devices correctly. Processors should obtain data from the manufacturer documenting that the devices provide adequate warning of toxin production. This data should compare the TTI's performance to the Skinner-Larkin curve we saw earlier, which predicts toxin production under various time and temperature exposures.

In all cases the TTIs selected should provide a margin of safety for the consumer. That is, they should reach their end point *before* toxin production is possible at proper storage and shipping temperatures up to and including moderate abuse temperatures.

In addition, if there's a limited shelf life for the TTI, then its shelf-life also serves as the safety shelf life of the product. In other words, the overall product safety shelf-life is dictated by the TTI shelf life even when the quality shelf-life of the product may be many more days or weeks.

Use of a TTI on consumer packages is *not* a substitute for ensuring the product is transported, shipped, or stored at the appropriate temperature or for the monitoring required under the Seafood HACCP Regulation. Processors should view *temperature* as the control, *not* the TTI. Ensuring that the recommended temperature control of below 38 degrees Fahrenheit is met also requires a continuous temperature record or icing the product. So, even with a TTI on the product, processors still need continuous temperature monitoring in the form of a digital time-temperature data logger or recording thermometer, or visual observations of the adequacy of ice or other cooling media during transport and storage.

Finally, TTIs aren't necessary for every type of ROP seafood product.

Remember, *C. bot.* does not grow in freezing temperatures. So, one alternative is to freeze the product and label it clearly to be kept frozen and thawed under refrigeration immediately before use.

There are several other strategies to control the non-proteolytic, less heat resistant forms of *C. bot.*, although they don't result in a "fresh" product. Thermal processing is used as a barrier to toxin production along with refrigeration in many ROP seafood products. Here we're talking about thermal processes other than canning, like pasteurizing in the final hermetically-sealed container. Other controls include acidification below pH 5, drying to a water activity level below 0.97, or water phase salt of greater than 5 percent, or some combination of these and other barriers, like smoke.

Remember, these processes and chemical inhibitors target the non-proteolytic, less heat-resistant forms of *C. bot.* that can grow at refrigeration temperatures like type E. When these levels are achieved, a TTI is not needed. These products should still be refrigerated at 40 degrees Fahrenheit or below to control other pathogens and the proteolytic types of *C. bot.* like type A.

Comparable controls for the more resistant proteolytic types of *C. bot.* require a pH below 4.6, water activity below 0.93 or more than 10 percent water phase salt. These products should be refrigerated at or below 40 degrees Fahrenheit in order to control other pathogens.

TTIs allow the consumer or other end users to determine whether or not these refrigerated products are safe. They provide the only indication that toxin may be present. They should be adequately designed and used correctly if they're to alert the consumer against botulism. Like any other tool used to maintain food safety, the function and limitations of TTIs should be clearly defined and monitored in the processor's HACCP program to ensure that they're performing as intended.