Chapter 8: Vacuum and Modified Atmosphere Packaged Fish and Fishery Products

Updated:

Potential Food Safety Hazard

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Clostridium botulinum toxin formation can result in consumer illness and death. This chapter covers the potential for *C. botulinum* growth and toxin formation as a result of time/temperature abuse during processing, storage and distribution.

When *C. botulinum* grows it can produce a potent toxin, which can cause death by preventing breathing. It is one of the most poisonous naturally occurring substances known. The toxin can be destroyed by heat (e.g. boiling for 10 minutes), but processors cannot rely on this as a means of control.

There are two major groups of *C. botulinum*, the proteolytic group (i.e. those that break down proteins) and the nonproteolytic group (i.e. those that do not break down proteins). The proteolytic group includes *C. botulinum* type A and some of types B and F. The nonproteolytic group includes *C. botulinum* type E and some of types B and F.

The vegetative cells of all types are easily killed by heat. *C. botulinum* is able to produce spores. In this state the pathogen is very resistant to heat. The spores of the proteolytic group are much more resistant to heat than are those of the nonproteolytic group. Table A-4 provides guidance about the conditions under which the spores of the most heat resistant form of nonproteolytic *C. botulinum*, type B, are killed. However, there are some indications that substances that may be naturally present in some products, such as lysozyme, may enable nonproteolytic *C. botulinum* to more easily recover after heat damage, resulting in the need for a considerably more aggressive process to ensure destruction.

Temperature abuse occurs when product is exposed to temperatures favorable for *C. botulinum* growth for sufficient time to result in toxin formation. Table A-1 provides guidance about the conditions under which *C. botulinum* and other pathogens are able to grow.

Packaging conditions that reduce the amount of oxygen present in the package (e.g. vacuum packaging) extend the shelf life of product by inhibiting the growth of aerobic spoilage bacteria. The safety concern with these products is the increased potential for the formation of *C. botulinum* toxin before spoilage makes the product unacceptable to consumers.

There are a number of conditions that can result in the creation of a reduced oxygen packaging environment. They include:

- Vacuum packaging or modified or controlled atmosphere packaging. These packaging methods directly reduce the amount of oxygen in the package;
- Packaging in hermetically sealed containers (e.g. double seamed cans, glass jars with sealed lids, heat sealed plastic containers), or packing in deep containers frosm which the air is expressed (e.g. caviar in large containers), or packing in oil. These and similar processing/packaging techniques prevent the entry of oxygen into the container. Any oxygen present at the time of packaging may be rapidly depleted by the activity of spoilage bacteria, resulting in the formation of a reduced oxygen environment.

Packaging that provides an oxygen transmission rate of 10,000 $cc/m^2/24hrs$ (e.g. 1.5 mil polyethylene) can be regarded as an oxygen-permeable packaging material for fishery products. This can be compared to an oxygen-impermeable package which might have an oxygen transmission rate as low as or lower than 100 $cc/m^2/24hr$ (e.g. 2 mil polyester). An oxygen permeable package should provide sufficient exchange of oxygen to allow aerobic spoilage organisms to grow and spoil the product before toxin is produced under moderate abuse temperatures. However, use of an oxygen permeable package will not compensate for the restriction to oxygen exchange created by practices such as packing in oil or in deep containers from which the air is expressed.

C. botulinum forms toxin more rapidly at higher temperatures than at lower temperatures. The minimum temperature for growth and toxin formation by *C. botulinum* type E and nonproteolytic types B and F is 38°F (3.3°C). For type A and proteolytic types B and F, the minimum temperature for growth is 50°F (10°C). As the shelf life of refrigerated foods is increased, more time is available for *C. botulinum* growth and toxin formation. As storage temperatures increase, the time required for toxin formation is significantly shortened. Processors should expect that at some point during storage, distribution, display or consumer handling of refrigerated foods, proper refrigeration temperatures will not be maintained (especially for the nonproteolytic group). Surveys of retail display cases indicate that temperatures of 45-50°F (7-10°C) are not uncommon. Surveys of home refrigerators indicate that temperatures can exceed 50°F (10°C) (FDA, 2001a).

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In reduced oxygen packaged products in which the spores of nonproteolytic *C. botulinum* are inhibited or destroyed (e.g., smoked fish, pasteurized crabmeat, pasteurized surimi), normal refrigeration temperatures of 40°F (4.4°C) are appropriate because they will limit the growth of proteolytic *C. botulinum* and other pathogens that may be present. Even in products where nonproteolytic *C. botulinum* is the target organism for the pasteurization process and vegetative pathogens, such as *Listeria monocytogenes,* are not likely to be present (e.g. pasteurized crabmeat, pasteurized surimi), a storage temperature of 40°F (4.4°C) is still appropriate because of the potential survival through the pasteurization process and recovery of spores of nonproteolytic *C. botulinum* aided by naturally occurring substances, such as lysozyme. In this case refrigeration serves as a prudent second barrier.

In reduced oxygen packaged products in which refrigeration is the sole barrier to outgrowth of nonproteolytic *C. botulinum* and the spores have not been destroyed (e.g. vacuum packaged raw fish, unpasteurized crayfish meat), the temperature must be maintained at 38°F (3.3°C) or below from packing to consumption. Ordinarily processors can ensure that temperatures are maintained at or below 38°F (3.3°C) while the product is in their control. However, current distribution channels do not ensure the maintenance of these temperatures after the product leaves their control. The use of time temperature integrators on each consumer package may be an appropriate means of enabling temperature control throughout distribution. Alternatively, products of this type may be safely marketed frozen, with appropriate labeling. For some products, control of *C. botulinum* can be achieved by breaking the vacuum seal before the product leaves the processor's control (FDA, 2001a).

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Refrigerated, reduced oxygen packaged smoked and smoke-flavored fish:

- For cold-smoked fish, the smoker temperature must not exceed 90°F (32.2°C).
- For hot-smoked fish, the internal temperature of the fish must be maintained at or above 145°F (62.8°C) throughout the fish for at least 30 minutes.
- Smoked fish must have not less than 3.5 percent water phase salt, or, where permitted, the combination of 3.0 percent water phase salt and not less than 100 ppm nitrite.
- Strict refrigeration control (i.e., at or below 40°F [4.4°C]) during storage and distribution must be maintained to prevent growth and toxin formation by *C. botulinum* type A and proteolytic types B and F and other pathogens that may be present (FDA, 2001a).

Refrigerated, reduced oxygen packaged, pasteurized fishery products, which are pasteurized in the final container:

- Strict refrigeration control (i.e., at or below 40°F [4.4°C]) must be maintained during storage and distribution to prevent growth and toxin formation by *C. botulinum* type A and proteolytic types B and F, and because of the potential survival through the pasteurization process and recovery of spores of nonproteolytic *C. botulinum* aided by naturally occurring substances, such as lysozyme (FDA, 2001a).

Refrigerated, reduced oxygen packaged, pasteurized fishery products, which are hot filled into the final container:

- Product temperature must be 185°F (85°C) or higher as the product enters the final container (FDA, 2001b).
- Containers must be sealed according to the container or sealing machine manufacturer's seal guidelines (FDA, 2001b).
- There must be a measurable residual of chlorine, or other approved water treatment chemical, at the discharge point of the container cooling tank; or the processor must follow the equipment manufacturer's UV light intensity and flow rate guidelines (FDA, 2001b).
- Strict refrigeration control (i.e., at or below 40°F [4.4°C]) must be maintained during storage and distribution to prevent growth and toxin formation by *C. botulinum* type A and proteolytic types B and F, and because of the potential survival through the pasteurization process and recovery of spores of nonproteolytic *C. botulinum* aided by naturally occurring substances, such as lysozyme (FDA, 2001a).

Refrigerated, reduced oxygen packaged "pickled" fish, caviar, and similar products:

- Growth and toxin formation by *C. botulinum* type E and nonproteolytic types B and F must be controlled by either:
    - Adding sufficient salt to produce a water phase salt level of at least 5 percent;
    - Adding sufficient acid to reduce the acidity (pH) to 5.0 or below;
    - Reducing the amount of moisture that is available for growth (water activity) to below 0.97; or
    - Making a combination of salt, pH, and/or water activity adjustments that, when combined, prevent the growth of *C. botulinum* type E and nonproteolytic types B and F.
- Processors should ordinarily restrict brining and pickling loads to single species and to fish portions of approximately uniform size. This minimizes the complexity of controlling the operation.
- Strict refrigeration control (i.e., at or below 40°F [4.4°C]) must be maintained during storage and distribution to prevent growth and toxin formation by *C. botulinum* type A and proteolytic types B and F (FDA, 2001a).

Refrigerated, reduced oxygen packaged raw, unpreserved fish and unpasteurized, cooked fishery products:

- For refrigerated, reduced oxygen packaged raw, unpreserved fish (e.g. vacuum packaged fresh fish fillets) and unpasteurized, cooked fishery products (e.g. vacuum packaged, unpasteurized crabmeat, lobster meat, or crayfish meat), the sole barrier to toxin formation by *C. botulinum* type E and nonproteolytic types B and F during finished product storage and distribution is refrigeration. These types of *C. botulinum* will grow at temperatures as low as 38°F (3.3C). As was previously stated, maintenance of temperatures at or below 38°F (3.3°C) after the product leaves the processor's control

cannot normally be ensured. Time temperature integrators on each consumer package may be an appropriate means of providing such control (FDA, 2001a).

Frozen, reduced oxygen packaged fishery products:

- If your product is immediately frozen after processing, maintained frozen throughout distribution, and labeled to be held frozen and to be thawed under refrigeration immediately before use (e.g. "Important, keep frozen until used, thaw under refrigeration immediately before use"), then formation of *C. botulinum* toxin may not be a significant hazard (FDA, 2001a).

Unrefrigerated (shelf-stable), reduced oxygen packaged fishery products:

- Spores of *Clostridium botulinum* types A, B, E and F must be destroyed after the product is placed in the finished product container or a barrier, or combination of barriers, must be in place that will prevent growth and toxin formation by *Clostridium botulinum* types A, B, E and F, and other pathogens that may be present in the product. Suitable barriers include:
    - Sufficient salt is added to produce a water phase salt level (the concentration of salt in the water-portion of the fish flesh) of at least 20 percent (Note: this value is based on the maximum salt level for growth of *S. aureus*.)
    - Sufficient salt is added to reduce the water activity to 0.85 or below;
    - Sufficient acid is added to reduce the pH to 4.6 or below;
    - The product is dried sufficiently to reduce the water activity to 0.85 or below (Note: this value is based on the minimum water activity for growth and toxin formation of *S. aureus*) (FDA, 2001a).

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Reduced Oxygen Packaging (ROP)

(A) Introduction

ROP which provides an environment that contains little or no oxygen, offers unique advantages and opportunities for the food industry but also raises many microbiological concerns. Products packaged using ROP may be produced safely if proper controls are in effect. Producing and distributing these products with a HACCP approach offer an effective, rational, and systematic method for the assurance of food safety. The purpose of this Annex is to provide guidelines for effective food safety controls for retail food establishments covering the receipt, processing, packaging, holding, displaying, and labeling of food in reduced oxygen packages.

(B) Definitions

The term ROP is defined as any packaging procedure that results in a reduced oxygen level in a sealed package. The term is often used because it is an inclusive term and can include other packaging options such as:

(1) *Cook-chill* is a process that uses a plastic bag filled with hot cooked food from which air has been expelled and which is closed with a plastic or metal crimp.

(2) *Controlled Atmosphere Packaging (CAP)* is an active system which continuously maintains the desired atmosphere within a package throughout the shelf-life of a product by the use of agents to bind or scavenge oxygen or a sachet containing compounds to emit a gas. Controlled Atmosphere Packaging (CAP) is defined as packaging of a product in a modified atmosphere followed by maintaining subsequent control of that atmosphere.

(3) *Modified Atmosphere Packaging (MAP)* is a process that employs a gas flushing and sealing process or reduction of oxygen through respiration of vegetables or microbial action. Modified Atmosphere Packaging (MAP) is defined as packaging of a product in an atmosphere which has had a one-time modification of gaseous composition so that it is different from that of air, which normally contains 78.08% nitrogen, 20.96% oxygen, 0.03% carbon dioxide.

(4) *Sous Vide* is a specialized process of ROP for partially cooked ingredients alone or combined with raw foods that require refrigeration or frozen storage until the package is thoroughly heated immediately before service. The sous vide process is a pasteurization step that reduces bacterial load but is not sufficient to make the food shelf-stable. The process involves the following steps:

(a) Preparation of the raw materials (this step may include partial cooking of some or all ingredients);

(b) Packaging of the product, application of vacuum, and sealing of the package;

(c) Pasteurization of the product for a specified and monitored time/temperature;

(d) Rapid and monitored cooling of the product at or below 3°C(38°F) or frozen; and

(e) Reheating of the packages to a specified temperature before opening and service.

(5) *Vacuum Packaging* reduces the amount of air from a package and hermetically seals the package so that a near-perfect vacuum remains inside. A common variation of the process is Vacuum Skin Packaging (VSP). A highly flexible plastic barrier is used by this technology that allows the package to mold itself to the contours of the food being packaged.

(C) Benefits of ROP

ROP can create a significantly anaerobic environment that prevents the growth of aerobic spoilage organisms, which generally are Gram negative bacteria such as Pseudomonads or

aerobic yeast and molds. These organisms are responsible for off-odors, slime, and texture changes, which are signs of spoilage.

ROP can be used to prevent degradation or oxidative processes in food products. Reducing the oxygen in and around a food retards the amount of oxidative rancidity in fats and oils. ROP also prevents color deterioration in raw meats caused by oxygen. An additional effect of sealing food in ROP is the reduction of product shrinkage by preventing water loss.

These benefits of ROP allow an extended shelf-life for foods in the distribution chain, providing additional time to reach new geographic markets or longer display at retail. Providing an extended shelf-life for ready-to-eat convenience foods and advertising foods as "Fresh-Never Frozen" are examples of economic and quality advantages.

(D) Safety Concerns

Use of ROP with some foods can markedly increase safety concerns. Unless potentially hazardous foods are protected inherently, simply placing them in ROP without regard to microbial growth will increase the risk of foodborne illnesses. ROP processors and regulators must assume that during distribution of foods or while they are held by retailers or consumers, refrigerated temperatures may not be consistently maintained. In fact, a serious concern is that the increased use of vacuum packaging at retail supermarket deli-type operations may be followed by temperature abuse in the establishment or by the consumer. Consequently, at least one barrier or multiple hurdles resulting in a barrier need to be incorporated into the production process for products packaged using ROP. The incorporation of several sub-inhibitory barriers, none of which could individually inhibit microbial growth but which in combination provide a full barrier to growth, is necessary to ensure food safety.

Some products in ROP contain no preservatives and frequently do not possess any intrinsic inhibitory barriers (such as, pH, a_w, or salt concentrations) that either alone or in combination will inhibit microbial growth. Thus, product safety is not provided by natural or formulated characteristics.

An anaerobic environment, usually created by ROP, provides the potential for growth of several important pathogens. Some of these are psychrotrophic and grow slowly at temperatures near the freezing point of foods. Additionally, the inhibition of the spoilage bacteria is significant because without these competing organisms, tell-tale signs signaling that the product is no longer fit for consumption will not occur.

The use of one form of ROP, vacuum packaging, is not new. Many food products have a long and safe history of being vacuum packaged in ROP. However, the early use of vacuum packaging for smoked fish had disastrous results, causing a long-standing moratorium on certain uses of this technology.

(1) *Refrigerated Holding Requirements for Foods in ROP*

Safe use of ROP technology demands that adequate refrigeration be maintained during the entire shelf-life of potentially hazardous foods to ensure product safety.

Bacteria, with the exception of those that can form spores, are eliminated by pasteurization. However, pathogens may survive in the final product if pasteurization is inadequate, poor quality raw materials or poor handling practices are used, or post-processing contamination occurs. Even if foods that are in ROP receive adequate thermal processing, a particular concern is present at retail when employees open manufactured products and repackage them. This operation presents the potential for post-processing contamination by pathogens.

If products in ROP are subjected to mild temperature abuse, i.e., 5°-12°C (41°-53°F), at any stage during storage or distribution, foodborne pathogens, including ***Bacillus cereus***, ***Salmonella*** spp., ***Staphylococcus aureus***, and ***Vibrio parahaemolyticus*** can grow slowly. Marginal refrigeration that does not facilitate growth may still allow ***Salmonella*** spp., ***Campylobacter*** spp., and ***Brucella*** spp. to survive for long periods of time.

Recent published surveys indicate that refrigeration practices at retail need improvement. Some refrigerated products offered in convenience stores were found at or above 7.2°C (45°F) 50% of the time; in several cases temperatures as high as 10°C (50°F) were observed. Delicatessen display cases have been shown to demonstrate poor temperature control. Foods have been observed above 10°C (50°F) and above 12.8°C (55°F) in several instances. Supermarket fresh meat cases appear to have a relatively good record of temperature control. However, even these foods can occasionally be found above 10°C (50°F).

Temperature abuse is common throughout distribution and retail markets. Strict adherence to temperature control and shelf-life must be observed and documented by the establishment using ROP. Information on temperature control should also be provided to the consumer. Currently these controls are not extensively used. Additionally, some commercial equipment is incapable of maintaining foods below 7.2°C (45°F) because of refrigeration capacity, insufficient refrigerating medium, or poor maintenance.

Most warehouses and transport vehicles in U.S. distribution chains maintain temperatures in the 0°-3.3°C (32°-38°F) range. It must be assumed, however, for purposes of assessing risk, that occasionally temperatures of 10°C (50°F) or higher may occur for extended periods. At retail, further temperature abuse must also be assumed. For instance, retail display cases can be as high as 13.3°C (56°F) for short periods and some refrigerated foods are provided no refrigeration for short periods of time. These realities point to the need for establishments to implement controls, such as buyer specifications, over refrigerated distribution systems so that better temperature control can be ensured.

(2) *Control of **Clostridium botulinum** and **Listeria monocytogenes** in Reduced Oxygen Packaged Foods*

Recently, there has been an increased interest in vacuum packaging or MAP at retail using conventional refrigeration for holding. Refrigerated foods packaged at retail may be chilled either after they are physically prepared and repackaged, or packaged after a cooking step. In

either case but primarily the latter, germination of ***Clostridium botulinum*** spores must be inhibited because spores are not destroyed by a heating step. Sanitary safeguards must be employed to prevent reintroduction of pathogens. Chief among these is ***Listeria monocytogenes***.

Clostridum botulinum is the causative agent of botulism, a severe food poisoning characterized by double vision, paralysis, and occasionally death. The organism is an anaerobic spore-forming bacteria that produces a potent neurotoxin. The spores are ubiquitous in nature, relatively heat-resistant, and can survive most minimal heat treatments that destroy vegetative cells. Certain strains of ***C. botulinum*** (type E and non-proteolytic types B and F), which have been primarily associated with fish, are psychrotrophic and can grow and produce toxin at temperatures as low as 3.3°C (38°F). Other strains of ***C. botulinum*** (type A and proteolytic types B and F) can grow and produce toxin at temperatures slightly above 10°C (50°F). If present, ***C. botulinum*** could potentially grow and render toxigenic a food packaged and held in ROP because most other competing organisms are inhibited by ROP. Therefore, the food could be toxic yet appear organoleptically acceptable. This is particularly true of psychrotrophic strains of ***C. botulinum*** that do not produce tell-tale proteolytic enzymes. Because botulism is potentially deadly, foods held in anaerobic conditions merit regulatory concern and vigilance.

The potential for botulism toxin to develop also exists when ROP is used after heat treatments such as pasteurization, or sous vide, processing of foods which will not destroy the spores of ***C. botulinum***. Mild heat treatments in combination with ROP may actually select for ***C. botulinum*** by killing off its competitors. If the applied heat treatment does not produce commercial sterility, the food requires refrigeration to prevent spoilage and ensure product safety. For this reason, sous vide products are frequently flash frozen in liquid nitrogen and held in frozen storage until use.

There is a further microbial concern with ROP at retail. Processed products such as meats and cheeses which have undergone an adequate cooking step to kill ***L. monocytogenes*** can be contaminated when opened, sliced, and repackaged at retail. Thus, a simple packaging or repackaging operation can present an opportunity for recontamination with pathogens if strict sanitary safeguards are not in place.

Processors of products using ROP should be cautious if they plan to rely on refrigeration as the sole barrier that ensures product safety. This approach requires very rigorous temperature controls and monitored refrigeration equipment. If extended shelf-life is sought, a temperature of 3.3°C (38°F) or lower must be maintained at all times to prevent outgrowth of ***C. botulinum*** and the subsequent production of toxin. ***Listeria monocytogenes*** can grow at even lower temperatures; consequently, appropriate use-by dates must be established and readily apparent to the consumer. Since refrigeration alone does not guarantee safety from pathogenic microorganisms, additional growth barriers must be provided. Growth barriers are provided by hurdles such as low pH, a_w, or short shelf life, and constant monitoring of the temperature. Any one hurdle, or a combination of several, may be used with refrigeration to control pathogenic outgrowth.

(3) *Design of Heat Processes for Foods in Reduced Oxygen Packages*

Heat processes for sous vide or cook-chill operations should be designed so that, at a minimum, all vegetative pathogens are destroyed by a pasteurization process. Special labeling of these products is necessary to ensure adequate warning to consumers that these foods must be refrigerated at 5°C (41°F) and consumed by the date required by the Code for that particular product.

The National Advisory Committee on Microbiological Criteria for Foods (NACMCF) chartered by the U.S. Department of Agriculture (USDA) and the Department of Health and Human Services (HHS) recently commented on the microbial safety of refrigerated foods containing cooked, uncured meat or poultry products that are packaged for extended refrigerated shelf-life and are ready-to-eat or prepared with little or no additional heat treatment. The Committee recommended guidelines for evaluating the ability of thermal processes to inactivate ***L. monocytogenes*** in extended shelf-life refrigerated foods. Specifically, it recommended a proposed requirement for demonstrating that an ROP process provides a heat treatment sufficient to achieve a 4 decimal log reduction (4D) of ***L. monocytogenes***.

Other scientific reports recommend more extensive thermal processing. Thermal processes for sous vide practiced in Europe are designed to achieve a 12-13 log reduction (12-13D) of the target organism ***Streptococcus faecalis***. It is reasoned that thermal inactivation of this organism would ensure destruction of all other vegetative pathogens.

Food manufacturers with adequate in-house research and development programs may have the ability to design their own thermal processes. However, small retailers and supermarkets may not be able to perform the microbiological challenge studies necessary to provide the same level of food safety. If a retail establishment wishes to use an ROP process, microbiological studies should be performed by, or in conjunction with, an appropriate process authority or person knowledgeable in food microbiology who is acceptable to the regulatory authority.

Finally, if foods are held long enough, even under proper refrigeration, extended shelf-life may be a problem. A recent study on fresh vegetables inoculated with ***L. monocytogenes*** was conducted to determine the effect of CAP on shelf life. The study found that CAP lengthened the time that all vegetables were considered acceptable, but that populations of ***L. monocytogenes*** increased during that extended storage.

(4) *Consumer Handling Practices and In-Home Refrigeration Temperatures*

Extended shelf-life provided by ROP is cause for concern because of the potential for abuse by the consumer. Consumers often can not, or do not, maintain adequate refrigeration of potentially hazardous foods at home. Foods in ROP that are taken home may not be eaten until enough time/temperature abuse has occurred to allow any pathogens present to increase to levels which can increase the chance of illness. Under the best of circumstances home refrigerators can be expected to range between 5° and 10°C (41°-50°F). One study reported that home refrigerator temperatures in 21% of the households surveyed were 10°C (50°F). Another study reported more than 1 of 4 home refrigerators are above 7.2°C (45°F) and almost 1 of 10 are above 10°C (50°F). Thus, refrigeration alone cannot be relied on for ensuring microbiological safety after foods in ROP leave the establishment.

Consumers have come to expect that certain packages of foods would be safe without refrigeration. Low-acid canned foods have been thermally processed, which renders the food shelf-stable. Retort heating ensures the destruction of ***C. botulinum*** spores as well as all other foodborne pathogens. Yet consumers may not understand that most products that are packaged in ROP are not commercially sterile or shelf-stable and must be refrigerated. A clear label statement to keep the product refrigerated must be provided to consumers.

The use of ROP has been extensively studied by regulators and the food industry over the past several years. Recommendations have been adapted from the Association of Food and Drug Officials "Retail Guidelines - Refrigerated Foods in Reduced Oxygen Packages" and New York State Department of Agriculture and Markets "Proposed Reduced Oxygen Packaging Regulations." As provided in the Food Code, some ROP operations may be conducted under provision 3-502.12 Reduced Oxygen Packaging, Criteria. Food that is packaged by an ROP method under these provisions is considered safe while it is under the control of the establishment and, if the labeling instructions are followed, while under the control of the consumer.

(E) Safety Barrier Verification

The safety barriers for all processed foods held in ROP at retail must be verified in writing. This can be accomplished through written certification from the product manufacturer. Independent laboratory analysis using methodology approved by the regulatory authority can also be used to verify incoming product and should be used to verify the barriers in a product that is packaged within the establishment by an ROP method. It should be noted that the Association of Food and Drug Officials (AFDO) guidelines recommend that laboratory analysis be conducted by official methods of the Association of Official Analytical Chemists (AOAC).

The multiple barrier or hurdle efficacy should be validated by inoculated pack or challenge studies. A product should be tested under abuse temperatures to demonstrate product safety during the food's shelf life.

Any changes in product formulation or processing procedures are cause for notification of the regulatory authority and a required approval of the revised ROP process. A record of all safety barrier verifications should be updated every 12 months. This record must be available to the regulatory authority for review at the time of inspection.

(F) USDA Process Exemption

Meat and poultry products cured at a food processing plant regulated by the U.S. Department of Agriculture using substances specified in 9 CFR 318.7 Approval of substances for use in the preparation of products and 9 CFR 381.147 Restrictions on the use of substances in poultry products are exempt from the safety barrier verification requirements. Other ROP operations may be developed that do not meet the provisions of Section 3-502.12 of the Code and that will require a variance and prior approval by the regulatory authority under Section 3-502.11.

(G) Recommendations for ROP Without Multiple Barriers

(1) *Employee Training*

If ROP is used, employees assigned to packaging of the foods must have documented proof that demonstrates familiarity with ROP guidelines in this Annex and the potential hazards associated with these foods. At the discretion of the regulatory authority, a description of the training and course content provided to the employees must either be available for review or have prior approval by the regulatory authority.

(2) *Refrigeration Requirements*

Foods in ROP that have only one barrier, i.e., refrigeration, to ***C. botulinum*** must be refrigerated to 5°C (41°F) or below and marked with a use-by date within either the manufacturer's labeled use-by date or 14 days after preparation at retail, whichever comes first. Alternatively, foods packaged by ROP may be kept frozen if freezing is used as the declared primary safety barrier. Any extension of shelf life past 14 days will require a further variance that considers lower refrigeration temperatures. Foods that are intended for refrigerated storage beyond 14 days must be maintained at or below 3°C (38°F).

(3) *Labeling - Refrigeration Statements*

All foods in ROP which rely on refrigeration as a barrier to microbial growth must bear the statement "Important - Must be kept refrigerated at 5°C (41°F)" or "Important - Must be kept frozen," in the case of foods which rely on freezing as a primary safety barrier. The statement must appear on the principal display panel in bold type on a contrasting background. Foods held under ROP which have lower refrigeration requirements as a condition of safe shelf life must be monitored for temperature history and must not be offered for retail sale if the temperature and time specified in the variance are exceeded.

(4) *Labeling - "Use-by date"*

Each container of food in ROP must bear a "use-by" date. This date cannot exceed 14 days from retail packaging or repackaging without a further variance granted by the regulatory authority. The date assigned by a repacker cannot extend beyond the manufacturer's recommended "pull date" for the food. The "use-by" date must be listed on the principal display panel in bold type on a contrasting background. Any label must contain a combination of a "sell-by" date and use-by instructions which makes it clear that the product must be consumed within 14 days of retail packaging or repackaging, as an acceptable alternative to a 14 day "use-by" date, i.e., for product packaged on November 1, 1999 - "Sell by November 10, 1999" - use within 4 days of sell-by date. Foods that are frozen before or immediately after packaging and remain frozen until use should bear a "Keep frozen, use within 4 days after thawing" statement.

(H) Foods Which Require a Variance Under Code Section 3-502.11 if Packaged in Reduced Oxygen Atmosphere

(1) Processed fish and smoked fish may not be packed by ROP unless establishments are approved for the activity and inspected by the regulatory authority. Establishments packaging

such fish products, and smoking and packing establishments, must be licensed in accordance with applicable law. Caviar may be packed on the premises by ROP if the establishment is approved by the regulatory authority and has an approved scheduled process established by a processing authority acceptable to the regulatory authority.

(2) Soft cheeses such as ricotta, cottage cheese, cheese spreads, and combinations of cheese and other ingredients such as vegetables, meat, or fish at retail must be approved for ROP and inspected by the regulatory authority.

(3) Meat or poultry products which are smoked or cured at retail, except that raw food of animal origin which is cured in a USDA-regulated processing plant, or establishment approved by the regulatory authority to cure these foods may be smoked in accordance with approved time/temperature requirements and packaged in ROP at retail if approved by the regulatory authority.

(I) Hazard Analysis and Critical Control Point (HACCP) Operation

All food establishments packaging food in a reduced oxygen atmosphere must develop a HACCP plan and maintain the plan at the processing site for review by the regulatory authority. For ROP operations the plan must include:

(1) A complete description of the processing, packaging, and storage procedures designated as critical control points, with attendant critical limits, corrective action plans, monitoring and verification schemes, and records required;

(2) A list of equipment and food-contact packaging supplies used, including compliance standards required by the regulatory authority, i.e., USDA or a recognized third party equipment by the evaluation organization such as NSF International;

(3) A description of the lot identification system acceptable to the regulatory authority;

(4) A description of the employee training program acceptable to the regulatory authority;

(5) A listing and proportion of food-grade gasses used; and

(6) A standard operating procedure for method and frequency of cleaning and sanitizing food-contact surfaces in the designated processing area.

(J) Precautions Against Contamination at Retail

Only unopened packages of food products obtained from sources that comply with the applicable laws relating to food safety can be used to package at retail in a reduced oxygen atmosphere. If it is necessary to stop packaging for a period in excess of one-half hour, the remainder of that product must be diverted for another use in the retail establishment.

(K) Disposition of Expired Product at Retail

Processed reduced oxygen foods that exceed the "use-by" date or manufacturer's "pull date" cannot be sold in any form and must be disposed of in a proper manner.

(L) Dedicated Area/Restricted Access

All aspects of reduced oxygen packaging shall be conducted in an area specifically designated for this purpose. There shall be an effective separation to prevent cross contamination between raw and cooked foods. Access to processing equipment shall be restricted to responsible trained personnel who are familiar with the potential hazards inherent in food packaged by an ROP method. Some ROP procedures such as sous vide may require a "sanitary zone" or dedicated room with restricted access to prevent contamination.

(M) References

1. Association of Food and Drug Officials, 1990. Retail Guidelines - Refrigerated Foods in Reduced Oxygen Packages. J. Assoc. Food Drug Offic. 54(5):80-84.

2. Berang, M.E., R.E. Brackett, and L.R. Beuchat., 1989. Growth of Listeria monocytogenes on fresh vegetables stored under controlled atmosphere. J. Food Prot. 52:702-705.

3. Brown, W.L., 1991. Designing Listeria monocytogenes thermal inactivation studies for extended-shelf-life refrigerated foods. Food Technol. 45(4):152-153.

4. Bryan, F.L., L.A. Seabolt, R.W. Peterson, and L.M. Roberts, 1978. Time-temperature observations of food and equipment in airline catering operations. J. Food Prot. 41: 80-92.

5. Conner, D.E., V.N. Scott, D.T. Bernard, and D.A. Kautter, 1989. Potential Clostridium botulinum hazards associated with extended shelf-life refrigerated foods: a review. J. Food Safety 10:131-153.

6. Daniels, R.W., 1991. Applying HACCP to new-generation refrigerated foods at retail and beyond. Food Technol. 45(4):122-124.

7. Davidson, W.D., 1987. Retail store handling conditions for refrigerated foods. Presented at a technical session "New extended shelf-life: low-acid refrigerated foods" at the 80th annual convention of the National Food Processors Association. Jan. 26, Chicago, IL.

8. Doyle, M.P., 1991. Evaluating the potential risk from extended-shelf-life refrigerated foods by Clostridium botulinum inoculation studies. Food Technol. 44(4):154-156.

9. Eklund, M.W., D.I. Wieler, and F. Polsky, 1967. Growth and toxin production of nonproteolytic type B Clostridium botulinum at 3.3 to 5.6C. J. Bacteriol. 93:1461-1462.

10. Harris, R.D., 1989. Kraft builds safety into next generation refrigerated foods. Food Proc. 50(13):111-112,114.

11. Hutton, M.T., P.A. Dhehak, and J.H. Hanlin, 1991. Inhibition of botulinum toxin production by Pedicoccus acidilacti in temperature abused refrigerated foods. J. Food Safety 11:255-267.

12. Kalish, F., 1991. Extending the HACCP concept to product distribution. Food Technol. 45(4):119-120.

13. Knabel, S.J., H.W. Walker, P.A. Hartman, and A.F. Mendonca, 1990. Effects of growth temperature and strictly anaerobic recovery on the survival of Listeria monocytogenes during pasteurization. Appl. Environ. Microbiol. 56:370-376.

14. Moberg, L., 1989. Good manufacturing practices for refrigerated foods. J. Food Prot. 52:363-367.

15. National Advisory Committee on Microbiological Criteria for Foods, 1991. Listeria monocytogenes. Int. J. Food Microbiol. 14:185-246.

16. National Advisory Committee on Microbiological Criteria for Foods, 1991. I HACCP Principles, II Meat and Poultry, III Seafood. Food Control 2(4):202-211.

17. New York Department of Agriculture and Markets, 1993. Proposed Reduced Oxygen Packaging Regulations. Division of Food Safety and Inspection, 1 Winners Circle, Albany, NY, 12235, 6 pp.

18. Nolan, D.A., D.C. Chamberlin and J.A. Troller, 1992. Minimal water activity of Listeria monocytogenes and Listeria innocua. Int. J. Food Microbiol. 16:323-335.

19. Palumbo, S. A., 1986. Is refrigeration enough to restrain foodborne pathogens? J. Food Prot. 49:1003-1009.

20. Refrigerated Foods and Microbiological Criteria Committee of the National Food Processors Association, 1988. Safety considerations for new generation refrigerated foods. Dairy Food Sanit. 8:5-7.

21. Rhodehamel, E.J., 1992. FDA concerns with sous vide processing. Food Technol. 46(12):73-76.

22.Schimdt, C.F., R.V. Lechowich, and J.F. Folinazzo, 1961. Growth and toxin production by type E C. botulinum below 40F. J. Food Sci. 26:626-630.

23. Scott, V.N., 1989. Interaction of factors to control microbial spoilage of refrigerated foods. J. Food Prot. 52:431-435.

24. Smith, J.P., C. Toupin, B. Gagnon, R. Voyer, P.P. Fiset, and M.V. Simpson, 1990. Hazard analysis critical control point approach (HACCP) to ensure the microbiological safety of sous vide processed meat/pasta product. Food Microbiol. 7:177-198.

25. Van Garde, S.J., and M. Woodburn, 1987. Food discard practices of householders. J. Am. Diet. Assoc. 87:322-329.

26. Wyatt, L.D., and V. Guy, 1980. Relationships of microbial quality of retail meat samples and sanitary conditions. J. Food Prot. 43:385-389.

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NMFS Guidelines

Inspection and certification of Cryovac vacuum packaged marine fresh fish products (salt water species only).

A. Qualification Requirements
    1. The plant must be an official establishment under a valid Type I Contract for product and sanitation inspection.
    2. A specification delineating the terms and conditions for approval of their Cryovac vacuum packaging system as set forth in B below.
    3. Labels for the vacuum packed fresh fish products to be used in labeling the inspected and certified products must be submitted for approval prior to production.

B. System approval requirements: the terms and conditions for approval are set forth as follows:
    1. The system must be described in a flow diagram and have accompanying fish processing guidelines (C below) for fresh fish.
    2. The process will be limited to marine species only. Fresh water species will not be approved.
    3. No fish older than 5 d after harvest will be subjected to the vacuum packaging or processing system.
    4. Consumer "Use By" dates will be placed on each package of fish subjected to the Cryovac processing and vacuuming procedures to ensure that the product is not consumed more than 10 d after packaging.
    5. Processors will be required to place appropriate time, temperature, or both, sensing devices on sufficient numbers of master cartons of product so that a receiver of the product can be assured that the product has not been subjected to temperature abuse.
    6. All labeling must bear the warning statement "Hold at Refrigerated Temperatures" or "Keep Refrigerated." This includes both primary and master cartons.

C. Proposed Cryovac Fresh Fish Processing Guidelines
    1. Cryovac recommends using fresh whole fish having been well iced on the fishing vessel and not more than 5 d from time of catch to provide user with maximum shelf life. Whole boxed fish must be iced effectively to insure maximum cooling efficiency. Whole fish are washed by an effective means to reduce the bacterial load on the surface of the fish prior to filleting. Good sanitation and handling practices are stressed during all operations. Fillets are given a brief rinse using

potable water, salt brine, or a chlorine solution (<15 ppm) on both sides, drained, then packaged on a vacuum chamber machine in the Cryovac E™ bag or comparable Cryovac flexible material. To increase the abuse resistance of the packaging material and to enhance its tight fitting appearance, the package is briefly exposed to hot water operating at a temperature of 185-190° F (85-87.8° C) for 1-2 s. Packages are rapidly "chilled" to an internal temperature of 34° F (1.1° C) ± 2° F (1.1° C) within 30-40 min without surface crust freezing. This can be accomplished by the use of refrigerated salt brine or glycol operating at 28° F (-2.2° C) ± 3° F (1.7° C), or a comparable air system. Liquid chilled packages are rinsed with potable water then placed in master shipping cases, palletized and all product stored at 30° F (-1.1° C) ± 2° F (1.1° C) for a minimum of 2 h. This enables the internal product temperature to equilibrate to 32° F (0° C) ± 2° F (1.1° C) prior to distribution (NMFS, 1983).

Inspection and certification of vacuum packaged hot-processed smoked or hot-processed smoke-flavored salmon

A. The processing facility must be in compliance with the requirements of 50 CFR Part 260.
B. Defrosting of eviscerated frozen fish shall be carried out in:
    1. Air at 45° F (7.2° C) or below until completely thawed and the internal temperature of any part of the fish does not exceed 45° F (7.2° C).
    2. A continuous water flow tank or spray system until thawed. Salmon shall not be mixed with other species during thawing. Fish shall not remain in the tank for over 4 h after they are completely defrosted and the temperature in any part of the fish shall not exceed 60° F (15.6° C) during this period. If longer delays and encountered, the fish shall be returned to temperatures of 38° F (3.3° C) or below until brined.
C. Both fresh and thawed fish shall be washed thoroughly with a vigorous chlorinated water spray or in a continuous water flow system prior to brining. (Chlorine concentration in water shall not exceed 50 ppm available chlorine).
D. All fish shall be dry-salted at a temperature not to exceed 38° F (3.3° C) throughout the fish, or if brined, the salmon shall not be mixed with other species of fish in the same tank and shall be brined in such a manner that the temperature of the fish and brine:
    1. Does not exceed 60° F (15.6° C) at the start of the brining, and
    2. If the brine time exceeds 4 h, the brining shall take place in a refrigerated room of 42° F (5.6° C) or lower. The product may not be held above 38° F (3.3° C) for more than 48 h.
    3. Brines may not be reused unless there is an adequate process (e.g., ultrafiltration) to return the brine to an acceptable microbiological level.

    [Note: Brine tanks that have been used to brine other species of fish shall be cleaned and sanitized before being used to brine salmon]

E. Hot-process smoked or hot-process smoke-flavored salmon shall be brined in such a manner that the final sodium nitrite content of the loin muscle of the finished product

shall be no less than 100 ppm and no more than 200 ppm (parts per million) after processing. These same products shall contain not less than the minimum concentrations of one of the following combinations of water phase salt (w.p. NaCl) and sodium nitrite in the deepest part of the loin:

% w.p. NaCl	ppm sodium nitrite
3.5	100
3.4	120
3.3	140
3.2	160
3.1	180

F. Hot-process smoked or hot-process smoke-flavored salmon shall be heated by a controlled heat process that provides a monitoring system (e.g., calibrated probes or dial thermometers) positioned in as many strategic locations in the oven as necessary to assure that all products reach the minimum internal temperature.

[Note: The temperature monitoring device shall be tested for accuracy against a known standard thermometer upon installation and at least once a year thereafter, or more frequently if necessary, to ensure their accuracy. Graduations on the temperature monitoring device shall not exceed 2° F (1.1° C) within a range of 10° F (5.6° C) of the processing temperature.]

Each fish or fish portion shall be heated to an internal temperature of 150° F (65.6° C) or higher and maintained at 150° F (65.6° C) for 30 min or longer.

G. Liquid smoke or generated smoke or a combination of liquid smoke and generated smoke shall be applied to the entire product. If only liquid smoke is used in the process it can be applied to the product prior to, at the beginning of, or during the process. Liquid smoke added during the process shall be applied before the internal temperature of the product exceeds 125° F (51.7° C). The liquid smoke shall be prepared by the aqueous process (e.g., U.S. Patent 3,106,473) and have a minimum of the following major constituents in its compositions: 1) 10% titratable acidity; 2) 9 mg phenol/g; 3) 12 g carbonyls/100 ml, and be used at concentrations of 50% or greater.

When only generated smoke is used in the process, dense smoke shall be applied to the fish for at least 90 min at the beginning of the process. If a drying cycle is used in the smoking process, the air temperature surrounding the product shall not exceed 110° F (43.3° C) and the time period shall not exceed 60 min at temperatures between 60° F (15.6° C) and 110° F (43.3° C) before the application of smoke. If lower temperatures (60° F [15.6° C] or below) are used, this time period shall not exceed 6 h. Generated smoke shall be produced from burning hardwood. If a combination of liquid smoke and generated smoke are used, the procedures for liquid smoke shall be followed and the generated smoke can be applied at any stage of the process.

H. The finished product shall be cooled to a temperature of 50° F (10° C) or below within 5 h after cooking and further cooled to a temperature of 38° F (3.3° C) or below within 12 h after cooking. The finished product shall be maintained at 38° F (3.3° C) or lower during all subsequent storage and distribution.
I. Primary packages and master cartons shall be clearly marked with a statement to maintain the product below 38° F (3.3° C). If fish are frozen, they shall be clearly labeled to thaw at refrigerated temperatures and that the thawed product must subsequently be stored below 38° F (3.3° C).
J. The finished product shall be analyzed chemically with sufficient frequency to assure that the required water-phase salt and sodium nitrite is obtained and that other chemical additives are present at authorized levels. To reduce the possibility of post processing contamination with food poisoning bacteria, the product shall be vacuum-packaged only within the facility in which it was processed.
K. Permanently legible code marks shall be placed on the outer layer of every finished product package and master carton. Such marks shall identify at least the plant where packed and the date of packing. Records shall be maintained at the processing facility for a minimum of 6 months from the date of processing as to provide positive identification (1) of the process procedures (including process/product time, temperature, sodium nitrite and water-phase salt levels) used for the manufacture of hot-process smoked and hot-process smoke-flavored fish and (2) of the distribution of the finished product (NMFS, 1984).

Inspection and certification of vacuum and modified atmosphere packaged (VAC and MAP) marine and estuarine bulk raw fishery products which are to be held at only refrigerated temperatures (non-frozen).

A. Product requirements: All NMFS bulk VAC and MAP raw products shall be in accordance with the following:
    1. Bulk packaged raw marine and estuary refrigerated fishery products are to be removed from their VAC or MAP packaging before being presented for retail sale. While it is not required, it is strongly recommended that retail or food service establishments selling NMFS inspected VAC and MAP packaged products operate under an approved NMFS HACCP-based inspection system.
    2. Non-bulk VAC or MAP packaged raw products are ineligible for DOC inspection unless they conform to the provisions of other manual releases on this subject.
B. Handling and storage: The handling, storage, and distribution systems shall have a demonstrated capacity to hold bulk VAC or MAP raw products at or below 40° F (4.4° C).
C. Labeling: All bulk VAC or MAP packaged marine or estuarine raw fishery products must bear the following statement on the label, "Hold at Refrigerated Temperatures 40° F (4.4° C) or Below" or, "Keep Refrigerated at or Below 40° F (4.4° C)." This label must be on all bulk primary and secondary containers and must be visible during shipment (NMFS, 1993).

State Guidelines

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New York: Smoked or Processed Fish (Corby, 1999) Top

Raw Materials

a. Fresh fish received shall be inspected and adequately washed before processing. Only sound, wholesome fish free from adulteration and organoleptically detectable spoilage shall be processed.
b. Every lot of fish that has been partially processed in another plant, including frozen fish, shall be adequately inspected and only clean wholesome fish shall be processed.
c. Fresh or partially processed fish, except those to be immediately processed, shall be iced or otherwise refrigerated to an internal temperature of 38 degrees Fahrenheit or below upon receipt and shall be maintained at that temperature until the fish are to be processed.
d. All fish received in a frozen state shall be either thawed promptly and processed or stored at a temperature that will maintain it in a frozen state.
e. The defrosting of frozen fish shall be conducted in a sanitary manner and by such methods that the wholesomeness of the fish is not adversely affected.
f. After thawing, fish shall be washed thoroughly with a vigorous potable water spray or a continuous water flow system.
g. All fish shall be free of viscera prior to processing, except:
    1. Small species of fish, such as anchovies and herring sprats, provided they are processed in an adequate fashion and will contain a water phase salt level of at least 10 percent, a water activity below .85 or a pH of 4.6 or less; and
    2. Fermented fish, provided they are processed in an adequate fashion and will contain a water phase salt level of at least 17 percent; and
    3. Fully cooked seafoods.
h. The evisceration of fish shall be conducted in an area that is segregated and separate from other processing operations. The evisceration shall be performed with minimal disturbance of the intestinal tract contents. The fish, including the body cavity, shall be washed thoroughly with a vigorous spray or a continuous water flow system following evisceration.

Operations and Controls for Processed Fish.

a. All operations involving the receiving, holding, processing and packaging of processed fish shall be conducted utilizing clean and sanitary methods and shall be conducted as rapidly as practicable and at temperatures that will not cause any material increase in bacterial or other microorganic content or any deterioration or contamination of such processed fish.
b. All processed fish shall be produced pursuant to a scheduled process established by a competent processing authority.
c. All processed fish shall be distributed and sold at temperatures that do not exceed 38 degrees Fahrenheit, except that:
    1. Processed fish that have a water phase salt level of at least 17 percent shall not require refrigerated storage and;
    2. Processed fish which contain a water phase salt level of at least 10 percent, a water activity of less than .85, or a pH of 4.6 or lower may be distributed or sold at refrigerated temperatures that do not exceed 45 degrees Fahrenheit.

d. Thermometers or other temperature monitoring devices accurate to within plus or minus 2 degrees Fahrenheit shall be installed in coolers, freezers, vehicles or other refrigerated areas where fish or processed fish are processed, held or displayed.
e. Processed fish shall be vacuum packaged or modified atmosphere packaged only if produced pursuant to a scheduled process which specifies vacuum packaging or modified atmosphere packaging.
f. The vacuum packaging or modified atmosphere packaging of processed fish shall be conducted only within the facilities in which the product was produced.
g. Processed fish to be vacuum packaged or modified atmosphere packaged shall be analyzed chemically with sufficient frequency to assure that the required water phase salt and sodium nitrite is obtained in every fish and that other chemical additives are present at acceptable levels.

Critical Aspects of Processes

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Critical aspects of handling vacuum packaged fish and fishery products may include:

- The temperature of the finished product cooler and of trucks or other carriers throughout transportation, or
- The quantity of ice or other cooling media at all times during storage and distribution (FDA, 1998a).
- A "Sell By" date on individual packages and master cartons
- Labels with "Keep Refrigerated" on individual packages and master cartons

Analytical Procedures

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Thermometer calibration

See Chapter 2.

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Vacuum Packaging Processes

Modified atmosphere and vacuum packaged salmon fillets

Aquacultured salmon fillets were inoculated on the surfaces with 100 spores/g of a *C. botulinum* type E spore mixture. The fillets were packaged in high-barrier film bags under selected atmospheres (100% air, a modified atmosphere containing 75% CO_2:25% N_2, and vacuum) and stored at 4ºC (39.2ºF), 8ºC (46.4ºF), and 16ºC (60.8ºF) (Reddy et al., 1997a).

Table 8-1. Days to sensory spoilage and onset of toxicity in fresh salmon fillets inoculated with *C. botulinum* type E (100 spores/g of fish), packaged under selected atmospheres, and stored at various temperatures.

Storage Temp. (ºC)	Atmospheres	Sensory Spoilage (d)	Toxin Detection (d)
16	100% air	4	4
	MA[a]	5-6	4
	Vacuum	3	3
8	100% air	13-17	17
	MA[a]	20-24	24
	Vacuum[b]	>6, <10	10
4	100% air	24-27	>66[c]
	MA[a]	55-62	>80[c]
	Vacuum	34-38	>66[c]

[a]MA = 75% CO_2: 25% N_2.
[b]No sample was taken between d 6 and 10.
[c]Last sampling was taken for toxin detection. No toxin was detected.

Modified atmosphere and vacuum packaged catfish fillets

Fresh catfish fillets (110-140 g) were surface inoculated on both sides with a *C. botulinum* type E spore mixture at 100 spores/g fish. Fillets were packaged in high-barrier film bags under selected atmospheres (100% air, a modified atmosphere containing 75% CO_2:25% N_2, and vacuum) and stored at 4ºC (39.2ºF), 8ºC (46.4ºF), and 16ºC (60.8ºF) (Reddy et al., 1997b).

Table 8-2. Days to sensory spoilage and onset of toxicity in fresh catfish fillets inoculated with *C. botulinum* type E (100 spores/g of fish), packaged under selected atmospheres, and stored at various temperatures.

Storage Temp. (ºC)	Atmospheres	Sensory Spoilage (d)	Toxin Detection (d)
16	100% air	3	3
	MA[a]	4	4
	Vacuum	3	3
8	100% air	6	9
	MA[a]	13	18
	Vacuum	6[b]	6[b]

4	100% air	13	>54[c]
	MA[a]	38-40	>75[c]
	Vacuum	20-24	46

[a]MA, 75% CO_2: 25% N_2.
[b]1st d of sampling.
[c]Last sampling was taken for toxin detection. No toxin was detected.

Modified atmosphere and vacuum packaged tilapia fillets

Fresh tilapia fillets (90-120 g) were surface inoculated on both sides with a *C. botulinum* type E spore mixture at 100 spores/g fish. Fillets were packaged in high-barrier film bags under selected atmospheres (100% air, a modified atmosphere containing 75% CO_2:25% N_2, and vacuum) and stored at 4ºC (39.2ºF), 8ºC (46.4ºF), and 16ºC (60.8ºF) (Reddy et al., 1997c).

Table 8-3. Days to sensory spoilage and onset of toxicity in fresh tilapia fillets inoculated with *C. botulinum* type E (100 spores/g of fish), packaged under selected atmospheres, and during 30 d and 90 d storage.

Storage Temp. (ºC)	Atmospheres	Sensory Spoilage (d)	Toxin Detection (d)
Preliminary 30 d study			
16	100% air	3-6	>3, <6
	MA[a]	3-6	>3, <6
	Vacuum	ND[b]	<6[b]
8	100% air	6	>9[c]
	MA[a]	13-16	>16
	Vacuum	9	>9, <16[d]
4	100% air	9	>13
	MA[a]	>30	>30[c]
	Vacuum	>30	>30[c]
Final 90 d study			
16	100% air	3	4
	MA[a]	4	4
	Vacuum	3	3

8	100% air	6	20
	MA[a]	17	40
	Vacuum	10	17
4	100% air	10	>47[c]
	MA[a]	80	>90[c]
	Vacuum	47	>90[c]

[a]MA = 75% CO_2: 25% N_2.
[b]ND, not determined. First day of sampling taken for toxin detection only.
[c]Last sample was taken for toxin detection. No toxin was detected.
[d]No sample was taken on d 13 for toxin detection.

Vacuum packaged cooked shrimp and Dungeness crabmeat

Vacuum packaged shrimp and crabmeat inoculated with about 300 *C. botulinum* type E spores/g and stored at 10ºC (50ºF), would have spoiled 14 d before it became toxic. At 4.4ºC (40ºF), the product would have spoiled 27 d before it became toxic (Lerke and Farber, 1971).

Vacuum packaged Dungeness crabmeat

C. botulinum type E spores (10,000 per sample) did not grow and produce toxin in crabmeat vacuum-packaged in oxygen impermeable films when stored at 12ºC (55ºF) for 20 d. When crabmeat was inoculated with 10,000 spores per sample and stored at 25ºC (77ºF), toxin was produced in one of the samples after 13 d of storage. However, toxin was not produced when the samples were inoculated with 1,000 type E spores per sample. Samples inoculated with 10,000 *C. botulinum* type A spores were not toxic after 3 d of storage, but were toxic after 6 d of storage at 25ºC (77ºF) in either oxygen-permeable or impermeable containers. All of the samples stored at 25ºC (77ºF) were grossly spoiled prior to the production of *C. botulinum toxin* (NMFS, 1977).

Time-Temperature monitoring tags

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Disclaimer Clause

Note: There are two types of temperature indicators: threshold and integrator. A threshold indicator monitors a product that has exceeded a given temperature. An integrator indicator monitors both time and temperature during a given period.

Full History Time/Temperature Integrators			
Product Name	**Threshold (Activation) Temperature**	**Cumulative Runout Time**	**Manufacturer/Supplier**

Fresh-Check®	39.2°F (4°C) - currently the lowest temperature	custom orders for times and temperatures	LifeLines Technology, Inc., 116 American Rd., Morris Plains, NJ 07950 Phone: 973/984-6000 Fax: 973/984-1520 e-mail: info@lifelinestechnology.com Web:www.lifelinestechnology.com
MonitorMark™ Time/Temperature Indicators	5°F (-15°C), 41°F (5°C), 50°F (10°C), 79°F (26°C), 88°F (31°C)	48 hrs, 48 hrs, 48 hrs, 1 wk, 48 hrs. (1 wk, 2 wks), 1 week	Thomas G. Goldkamp, Inc., 186 South Main St., Ambler, PA 19002 Ph: 215/646-7220 Fax: 215/646-0148
Vitsab® TTI, M2-51015 Fresh White Fish (cultured)	35°F (2°C)	5, 10, 15 days (3 dot label); custom orders available for time and temperature.	Cox Technologies, Inc., Vitsab® Division, 71 McAdenville Rd., Belmont, No. Carolina 28012 Ph: 704/825-8146, Fax:704/825-4368, E-mail: sales@vitsab.com, Web: www.vitsab.com
Vitsab® TTI, M5-469 Fresh Salmon (shipped chilled)	41°F (5°C)	4, 6, 9 days (3 dot label); custom orders available for time and temperature.	Cox Technologies, Inc., Vitsab® Division, 71 McAdenville Rd., Belmont, No. Carolina 28012 Ph: 704/825-8146, Fax:704/825-4368, E-mail: sales@vitsab.com, Web: www.vitsab.com
Vitsab® TTI, MO-4710 Fresh White Fish (chilled)	32°F (0°C)	4, 7, 10 days (3 dot label); custom orders available for time and temperature.	Cox Technologies, Inc., Vitsab® Division, 71 McAdenville Rd., Belmont, No. Carolina 28012 Ph: 704/825-8146, Fax:704/825-4368, E-mail: sales@vitsab.com, Web: www.vitsab.com
Partial History Time/Temperature Integrators			
Product Name	**Threshold (Activation) Temperature**	**Cumulative Runout Time**	**Manufacturer/Supplier**

ColdMark (2 models available)	32°F (0°C) or 26°F (-3°C)	≈ 30 min.	Delta TRAK, Inc., P.O. Box 398, Pleasanton, CA 94566 Ph: 925/249-2250 Fax: 925/249-2251 E-mail: salesinfo@deltatrak.com Web: www.deltatrak.com
TempDot (4 models available)	41°F (5°C), 46°F (8°C), 50°F (10°C) and 77°F (25°C)	Up to 60 min. (cumulative degree minutes)	Delta TRAK, Inc., P.O. Box 398, Pleasanton, CA 94566 Ph: 925/249-2250 Fax: 925/249-2251 E-mail: salesinfo@deltatrak.com Web: www.deltatrak.com
WarmMark (10 models available)	0°F (-18°C) to 99°F (37°C)	brief, moderate or prolonged	Delta TRAK, Inc., P.O. Box 398, Pleasanton, CA 94566 Ph: 925/249-2250 Fax: 925/249-2251 E-mail: salesinfo@deltatrak.com Web: www.deltatrak.com

References

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Corby, J. 1999. Personal communication, Director, Division of Food Safety and Inspection, Department of Agriculture and Markets, State of New York, Albany, NY.

FDA. 1999. Annex 6: Food Processing. Food Code 1999. p. 414-425. Department of Health and Human Services, Public Health Service, Food and Drug Administration, Center for Food Safety and Applied Nutrition, Office of Seafood, Washington, DC.

FDA. 2001a. *Clostridium botulinum* toxin formation. Ch. 13. In *Fish and Fishery Products Hazards and Controls Guidance*, 3rd ed., p. 167-190. Food and Drug Administration, Center for Food Safety and Applied Nutrition, Office of Seafood, Washington, DC.

FDA. 2001b. Introduction of pathogens after pasteurization and specialized cooking processes. Ch. 18. In *Fish and Fishery Products Hazards and Controls Guidance*, 3rd ed., p. 227-236. Food and Drug Administration, Center for Food Safety and Applied Nutrition, Office of Seafood, Washington, DC.

Lerke, P. and Farber, L. 1971. Heat pasteurization of crab and shrimp from the Pacific coast of the United States: Public health aspects. J. Food Sci. 36:277-279.

NMFS. 1977. Fresh and frozen Dungeness crab meat – handling practices. Weekly Activities Report, March 18, 1977 (F118), Utilization Research Division, Northwest and Alaska Fisheries Center, National Marine Fisheries Service, Seattle, WA.

NMFS. 1983. Inspection and certification of Cryovac vacuum packaged marine fresh fish products (salt water species only). *NMFS Fishery Products Inspection Manual*, Part III, Ch. 6, Sec. 01. National Marine Fisheries Service, Washington, DC.

NMFS. 1984. Inspection and certification of vacuum packaged hot-processed smoked or hot-processed smoke-flavored salmon. *NMFS Fishery Products Inspection Manual*, Part III, Ch. 6, Sec. 2. National Marine Fisheries Service, Washington, DC.

NMFS. 1993. Inspection and certification of vacuum and modified atmosphere packaged (VAC and MAP) marine and estuarine bulk raw fishery products which are to be held at only refrigerated temperatures (non-frozen). *NMFS Fishery Products Inspection Manual*, Part III, Ch. 6, Sec. 03. National Marine Fisheries Service, Washington, DC.

Reddy, N.R., Solomon, H.M., Yep, H., Roman, M.G., and Rhodehammel, E.J. 1997a. Shelf life and toxin development by *Clostridium botulinum* during storage of modified-atmosphere-packaged fresh aquacultured salmon fillets. J. Food Protect. 60(9):1055-1063.

Reddy, N.R., Roman, M.G., Villanueva, M., Solomon, H.M., Kautter, D.A., and Rhodehammel, E.J. 1997b. Shelf life and *Clostridium botulinum* toxin development during storage of modified atmosphere-packaged fresh catfish fillets. J. Food Sci. 62(4):878-884.

Reddy, N.R., Paradis, A., Roman, M.G., Solomon, H.M., and Rhodehammel, E.J. 1997c. Toxin development by *Clostridium botulinum* in modified atmosphere-packaged fresh tilapia fillets during storage. J. Food Sci. 61(3):632-635.