Safety of Modified Atmosphere and Vacuum Packaged Products

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Microbial activity limits the shelf-life of packed as well as unpacked fish products. Packaging of fresh seafood products under vacuum and modified-atmosphere coupled with refrigerated storage increases their storage shelf-life. These packaging methods enhance product quality and appearance by suppressing normal spoilage bacteria in products stored at refrigeration temperatures. Modified Atmosphere Packaging (MAP) is highly specific for each fish product, and for each packaging system. The process of vacuum packaging (VP) removes air and prevents its return by an airtight seal of the packaging material. With modified atmosphere or "gas" packaging, air is again removed and is replaced by a strictly controlled mixture of gases chosen from carbon dioxide, oxygen and nitrogen.

Potential food safety hazards

Concerns have been expressed that the increase in shelf life of VP/MAP product may provide sufficient time for human pathogens to multiply to levels which render the food unsafe while still edible organoleptically particularly when storage temperatures are not strictly controlled. A limiting factor to a wider application of MAP or VP to fresh fish storage is the potential risk from psychrotrophic *Clostridium botulinum* (type E and non-proteolytic types B and F). Hence, the adequacy of refrigeration in preserving the safety of these refrigerated foods has been questioned. New safety issues have been raised due to the emergence of other psychrotrophic pathogens like *Listeria monocytogenes, Aeromonas hydrophila* and *Yersinia enterocolitica*. *Y. enterocolitica* can grow at temperatures as low as -2°C.

Growth of *Y.enterocolitica* –like organisms have been reported on vacuum-packaged refrigerated beef and lamb. When the beef was packaged in CO_2, *Yersinia* grew at 5°C after a prolonged lag period. Studies on cod and trout have shown that storage temperature below 5°C with packaging in CO_2 seem to be effective in reducing the risk from these cold tolerant pathogen. *Aeromonas hydrophila* is a psychrotrophic pathogen and appears to be part of the normal intestinal flora of healthy fish. Greater reductions were noticed in the *Aeromonas* growth in cod and trout under the atmosphere richest in CO_2 and at the lowest temperatures. Total inhibition of growth of *A. hydrophila* occurs at 5°C.

If products in VP/MAP 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, Vibrio parahaemolyticus* and *Clostridium perfringens* can grow slowly. Inhibition of *S. aureus* under the MA was much greater at 2°C than at 7°C Growth of Enterobacteriaceae, including *Salmonella* spp., is reduced with increasing concentration of CO_2, but storage temperature is also a factor. The ability of *B.cereus* spores to germinate in the presence of 100% CO_2 was reduced by 70- 90% as compared to germination under 100% N_2. A combination of low temperature (12.8°C) and MA (75% CO_2: 25% O_2) could prevent

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the outgrowth of *C.perfringens*. At 4°C, *C.perfringens* was unable to grow in cooked roast beef either stored under air or the MA (75% CO_2: 25% O_2). Marginal refrigeration that does not facilitate growth may still allow *Salmonella* spp., *Campylobacter* spp., and *Brucella* spp. to survive for long periods of time. *Campylobactor* spp do not grow at or below 30 °C. However, the organisms may be able to survive the low oxygen conditions of MAP. Other potential microbial hazards include histamine formation and antimicrobial treatments. Low temperatures also effectively inhibit histamine producers, irrespective of the gaseous composition used. Histamine is produced primarily by aerobic and mesophilic microorganisms.

Safety concerns and risks

Psychrotrophic pathogenic organisms of primary concern in MA/ VP-packaged products are *C. botulinum* and *L. monocytogenes*. Although VP/MAP techniques can protect food products from external contamination and increase the shelf-life, under certain circumstances *C. botulinum* may grow.

Psychrotrophic *Clostridium botulinum*

C. botulinum is the causative agent of botulism, a severe food poisoning characterized by double vision, paralysis, and occasionally death. It is a spore-forming, anaerobic bacterium which can be grouped into seven different types A to G, each type producing a serologically distinct neurotoxin. Types A to F have been identified in marine environments. The spores of *C. botulinum* are 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 group II), 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). These strains are the major concern in MAP. Other strains of *C. botulinum* (type A and proteolytic types B and F group I) can grow and produce toxin at temperatures slightly above 10 °C (50 °F). Groups I and II have been associated with human botulism. The biological properties of *C.botulinum* Groups I and II are given in Table 1. A few ng of toxin is sufficient to cause intoxication in humans.

Table 1. Characteristics of *C.botulinum* Groups I and II

Properties	Group	
	I	II
Proteolytic	+	-
Association with human botulism	+	+
Toxin types	A,B,F	B,E,F
Inhibitory pH	4.6	5.0
Inhibitory NaCl concentration	10%	5%
Minimal Water activity	0.94	0.97
Temperature range for growth and toxin production	10-48 °C	13.3- 45 °C
D_{100} of spores	25 min	<0.1 min

For food borne botulism to occur, basically four conditions must exist: the food must be contaminated with C. botulinum, inadequate processing permitting survival of *C. botulinum* already present or postprocessing contamination of adequately processed foods, a food must support toxin formation when stored under temperature conditions exceeding 3.3 °C, and the food must be acceptable to the consumer and consumed without enough

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heating to inactivate botulinal toxin. Some of these conditions could occur in MA-packaged fish products.

Pychrotrophic *C.botulinum* strains which are able to grow and produce toxin at chill temperatures is considered a risk. Mesophilic *C.botulinum* is not considered a risk with respect to VP/MAP chilled foods as it does not grow below 10°C. However, both organisms may cause safety problems if the foods are stored above 10°C, as the controlling factors may not be adequate. In an unpreserved VP/MAP food stored at chill temperature, growth of *C.botulinum* will be slow. Under normal conditions it is assumed that the food is contaminated unless there is a specific step which removes this possibility. It is on this basis that specific requirements for shelf-life are proposed to assure the safety of food, even though some limited growth of the food poisoning organismmay be possible.

For VP/MAP foods with a shelf-life of greater than 10 days at chill temperatures <8°C, where there are no other controlling factors, the minimum heat treatment required is that the slowest heating part of the food should be held at 90 °C for 10 minutes or equivalent. The level of acid in a food is a controlling factor in the growth of microorganisms and a pH of 5.0 or below throughout a food stored at chill temperatures < 8°C is sufficient to inhibit the growth of psychrotrophic *C.botulinum*. A level of 3.5% salt throughout the aqueous phase of a food stored at chill temperatures <8°C is sufficient to inhibit the growth of psychrotrophic *C.botulinum*. For foods with salt or other solutes as the main a_w depressant, an a_w of 0.97 should be achieved throughout the food stored at chill temperatures <8°C to inhibit the growth of psychrotrophic *C.botulinum*.

Combinations of a lower level of the specific controlling factors described above may be able to prevent growth of psychrotrophic *C. botulinum*. Where a lower level of factors is used, each factor is not able to inhibit the growth of *C. botulinum* on its own but is reliant on the combined effect of all factors. It is necessary to illustrate that the preservation system chosen can consistently prevent growth and toxin production by psychrotrophic *C. botulinum*: this may be done by challenge testing and possibly predictive models, providing that sufficient validation data are available to substantiate the reliability of predictions.

Vacuum packaging is not a requirement for *C. botulinum* growth in fish. It has been shown that reduced oxygen pressure like vacuum packaging in air tight bags and prolonged cold storage will increase the risk of *C. botulinum* toxin production. Inclusion of oxygen in the gas mixture did not provide more safety than the elevated CO_2 atmosphere. A 100% CO_2 atmosphere has shown to inhibit *C. botulinum* at chilled temperature. At temperature of 4 to 12 °C organoleptic spoilage of fish precedes toxin production where as at ambient temperatures (20 °C and above) toxin production precedes organoleptic spoilage. For studies that investigated microbiological safety, *C. botulinum* outgrowth and toxin production were demonstrated in some inoculated packs (Table 2). However, experimental designs of these studies have varied widely. Variables include species of fish, inoculum size, method of inoculation, site of inoculation (surface or intramuscular), storage times and temperatures, gas composition, additives, and analyses for product decomposition and toxin production. Because of the conflicting results from these studies, it is not possible to draw definitive conclusions regarding the safety of this packaging technology applied to refrigerated raw fishery products. *C. botulinum* toxin production was not reported in uninoculated pack studies and

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in very few uninoculated controls from other research. Some of the inoculated pack studies used inoculum sizes of 10^4- 10^6 spores/g. These inoculum sizes appear unrealistically high for purposes of evaluating risk even under "worst case scenarios". Fish inoculated with high numbers of spores have been shown to become toxic after 6-8 days as temperatures increase to 50°F(10 °C). Therefore, storage temperatures of 40-50 °F and temperatures which fluctuate in that range and even higher are a cause for concern. These product temperatures can be found during distribution and retail storage. Extended storage under these conditions is hazardous.

Table 2. Organoleptic spoilage and botulinal toxin production in fish products inoculated with *C. botulinum* and packaged under various modified atmospheres at 4 and 8 °C.

Storage temperature °C	Type of fish	Modified atmosphere	Inoculum level (spores / sample)	Toxin detection(d)	Organoleptic spoilage (d)
8.0	Salmon	vacuum)	10^4/ 2fillets	6	-
	Salmon	CO_2	10^4/2	12	—
	Red snapper	(100%)	fillets	9	
	Salmon	CO_2 Air	10^4/2	12	
	Red snapper	(70;30)	fillets	9	—
4.0	Salmon	Vacuum	10^2/ 2 fillets	>60	42
	Red snapper		10^4	21	—
	Salmon	CO_2	10^4/	>60	—
	Red snapper	(100%)	2fillets	21	—
	Salmon	CO_2 Air	10^4/2	>60	—
	Red snapper	(70;30)	fillets	21	—

Listeria monocytogenes

Listeria monocytogenes is a psychrotrophic pathogen and thus can grow and able to multiply at chill temperature such as the new generation' of foods including Cook–chill MAP products with extended shelf life. *L. monocytogenes* cannot survive well in a anaerobic MA (75% CO_2, 25% N_2) however, if a little O_2 (5%) is added *L. monocytogenes* can grow in the MA at 4°C .

Determination of the Safety of Chilled VP/MAP Foods

The shelf-life of a chilled VP/MAP food (held at 3-8°C should never exceed 10 days unless its safety under expected storage conditions can be demonstrated. In order to determine whether a chilled VP/MAP food is safe and to determine when challenge testing is appropriate, the 3-Step Principle should be followed.

*Step*1. *Determine whether shelf- life of* chilled food is short or long.

Step 2. Determine whether the product is chilled at 3-5°C or 5-8 °C.

Step 3. Determine whether, in combination with storage at < 8 °C, one or more of the controlling factors (heat treatment, pH 5.0 or less , a_w 0.97, salt 3.5%)

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are demonstrated : if not, the product should be challenge tested.

For long shelf-life foods(> 40 days) stored at chill temperarure <8°C, in addition to heat treatment of 90°C for 10 min (or equivalent), challenge testing may be needed to establish the maximum shelf-life.

Challenge Testing

To establish the potential risk from growth and toxin production by *C.botulinum* in chilled VP/MAP foods with a long shelf-life (>10 days) which do not meet the specific controlling factors, challenge test studies should be carried out; direct microbiological testing for the organism in a product is inappropriate.

- Where the specific controlling factors have not been demonstrated, a good safety record for the product cannot be relied upon; challenge testing must be carried out.
- Where the specific controlling factors have not been demonstrated and where there is no challenge test data to show that psychrotrophic *C.botulinum* will not grow in the food within the specified shelf-life, then the shelf-life of the food should be reduced to 10 days (or the specific control factors implemented).

Due to the nature of the hazard, challenge testing must be conducted with the necessary expertise to safely handle the organism.

Several points should be considered in designing *C. botulinum* challenge study.

1. Types and number of strains of C. *botulinum* to be used.
2. Methods for spore production, preparation, and enumeration.
3. Number of spores to be inoculated.
4. Methods for inoculating product with spores.
5. Packaging of product.
6. Time(s) and temperature(s) of product incubation.
7. Sample size, sampling times, number of samples to test.
8. Botulinal toxin testing procedure.
9. Product analyses to be performed during the study.

1. Types and Number of *C. botulinum* strains

Nonproteolytic types of *C. botulinum* are used in challenge studies (Table 3). A mixture of minimum five strains of nonproteolytic types B and E, and one strain o f nonproteolytic type F is suggested. Strains should be selected from as many different sources as possible and may include strains shown in Table 3.

Table 3. Nonproteolytic types of *C. botulinum* used in challenge studies.

Nonproteolytic Type B	Nonproteolytic Type E	Nonproteolytic Type F
2B	Beluga	83
17B	Saratoga	187
2129B	Minnesota	202
17844B	Iwanii	3194
KAPI-B	Alaska	
25765B	Birmingham	
	070	
	G21-5	

Strains 17B and Beluga should be used in all studies. Each strain of *C. botulinum* used for inoculated pack studies periodically (yearly) should be assayed by the mouse bioassay for toxin production. Any culture producing less than 1000 MLD/ml should not be used; either a productive culture of the same strain should

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be obtained or a different strain should be used in lieu of the nonproductive culture.

2. Methods for spore Production, Preparation, and Enumeration

Spore crops may be produced by a variety of methods. (1) the use of many different sporulation broths (2) biphasic methods using different types of liquid media over different types of agar media; and (3) agar media (such as anaerobic egg yolk agar) held under anaerobic conditions. The best spore production method for nonproteolytic strains is in liquid medium (e.g., TPGY medium) incubated at 26 to 30/36 °C for 10 to 12 days.

During harvesting, spores should be washed three times with sterile distilled water and appropriate centrifugation. The spore suspension in sterile distilled water should be stored in vials at refrigeration temperatures at or below 38 °F (3.3 °C). Spores may be enumerated by 3 or 5-tube most probable number (MPN) procedure. Alternatively, spore crops with sufficient numbers of spores can be enumerated by direct plating procedures.

3. Number of Spores Inoculated

Spore mixtures to be used in inocula should contain an approximately equivalent number of spores of each strain of *C. botulinum* in the cocktail. Spores should be diluted appropriately in sterile distilled water and stored at refrigeration temperatures at or below 38 °F (3.3 °C).

Raw fish require surface inoculation. Since spores cannot be uniformly distributed throughout, an inoculum level of 10 to 100 spores per gram is recommended.

4. Methods of Inoculation

Only high quality raw fish should be used in inoculation studies. Samples should be surface inoculated by dropwise (up to 0.1 ml per drop) addition of inoculum that is spread out in a thin layer using sterile utensils (e.g., sterile gloves or bent glass rods).

5. Packaging of Product

When fish are packaged under special conditions such as VP/MAP, a packaging scheme should be used which duplicates the condition of the product as it is normally packaged. Such products should be packaged in a manner that does not affect the normal course of changes within the package relative to the gas mixture. Alternative packaging may be used provided conditions within the package approximate the conditions within commercially packaged product.

6. Time and Temperatures of Incubation of Product

The recommended incubation temperatures for inoculated pack studies with nonproteolytic *C. botulinum* is 50 °F (10 °C). Incubation time should be one and one-half times the product's intended shelflife or up to the time when the product is overtly spoiled (unfit for human consumption).

7. Sample Size, Sampling Times, and Number of Samples to Test

Ideally, the entire sample should be homogenized or extracted for botulinal toxin testing. If samples are large (> 300 g), a minimum sample size of 50 g is recommended.

Sampling times should be adjusted according to the expected shelflife of the product. Samples for botulinal toxin assay should be taken at "0"-time (day of inoculation) and at a minimum of four additional times, with at least three sampling times between halfway and the final testing time. The recommended minimum number of samples assayed for botulinal toxin at each sampling time is five. A minimum of three samples taken at "0"-

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time and at the final sampling time is recommended for *C. botulinum* enumeration. It is not necessary to continue sampling after two consecutive positive sampling times.

8. Botulinal Toxin Testing Procedure

The mouse bioassay procedure as described in the U.S. Food and Drug Administration Bacteriological Analytical Manual is the recommended method for botulinal toxin testing. Only individuals properly immunized with botulinal toxoid should perform these tests. Samples inoculated with nonproteolytic *C. botulinum* spores should be tested first without trypsinization. If mice survive, then tests should be done on trypsinized samples. Preferably, toxin analysis should be done on the day of sampling, but if this is not possible, samples should be homogenized, extracted in gel phosphate buffer (pH 6.2), sedimented by centrifugation (27,000 x g, 20 min, 4 °C), and the supernatant fluids (at least 10 ml;. adjusted to pH 6.2 if necessary) stored at refrigeration temperatures at or below 38°F (3.3 °C), not to exceed 3 days. An alternate procedure (such as an ELISA method) for the mouse bioassay test may be used providing the alternate method has been documented to be of equal or greater sensitivity than the mouse bioassay.

9. Product Analyses

In addition to botulinal toxin testing and *C. botulinum* spore enumeration, the product (duplicate samples) at "0"-time should be assayed for moisture, fat, pH, and aerobic plate count. Depending on the type of product, other analyses (such as protein, salt content, water activity, titratable acidity, nitrite content, sorbate content, gas analysis, psychrotroph count, spore count, lactic acid bacteria count, anaerobe count) also should be used. Visual appearance (including gas formation [puffiness of samples]) and odor of samples should be determined and recorded at each sampling time. A sensory evaluation method using standard odor rejection criteria must be used.

Control Measures

Safe use of MAP technology demands that adequate refrigeration be maintained during the entire shelf-life of potentially hazardous foods to ensure product safety. Minimum conditions for VP/MAP technology control are: 1. Raw Fish Quality 2. Hazard Analysis Critical Control Point Plan 3. Hazard Analysis/Risk Assessment. Prior to packaging, proper handling of raw fish must be assured from the point of harvest. Vacuum or modified atmosphere packaging (VP/MAP) must not be used to extend the shelflife of fish whose quality has deteriorated. A Hazard Analysis Critical Control Point (HACCP) plan from point of packaging through retail sale must be developed for VP/MAP, recognizing that rigid temperature control is the primary preventive measure to ensure safety.

To ensure that safe processes are implemented, good manufacturing practice should be followed.

Key Points of Good Manufacturing Practice

- Keep evidence to show that all Critical Control Points are under control.
- Check all raw materials are delivered under appropriate conditions
- Poor quality raw materials should not be used.
- Transfer chilled ingredients to chilled storage areas on receipt and monitor
- Keep food cool during processing if possible - below 5 °C.
- Cooked ingredients must be kept apart from raw ingredients and ideally stored in a separate chiller. Equipment, used for handling raw ingredients, must not be used for handling cooked food.
- All preservation factors, e.g. acidity, pH, salt levels and cooking processes, are

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controlled and meet the specified requirements for every batch of product produced.

- After cooking, cool the product as possible and transfer to chilled storage.
- Check that all seals are intact.
- Control the temperature during storage and distribution.
- Base the shelf-life of the product on the preservation factors used.
- There should be an effective withdrawal procedure system to control any faulty product and to allow it to be effectively traced and returned.

The studies to support hazard analysis/ risk assessment must be completed by food safety experts who are competent in HACCP systems, *Clostridium botulinum* methodology, sensory evaluations, and statistical procedures. To ensure safety of VP and MAP foods, customers and suppliers must be given information on the product type and handling and usage instructions given below.

- Refrigeration instructions.
- Storage temperature.
- Use by date.
- Partial use of products, e.g. "Consume within X days of opening."
- Cooking or reheating instructions if appropriate, which must be based on scientific evaluation of product safety.
- Ingredients.
- Manufacturer's name and address.

The other methods for minimizing or eliminating safety risks include pretreatment of MA packaged fresh fish products with potassium sorbate, tripolyphosphate and nisin which delay the growth and toxin production by *C. botulinum*. Because strict temperature control is critical in minimizing safety risks of vacuum and MA packaging, various attempts have been made to ensure continued refrigerated conditions. One method has been the addition of a time-temperature indicator (TTI) on each consumer package.

The TTI involves a visual colour change once a certain cumulative time/ temperature threshold has been exceeded. 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. These indicators would give easily discernible warnings and messages to the consumer if the product was improperly handled at some point. Ultimately, consumers will have to decide whether to consume the product thus they must assume partial responsibility for their own safety.

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