*Research Summary*

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# Reduced Oxygen Packaging and Safety of Refrigerated Raw Flounder and Refrigerated Fully Cooked Battered and Breaded Fish Portions

## WFLO Research Project #147

By

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# Executive Summary

New refrigerated foods with extended shelf life present a highly profitable opportunity for food processors. Packaging products under vacuum (VAC) and modified atmosphere packaging (MAP) conditions can significantly extend shelf life of refrigerated raw and fully cooked fishery products. However, several pathogens (such as *Clostridium botulinum*) can grow at refrigerated temperatures, and for some pathogens ingestion of only a few cells can cause illness.

This research project sought to determine the safety of refrigerated raw flounder fillets and fully cooked battered and breaded Pollock portions in reduced oxygen packaging (ROP).

A sensory panel rejected many of the tested samples because of odor or appearance before dangerous toxins were formed. Some of the samples did not form toxin in the 35-day testing period. However, toxin formed in some of the samples before or at about the same time as sensory rejection. This means that consumers could potentially think that the fish is wholesome when dangerous toxins are already present. These data can help establish handling temperatures and sell-by or use-by dates to ensure the safety of these type products to consumers.

**Table 1. Summary of results**

| Type of Fish | Temperature | Packaging | Result |
|---|---|---|---|
| Raw flounder fillets | 39°F (4°C) | Aerobic | Toxin did not form |
| | | VAC | Toxin formed before sensory rejection |
| | | MAP | Toxin did not form in samples held at a constant 39°F (4°C) temperature |
| | 50°F (10°C) | Aerobic | Toxin formed after sensory rejection |
| | | VAC | Toxin formed before and/or at about the same time as sensory rejection |
| | | MAP | Toxin formed before and/or at about the same time as sensory rejection |
| Fully cooked Pollock portions | 47°F (8°C) | Aerobic | Toxin did not form |
| | | VAC | Toxin formed before sensory rejection |
| | | MAP | Toxin did not form |
| | 53°F (12°C) | Aerobic | Toxin formed after sensory rejection |
| | | VAC | Toxin formed before sensory rejection |
| | | MAP | Toxin did not form |

# Introduction

## Overview

The refrigerated and frozen food segment is gaining an increasing share of the consumer meal consumption business. Figueiredo (2000) stated that today's customer is demanding speed, ease and convenience along with nutrition, health, and food safety. Goodburn (2000) reported, that during the last twenty years the chilled prepared food product area has rapidly developed into a major segment in Europe. However, ensuring the safety of these products requires the

establishment of an effective temperature protocol, shorter product shelf life, and strict control of hygienic measures and procedures.

Consumers are demanding fresh, easy to prepare products that are safe and wholesome. New refrigerated foods with extended shelf life present a highly profitable opportunity for food processors. Extended shelf life refrigerated foods are those that have received minimal processing or pre-cooking and have an enhanced but limited shelf life, with refrigeration being a key preservation technique (Marth, 1998). Packaging products under vacuum (VAC) and modified atmosphere packaging (MAP) conditions can significantly extend shelf life of refrigerated raw and fully cooked fishery products. Such products, however, require temperature control of 38°F (3.3°C) or less throughout handling, storage, and distribution to reduce the likelihood of pathogen growth and possible toxin formation.

Microbiological concerns with VAC and MAP refrigerated fishery products focus on psychrotrophic and mesophilic pathogens that have an opportunity to grow at refrigerated temperatures during extended shelf life. Several pathogens can grow at refrigerated temperatures, and for some pathogens ingestion of only a few cells can cause illness (Marth, 1998). Common bacterial pathogens that can grow at refrigerated temperatures and are associated with fishery products include *Clostridium botulinum* Type E, *Yersinia enterocolitica,* and *Listeria monocytogenes. Salmonella* spp. and *Staphylococcus aureus* are two other common foodborne pathogens that can grow at 45°F (7.2°C) and higher temperatures. Good personnel hygienic practices, following Good Manufacturing Practices (GMPs), and adequate cooking will control several of these pathogens. However, the primary food safety concern of the US Food and Drug Administration (FDA) for VAC and MAP refrigerated fishery products is the possible growth of *C. botulinum* and subsequent toxin production during prolonged refrigerated storage and distribution.

Storage at 38°F (3.3°C) or less will prevent the growth of *C. botulinum* in these products. However, Kalish (1991) checked approximately 2,000 retail stores, including back-room storage facilities and chill cases, and found that only 37% of the products were stored within the recommended 32-38°F (0-3.3°C) range. Many refrigeration case temperatures averaged 44°F (6.7°C) with some as high as 55°F (12.8°C). Products were also found stacked on the floor without any refrigeration. Studies at the retail level have also demonstrated that product rotation procedures are sometimes inadequate, with product sales dictating product rotation frequency (Kalish, 1991). Consumers are also at risk, since refrigerator temperatures in consumer homes can range from 32 to 55°F (0-12.8°C) (Beard, 1991).

## *Clostridium botulinum*

*Clostridium botulinum* type E is a Gram positive, anaerobic, spore-forming foodborne pathogen. It is a member of the non-proteolytic, psychrotrophic group comprised of *C. botulinum* B, F, and E. *C. botulinum* Type E is isolated from water, aquatic sediments, and organisms. Estimated concentrations of *C. botulinum* Type E range from 1-200 per kg, although some studies have indicated 2,000-3,000 spores/kg (Lund and Peck, 2000). This organism is indigenous to the aquatic environment, and fish is an excellent substrate for growth. Spores can survive cooking or adequate heating. The organism also has ability to grow at temperatures as low as 38°F (3.3°C) (Eklund et al., 1982; 1992) or even down to 37°F (3.0°C) (Graham et al., 1997). Eklund (1992) identified the following conditions for foodborne illness from *C. botulinum* to occur:

1. The food must be contaminated with spores or vegetative cells.

2. The processing treatment must be inadequate to inactivate spores, or the product must be recontaminated after processing.
3. The food must support growth and toxin formation when temperatures exceed 38°F (3.3°C).
4. The food must be consumed without cooking or after inadequate heating to inactivate preformed toxin.

The use of MAP and VAC can increase the shelf life of refrigerated fish products (Reddy et al., 1992) by inhibiting aerobic spoilage bacteria. However, VAC and MAP will not inhibit the growth of *Clostridium botulinum* (ICMSF, 1996). Fish inoculated with high numbers of spores and stored under VAC or MAP conditions have become toxic within 6-8 days during refrigerated storage when temperatures approached 50°F (10°C) (NACMCF 1992). This is a concern, since in distribution and retail storage, product temperatures have been found to fluctuate between 40 and 50°F (4.0-10°C) (NACMCF 1992).

## FDA Position on Reduced Oxygen Packaging (ROP)

The FDA currently requires that ROP un-pasteurized refrigerated fishery products must be packaged using films with a minimum OTR of 10,000 cc/$m^2$/24h@70°F, unless time/temperature indicators are used as a control procedure or inoculated pack studies have been conducted under moderate temperature abuse conditions showing that the product is safe when using a higher barrier film.

# Objectives

1. Determine the safety of refrigerated ROP raw flounder fillets packaged using films with an oxygen transmission rate (OTR) of 3,000 cc/$m^2$/24h@70°F and 7.3 cc/$m^2$/24h@70°F.
2. Determine the safety of refrigerated ROP fully cooked battered and breaded Pollock portions packaged using films with an OTR of 6,000 cc/$m^2$/24h@70°F and 7.3 cc/$m^2$/24h@70°F.

# Materials and Methods

For the aerobically packaged refrigerated raw flounder fillets, a film with an OTR of 3,000 cc/$m^2$/24h@70°F was used. For the aerobically packaged refrigerated fully cooked battered and breaded Pollock portions a film with an ORT of 6,000 cc/$m^2$/24h@70°F was used. (Note: The FDA considers these films as ROP).

For the vacuum (VAC) and modified atmosphere packaged (MAP) (100% $CO_2$) refrigerated raw flounder and refrigerated fully cooked Pollock portions, a film with an OTR of 7.3 cc/$m^2$/24h@70°F was used.

Refrigerated storage temperatures used in the study were 39°F (4°C) and 50°F (10°C) for the raw flounder fillets and 47°F (8°C) and 53°F (12°C) for the fully cooked battered and breaded Pollock portions.

Microbiological analyses included psychrotrophic plate counts, aerobic plate counts, and anaerobic plate counts. Analytical procedures followed the Food and Drug Administration *Bacteriological Analytical Manual* (FDA/BAM 1998).

Five strains of nonproteolytic *C. botulinum* (Type B 17B, Type E Beluga, Minnesota, Alaska, and Type F 83) were cultured anaerobically and spore crops were created using procedures as described in Anellis, et. al (1972). Spores were enumerated using serial dilutions in buffered peptone water and anaerobically incubated in TYGPT roll tubes, then diluted to a final concentration of $10^2$-$10^3$/ml with sterile buffered peptone water, then stored in vials at -112°F (-80°C) until needed.

Detection of *C. botulinum* toxin followed the general protocol as outlined in the FDA *Bacteriological Analytical Manual* (FDA/BAM 1998). Mice were observed periodically for symptoms of botulism for 48 h. Botulism signs typically began within the first 24 h with ruffling of fur, followed in sequence by labored breathing, weakness of limbs, total paralysis with gasping for breath, and finally death due to respiratory failure.

## Sensory Evaluation

Sensory analysis was performed in conjunction with the microbiological analysis and toxin detection. The fillets were held frozen at -112°F (-80°C) until the day of the sensory analysis. Samples were thawed at room temperature, allowed to equilibrate at 39°F (4°C), and presented as whole fillets to three untrained panelists, who were vaccinated against *C. botulinum* toxin. The panelists held packaged fish samples a minimum distance of 8 in. from the nose. If an odor could not be identified, the package was brought closer to the nose. A 5-point hedonic scale was used to determine characteristics of odor and appearance (1-little or no odor; 5-putrid, trash like odor, musty off odor). Normal rejection was given a numerical value of 4 while a score of a 5 was reserved for a product that had deteriorated to the point of absolute rejection (i.e., if the consumer had purchased it and were starving to death they would not consider consuming it) (Post, et. al.,1985).

# Results and Discussion

## Study 1: Refrigerated Raw Flounder

### Aerobically Packaged Flounder

At 39°F (4°C) the flounder spoiled in the aerobic package (i.e., 3,000 cc/$m^2$/24h@70°F) after 15 days, but no toxin was formed even after 35 days of refrigerated storage (Table 2).

In inoculated aerobically packaged refrigerated raw flounder fillets stored at 50°F (10°C), absolute sensory rejection (a score of 5-putrid rejection) occurred on day 5, while toxin formation occurred on day 8 (Tables 2 and 3). These data indicate that refrigerated raw flounder fillets are safe when packaged using a film with an OTR $\geq$3,000 cc/$m^2$/24h@70°F.

### Vacuum and Modified Atmosphere Packaged Flounder

At 39°F (4°C) microbial shelf life for the VAC product was extended to more than 22 days, but toxin developed on day 20 (Table 2). (Note: These aquacultured fish were extremely high quality. They were removed directly from the culture tanks and processed immediately. It is unlikely that commercial fish fillets would have a shelf life of >22 days).

At 39°F (4°C) for MAP (100% $CO_2$) fillets, microbial shelf life was extended for more than 37 days. Toxin formed in one MAP replication where the incubator malfunctioned and the initial temperature was 48-52°F (9-11°C) for 48h followed by 41°F (5°C) for 48h prior to continued storage at 39°F (4°C). Toxin did not form in any other MAP replication where the temperature was maintained at 39°F (4°C) (Table 2).

At 50°F (10°C), the VAC fillets were toxic at day 9 (Tables 2 and 3). The aerobic plate counts (APC) indicated microbial spoilage, but the mean sensory odor score was 2.7 and the mean sensory appearance score was 1.0 (Table 3). For the MAP (100% $CO_2$) fillets, at 50°F (10°C) microbial spoilage occurred on day 9, and toxin was formed by day 9 (Tables 2 and 3), but the mean sensory odor score was 2.0 and the mean sensory appearance score was 1.7 (Table 3). A summary of sensory scores, toxin formation and flounder spoilage at 50°F (10°C) are presented in Table 3.

**Table 2. Raw flounder fillets**

| Storage Temperature | Packaging | Approximate Days to Microbial Spoilage | Initial Days of Toxin Formation |
|---|---|---|---|
| 39°F (4°C) | Aerobic | 15 | >35* |
| | VAC | >22 | 20 |
| | MAP | >37 | 25** |
| 50°F (10°C) | Aerobic | 5 | 8 |
| | VAC | 9 | 9 |
| | MAP | 9 | 9 |

*No samples were analyzed for toxin after day 35. ** Toxicity occurred in one replication (out of 7 replications) when the initial 48 h of incubation was at 48-52°F (9-11°C) followed by 48 h at 41°F (5°C).

**Table 3. Sensory scores, toxin formation and aerobic plate counts[a] in flounder stored at 50°F (10°C)**

| Packaging Type | Days of Storage | Toxin Detected | Mean Odor Score[d] | Mean Appearance Score[d] | Mean APC Log CFU/g |
|---|---|---|---|---|---|
| Aerobic[b] | 4 | No | | | |
| | 6 | No | | | |
| | 7 | No | | | |
| | 8 | Yes | 5.0 | 3.0 | 8.3 |
| | 9 | Yes | | | |
| Vacuum[c] | 7 | No | | | |
| | 9 | Yes | 2.7 | 1.0 | 7.8 |
| | 10 | Yes | 3.3 | 1.3 | 8.2 |
| | 11 | Yes | 4.2 | 1.5 | 8.1 |
| 100% $CO_2$[c] | 9 | Yes | 2.0 | 1.7 | 7.8 |
| | 12 | Yes | | | |
| | 15 | Yes | 1.7 | 1.0 | 7.3 |
| | 17 | Yes | 2.0 | 1.0 | 7.0 |
| | 18 | Yes | 1.5 | 1.8 | 6.8 |

[a] 5 samples were used to determine sensory and APC mean values.
[b] OTR 3,000 cc/$m^2$/24h@70°F.

[c] OTR 7.3 cc/m$^2$/24h@70°F.
[d] Hedonic rating 1-5, where 1 is best quality and 5 is spoiled putrid product.

## Study 2: Refrigerated Fully Cooked Pollock Portions

Unlike the raw flounder, the fully cooked battered and breaded Pollock became moldy, but never developed typical spoilage odors. At 47°F (8°C), after 35 days of refrigerated storage, the aerobically packaged refrigerated Pollock portions (i.e., 6,000 cc/m$^2$/24h@70°F) developed yeast-like odors and mold was present on the portions, but toxin did not form (Table 4). The APCs for the aerobically packaged portions were 7.6 log CFU/g (microbial spoilage), and mean appearance scores were 3.9, which is just shy of normal sensory rejection (a score of 4-normal rejection) (Table 5). No yeast-like odors or mold were present on the VAC and MAP (100% $CO_2$) portions. Toxin did not form in the MAP product at the storage temperature of 47°F (8°C) (Table 4). In VAC portions stored at 47°F (8°C), however, toxin formed on day 25 when the mean sensory appearance and mean sensory odor scores were still acceptable at 1.8 and 3.8, respectively (Tables 4 and 5).

**Table 4. Fully cooked Pollock portions**

| Storage Temperature | Packaging | Approximate Days to Spoilage (Mold) | Initial Days of Toxin Formation |
|---|---|---|---|
| 47°F (8°C) | Aerobic | >35 | >35* |
| | VAC | >35 | 25 |
| | MAP | >35 | >35* |
| 53°F (12°C) | Aerobic | 20 | 25 |
| | VAC | >35 | 25 |
| | MAP | >35 | >35* |

*No samples were analyzed for toxin after day 35 (aerobic and MAP)

At 53°F (12°C) mold developed on the aerobically packaged refrigerated Pollock portions (day 20), but not on the VAC and MAP products (>35 days) (Table 4). At 53°F (12°C), toxin formed (day 25) in the aerobically packaged after mold development (day 20) (Tables 4 and 6). In contrast, the VAC product developed toxin (day 25) when the mean sensory appearance score (2.3) and sensory odor score (2.0) still indicated an acceptable product (Tables 4 and 6).

At 53°F (12°C), toxin did not form in the MAP (100% $CO_2$) Pollock portions during refrigerated storage (>35 days) (Table 4). On day 25, mean sensory appearance and mean sensory odor scores for these MAP Pollock portions were 1.2 and 1.5, respectively, indicating an acceptable product (Table 6).

**Table 5. Sensory scores, toxin formation, and aerobic plate counts[a] in refrigerated breaded and battered Alaskan Pollock stored at 47°F (8°C)**

| Package Type | Days of Storage | Toxin Detected | Mean Odor Score[d] | Mean Appearance Score[d] | Mean APC Log CFU/g |
|---|---|---|---|---|---|
| Aerobic[b] | 20 | No | 2.0 | 2.7 | 5.0 |
| | 25 | No | 1.9 | 3.1 | 4.5 |
| | 30 | No | 2.0 | 3.9 | 7.3 |
| | 35 | No | 2.7 | 3.9 | 7.6 |

| | | | | | |
|---|---|---|---|---|---|
| Vacuum[c] | 20 | No | 1.8 | 1.7 | 5.1 |
| | 25 | Yes | 3.8 | 1.8 | 6.0 |
| | 30 | Yes | 3.3 | 1.8 | 7.3 |
| | 35 | No | 1.5 | 2.0 | 6.2 |
| 100% $CO_2$[c] | 20 | No | 1.3 | 1.3 | 4.6 |
| | 25 | No | 1.1 | 1.2 | 4.9 |
| | 30 | No | 1.6 | 1.4 | 2.7 |
| | 35 | No | 1.7 | 1.1 | 2.0 |

[a] 5 samples were used to determine sensory and APC mean values.
[b] OTR 6,000 cc/$m^2$/24h@70°F.
[c] OTR 7.3 cc/$m^2$/24h@70°F.
[d] Hedonic rating 1-5, where 1 is best quality and 5 is moldy product.

**Table 6. Sensory scores, toxin formation and aerobic plate counts[a] in refrigerated fully cooked breaded and battered Alaskan Pollock stored at 53°F (12°C)**

| Packaging Type | Days of Storage | Toxin Detected | Mean Odor Score[d] | Mean Appearance Score[d] | Mean APC Log CFU/g |
|---|---|---|---|---|---|
| Aerobic[b] | 20 | No | 2.9 | 5.0 | 4.5 |
| | 25 | Yes | 3.0 | 5.0 | 4.0 |
| | 30 | Yes | 2.0 | 5.0 | 9.1 |
| | 35 | No | 2.6 | 5.0 | 8.1 |
| Vacuum[c] | 20 | No | 1.8 | 1.9 | 5.5 |
| | 25 | Yes | 2.0 | 2.3 | 5.8 |
| | 30 | Yes | 2.3 | 2.6 | 6.7 |
| | 35 | Yes | 3.0 | 2.3 | 7.0 |
| 100% $CO_2$[c] | 20 | No | 3.2 | 1.0 | 4.3 |
| | 25 | No | 1.2 | 1.5 | 3.1 |
| | 30 | No | 1.8 | 1.3 | 5.3 |
| | 35 | No | 1.5 | 1.2 | 6.1 |

[a] 5 samples were used to determine sensorial and APC means.
[b] OTR 6,000 cc/$m^2$/24h@70°F.
[c] OTR 7.3 cc/$m^2$/24h@70°F.
[d] Hedonic rating 1-5, where 1 is best quality and 5 is moldy product.

# Conclusions

## Refrigerated Raw Flounder Fillets

1. Absolute sensory spoilage and microbial spoilage occurred prior to toxin formation in refrigerated raw flounder fillets packaged using a film with an OTR of 3,000 cc/$m^2$/24h@70°F and stored at 39°F (4°C) or 50°F (10°C).
2. Toxin formed in VAC fillets (OTR 7.3 cc/$m^2$/24h@70°F) on day 20 and microbial spoilage occurred >22 days at 39°F (4°C).
3. Toxin formed in VAC fillets (OTR 7.3 cc/$m^2$/24h@70°F) on day 9 and microbial spoilage occurred on day 9 at 50°F (10°C).
4. Toxin did not form in MAP fillets (100% $CO_2$ and OTR 7.3 cc/$m^2$/24h@70°F) when held at a constant temperature of 39°F (4°C).

5. Toxin formed in MAP fillets (100% $CO_2$ and OTR 7.3 cc/$m^2$/24h@70°F) on day 9 and microbial spoilage occurred on day 9 at 50°F (10°C).

## Fully Cooked Refrigerated Battered and Breaded Pollock Portions

1. There was no correlation between APC and toxin formation for fully cooked refrigerated battered and breaded Pollock portions.
2. Mold did not form and toxin was not present in the aerobically packaged product (OTR 6,000 cc/$m^2$/24h@70°F) stored at 47°F (8°C) for 35 days.
3. Mold formed (absolute sensory rejection for appearance) on day 20 and toxin was present on day 25 in the aerobically packaged Pollock portions (OTR 6,000 cc/$m^2$/24h@70°F) at 53°F (12°C).
4. Toxin formed in the VAC Pollock portions (OTR 7.3 cc/$m^2$/24h@70°F) on day 25 when stored at 47°F (8°C) and 53°F (12°C). Mold formation did not occur in the VAC Pollock portions (OTR 7.3 cc/$m^2$/24h@70°F) even after 35 days at 47°F (8°C) and 53°F (12°C).
5. Toxin never formed in MAP (100% $CO_2$) Pollock portions (OTR 7.3 cc/$m^2$/24h@70°F) when stored for more than 35 days at 47°F (8°C) and 53°F (12°C).

## General Guidelines

The following general guidelines are appropriate for all ROP refrigerated fishery products:

1. Incorporate ROP processes for refrigerated fishery products into HACCP plans.
2. Refrigerated storage of ROP fishery products requires storage temperatures of 38°F (3.3°C) or less to ensure product safety from the time of packaging through distribution and storage by the consumer.
3. Safe handling of all ROP refrigerated fishery products requires the maintenance of proper product temperatures from packaging through consumption.
4. A "Keep Refrigerated" label is required on each master carton and on each individual package.
5. A "Use By Date" on each package is highly recommended.

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