ARROWTOOTH FLOUNDER
PROTEASE INHIBITOR INJECTION PROJECT

FINAL REPORT

October 1, 1990

ABSTRACT: The estimated potential annual yield of arrowtooth flounder *Atheresthes stomias* off of Alaska is 300,000 mt per year. Yet this species is not harvested in any significant amount because of an enzyme related flesh softening problem. Additives formulated to inhibit these troublesome enzymes and methods to apply the additive were developed and tested on arrowtooth fillets. The results, although inconclusive, indicated that both the inhibitor and the application methodology can produce a marketable fillet. The main outstanding concern is whether or not the method is commercially viable.

Prepared for:

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by:

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Kodiak, Alaska 99615
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Arrowtooth Flounder Protease Inhibitor Project

I. Introduction

AFDF and the Alaska seafood industry are currently involved in a project to demonstrate the feasibility of harvesting, processing and marketing Alaska flatfish (flounder and sole). The application of existing processing technologies to Alaskan flatfish harvests has resulted in successful flatfish processing by Eagle Fisheries and two other Alaskan seafood processors.

Despite this success, a major portion of the Gulf of Alaska flatfish resource remains underutilized. Sixty percent of the flatfish biomass is made up of arrowtooth flounder (*Atheresthes stomias*) which have an inherent flesh softening tendency caused by a myxosporean parasite. This parasite triggers an enzymatic degradation of the fish flesh at certain temperatures.

This project was designed to attempt one approach to solving this softening problem using a chemical injection process. The use of Food and Drug Administration (FDA) approved flavorings containing carbonic acid (a source of ionic-active polysaccharides including a soya protein isolate, plasma, and hydrolyzed plant or animal muscle extracts known for their enzyme blocking capabilities) was the key to this project.

Arrowtooth flounder is the third largest biomass among the flatfishes of the North Pacific. About 300,000 metric tons (mt) of arrowtooth are available for commercial harvest per year off of Alaska. Approximately 5,000 mt are harvested as by-catch every year, and, of that amount, 720 mt are used in various specialty foods. Far fewer arrowtooth are used than potentially could be processed into wholesome, acceptable foods. Given no other limitations such as harvest restrictions, the arrowtooth resource conceivably could produce more than 100,000 mt of finished product.

Arrowtooth flounder are harvested at sizes up to two and a half feet, though 18-20" is the average. Fillets average 8-10 oz., and the fishing season is year-round. Obviously, arrowtooth flounder has the potential to be an important resource for the fisheries of Alaska.[1]

But like many fish, arrowtooth flounder have a protease in their muscle that is released soon after the death of the fish. The extent to which the muscle texture breaks down due to this protease depends on the species of fish. Myxosporean parasites and the bacteria that are present on the skin and in the stomach lining penetrate into the flesh and cause the flesh to deteriorate.

In arrowtooth flounder, the inherent protease causes a more extreme breakdown of muscle texture than occurs in other flatfish. However, the breakdown that occurs naturally due to the myxosporean parasites and bacteria present is quite minimal compared to the rapid textural softening that results from protease activity during cooking.[2] Primarily as a result of this latter effect, the arrowtooth muscle has a poor reputation in the market place. Because the fish texture softens so much during cooking, arrowtooth is unusable as a mince or surimi raw material. At present, arrowtooth flounder caught by flatfish bottom trawlers are either discarded or sold at a very low price.

It has been reported that the activity of alkaline proteases in shrimp was somewhat identical with trypsin breakdown of proteins.[3] It also has been recognized in previous studies that alkaline proteases are cysteine site protease.[2,3] Reports also indicate that serine protease inhibitors also display inhibitory activity beneficial to the muscle texture of seafoods.[2,3]

II. Methods

Phase I: McFarland's Foods, January/February 1990

Because of the information on the enzyme's activities, efforts were made to identify ingredients for inhibition which have been documented to block both the serine and cysteine site. Several food grade ingredients (GRAS) commonly used in the food industry are known to contain the serine-site and cysteine-site protease inhibitors. In addition, other properties of ingredients were considered, such as water holding capacity, binding (external and internal) properties with muscle proteins, osmotic strength, freeze-thaw stability, flavor and antioxidant properties.

A colorimetric method was modified to test the effectiveness of selected ingredients and trypsin inhibition.[3,4,5,6,7] Various blends of natural flavoring consisting of beef extract, albumin proteins and egg white proteins blended in an acidic sterile solution of chicken broth containing various phosphates and EDTA were used. Fresh fish were soaked in and/or injected with different quantities of each ingredient, both singly and in combination, and were tested for alkaline protease inhibition in the fish muscle. In addition, the inhibitor-treated fish were evaluated organoleptically for texture, flavor and smell, in both fresh and frozen condition.

Preliminary soak tests were conducted by marinating the fish in the ingredient; the product was then evaluated for organoleptic properties of fish. It was found that proper osmotic strength and pH were necessary for the inhibitor to penetrate into the muscle to reach the protease and thereby inhibit the enzymes and preserve the firmness in fish muscle.

The tests were conducted as follows:

1/22/90 - Ingredients were tested on trypsin inhibition (tryspin was obtained from Sigma Chemical Co.) both singularly and in various combinations.

1/25/90 - Protease inhibition tests were conducted by soaking the fish in solutions made with the ingredients selected.

2/2/90 - Protease inhibition tests were conducted by injecting the ingredients into fish. Then alkaline protease was extracted by methods similar to those used in (2), and percentage of inhibition was evaluated.

2/15/90 - Protease inhibition tests were conducted by injecting the ingredients at different levels singly and in combination, and the effect was evaluated by percentage of inhibition.

2/23/90 - Tests were conducted to speed up digestion of the inhibitor by the fish flesh using catalysts. This was done during the evaluation of the protease inhibition.

Since the results revealed no major concerns, four formulas were selected which combined the optimum ingredients considering the limited budget and limited processing window of Kodiak in February and March. It was also determined from efforts that injection of the fish with the protease inhibiting ingredients was better than the soaking method.

Four experimental blends of ingredients and the processing equipment were sent to All Alaskan Seafoods. This equipment and process of injecting and vacuum tumbling, which we proposed to use, has been proven for more than forty years in the ham and bacon industry to disperse materials such as sodium nitrite accurately and safely throughout the muscle structure. This process has become the worldwide commercial method for quick curing. Also, it is the only commercial method known to accomplish the dispersion of meat cures in minutes. Worldwide, injection and tumbling is the preferred quick method of preparation for solid protein stabilization and it was determined that it was likely to be applicable for fish fillets.

Phase II: All Alaskan Fisheries, March 1990

Whole arrowtooth flounder were delivered to All Alaskan Seafoods by several large trawlers. The holding medium was refrigerated sea water. The arrowtooth flounder were caught in the last few hours of each vessel's trip and delivered to the plant with a core temperature of 31 to 33 degrees F. After unloading and sorting, arrowtooth were layer iced in totes and then processed. No fish used in this project were more than 24 hours old.

Each day that arrowtooth flounder were processed, they would first be size graded so anything that could be cut on the Baader 175 would be processed on that line and anything over 19" would be hand filleted. After the filleting was accomplished, the fillets were skinned on either a Baader 47 Skinner or a Baader 52 Skinner. After skinning, all fillets were candled for parasites.

After candling and trimming were completed, the fillets were run through the Fomaco 2080 injector and injected with the inhibitor solutions. The seasoning inhibitor concentrates were reconstituted with 4 ppm. chlorine. The solutions were prepared in a Hobart mixer and filtered prior to injection. After they were injected, the fillets were batched in 100/lb lots and placed in the Varico 75 vacuum tumbler to evenly distribute the seasoning inhibitor throughout the fillet. Upon completion of the vacuum tumbling, the fillets were then IQF frozen in a Sanvick IQF tunnel freezer or block frozen in 16.5/lb blocks in a plate freezer (Figure 1).

In addition to this procedure, some fillets were run through the identical process with the exception of skinning. This method was proposed as the method to produce minced arrowtooth flounder by running the skin on fillet through a Baader 697 deboner. A small portion of lot one was garnished with the sauces and vacuum packed on typical grocery store trays. The sauces included hollandaise, cajun, barbecue and Dijon. The sauces, packaging and portioning were designed to duplicate typical retail products which hace been secondarily processed.

Phase III: All Alaskan Fisheries, March 1990

A large batch of a modified and improved inhibitor was premixed. This produced approximately 10,000 lbs. of injected fillets. The inhibitor was mixed and applied with flavor additives. Halibut flavoring and two commercially available fish flavors (FCI-66 and FCI-11017 from Flavor Consortium, Inc.) were tested. The percentage of flavoring known to contain enzyme inhibitors was increased about 15% and the buffer's pH raised.

III. Results

During the Phase I development of inhibitor solutions the results indicated no problems with the solutions. Four formulations were selected for production based on performance on small scale samples. Percent enzyme inhibition varied from zero to ninety-four for various ingredients. Results also indicated that injection was a better application method than a simple soak in terms of inhibition of the enzme (Table 1).

Figure 1. Process Flow for Injected Arrowtooth Flounder Fillet Production

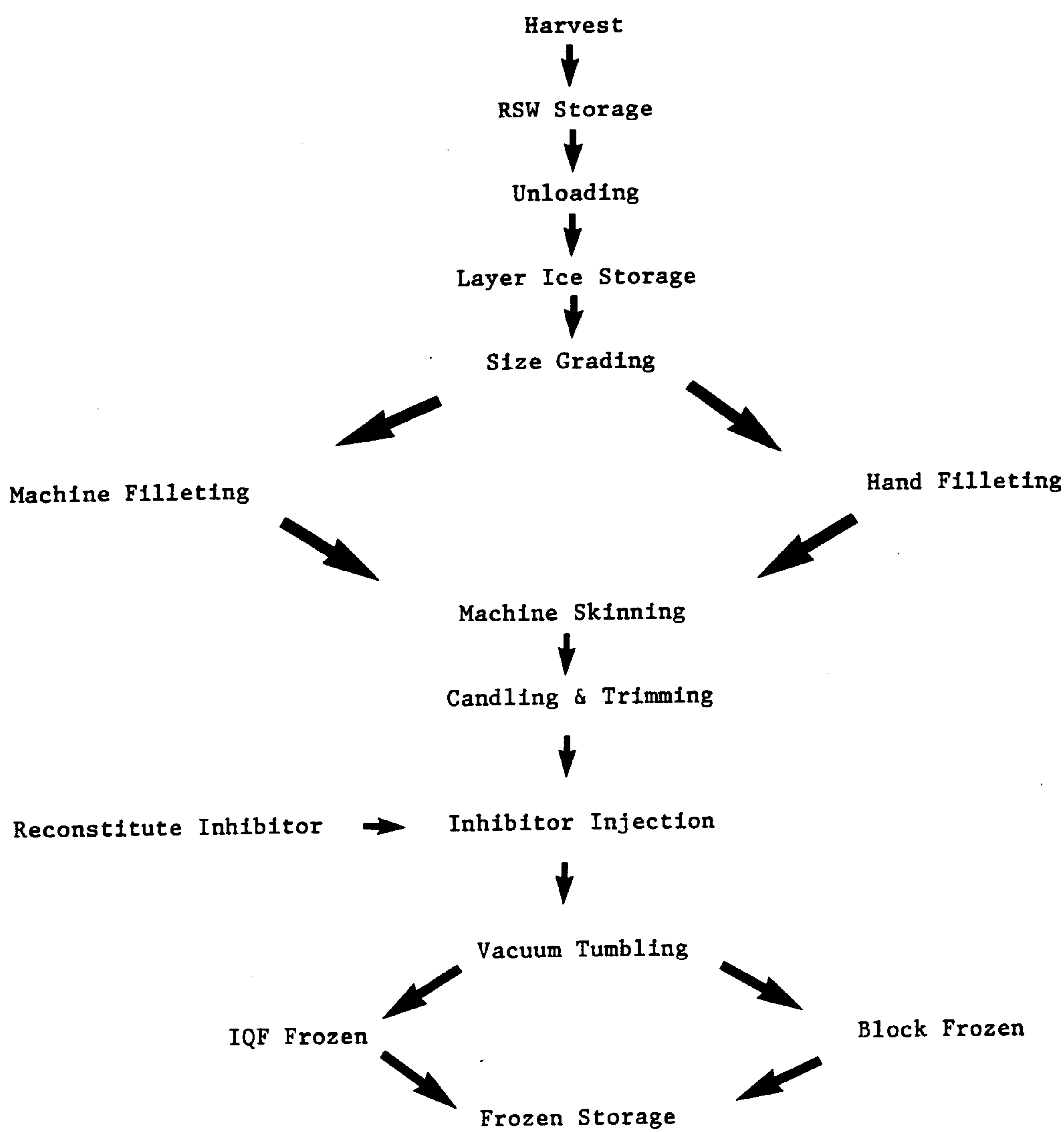

Table 1. Phase 1 Inhibitor Test Results

	% Enzyme Inhibition Soak Method*	% Enzyme Inhibition Injection Method*
Ingredient 1		
Concentration A	60	94
Concentration B	—	85
Concentration C	—	93
Concentration D	—	89
Ingredient 2		
Concentration A	—	94
Concentration B	—	45
Concentration C	—	70
Concentration D	—	74
Ingredient 3		
Concentration A	60	60
Concentration B	—	74
Concentration C	—	60
Ingredient 4		
Concentration A	—	16.7
Concentration B	—	58.4
Ingredient 5		
Concentration A	—	0
Concentration B	—	34
Concentration C	—	56
Ingredient 6		
Concentration A	—	21

* Procedure adapted from: Characteristics of an alkaline proteinase and exopeptidase from shrimp (<u>Penaeus indius</u>) muscle. S.N. Doke and V. Ninjoor. Vol. 52, #5. 1987, pp.1203 -1208, Journal of Food Science.

During Phase II several pieces of new and valuable information were collected. Arrowtooth flounder landings were over maximum size range of the machine filleter (19 inches) up to eighty percent of the time. This forced a lot of the filleting to the hand line which was time consuming and labor intensive. In addition, arrowtooth flounder carry a higher number of parasites than other smaller flatfish. They are similar to Pacific cod in the amount of time required for adequate candling.

Recovery rates for arrowtooth flounder were much higher than the typical rates for flatfish which range around 25 - 28%. The results indicate a yield of 36 - 38% for untreated fillets and up to 44% for injected fillets.

Despite the good recovery rates plant labor costs were high because of the required sorting, hand filleting, candling, hand feeding of the injector and batch feeding of the vacuum tumbler. Labor costs were $.68/lb before freezing and totaled nearly $.78/lb through shipping. Costs could certainly be lowered if this process was used on a regular basis, but with current market prices for this product it is an obstacle.

During the injector needles would occasionally plug. Small meat particles from the fillets over flowed the filter into the pump. The needles can be kept clean easily with close attention to the filter. However, in an industrial installation, a large automatic self cleaning filter would be used to completely eliminate the needle plugging. Also, a normal HACCP quality controlled operation would include removing all needles for inspecting and cleaning daily.

One other problem during phase I was inadequate dissolution of the inhibition ingredients in the injection solution. Because of this, ingredient modifications were made for Phase II. The cost of the ingredients was approximately $.50/lb. during Phase I.

Of the formulation lots tested under Phase II only two lots were determined to be worthy of analysis. They had both positive and negative results.

Arrowtooth flounder fillets picked up between five and six percent gain in weight during the injection process. Fillets coming out of the vacuum tumbler had a typical fresh fish surface shine, intact muscle bundles, and resulted in significantly preserved flake structure in cooked flesh.

Shelf life tests were very positive in both fresh and frozen forms. Fresh product was held at 40 degrees F for up to ten days with no odor or loss of moisture. Flavor was maintained at an acceptable level. Frozen shelf life tests conducted over three months were also encouraging. Treated samples had less than three percent freezer burn while untreated samples had twelve to fifteen percent freezer burn. Frozen samples were held in a small holding freezer which is very susceptable to temperature fluctuations and, because it is a blast freezer, it often causes rapid dehydration.

Evaluations of the phase II injected arrowtooth flounder consisted of casual in-house cooking and testing evaluations, formal texture and sensory panel analyzes and sample distributions to seafood buyers. The in-house cooking included microwave and frying pan preparation and had very piositive results. A blind test was set up in All Alaskan's quality control laboratory in Kodiak. Four frozen fillets; including Dover, yellow fin, rock and treated arrowtooth were retrieved from the freezer. The fillets were marked and a new teflon coated pan was used and heated to 350 deg. F. for the test. Each fillet was cooked in a hot pan to an internal temperature 180 deg.F. and placed under a paper towel to keep the identities confidential. The plant manager, the production supervisor, the flatfish supervisor, and staff form McFarlands' Foods tested the products. The rating was unanimous with arrowtooth first, rock second; and Dover and yellowfin, third and fourth. Similar tests were performed with the microwave cooked fillets and the results were similar, good texture and flavor but still soft by All Alaskan standards.

The sensory and textural evaluation were reported on June 8, 1990 by Dr. Jerry Babbitt Laboratory Director of National Marine Fisheries Service (NMFS) Utilization Research Laboratory in Kodiak (see attachment 1). No substantial differences in texture or sensory properties between treated and untreated arrowtooth flounder fillets were found.

Samples were sent to 16 buyers of flounder and sole. Both IQF and block frozen samples were sent for the buyers evaluation and comments. Of the 16 buyers that samples were sent to, Globe Seafoods Inc in Odgen, Utah was the only favorable response and the only buyer that showed any interest in the product.

A list of sample recipients is attached to this report (attachment 2). The major buyers Gortons, Mrs. Pauls and Fishery Products International reported mixed results. Mrs. Pauls was the most critical of the three majors and said not to send any more arrowtooth samples because of the cost to go through their sampling procedure, and the lack of any positive results. Gortons response stated that microwave cooking had a acceptable "mouth feel" although they still classified it as soft, and oven baking was softer yet, more typical of untreated samples sent along with treated samples for control purposes. Fishery Products simply classified it as soft and unacceptable for their use.

The fillets from Phase II which packaged with sauces were sampled informally in house, after frying and were found to be very acceptable.

Finally, the injected skin-on fillets which were minced showed promise. The resulting mince maintained good texture after short term frozen storage. However, the skins from the injected fillets stuck to the machine rollers and this may limit the feasibility of this approach.

Phase III results were much more positive and signalled a significant step forward toward utilizing arrowtooth flounder. The inhibitor solution was much more easily dissolved for injection and the cost for the formulation was reduced by sixty percent to $.20/lb. Evaluations of the product were again completed in both casual and formal settings. The informal evaluations occurred in-plant, at a fisheries conference, and with various seafood buyers. All results were positive including samples evaluated nearly six months after preparation. The sensory panel evaluation was once again completed by Dr. Jerry Babbitt of NMFS, (attachment 3). The tests were reported on September 7, 1990 and the appearance, odor, and flavor results were very positive. Texture appearance and mouth feel were still in the soft range but overall the fillets were considered to be quite acceptable.

IV. Discussion

This project effectively verified the problems with arrowtooth flounder and documented an inhibitor blend and an application method which has the potential to make this large resource available for development. Texture even under the best case was never firm although the best inhibitor and/or microwave cooking certainly bring it up to an acceptable firmness. The application method seems adequate although full production would require larger capacities and less labor to be cost effective. The automated filleting process may need to be improved so that speed and fish size ranges are increased. Harvesting of arrowtooth flounder seems to be restricted only by potential bycatch of halibut as populations and availability are both extremely high.

One major hurdle for arrowtooth flounder is its name. Buyers have long associated it with soft flesh from past experiences. A name change, use of a regionally recognized name, or selling directly to secondary processors have all been suggested as solutions to this problem. Certainly, blind taste tests and recent demonstrations indicated the injected fillet are comparable to other flatfish fillets. The fact that all 10,000 lbs. of treated fillets have been marketed indicates a potential. One prime example is a test by an IGA store which sold individually wrapped fillets for $2.98/lb. and individually wrapped fillets in sauce with lemon and parsley for $5.98/lb. One other approach, which is not feasible at this time, would be a consumer education program at introducing treated arrowtooth and maintaining interest.

Future efforts should include a full scale production effort, creative secondary processing for retail markets, production cost reductions, and increased recovery efforts. Before these steps are taken, however, it is important that laboratory evaluation of the effectiveness of this approach is completed. This will enable more effective formulation of the inhibitor and, hopefully, another order of magnitude improvement in arrowtooth flesh texture and marketability.

ATTACHMENT 1

by the panelists to rate the coded fillet samples has been enclosed and the results are shown in Table 1.

Table 1. Sensory Results For Untreated And Treated Arrowtooth Flounder Fillets.

Property	Untreated	Treated
Flavor	3.2 ± 1.0	3.1 ± 1.0
Physical Texture	2.3 ± 0.9	2.7 ± 1.1
Mouthfeel Texture	2.0 ± 0.9	2.2 ± 1.3
Odor	3.8 ± 1.4	3.2 ± 1.3
Overall Rating	2.1 ± 0.8	1.9 ± 0.8

No significant differences in sensory properties were observed between the treated and untreated arrowtooth flounder fillets.

Summary.

Using standard methods to determine the textural and sensory properties of foods, no differences in texture or sensory properties of untreated and treated arrowtooth flounder fillets were found.

Instron stress at failure values for the untreated arrowtooth fillets were similar to those values reported in previous work.

Instron stress at failure values were in good agreement with sensory textural scores for untreated and treated arrowtooth flounder fillets.

Sincerely,

Jerry Babbitt

Jerry Babbitt, Ph.D.
Laboratory Director

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RATING DESCRIPTORS DEFINITION SHEET

(Score) FLAVOR (Rate each sample individually, not compared to control)

5 Sweet and/or full flavor

4 Less intense but flavor present is still characteristic of species (less meaty, but this fish still has flavor)

3 Bland, no follow through (we're just eating protein here)

2 Slightly disagreeable (faint off taste, chemical or medicinal

1 Disagreeable (would not eat this again if you could help it)

PHYSICAL TEXTURE (APPEARANCE)

5 Moderately firm flakes(smooth, concave, shell-like)

4 Soft flakes (smoth, concave, but don't hold up well after poking around with a fork)

3 Breaks along fibers (no flakes, but breaks into jagged chunks

2 Breaks across fibers (no flakes, can easily mash into a paste with fork

1 Disintegrates (no definable break)

TEXTURE IN THE MOUTH

5 Moderately firm (offers resistance to bite, fels like you are eating something "substantial") 4 Soft, but still a clean bite (you would call this fish "delicate") 3 Soft, mushy (no resistance upon impact with teeth)

2 Soft, gumy (sticks to teeth slightly after firm bite)

1 Soft, pasty (has a drying effect, seems to absorb saliva, floury)

ODOR

5 Intense briny (pleasant sea water smell, like hot salt water) 4 Faint briny

3 Neutral (no outstanding characteristic; just hot water)

2 Slightly disagreeable (please note on ballot why)

1 Medicinal or tainted

OVERALL RATING

5 Like very much

4 Like slightly

3 Neutral (wouldn't go out of the way to order or buy it)

2 Dislike slightly

1 Dislike very much

ATTACHMENT 2

Sample Recipients

Mr. John Astma, Director
Meat and Seafood Sales
D and W Food Centers
3966 44th Street
Grandville, Mi 49418

Mr. Dan Householder
Frozen Seafood Buyer
The Kroger Company
1014 Vine Street
Cincinati, Ohio 45202-119

Mr. Jim Hernandez
Frozen Buyer
Chicago Fish House
1250 W. Division
Chicago, Ill 60622

Poseidon Fisheries International Inc
P.O. Box 279
Mill Neck New York 11765

Mr. Michael E. Sobieszyk, Manager
Procurement/Marketing
Kraft, Inc.
Kraft Court
Glenview, Illinois 60025

Ms Pam Malone
Seafood Buyer
Schnucks Markets
12921 Enterprise Way
Bridgeton, Missouri 63044

Mr. Brian Stengler
Senior Seafood Buyer
Red Lobster
6770 Lake Ellenor Drive
Orlando, Florida 32809

Mr. John Gordon Jr.
Gordon Food Services
333 50th Street, P.O. Box 1787
Grand Rapids, Michigan 49501

Mrs, Pauls
Ms Barbara Belkin, Buyer
Minneapolis, Minn

The Gorton Group
Robert Gilinger/Techical Representative
International Division
327 Main Street
Gloucester, Ma 01930

Fishery Products Inc.
Wanda Landry/Manager Ineternational Sourcing Division
18 Electronics Ave, Danvers, Ma 01923

Mr. Stephen Green
Clearwater Fine Foods Inc
757 Bedford Highway
Bedford, Nova Scotia, Canada

Mr. Stephen Thompson
Jerrico
101 Jerrico Drive
P.O. Box 11988
Lexington, Ky 40579

Big Island Seafoodss
Ms Iris Newman/Director of Technical Operations
520 112th Ave N.E.
Suite 203
Bellevue Wa 98004

Fisheries Resource Development Ltd
Mr. Andrew Stark/Manager Fishery Process Technology
2021 Brunswick Street, Suite 317
Halifax, Nova Scotia
Canada B3K2Y5

Globe Seafoods Inc
Garth Christonsen/President
3287 South 1800 West
Ogden, Utah 84401

ATTACHMENT 3

TG RF

UNITED STATES DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
NATIONAL MARINE FISHERIES SERVICE

Northwest Fisheries Science Center
Utilization Research Laboratory
P. O. Box 1638
Kodiak, AK 99615
907-487-4961/Telefax 907-487-4960

September 7, 1990

CC: MEL MONSON

Mr. Gary Taylor
All Alaskan Seafoods, Inc.
101 Marine Way
Kodiak, AK 99615

RE: Shelf-Life Study of Frozen Arrowtooth Flounder Fillets.

Dear Gary:

Enclosed are the results of our evaluation of the frozen fillets I received from you on August 22, 1990. The nine fillets were packed in unmarked zip-lock bags. Thus, all the fillets were assumed to be from the same treatment and no comparisons between fillets were made. The evaluations were conducted on September 5th. The visual appearance of the fillets was noted and quality as perceived by a buyer or consumer was determined using sensory evaluations and by determination of thaw drip.

Thaw Drip.

The fillets were placed on a rack and thawed at ambient temperature for 90 min. Thaw drip was expressed as the per cent difference between the frozen and thawed weights of the fillet.

Sensory Evaluations.

Fillets were cooked in sealed aluminum pans for 15 min. in a steam cooker. Six panelists evaluated the fillets immediately after cooking for flavor, texture, odor, and overall desirability (see attached copy of sensory ballot).

RESULTS

The visual appearance of the frozen fillets was very good. The average thaw drip value of 5.8 ± 2.5 was indicative of well handled fillets. The results of the sensory evaluations are shown in Table 1. The results indicated that the fillets were flavorable, possessed very good odor, and although the texture was fai^r^ly soft (3.3), the fillets were rated by all panelists to be very good (overall rating- 4).

Table 1. Sensory Values Of Frozen Fillets.

	Mean ± Standard Deviation
Flavor	4.2 ± 0.4
Physical Texture (Appearance)	3.3 ± 0.5
Texture In The Mouth	3.3 ± 0.8
Odor	4.2 ± 0.8
Overall Rating	4.0 ± 0

Thus, from a consumers view, the sensory attributes (appearance, odor, flavor) would "over-ride" the "soft" textural properties of the fillet and even though the overall rating may not be as high as given to say halibut, the fillets would still be considered to be quite acceptable.

Sincerely,

Jerry Babbitt

Jerry Babbitt, Ph.D.
Laboratory Director

RATING DESCRIPTORS DEFINITION SHEET

(Score) FLAVOR (Rate each sample individually, not compared to control)

5 Sweet and/or full flavor
4 Less intense but flavor present is still characteristic of species (less meaty, but this fish still has flavor)
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1 Disagreeable (would not eat this again if you could help it)

PHYSICAL TEXTURE (APPEARANCE)

5 Moderately firm flakes(smooth, concave, shell-like)
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3 Neutral (no outstanding characteristic; just hot water)
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OVERALL RATING

5 Like very much
4 Like slightly
3 Neutral (wouldn't go out of the way to order or buy it)
2 Dislike slightly
1 Dislike very much