

ARROWTOOTH FLOUNDER MICROWAVE PROJECT

EAGLE FISHERIES, L.P.

FINAL REPORT

June 25, 1990

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This information was produced with funds provided through the Saltonstall-Kennedy program administered by the National Marine Fisheries Service under Cooperative Agreement #87-ABH-SK-020.

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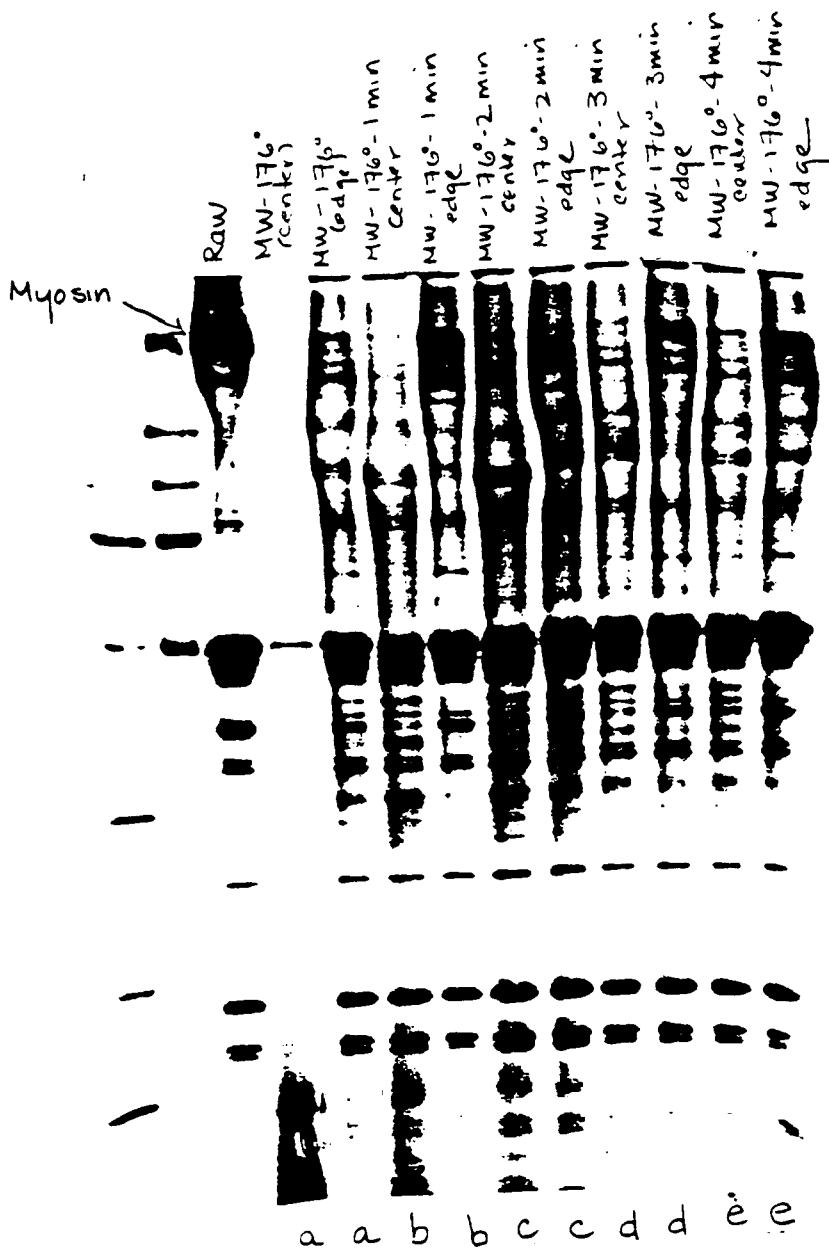
All research objectives outlined in the project extension proposal have now been completed. There is no doubt that microwaving is an effective method of inactivating the arrowtooth muscle protease. Extensive experiments at the NMFS Kodiak Laboratory using polyacrylamide gel electrophoresis (see accompanying gel photocopies) demonstrated that microwaved arrowtooth has improved texture and shows limited evidence of proteolytic activity. In the course of completing the study, several aspects regarding the use of microwaves to cook arrowtooth flounder were investigated.

1) The arrowtooth protease was reported to be inactivated at a temperature of 176° F or 80° C (Greene, D.H. and Babbitt, J.K. 1990. Control of muscle softening and protease-parasite interactions in arrowtooth flounder *Atheresthes stomias*. J. Food Sci. 55: 579-580). As we stated in the first progress report, however, simply reaching this internal temperature did not appear to be sufficient to inactivate the enzyme, so we looked at the time required to hold the product at 176° F or above in order to achieve full denaturation of the enzyme. Fresh filleted and skinned fillets were ground and formed into 100 gram (3.5 ounce, portions (standard food service size) and microwaved in C-PET trays loosely covered with 4 mil Trigon barrier film according to the following schedule:

- a) Microwave on HI until internal temperature reaches 176° F then remove from oven.
- b) Microwave on HI until internal temperature reaches 176° F then hold 1 more minute at that temperature before removing from oven.

- c) Microwave on HI until internal temperature reaches 176° F then hold 2 more minutes at 176° F.
- d) Microwave on HI until internal temperature reaches 176° F then hold 3 more minutes at 176° F.
- e) Microwave on HI until internal temperature reaches 176° F then hold 4 more minutes at 176° F.

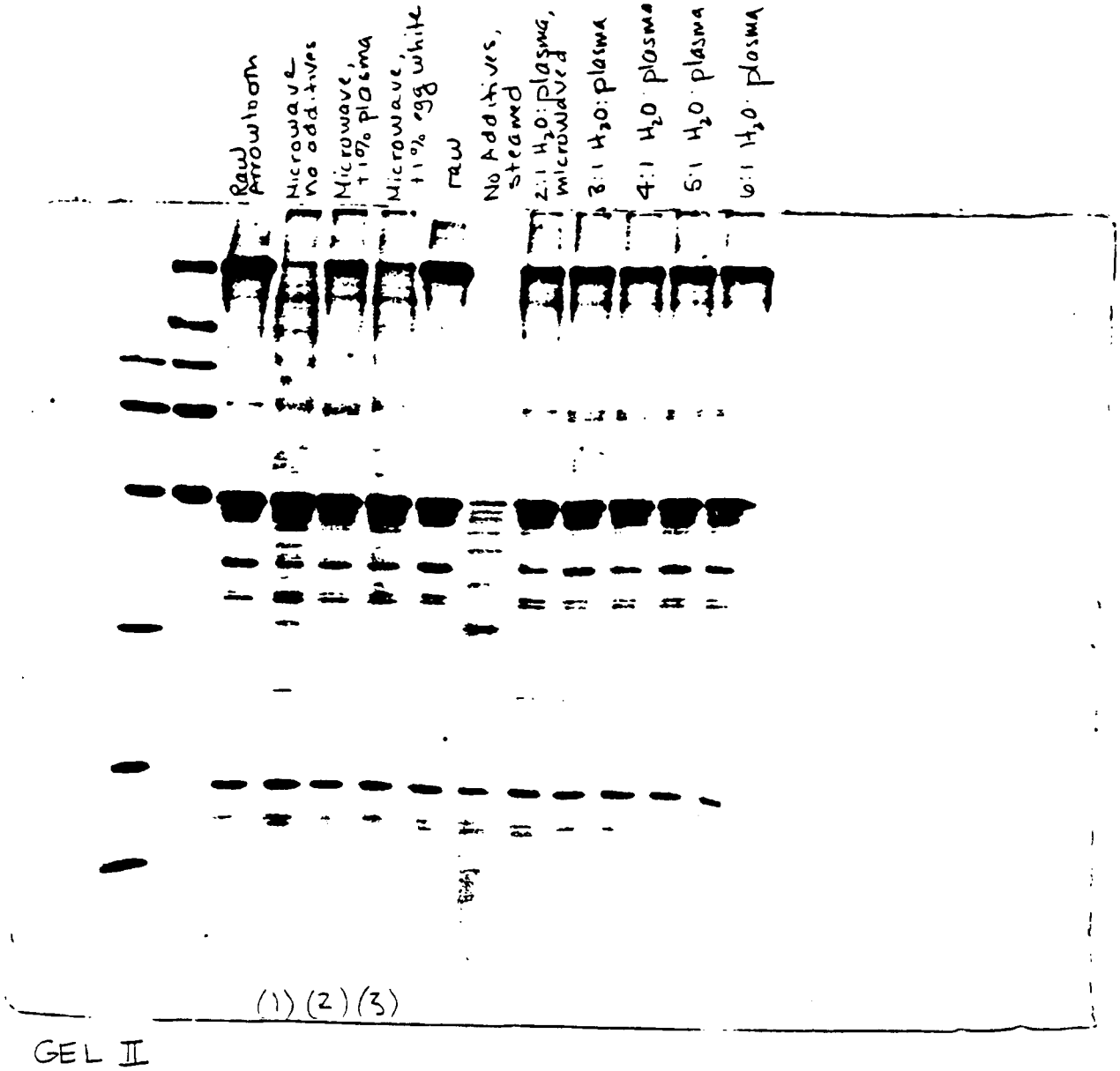
The ground portions were allowed to cool to room temperature, then three gram samples were removed from the center and edge of each portion for SDS-PAGE analysis. The important band to observe in the accompanying photocopy (I) is the one at the top, which represents myosin, the protein that is largely responsible for firm texture in "normal" fish. It is always present intact in raw arrowtooth muscle, and does not begin to degrade until heat is applied. It was clear from the electrophoresis gels that 1) it was necessary to hold the fish for at least 1 minute at 176° F and 2) the center of each fish portion was cooking a lot more slowly than the edges. It was also apparent that if the fish were allowed to go to a temperature higher than 176° F, there would be less time spent in the "proteolytic zone," or that time period when the enzyme is most active.



GEL I

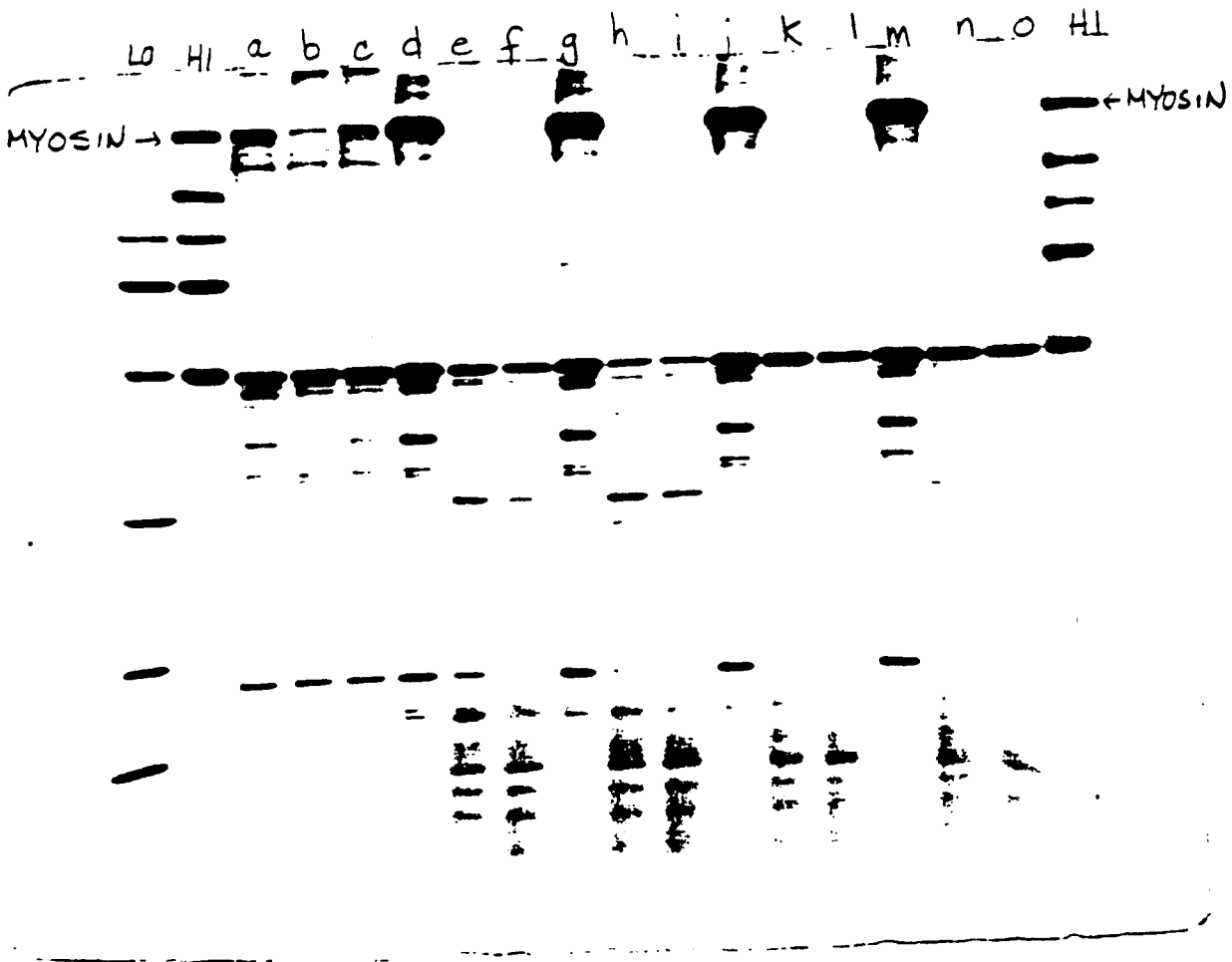
2) Realizing that the use of full power in the microwave oven was necessary, we then compared cooking times for 100 gram portions of ground rock and Dover soles. These fish were fully cooked within 1.5 to 2 minutes on HI power. Since arrowtooth flounder muscle generally contains less moisture than rock or Dover soles, it takes a little longer to cook, but after some experimentation we found that a 3.75" x 0.75" portion of ground fish will cook in 2 minutes. We then compared ground arrowtooth 1) with no additives 2) with 1% by weight powdered egg white and 3) with 1% by weight powdered beef plasma. These samples were microwaved on HI and allowed to cool to room temperature before sub-samples from the center of each fish portion were solubilized and analyzed by SDS-PAGE. The following photocopy (II) of the resulting gel (again, observe the top band, myosin) shows that microwaving alone partially halts the degradation of myosin, but that when plasma is added the myosin is better protected. The plasma was also more effective than egg white when added to ground fish. The right half of the gel compares treatments adding the same amount of plasma (1%), but adding more water to the ground fish. Increasing the plasma to water ratio from 2:1 to 3:1 improved the effectiveness of the plasma in inhibiting proteolysis, as can be seen from the darker band for myosin. There did not appear to be any additional gain by increasing the ratio of water to ground fish. The microwaved arrowtooth with plasma added at the 1% level was extremely firm, in fact more firm than the ground rock and Dover soles. It is possible that the combination of microwaving and plasma "overcompensates" for the possibility of proteolytic degradation, and less plasma is required. On the other hand, the samples with the greater water:plasma ratio were more juicy due to the incorporated water. This made the texture seem more tender, although by no means soft, and for this reason the greater

water:plasma ratio may be desirable.



3) As outlined in the project extension agreement, we also examined arrowtooth flounder that had been injected with McFarland's Foods ALAK compared to plasma and egg white treated samples. Nontreated fillets that were frozen by All Alaskan at the same time as the treated fillets were used as a "control." As explained above and in other AFDF publications (Lodestar, Spring 1990, Vol. 8, No. 1; ASTF First Quarterly Report) the textural softening of arrowtooth flounder muscle when cooked is due primarily to proteolytic degradation of myosin, the major myofibrillar protein of fish muscle. The best way to check if an ingredient is in fact a protease "inhibitor" is to incubate that substance with the fish muscle at a temperature optimum for the enzyme in the fish muscle, then check to see what, if anything, has happened to the myosin. The most definitive method available to do this is gel electrophoresis. After the fish is incubated, a reagent called sodium dodecylsulphate (SDS) is used to solubilize all the proteins in the sample (the fish "dissolves" in the SDS solution). The dissolved proteins are then applied to a polyacrylamide gel, and an electric current is then run through the gel. The proteins will migrate by molecular weight, and after a set period of time the gel is stained. Only the proteins will retain the stain, and so we can see exactly what the composition of the muscle is before, during and after incubation. When the gels are run, it is also normal practice to run what are called "standards," known proteins of known molecular weights to serve as a reference on the gel (these are labelled HI and LO for high molecular weight and low molecular weight standards, respectively). When the polyacrylamide gels are made according to a formula for a "12%" gel, myosin is always the band at the top of the gel because it is a large molecule and travels very slowly in a 12% gel. If we were to change the percent of acrylamide in the gel, we could get myosin to migrate further down the gel, but since very

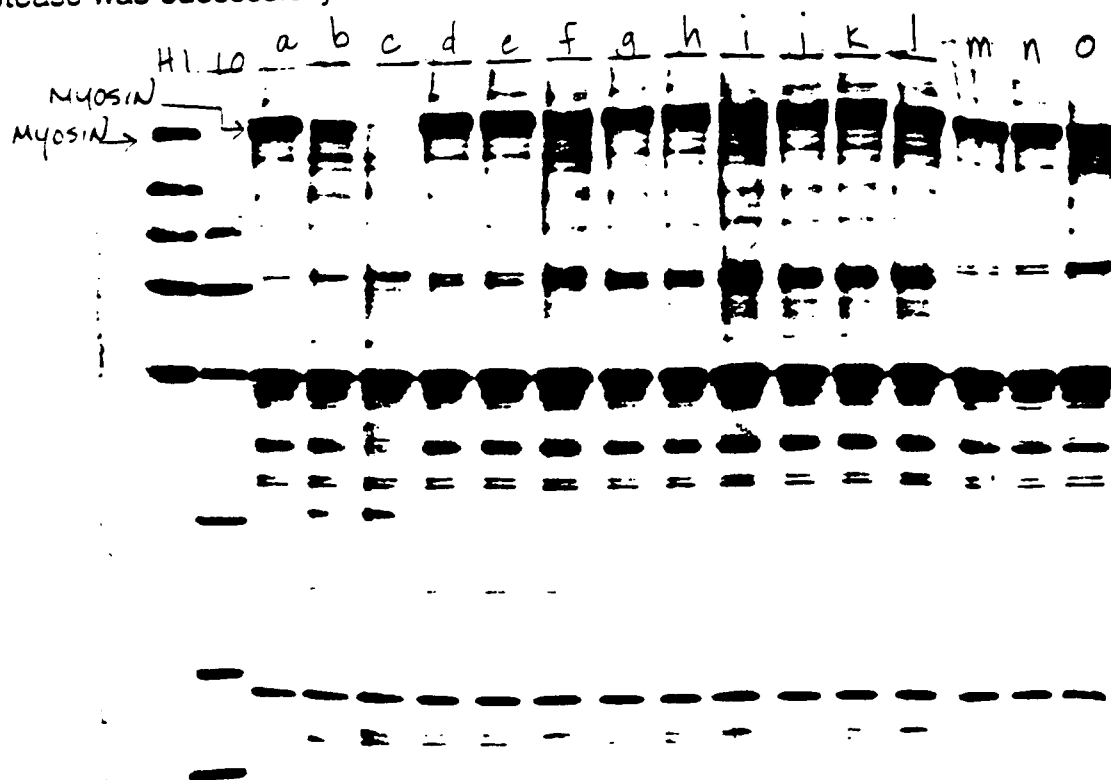
small protein fragments that migrate very quickly down to the bottom of the gel are produced by the active protease, we want to be able to observe these as well. For these reasons, the 12% formula gives the best "resolution" of what is happening in the fish muscle. If a substance is an effective inhibitor of a protease that "attacks" myosin, the myosin band on the gel should remain the same throughout the incubation period. If the ingredient is not inhibiting the protease, then the sample will look the same on the gel as an untreated fish sample, where we can expect that the myosin band will disappear with time. This is illustrated very clearly on the following two gels (III & IV).



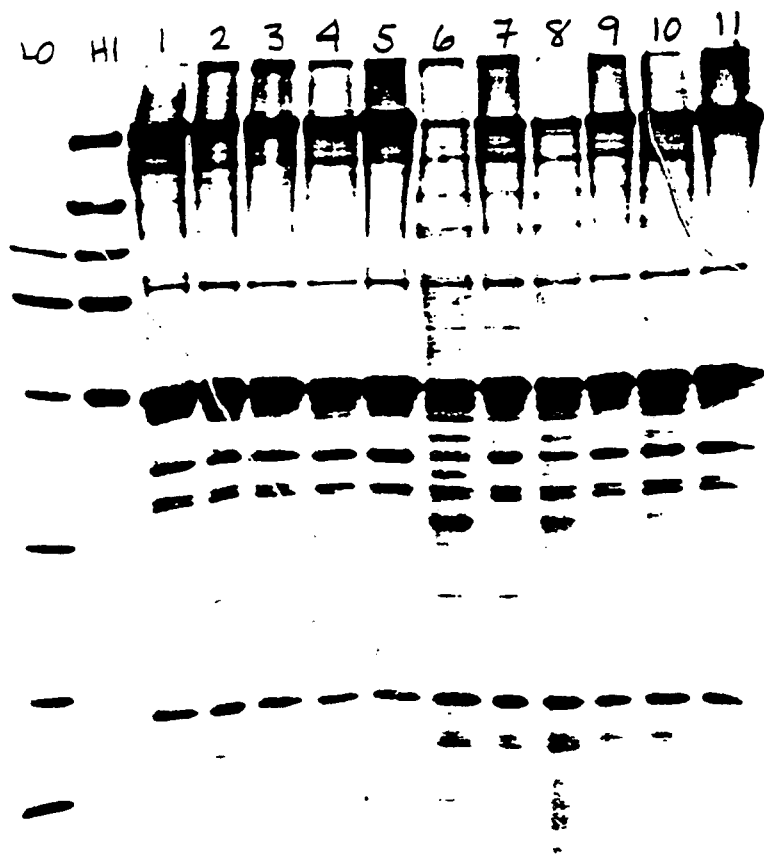
GEL III

Lanes d and g represent raw arrowtooth flounder fillets from two different fish. Likewise, lanes j and m represent two different fillets treated with ALAK. None of the samples in these four lanes was subject to heat. They were kept at 4° C (39.2° F) until solubilized. In all cases, the myosin band is very dark, and there are no small peptide fragments, which would be represented by stained bands at the bottom of the lanes. Lanes e, h, k and n represent what those same fish look like after being held 30 minutes at 55° C. In all cases, the myosin band has disappeared completely from the gel; instead of myosin, now we find a large number of small peptides at the bottom of the gel, which indicate that the myosin has been thoroughly digested by the protease. Similarly, lanes f, i, l, and o represent the respective fish samples after 60 minutes at 55° C. The gel indicates that ALAK does not contain any effective inhibitors. The same fish that were incubated in lanes d, e and f (untreated) and j, k and l (treated) were also ground and microwaved. Lane a represents the untreated arrowtooth, while lanes b and c represent the treated. From these gels it appears that microwaving was more effective for the untreated sample than the treated sample. Further research would be needed to determine why this happened. At this point we can only speculate that either the vacuum injection method itself may have had a deleterious effect on the fish, *i.e.* possibly cell membranes are ruptured in the process liberating proteolytic enzymes or the addition of water as the injection medium may have assisted the enzyme in gaining access to more of the fish muscle. It is important to remember that although we speak of the protease as though it were an entity, actually we are referring to thousands of molecules. Adding water without an inhibitor just makes it easier for those molecules to diffuse throughout the medium.

In contrast, the following gel (IV) demonstrates the inhibitory effect of plasma on arrowtooth flounder muscle. Again, the "standard" proteins are in the first two lanes on the left, labelled H1 and LO. Lane a is a sample of untreated arrowtooth flounder that has not been subject to heat, but solubilized at 4°C. Lane b represents that same sample after 30 minutes of heat and lane c represents 60 minutes at 55° C. The myosin gradually fades out with time, as expected. Lanes d-f, g-i and j-l represent the addition of powdered beef plasma at the 0.5%, 1% and 1.5% levels, respectively, while in lanes m-o powdered egg white has been added at the 1% level. The first lane in each series (d, g, j and m) represents the unheated sample, the second lane in each series (e, h, k and n) represents 30 minutes at 55° C and the third lane (f, i, l and o) represents 60 minutes. While it is not easy to see from the photocopy, some proteolytic degradation of myosin is visible after 60 minutes in the case of the 0.5% plasma addition (lane f). However, in all other cases the myosin band retains its original intensity despite heat treatment, indicating that the protease was successfully inhibited from degrading myosin.



4) In the last phase of the microwave cooking project we investigated the effects that "hot spots" in microwave ovens might have on the effectiveness of microwaving in preserving fish texture. All of our tests were conducted using a General Electric model No. JEM 31H Spacemaker II microwave oven. The electric output of this model is 700 watts, and it was rated several years ago in Consumer Reports as being the least troubled by hot spots of the ovens then on the market. We compared thawed 100 gram sections of arrowtooth fillets that had been frozen as IQF fillets. The fillets were selected to be of comparable length, width and thickness. Once thawed the fillets were placed in C-PET trays, loosely covered with Handi-wrap and microwaved in the center, back center, left and right sides of the oven. Lane 1 of the following gel (V) (immediately to the right of the standard LO and HI lanes) represents a raw fillet. Lanes 2 and 3 were taken from the center and edge, respectively, of a fillet cooked for 2 minutes in the center of the oven on HI power. Lanes 4 and 5 were taken from the center and edge, respectively, of a different fillet also cooked in the center of the oven, but for 1.5 minutes. Since the fillets were thinner than the ground portions discussed above, after 1.5 minutes on HI the edges of the fillet became overcooked and were not truly representative of a normally cooked fillet. For this reason we cut back the cooking time to 1.5 minutes. Lanes 6 and 7 are samples taken from the center and edge of a fillet cooked at the back center of the oven. Lanes 8 and 9 were likewise taken from a fillet cooked on the left side of the oven, while samples for lanes 10 and 11 were taken from a fillet cooked on the right side of the oven. From the gel we can see that the fillets cooked in the center of the oven cooked uniformly and that there was no degradation of myosin. In fact, it appears that microwaving is even more effective for the undisturbed fillet than for ground fish (compare electrophoresis gels discussed above).



GEL V

The same element possibly at work with the injection procedure, namely, disruption of cell membranes during treatment, might explain this difference. In lanes 6 through 11, however, we can see that in all cases the center was not being cooked as effectively as the edge (compare lanes 6 & 7, 8 & 9 and 10 & 11). Further, with the exception of the fillet cooked in the right side of the oven, there was more overall destruction of myosin even at the edges of fillets that were not cooked directly in the center of the oven. Extensive myosin degradation is evident in lanes 6 and 8, where the appearance of numerous small protein fragments is visible as stained bands at the bottom of the gel. Due to the variability among individual fish, and the possible severity of the softening that can occur during cooking, we believe that power variabilities among different microwave oven models and manufacturers in addition to variability within a given oven, can be a serious concern in trying to put a microwavable product on the market. Given the proliferation of microwave ready entrees on the market, it would appear that product uniformity is of paramount importance. With a product that is going to receive final preparation in the home, this simply cannot be guaranteed.

Microwaving is clearly an effective method of enhancing cooked arrowtooth flounder texture. However, its application at this time appears to be most appropriate to controlled production conditions, *i.e.*, secondary processors with continuous microwave ovens where the fish can be "pre-cooked" before freezing to assure no further degradation when the product is subject to a secondary cooking step.

ARROWTOOTH FLOUNDER MICROWAVE PROJECT
Project Report
26 March 1990

The following project objectives have been met:

1) The optimum temperature for activity of the arrowtooth flounder muscle protease was documented by Greene & Babbitt (Control of muscle softening and protease-parasite interactions in arrowtooth flounder (*Atheresthes stomias*). Journal of Food Science, 1990). Their research showed that the protease(=proteolytic enzyme) was most active between 55°C and 60°C (131° F - 140°F) but that the protease was inactivated by temperatures in excess of 80° C (176° F). The attached chart illustrates the time temperature relationship for degradation of the muscle tissue and consequent loss of texture. Initial studies were therefore conducted to determine the microwave cooking times required to achieve a minimal internal temperature of 176° F in fillets of various thickness.

The following numbers show clearly that there is an obvious advantage to using thinner fillets. We also observed that the time required to get through the critical temperature range also varied from fish to fish and appeared to be dependent upon the apparent moisture content of the fillet (*i.e.*, the higher the % moisture, the longer it took a given thickness fillet to reach temperature). The critical temperature range is 120° F to 176° F, and the faster the fish gets through this range, the less damage is done to the muscle fibers.

Length x Thickness (inches)
(All pieces were 1 1/2" wide)

time (sec) from 120° F to 176° F

3 x 1/2	18
2 1/4 x 5/8	18
4 x 1/2	18
3 1/2 x 1/2	19
4 x 1/2	17
3 1/2 x 3/8	17
4 x 3/4	39
4 1/2 x 3/4	39
4 1/4 x 5/8	39
3 1/4 x 3/8	16
3 1/2 x 1/2	22
3 1/4 x 3/8	25
3 1/2 x 1/2	19
4 x 5/8	27
3 3/4 x 1/2	24
4 1/4 x 3/8	29
4 1/2 x 1/2	35
4 1/2 x 5/8	32

All of the above fillet pieces were removed from the microwave oven when an internal temperature (using a probe) registered 176° F. They were assessed for texture at that time by an informal panel and then again when all of the samples had been microwaved. While the texture of all samples was judged to be acceptable when the fish were removed

from the microwave, the texture at the center of each piece continued to deteriorate as the samples were left standing at room temperature. This indicates that not all of the enzyme in the muscle was inactivated and that it is necessary to reach a higher final temperature or at least hold at 176° F for a longer time in order to effect a permanently firm texture. In the case of microwaving, of course, the simplest remedy is to cook to a higher temperature and protect the thinner portions of the fillet piece with a sauce. Areas of the fish pieces which were heated to temperatures higher than 176° remained "firm" upon standing. The term "firm" in this case is relative, in that the arrowtooth when microwaved is more firm than when baked, steamed or sauteed (see data below), but it is still less firm than other commercially important flatfish species, such as rock, Dover, rex and flathead soles.

In comparison tests of microwaved arrowtooth, Dover, rock and rex soles (conducted at the National Marine Fisheries Service lab), fillet pieces measuring roughly 4" x 1 1/2" x 3/8" were microwaved on HI setting (1400 watts power) for 1.5 minutes to insure total inactivation of the arrowtooth enzyme. The samples were allowed to reach room temperature before testing. An Instron model 1000, which measures the force required to compress a sample up to its "yield point," (=the point at which a constant speed flat surface probe will break through the surface) was used to assess the comparative textural firmness of the four species. The yield point is expressed as kilograms force. As measured by the Instron, microwaved arrowtooth was 2.5 times more firm than baked arrowtooth and 1.5 times more firm than steamed arrowtooth. The average force values for 20 arrowtooth samples taken from 10 different fish for baking, steaming and microwaving were 0.26 ± 0.05 , 0.45 ± 0.11 , and 0.67 ± 0.17 , respectively. Average values for microwaved Dover, rex and rock soles, however, were 1.32 ± 0.42 ,

0.35 ± 0.10, and 1.33 ± 0.53, respectively. While the rex sole samples gave very little resistance to force, in this case less than the cooked arrowtooth, sensory analysis indicated that this was a function of the peculiar parallel structure of rex sole muscle fibers, since the rex was still quite firm to chew even though it readily gave way to puncture compression on the Instron. Under the best of circumstances, then, arrowtooth itself will never be as firm as the other flatfish, but microwaving effected enough of a textural enhancement to bring the arrowtooth within the realm of acceptable palatability. Preliminary experimentation with sauces to date suggests that thin fillets or fillet slices in an acid pH sauce (e.g. tomato) may attain textural values closer to those measured for other flatfish that are not subject to enzymatic softening than thick pieces of arrowtooth cut from large fillets.

2) Choice of suitable packaging requirements has been the most time consuming phase of the project. It was decided at the outset of the project that a Trigon Intact packaging machine would be used. The bench top model (RM331) that we have been using creates a "fresh look" vacuum seal and the finished product appearance is certainly competitive with other fish products that have been shown at trade shows in Boston and Long Beach over the past two years. However, the number of variables involved in putting together a complete packaging program are enormous. The major options for microwaveable trays in order of increasing cost are primarily polyethylene, polypropylene, crystalline polyester and co-extruded polycarbonate. For proper use of the Intact machine, it is necessary to use a tray magazine in the vacuum chamber. The tray magazine supports the microwaveable trays while the chamber is evacuated and during heat sealing. The magazines are cut to the specific shape of the trays being used, and it appears that no

two companies produce precisely the same tray sizes (see attached list of suppliers). Since the cost of the magazines is approximately \$150 each, we were confronted with the dilemma of not wanting to order the magazines before deciding on the optimum tray while not being able to properly evaluate different tray types without using a magazine. The compromise solution was one of using a temporary adjustable styrofoam support in the chamber to raise each of the different tray types and sizes to the approximate height required for sealing.

Most of the more attractive and upscale microwave entrees in other food categories are using crystallized polyester, or "C-PET," trays. These trays have a temperature range of -40 °F to +400°F. We were advised by one of the Trigon representatives that a new type of microwaveable foam tray was being introduced on the market, and after several months' delay we were able to acquire samples of these noryl EFC resin/styrene blend trays. Although the foam trays are cheaper than C-PET, we decided against using them for the following reasons: 1) it was very difficult to peel off the sealing film from the tray before cooking, 2) the trays gave off a "plastic" odor when exposed to the relatively long-time high temperature cook required for the arrowtooth product (5 minutes on HI power for a 4 ounce portion starting at refrigeration temperature), and 3) when panelists were asked to evaluate the appearance of the tray the majority voiced concern that they looked cheap and reminiscent of meat packaging trays found in supermarkets. The C-PET trays were preferred by everyone, although the various shapes were likewise subject to "other-product" associations. Panelists said they preferred shapes that tended toward oval for fish dishes and that the trays should not be too deep. This narrowed down the choices considerably, and we are currently experimenting with C-PET trays in the depth range of 1 3/8" to 1 5/16". This size will readily hold a single 3 1/2 to 4 ounce fillet or two

1 1/2 to 2 ounce fillets plus sauce and a topping (crumbs, cheese, diced vegetables, etc.). This constitutes a normal dinner portion.

The second major consideration after the tray itself is the choice of film. The two options are gas (oxygen) permeable and oxygen barrier. The gas permeable film is advisable for products that are on the fresh market in order prevent the growth of the pathogen *Clostridium botulinum*, which will grow at refrigeration temperature in the absence of oxygen. This is not a major concern for products that will be frozen, such as the entrees being developed in this project. However, the microwave requirement for arrowtooth precludes the use of permeable film, since it is composed only of 2 layers and tends to melt under prolonged exposure to microwaves. The barrier film, on the other hand, is composed of 5 layers and is much more heat resistant. However, the cost is significantly higher. For example, if the chamber can accomodate 4 trays at once, the cost differential for 4, 6 and 8 mil barrier films is approximately 45%, 43% and 24% higher, respectively. We initially evaluated the 8 mil barrier film to insure heat resistance in microwaving arrowtooth (other flounder species can be microwaved in half the time), but have encountered problems in sealing due to film thickness. Because steam pockets tend to develop in microwaved fish, often causing "blow-outs" where portions of fish can actually splatter the oven walls, it is necessary that the plastic film be at least loosely draped over the product during cooking. The normal procedure is to peel it part way off the frozen entree and then lay it back over the top during cooking. This way the entree is adequately vented and "blow-outs" also seem to be less frequent. We currently have 4 mil barrier film on order, and will test its usefulness in a microwaveable frozen flounder entree.

Several other factors influence the presentation of the product. At the start we

experimented with raw fillets (actually 3 1/2 ounce pieces of large arrowtooth fillets and 4 ounce whole rock sole fillets for comparison). While they tend to flatten somewhat with vacuum packaging, the appearance is still attractive. When sauce is added, whether to a flat or rolled fillet, if the tray is sealed at refrigeration temperature, the surface becomes concave and is less attractive. This problem is easily solved by partially freezing the entree before sealing.

3) We have not yet attempted any commercial distribution of microwaveable arrowtooth entrees for the following reasons. As indicated above, the packaging component of the product consumed a larger portion of our R&D time than initially anticipated. Now that we have zeroed in on the most appropriate tray sizes and film types for our application, the die maker at Trigon is on vacation. We have to send down sample trays to have the magazines made, which will take several more weeks when all the transportation time is counted in. When we get the go-ahead on the tray size we've selected, we will need to place a large order for trays. Up to this point, we have been working with sample trays supplied to us by various manufacturers. At that point we will test out the sauces we have formulated for the arrowtooth product and ask NMFS to run a sensory panel for us.

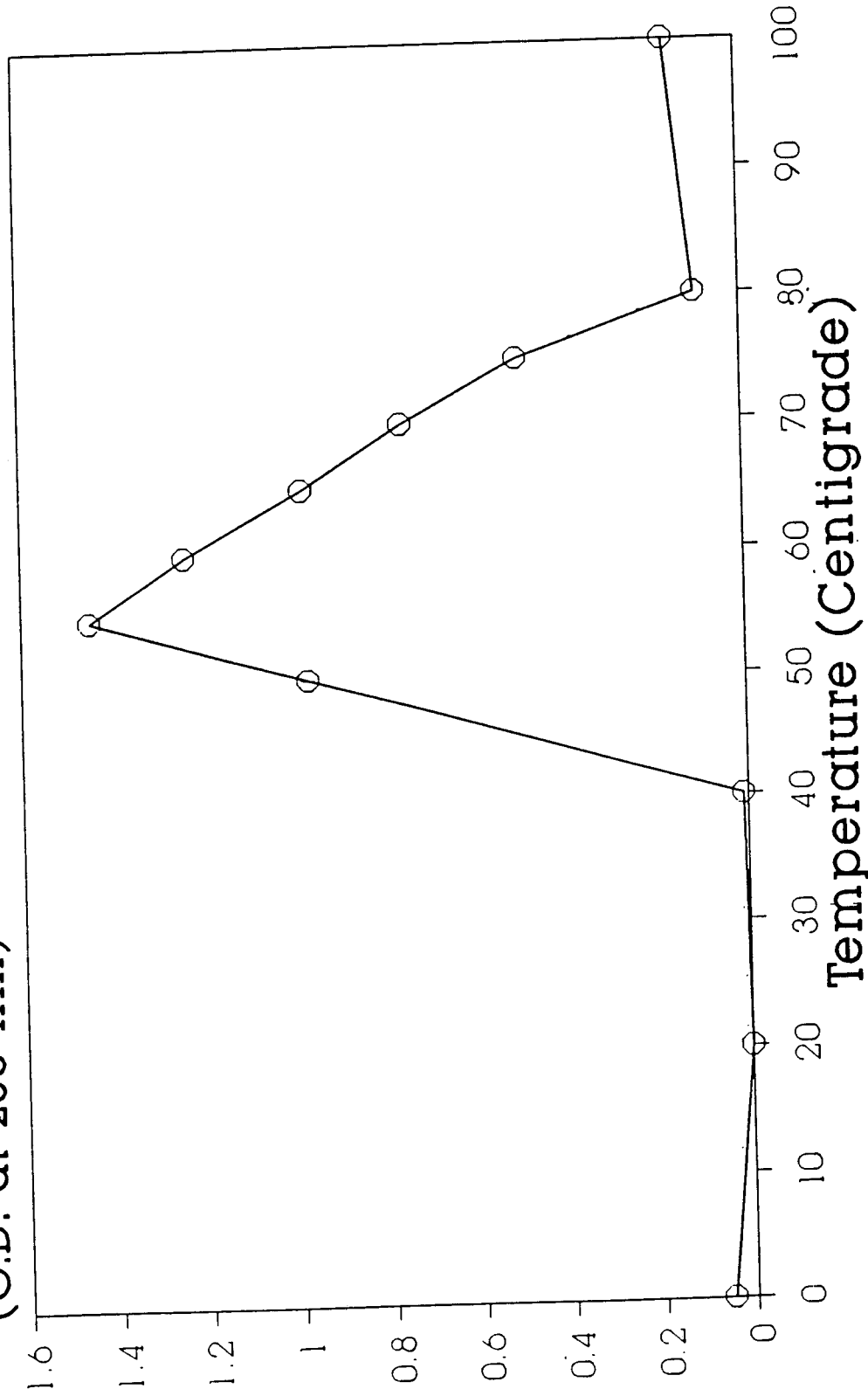
In the budget for this project \$1,000.00 is allocated for purchasing labels. However, the stiff paper sleeves that are sold to fit the trays we have ordered can only be purchased in a minimum \$3,500 order. We are exploring other options with Anchorage printers, but to date have not found a price quotation within the price range of the budget.

Recent developments in arrowtooth research at the NMFS lab in Kodiak (Dr. D. Wasson--see recent Lodestar) also suggest that a retail fillet product could be greatly enhanced by applying the vacuum injection technique using a food grade enzyme blocker

such as bovine plasma. Depending on the thoroughness of the vacuum injection, we believe that by pretreating the fillet we could have a much more competitive product that would not be as subject to variabilities in fillet thickness or the power output of home microwave ovens.

Dr. Wasson has also offered to conduct a study monitoring the actual breakdown of myosin during microwaving, comparing untreated fillets and those that have been vacuum injected with 1) a solution of bovine plasma alone and 2) a solution of bovine plasma plus other seasoning ingredients of our choosing. Using gel electrophoresis she would be able to provide us with a definitive answer on the comparative effectiveness of the two techniques, which we believe would be most useful for anyone considering entering the retail market with an arrowtooth product.

Proteolytic Activity of Arrowtooth Flounder As Measured by Autolysis (O.D. at 280 nm)



$$\text{O.D.} = t(60) - t(30)$$

MICROWAVE TRAY MANUFACTURERS

Crystallized Polyester Resin (CPET)

Amoco Foam Products, Inc.
3803 North Fourth Avenue
Sioux Falls, SD 57104
(800)642-7654

Mankato Corp.
P.O. Box 1030
Mankato, MN 56001
(507)625-1131

VP Sales and Marketing
Comet Products, Inc.
6 Stuart Rd.
Chelmsford, MA 01824
(508)256-6551

MCP Performance Plastics
P.O. Box 542
Janesville, WI 53547
(608)756-7400

DRG Plastics
329 Stirling Avenue South
Kitchener, Ontario
Canada, N2M 3H6

Micro-Qwik
P.O. Box 475
Middleton, WI 53562
(608)831-0475

PCA/EKCO Products, Inc.
2100 Sanders Road
Northbrook, IL 60065-3080
(312)205-2362

Therma-Plate Corp.
801 Montrose Avenue
South Plainfield, NJ 07080
(201)561-8111

Genpak Corp.
68 Warren Street
Glens Falls, NY 12801
(518)798-9511

Hopple Plastics
7430 Empire Drive
Florence, KY 41042
(606)283-1570

ITW/High Performance Plastics
3600 West Lake Avenue
Glenview, IL 60025

Microwaveable Foam (Noryl-ESC Resin)

Tekni-Plex, Inc.
201 Industrial Pkwy.
Somerville, NJ
(201)722-4800

Microwaveable Molded Fiber/Polyester Laminate

Keyes Fibre Company
3003 Summer Street
P.O. Box 3861
Stamford, CN 06905