Appendix II
Progress report: Understanding chalky halibut

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Recent activities

In mid-November, 2002, we caught 75 halibut north of Afognak Island using longline gear. The fish were held in insulated totes with continuous flow seawater, brought back to Kodiak and placed in seawater tanks located at the Kodiak Island Research facility. Other fish were stunned and blood samples taken to determine at-sea conditions. For the next three weeks, halibut slowly acclimated and did not eat. It took approximately four weeks for the first fish to start eating again. Since that time, more halibut are eating the herring provided each morning.

In mid-December, we sacrificed twenty fish to practice techniques for drawing blood, taking muscle samples and laboratory analyses. The information obtained from these fish will serve as one set of baseline data.

Currently, all fish are eating, adjusting to the tanks and returning to "normal"/unstressed state. We anticipate that the project will start approximately January 20, 2003.

Research outline and planned experiments

The objective of this project is to evaluate the conditions leading to chalky halibut during catch and to identify possible measures that may reduce or treat its incidence. Specific goals include:

1. Summarizing research on chalky-like conditions that exist in other food products, primarily PSE pork and other meats;

2. Observing physical and biochemical changes in halibut muscle as it is stressed and becomes chalky; and

3. Evaluating experimental results and recommending potential measures to avoid or mitigate occurrence of chalkiness.

Research review

There are many parallels to chalky halibut found in other muscle foods. Most familiar is the condition of pale, soft and exudative (PSE) pork (Ing et al. 2000) and "burnt" tuna (Watson et al. 1989). Acid conditions in beef muscle have also been reported in the literature causing undesirable texture changes (Motilva 1993; Bayliss 1995). Other research has focused on the effect of lactic acid on myofibrillar proteins and acceleration of post mortem changes in poultry and pork (Rong et al. 1997). A review of selected papers will be prepared as part of this project that may help clarify the biochemical processes that occur in chalky halibut.

Physical and biochemical changes

Monitoring physical and biochemical changes that occur as lactic acid builds in halibut muscle is key to understanding the development of chalkiness. Two approaches for inducing chalkiness will be tested in this project - stress during catch and influence of water temperature.

One of the first tasks will be high quality microscopy to observe the differences in muscle structure between normal and chalky halibut. It is anticipated that myofibrillar destruction would be present in chalky fish. Samples of chalky and normal fillets will be obtained for evaluation. The pH of chalky and normal fillets will be determined and samples will be fixed and stained for microscopy (Ushio et al. 1991). Any differences in muscle structure will be noted and evaluated as to potential causes.

Measuring changes that occur in stressed halibut will lead to a better understanding of the process of chalkiness. Halibut will be caught by jigging or longlining currently being conducted for other projects at the Fishery Industrial Technology Center (FITC) in Kodiak. Up to forty-five halibut weighing between 20 and 30 pounds will be selected, returned live to FITC, placed in seawater holding tanks available at the Kodiak Fishery Research Center (KFRC) and allowed to acclimate. We currently have three tanks, one at 1,500 gallons capable of holding thirty fish and two at 800 gallons that will hold five to seven fish. The large tank will be used for testing the effects of stress during catch and the smaller tanks will be used to evaluate stress from increased water temperature. Water temperature in these tanks can be controlled through the use of portable refrigerated seawater systems.

Capture induced chalkiness

Once the halibut have acclimated to the holding tanks, the first series of experiments to observe changes during capture stress will be conducted. Tank temperature will remain constant at ambient seawater temperature determined from the halibut capture site. Three fish will be selected as controls. These fish will be sacrificed at the end of the tests to provide baseline bioenergetic and biochemical information. To simulate commercial catching and landing conditions, the halibut will be starved for two days prior to testing. Our current plan is to catch the fish with a baited longline hook and then allow it to struggle on the line for fixed time periods. We will consult with IPHC staff, fishery biologists, and Alaska fishermen to develop a protocol that can be related to actual catch conditions. It is anticipated that stress conditions will be a combination of exercise and time. At 120, 180 and 240 minutes of "soak time," three fish will be taken, stunned and immediately sampled. Periods of activity and rest will be recorded to determine accumulated struggle time. Each line will be tagged and data on the fish recorded that will include size, weight, sex, any observable defects and other noteworthy attributes. Blood and muscle samples will be taken for analysis. These treatments will be repeated three times for a total of nine fish per treatment.

Analysis will include physical and biochemical measures and may include pH measurement (AOAC 1984) amounts of cortisol (Lowe et al. 1993), lactic acid (Boyd et al. 1989) and glycogen (Keppler and Dekker 1974) in blood and muscle, changes in the nucleotide levels (Greene and Babbitt 1990), and changes and denaturation in myofibrillar proteins (Ofstad 1996; Yamanaka, 2002) using electrophoresis (BioRad 1999), solubility measures (AOAC 1984), differential scanning calorimetry (DSC, Perkin Elmer 2001), and high performance liquid chromatography (HPLC, Waters 2002) as appropriate. Color values (Minolta 2000) and water holding capacity (Regenstein et al. 1979) will be determined as a measure of quality. Samples will also be taken from control (non-chalky) halibut that will serve as controls.

In addition of immediate sampling of blood and muscle, fish will be filleted and held at refrigerated storage (3°C) for as long as five days. During this period, additional measurements will be made to monitor development of chalkiness and acquire some data on post mortem metabolism. As indicated in earlier research (Kaimmer 2000), muscle pH starts to drop and chalkiness appears up to five days post mortem. Sampling during this period will include muscle pH and amounts of lactic acid, nucleotide degradation and perhaps, glycogen levels.

For an additional three fish, once muscle pH has reached the "chalky" state, the stressed halibut will be released from the longline and allowed to rest to determine how long it will take before the lactic acid is removed from the muscle and the fish return to its normal state. Blood and muscle samples will be taken and tested as described above. These experiments should reveal how the buildup of lactic acid affects the biochemical properties of halibut muscle and how long it takes fish muscle to return to normal.

Temperature induced chalkiness

Kaimmer (2000) suggested that water temperature may have a significant effect on the development of chalky halibut. Davis and Olla (2001) describe increased stress in landed Pacific halibut related to increased water temperature. A second set of experiments will be conducted to determine effects of elevated temperature on chalkiness in halibut. Using the smaller seawater tanks, twelve fish will be captured and placed in these tanks. The tanks will each contain six fish and be held at ambient temperature. Assuming this project is conducted during Fall 2002 and Winter 2003, the seawater temperature should be about 5°C. After halibut have acclimated to the holding tanks, temperatures of the tanks will be slowly increased. Initially, each tank will be increased to 8°C and held at this temperature for 48 hours. At the end of that period, three fish will be captured, stunned and immediately sampled. The tanks will then be warmed to 11°C, then 14°C for 48 hours and fish sampled at each temperature increase. Analysis of muscle, blood and processed fillets will be determined as described above.

Control samples obtained from the earlier study also will serve as control samples for this experiment providing baseline data for assessing biochemical changes that occur. This experiment should provide evidence of effect of temperature on stress and development of chalky halibut.

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