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Cysteine significantly reduces mercury content in sablefish without altering taste or texture.

Reduction of Mercury in Sablefish (*Anoplopoma fimbria*) and the Use of the Treated Flesh in Smoked Products

F. M. TEENY, ALICE S. HALL, and E. J. GAUGLITZ, JR.

ABSTRACT

*A significant number of sablefish (*Anoplopoma fimbria*) caught in certain areas of the west coast of North America have a mercury content in the flesh that exceeds the 0.5-parts per million guideline established by the United States Food and Drug Administration.*

This study was conducted to determine the feasibility of cysteine treatment to reduce the mercury content of sablefish and of subsequent utilization of the treated flesh in preparing hot-smoked products. This fish tissue was extracted with water containing cysteine hydrochloride in concentrations up to 1.0 percent. When comminuted flesh was extracted with 1.0 percent cysteine solutions, up to 80 percent of the mercury present initially in the flesh was recovered in the wash solutions. The amount of mercury that was removed from the flesh was related to: (a) pH of the cysteine-tissue mixture, (b) concentration of the cysteine solution, (c) volume of extracting solution, (d) number of extractions, (e) contact time, (f) processing temperature, and (g) tissue particulate size. Smoked products of good texture and flavor were prepared from the cysteine-treated flesh. Yields (dry basis) of 96, 81, and 73 percent were obtained as smoked products prepared from chunks, slices, and comminuted flesh, respectively.

INTRODUCTION

Trace metal analyses have shown that a significant number of sablefish (*Anoplopoma fimbria*) caught in certain areas off the west coast contained mercury that exceeded 0.5 parts per million (ppm). The United States Food and Drug Administration has established a guideline of 0.5 ppm of mercury as the maximum allowable level in fish (Edwards, 1971). Decreased fishing in those areas for sablefish places a heavier demand on the limited number of other species of low mercury content caught in those areas. The successful reduction of mercury from this fatty fish, and the utilization of the flesh in further processing into foods that are new or that would simulate those normally produced from this species could set a pattern to follow in dealing with other species with similar problems.

F. M. Teeny, Alice S. Hall, and E. J. Gauglitz, Jr. are members of the staff of the Pacific Fishery Products Technology Center, National Marine Fisheries Service, NOAA, Seattle, WA 98112.

Studies on the reduction of mercury from fish have shown that a significant percentage of the mercury present in the muscle was removed by chemical means. Spinelli et al. (1973) used cysteine to reduce the mercury content of halibut and fish protein concentrate by up to 50 percent; therefore, the possibility of using cysteine to reduce the mercury content from the muscle of sablefish was studied.

This report discusses the experimental use of cysteine in reducing the mercury content of sablefish and the utilization of the treated flesh in preparing organoleptically acceptable smoked products.

MATERIALS AND METHODS

Materials

The sablefish used in these experiments were caught commercially off the west coast of the United States and held in frozen storage at $-18°C$ for up to 6 months prior to use. The cysteine used was L-cysteine, HCl, H_2O. All percentages of cysteine are based on the weight of the monochloride, monohydrate form of the acid. All other chemicals were reagent grade.

Methods

Preparation of fish tissue and extraction procedures

The samples of fish used in the extraction procedures described below were either comminuted, sliced, or cut into chunks just before the extraction steps. Control samples were prepared in the same manner as the cysteine-treated samples except no cysteine was used in the extraction steps.

The comminuted flesh was prepared by passing chunks of flesh once

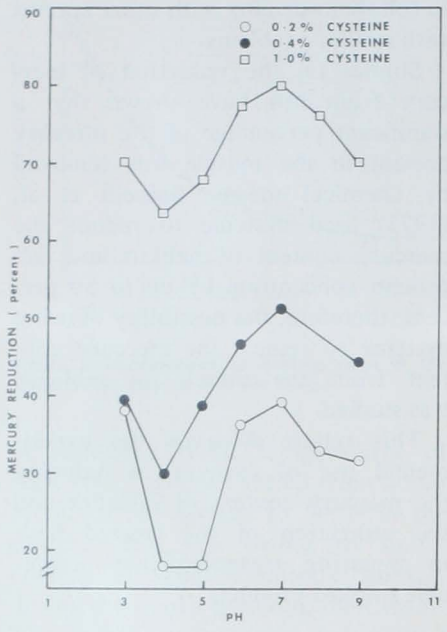

Figure 1.—Relation of total mercury reduction to pH of the comminuted flesh extracted with several levels of cysteine.

through a Hobart[1] grinder equipped with ¼-inch plate. The flesh was then mixed with aqueous cysteine solution at a ratio of one part flesh to two parts cysteine solution (w/v). The mixture was slowly stirred with a magnetic stirrer for the desired length of time. After stirring, the mixture was centrifuged for 15 minutes at 2,500 rpm. The solids were then resuspended in water containing no cysteine and the stirring and centrifugation repeated. The solids were finally resuspended in 0.1*M* NaCl and the stirring and centrifugation repeated once more.

The chunks (150-250 grams) of flesh were immersed in a cysteine solution in the same ratio as described for comminuted flesh. After holding the flesh in the solution for the desired length of time, the cysteine solution was decanted and flesh was washed under running tap water for 1 hour. The water was then drained, and 0.1*M* NaCl was added at a ratio of two parts of NaCl solution to one part flesh (v/w), recirculated for 1 hour, and drained.

The sliced flesh (approximately ¼-inch-thick sections) was treated in the same manner as the chunks.

After extraction, the washed comminuted flesh was mixed for 2 minutes in a blender (Osterizer Model B) with 2.5 percent NaCl and 0.2 percent sodium tripolyphosphate (STPP) in water. The flesh was then formed into blocks (approximately 200 grams each) for hot smoking.

The washed sliced flesh was formed into blocks of approximately 200 grams (cheesecloth and wire screens were used as molds for making the blocks) and soaked at a 2:1 ratio (weight of brine to fish) for ½ hour in 23 percent NaCl solution containing 0.2 percent STPP. The washed chunks of flesh were soaked in the concentrated brine in the same way.

Hot smoking

All samples were smoked for 7 hours in an electrically-heated smokehouse. During the final hour of processing, the samples were hot-smoked at a maximum smokehouse temperature of 100°C to obtain an internal flesh temperature of 75°C. Temperatures were measured by thermocouples imbedded in the flesh. After processing, the fish were cooled and weighed to determine yield.

Mercury analysis

Mercury was determined by the method of Malaiyandi and Barrette (1970) as modified by Munns[2]. Mercury reduction was based upon total mercury found in the flesh before and after processing.

Total solids and fat

Total solids and crude fat were determined on the raw and smoked products. For total solids, about 10-gram samples of prepared wet fish were weighed into flat bottom aluminum weighing dishes, about 5-cm diameter, heated for 16 hours at 100°C, cooled in a desiccator, and weighed. Crude fat was determined as described by the Association of Official Analytical Chemists (Horwitz, 1960) method.

Yield

Yield was determined for the raw and smoked products prepared from the treated flesh. Yields were determined on both dry (moisture-free) and wet basis for comparative purposes. Calculations were made as follows: Percent yield (dry basis) = dry weight of the flesh after either extraction or smoking divided by the dry weight of the raw flesh times 100. Percent yield (wet basis) for a smoked product = wet weight of the smoked product prepared from the extracted flesh divided by its wet weight prior to extraction times 100.

Sensory evaluation

The smoked products were coded and evaluated for quality by a panel of 10 experienced judges who compared differences in flavor, texture, and preference between the various test samples and two control samples. One of these control samples contained a sufficient amount of cysteine added just before smoking to ascertain the effect of a high level of added cysteine on the flavor. The other control sample was treated the same as the experimental samples but without cysteine in the extracting solution.

EXPERIMENTAL RESULTS

The feasibility of cysteine treatment to reduce the mercury content of sablefish was studied primarily with comminuted flesh in order to provide for the most effective contact of the cysteine solution with the flesh. Experimental variables included the pH of the extraction solution, the number of extractions, the concentration of cys-

[1] Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

[2] R. K. Munns, Food and Drug Administration, Denver, Colo., pers. commun.

teine in the extraction solution, the ratio of extraction solution to fish weight, contact time during extraction, temperature of the extraction mixture, and the effect of using water compared to 0.1*M* NaCl for the cysteine solution. Except in those tests in which pH and temperature were altered, the pH of the mixture was that of the fish-cysteine solution or pH 7, and the temperature was ambient.

Reduction of mercury

The effect of pH on the efficiency of cysteine to remove mercury from the comminuted flesh was determined. Extracting solutions of 0.2, 0.4, and 1.0 percent cysteine in water were mixed with the comminuted flesh and adjusted with either dilute hydrochloric acid or sodium hydroxide to pH values from 3 to 9. The results of these experiments (Figure 1) show that minimum reduction of mercury was obtained at pH 4 and maximum reduction at pH 7. Regardless of the cysteine concentration, there was an increase in total mercury reduction at pH 3 but a corresponding decrease in percent yield (Figure 2). The increase in total mercury reduction at pH 3 may be ascribed to the partial solubilization and subsequent loss in the myofibrillar portion of the muscle that binds mercury in fish flesh (Spinelli et al., 1973; Spinelli, Koury, and Miller, 1972).

Extractions of the comminuted flesh with various concentrations of cysteine up to 1.0 percent of fish weight, showed that total mercury removed increased with increasing cysteine concentration, but at a reduced rate. Approximately 40-50 percent of the mercury present in the flesh was removed with 0.2 percent cysteine, whereas only 60-80 percent of the mercury present in the flesh was removed with 1.0 percent cysteine (Figure 3). Two successive extractions with fresh solution of the same cysteine concentration removed only about 10 percent more mercury than with a single extraction of a solution of twice the concentration (Table 1).

Other experiments were conducted wherein the ratio of the cysteine to fish was kept constant (0.2 gram cysteine to 100 grams fish), but the volume in which the cysteine was dissolved ranged between 50 and 500 ml. Results showed that cysteine was most effective (Table 2) where the cysteine solution was most concentrated.

The effect of contact time between the flesh and the cysteine extracting solution upon mercury reduction was determined. With comminuted flesh, extractions were performed in which the contact time between flesh and solution varied from 2 to 60 minutes. Results (Figure 4) show no increase in mercury reduction beyond 2 minutes. This indicates the rapidity of mercury displacement from the flesh in contact with the cysteine solution.

Comminuted flesh was extracted with various concentrations of cysteine solutions at 2°C and at 20°C. The results (Table 3) show that for any given concentration of cysteine, higher mercury reduction and lower fish weight (dry basis) and fat were obtained at 20°C than at 2°C. The overall lower weight yield at 20°C could be ascribed to the greater losses of fat. In these experiments the fat accounted for 57 percent of the dry weight.

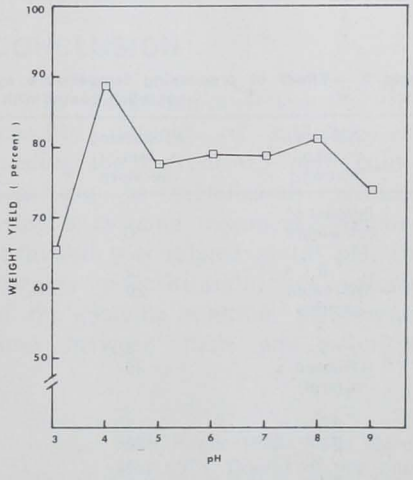

Figure 2.—Relation of total weight recovered to the pH of the comminuted flesh-cysteine mixture.

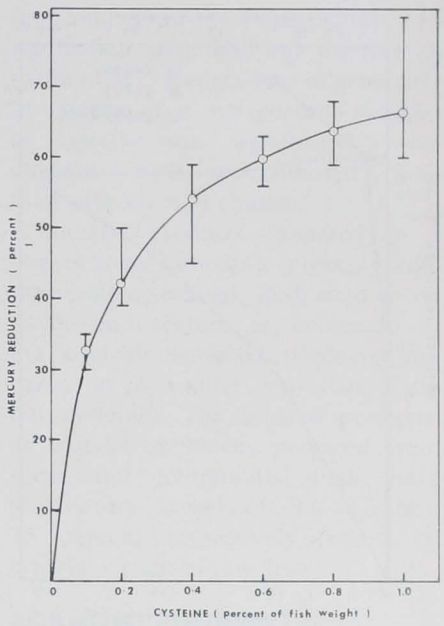

Figure 3.—Relation of mercury reduction to cysteine concentration.

Table 1.—Relation of mercury reduction to number of extractions with cysteine solution of several concentrations.

Number of extractions	Cysteine concentration	Total mercury reduction
	Percent	*Percent*
1	0	6.0
2	0	13.0
1	.2	42.0
2	.2	67.3
1	.4	51.9
2	.4	71.2
1	.8	65.3

The following variables were studied and found to be ineffective in improving the effectiveness of cysteine to remove mercury from sablefish: Heat denaturation prior to extraction, pressure (up to 2,000 pounds per square inch), recirculation of the cysteine solution, and preextracting the flesh with water, 0.1*M* NaCl and EDTA (tetra sodium or calcium disodium). Other additives

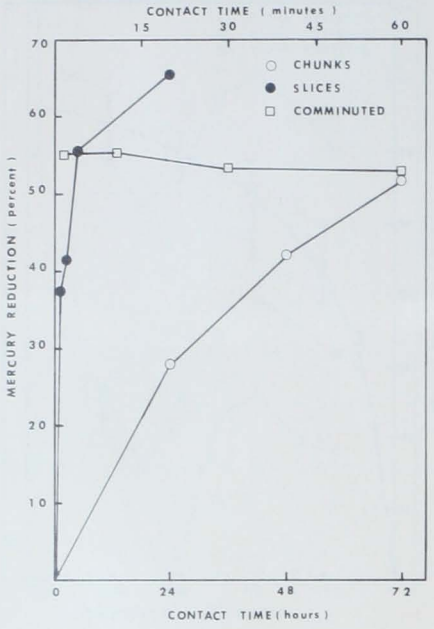

Figure 4.—Relation of mercury reduction to contact time between chunks, slices, and comminuted flesh of sablefish and the extracting cysteine solution (contact time is the hours for chunks and slices and minutes for comminuted flesh).

tried including citric acid, thiourea, nonfat dry milk solids, and fresh whole milk were ineffective, either alone or in conjunction with cysteine. Extracting the flesh with cysteine in an atmosphere of nitrogen and carbon dioxide did not improve the effectiveness of cysteine to remove mercury from the flesh.

Mercury Distribution in the Flesh Prior to and After Cysteine Extraction

Extraction of the comminuted flesh with 1.0 percent cysteine followed by one water and one NaCl wash resulted in mercury reduction of 60 to 80 percent. In a typical experiment where 100 grams of raw flesh contained 100 μg mercury, we found that after extraction the washed flesh contained about 23 μg mercury or 23 percent of that originally present in the flesh. The cysteine, water, and NaCl washes contained 45, 15, and 17 percent of the total mercury, respectively. Mercury appeared to be associated only with the myofibrillar portion of the flesh (Spinelli et al., 1973). The apparent loss of mercury from controls (Table 3) can be attributed to losses of very fine protein particles during the washing process.

Table 2.—Effect of volume of the cysteine solution on mercury reduction.

Cysteine (percent of fish weight)	Ratio of fish weight to volume of solution	Total mercury reduction	Mercury (ppm dry basis)	Total weight yield (dry basis)
		Percent		*Percent*
0	0	—	3.08	100.0
0	1:2	5.5	3.58	80.3
0.2	1:½	44.3	2.03	84.5
0.2	1:1	39.9	2.16	85.6
0.2	1:2½	37.7	2.31	83.0
0.2	1:5	36.5	2.35	83.2

Removal of Organoleptically Detectable Cysteine

The level of cysteine in the smoked products that could be detected organoleptically was determined by adding various amounts of cysteine to the comminuted, washed flesh prior to smoking and evaluating the smoked products for cysteine flavors. Results showed no detectable cysteine off-flavors at the 0.01 percent level, whereas 30 percent of the panel members detected cysteine at the 0.05 percent level. Thus we estimate that the cysteine remaining in the tissue after extraction to be less than 5 percent of the cysteine added for mercury reduction purposes.

Tests with Slices and Chunks

Two variables, contact time with the extraction solution and a method of increasing surface contact of chunks with the solution, were evaluated to demonstrate the feasibility of using intact slices or chunks of sablefish as compared to the process of comminution, treatment, and preparation of a reformed block. The limited tests showed that extraction of mercury from sablefish flesh was much less efficient when either slices or chunks were used. With slices and chunks, for example, the contact time between the cysteine solution and flesh varied from 1 to 24 hours, and 1 to 3 days, respectively (Figure 4). The data show that in

Table 3.—Effect of processing temperature upon mercury reduction and yield from comminuted sablefish treated with various levels of cysteine.

Cysteine concentration	Processing temperature	Total weight yield	Total fat yield	Total mercury reduction
Percent of fish weight	°C	*Percent*	*Percent*	*Percent*
0 (Untreated control)	2	100.0	100.0	—
	20	100.0	100.0	—
0 (Treated control)	2	87.3	92.5	8.3
	20	71.1	65.8	9.4
0.2	2	85.7	93.8	31.1
0.2	20	75.2	69.2	39.6
0.4	2	85.8	93.8	34.1
0.4	20	65.5	61.0	51.2
1.0	2	88.4	97.3	46.7
1.0	20	72.5	63.0	68.0

1.0 percent cysteine solution it required about 4 hours for slices and 3 days for chunks to lose approximately 50 percent of the mercury originally present in the flesh. Extracting the flesh with cysteine in 0.1*M* NaCl showed no advantage over cysteine in water.

Increasing the surface area of the chunks of flesh in contact with the cysteine solution by perforation (about 25 holes to the square inch) resulted in increased reduction of approximately 10 percent.

Yield of Smoked Products

The extraction operation resulted in yields (dry basis) up to 70, 80, and 95 percent as raw washed flesh prepared from comminuted flesh and flesh cut into slices and chunks, respectively. Losses can be attributed mainly to losses in fat and sarcoplasmic proteins. Results from a typical experiment on comminuted flesh showed that 70 percent of the dry weight of the flesh was obtained as washed flesh, 25 percent as fat floating on top of the extracting solutions, and the rest consisting mainly of the soluble sarcoplasmic proteins. The fat floating on top of the extracting solutions could be recovered and added back to the washed flesh.

Yields (dry basis) as smoked products, varied with the type of flesh used. Yields of about 96, 81, and 73 percent were obtained from chunks, slices, and comminuted flesh respectively. No drip was observed from blocks made from the comminuted flesh, but some drip was observed from chunks and slices. The salt compensated for some of the moisture lost during the smoking process.

The yield of the smoked product on wet basis was 70 percent for chunks and 68 percent for blocks made from sliced flesh. Inasmuch as the smoked products prepared from the comminuted flesh were prepared by blending the washed flesh with a definite amount of salt dissolved in water, recovery was on the dry-weight basis described earlier.

Table 4.—Sensory evaluation of smoked sablefish prepared from sliced flesh treated with various levels of cysteine.

	Flavor[1]	Texture[1]	Preference[2]
Control (no cysteine treatment)	3.8	4.2	6.6
Extracted with 0.2% cysteine	3.9	4.1	6.9
Extracted with 1.0% cysteine	3.6	4.0	6.4

[1] 5-point scale: 5, very good; 1, poor.
[2] 9-point hedonic scale: 9, like extremely; 5, neither like nor dislike; 1, dislike extremely.

Sensory Evaluation of the Finished Smoked Products

Sensory evaluation of the finished smoked products prepared from the cysteine-treated flesh showed the samples to have a delicate smoked flavor typical of the smoked sablefish. Blocks made from comminuted flesh had moist spreadable texture. The blocks made from slices had flaky and moist texture such as found in the chunks, but the flakes were of slightly smaller size (due to slicing). Typical results (Table 4) showed no significant differences in flavor, texture, or preference between the cysteine-treated samples and the untreated controls. Similar results were obtained from the evaluation of treated and smoked chunks.

CONCLUSION

This work demonstrated that the mercury content of sablefish exceeding the current 0.5 ppm guideline can be significantly reduced using a cysteine treatment. Mercury reduction was related to (a) pH, (b) cysteine concentration, (c) volume of the cysteine solution, (d) contact time between flesh and solution, (e) temperature of extraction, (f) particulate size, and (g) number of extractions. Reduction of mercury by means of a 1.0 percent solution of cysteine was significantly more efficient with comminuted flesh than with slices or chunks.

Smoked products prepared in a conventional smoking process from the cysteine-treated flesh had good flavor and texture as compared to the controls (smoked products prepared in a similar way but without cysteine). The smoked products, as chunks or blocks prepared from slices and comminuted flesh, have yields (dry basis) of 96, 81, and 73 percent respectively, based on original weight of raw flesh.

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