Reduction of Mercury with Cysteine in Comminuted Halibut and Hake Fish Protein Concentrate

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A study was made to determine the effectiveness of cysteine in reducing the mercury content of comminuted fish and fish protein concentrate (FPC). Comminuted fish tissue was extracted with 0.1 *M* NaCl containing cysteine $HCl \cdot H_2O$ in concentrations ranging from 0 to 0.5%. The amount of Hg that could be extracted from the fish was related to the concentrations of cysteine and the pH of the tissue, but the relation was not linear. Fish blocks were prepared from the cysteine-extracted comminuted tissue and did not develop off-flavor during storage. When cysteine was added to isopropyl alcohol to prepare FPC, the Hg in the FPC was reduced up to 50%.

To ensure compliance with the guideline of 0.5 ppm of mercury in fishery products (Edwards, 1971), industry has developed extensive monitoring systems to sort out those fish that exceed the guideline. The analysis for Hg in fish is now routinely performed on some species from areas previously shown to produce fish over the guideline or on fish of certain sizes where the incidence of mercury has been found to be high. Unacceptable fish are withheld from U. S. food channels and constitute a significant economic loss to industry. Conceivably, these losses could be reduced if, during processing, a means could be found to lower the Hg content of the fish.

It is well known that sulfhydryl compounds react with mercury and that some of these compounds, such as British anti-Lewisite (2,3-dimercapto-1-propanol), have been used as detoxicants in cases of Hg poisoning (West and Todd, 1955). More recent reports (Industrial Research, 1971) have pointed to the use of synthetic resins containing SH groups to reduce the amount of methyl mercury absorbed by the body when both the resin and foods containing methyl mercury are fed to rats.

One of the most abundant naturally occurring sulfhydryl-containing compounds is the amino acid, cysteine. The affinity of this compound for mercury has been demonstrated by Westöö (1967), who used it as a reagent in isolating methyl mercury from foods. To determine the usefulness of cysteine in reducing the Hg in some fishery products, an investigation was made to examine some of the process variables concerning its use. Specifically, this report deals with the use of cysteine in reducing the Hg content of comminuted halibut and hake fish protein concentrate. It also reports on the organoleptic properties of the treated products.

MATERIALS AND METHODS

Fish. *Pacific Halibut (Hippoglossus stenolepis).* All halibut used in these experiments were commercially caught. In laboratory experiments designed to determine the effect of cysteine in reducing the Hg content of the flesh, two fish weighing approximately 120 lb each were used. They had been in frozen storage (−20°F) for 6 months prior to use.

In experiments designed to assess the organoleptic properties of fish treated with cysteine, freshly caught halibut weighing approximately 80 lb were used.

Hake (Merluccius productus). All hake used were caught in Puget Sound. Except where indicated, fish protein concentrate was made from frozen hake stored at −20°F.

Comminution of Fish and Extraction Procedure. *Laboratory Batch Procedures.* Halibut flesh was comminuted by passing it once through a Hobart grinder equipped with ⅛-in. plate. The comminuted tissue (200 g) was mixed in a beaker containing cysteine and 0.1 *M* NaCl at a ratio of 2 parts of solution to 1 part of fish (v/w). The mixture was slowly stirred with an overhead stirrer for the desired length of time. After stirring, the mixture was centrifuged in 250-ml centrifuge bottles for 10 min at 2500 rpm. The solids were then resuspended in the saline solution containing no cysteine, and the stirring and centrifugation were repeated. The desired amount of cysteine was always added in the first extraction stage.

Laboratory Column Procedure. The fish was comminuted as described and 200 g mixed with 200 ml of 0.1 *M* NaCl were poured into a borosilicate glass test tube (1⅞-in. i.d.) that was tapered at one end to a ¼-in. opening. Two-hundred milliliters of 0.1 *M* salt solution containing 0.2% cysteine was passed through the column of fish solids. This was followed by 400 ml of 0.1 *M* NaCl containing no cysteine. The flow rate of the solutions through the column was approximately 20 ml/min at ambient temperature (~25°). After extraction, the fish solids were removed from the column and thoroughly mixed, and an aliquot was taken for Hg analysis. Another aliquot was dried for the determination of solids.

Preparation of Modified Fish Blocks. Approximately 15 lb of comminuted halibut was mixed with 35 gal of 0.1 *M* NaCl containing 0.2% cysteine. The mixture was stirred for 15 sec at low speed in a vertical Hobart cutter-mixer. It was allowed to settle for 10 min and the free liquor was decanted. The solids were put into a double thickness of cheesecloth and centrifuged in a Bock (Model No. 15-RC) centrifuge for 5 min at 1750 rpm. The solids were immediately resuspended in 0.1 *M* NaCl containing no cysteine and the centrifuging procedure was repeated twice with fresh NaCl solution. An equal weight of fish was similarly extracted with no cysteine in the extracting solution and modified fish blocks were prepared according to the procedure described by Teeny and Miyauchi (1972) and stored at −20°F.

Preparation of Portions and Organoleptic Evaluation. The blocks were cut into 1½ × 1½ × ⅝-in. pieces which were breaded and deep-fat fried for 5 min at 370°F. The product was evaluated organoleptically by a panel of six experienced judges using a 5-point hedonic scale.

Preparation of Fish Protein Concentrate (FPC). FPC was prepared from fresh hake as described by Ernst (1971) at the Experimental Demonstration Plant located at Aberdeen, Wash. Cysteine was added to the first extraction stage.

Analytical Methods. *Mercury.* Except where indicated, fish samples were freeze dried prior to analysis. Freeze drying was done by spreading about 10 g of wet fish on a styrene Petri dish and drying for 16 hr in a Thermavac freeze dryer (Model·FDC·IND·32F) at a plate temperature of 20 ± 5°. Analyses of several samples of fish for Hg

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Table I. Effect of Freeze Drying on the Mercury Content of Comminuted Halibut

Sample and Hg content			
Wet, ppm	After freeze drying, ppm	Solids content after drying, %	Gain or loss of Hg after freeze drying, ppm
1.20	6.0	20.0	0
1.20	6.0	20.0	0
1.20	6.0	19.5	−0.03
1.06	5.3	19.7	−0.02
1.08	5.4	19.7	−0.02

before and after freeze drying established that there was no significant Hg loss (Table I) using this procedure, and agrees with the work of Tanner *et al.* (1972). One-gram samples of dried fish, 5-g samples of wet fish, and 0.5- to 5.0-g samples of desolventized still bottoms were analyzed by the method of Munns and Holland (1971).

Sulfhydryl. Aliquots (0.5-ml) of the extracting solution were analyzed by the method described by Dubé *et al.* (1972).

Cysteic Acid. FPC samples were milled to less than 100 mesh. Approximately 25 mg were oxidized with performic acid, hydrolyzed with 6 *N* HCl, and then evaporated to dryness. Cysteic acid was determined by column chromatography by the method of Spackman *et al.* (1958) using a Spinco Model 120B amino acid analyzer.

Reagents. *Cysteine.* All cysteine used in laboratory experiments was obtained from either Nutritional Biochemical Company, Cleveland, Ohio, or Sigma Chemical Company, St. Louis, Mo. The cysteine used at the EDP was obtained from SST Chemical Company, N. Y. All the cysteine used was L-cysteine·HCl·H_2O. Throughout the paper, all given percentages of cysteine are based on the weight of the monochloride,monohydrate form of the acid.

EXPERIMENTAL AND RESULTS

Reduction of Mercury in Comminuted Fish. *Batch Extractions.* The effectiveness of different concentrations of cysteine in lowering the mercury content of comminuted halibut muscle is shown in Figure 1. In these experiments, 0.1 *M* NaCl was the solvent for cysteine at the levels of 0, 0.1, 0.2, and 0.5%. With 0.1 and 0.2% cysteine in the extracting solution, the amount of Hg removed after each stage of extraction is fairly constant. After three extractions, about 22% of the Hg was removed in the fish treated with the 0.1% cysteine solution and 40% was removed in the fish treated with 0.2% cysteine solution. When the cysteine content of the extracting solution was increased to 0.5%, about 40% of the Hg was removed in the first extraction, but only an additional 10% was removed in the combined second and third extractions.

The apparent increase in Hg content in the fish extracted with 0.1 *M* NaCl alone indicated that the Hg is associated with the myofibrillar or other insoluble proteins but not with the soluble sarcoplasmic proteins that are removed by the 0.1 *M* NaCl solution. This was verified when we found no detectable quantities of Hg in the sarcoplasmic fractions extracted with 0.1 *M* NaCl containing no added cysteine.

Column Extractions. When the comminuted fish was extracted in a column, we again found that the reduction of Hg was related to the concentration of cysteine in contact with the tissue. A series of six column extractions were made in which the concentration of cysteine was varied from 0 to 0.5%. The pattern of these experiments (Figure 2) is similar to that of the batch operation in that optimal reduction is achieved when the extractant contains 0.3% cysteine. As is to be expected, the column extraction is more efficient than batch extraction. These reductions were achieved with only half the volume of cysteine solution

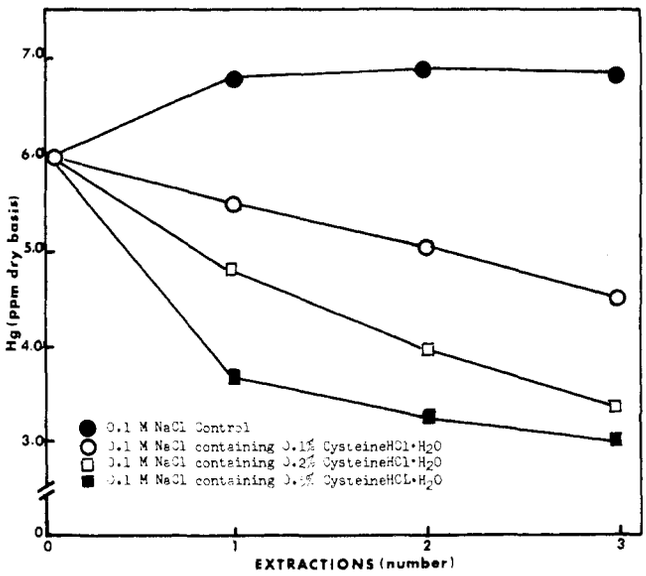

Figure 1. Relation of Hg reduction to the concentration of cysteine in a batch extraction procedure. The ratio of fish to extracting solution was 1:2 and the cysteine was added to only the first stage. Extraction on the remaining stages was done with azeotropic isopropyl alcohol.

needed in the batch extractions. Again, the apparent increase in Hg in the saline-extracted fish containing no cysteine shows that the Hg is associated with the insoluble protein fractions.

Effect of pH. Halibut has a normal pH of about 6.5 and can vary from 5.5 to 7.0 (Patashnik, 1966). Experiments were conducted to determine the effect of pH in roughly that range on the efficacy of cysteine in removing Hg from comminuted halibut muscle. Extracting solutions of 0.1 *M* NaCl and 0.2% cysteine were adjusted with either sodium citrate buffers, citric acid, or sodium bicarbonate to pH's of 4.5, 5.0, 5.5, 6.0, 6.5, and 7.0. The results of these experiments (Figure 3) show that only a 15–20% reduction was achieved when the extractions were carried out be-

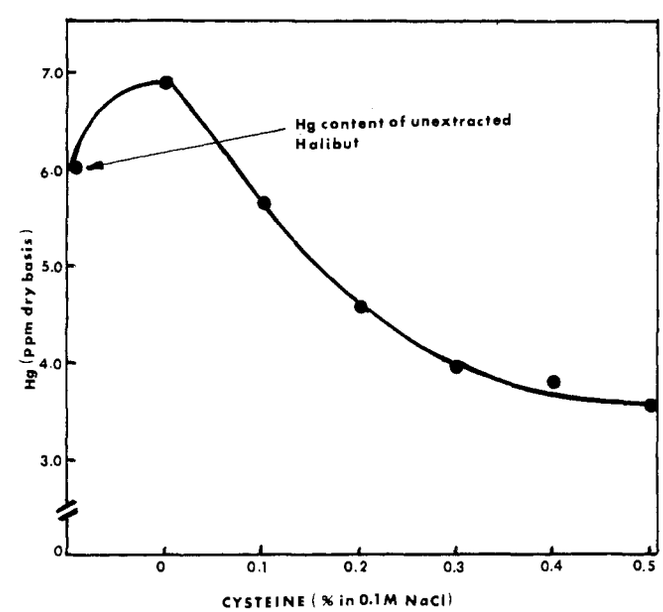

Figure 2. Relation of Hg reduction to the concentration of cysteine in a column extraction procedure. The ratio of fish to extracting solution to start the extraction was 1:1. Two additional volumes of 0.1 *M* NaCl extracting solution were used to complete the extraction.

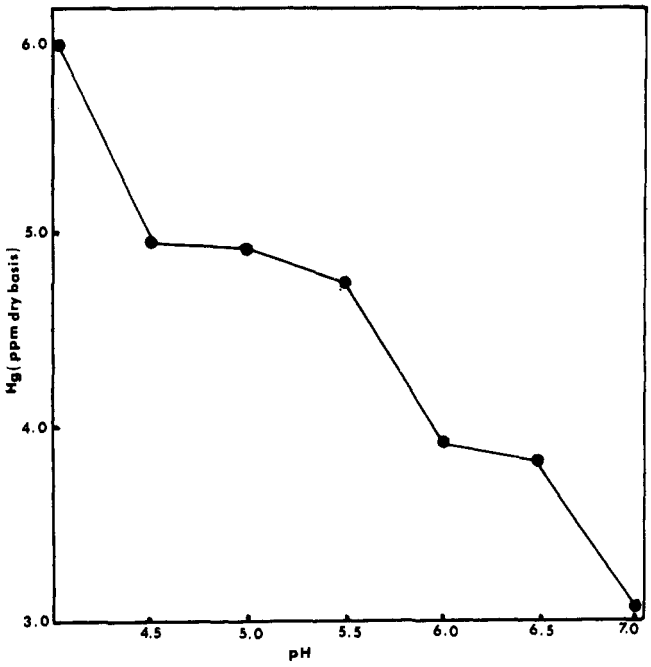

Figure 3. Relation of pH to Hg reduction in a batch extraction procedure.

tween pH 4.5 and 5.5. The amount of Hg removed increased sharply as the pH was raised toward 7.0, where almost 50% of the mercury was removed. Extractions at pH's higher than 7.0 or lower than 4.5 were not investigated because myofibrillar proteins are solubilized outside these limits (Spinelli *et al.*, 1972). Neither citrate nor bicarbonate had any detectable effect on the removal of mercury when they were used to adjust the pH of the fish from 6.5 to 7.0.

Effect of Contact Time and Temperature. Extractions were performed in which the contact time of the extracting solutions was varied from 5 to 120 min. No significant differences were found in the amount of Hg that could be removed when the contact time was increased. No difference in Hg removal was found when the extractions were carried out at either room temperature or at 3°. These observations indicate that rate of reactions or displacement of the Hg from the fish tissue is rapid and that, of the parameters investigated, only pH and concentration of cysteine influence this reaction.

Cysteine Residue in the Extracted Tissue. The amount of cysteine remaining in the tissue extracted with cysteine was estimated by measuring the sulfhydryl content in 100 ml of the extracting solution after each stage of extraction. Corrections were made for naturally occurring sulfhydryls by substracting the sulfhydryl content of the 0.1 *M* NaCl extracting solution containing no cysteine from that of the 0.1 *M* NaCl extracting solution containing cysteine. A direct measurement was also made by determination of the cysteic acid content of comminuted tissue that was extracted with 0.1 *M* NaCl containing no cysteine and 0.2% cysteine. Results of this experiment (Table II) show that 92% of the added cysteine can be accounted for by totaling the amount of cysteine found in the three extraction solutions. Direct analysis of the freeze-dried extracted tissue showed 0.083 $\mu M/g$ of cysteic acid in the sample extracted with 0.1 *M* NaCl containing 0.2% cysteine and 0.080 $\mu M/g$ in the sample extracted with 0.1 *M* NaCl. These results show that the added cysteine does not form a strong bond with the fish protein and that when the comminuted fish is treated as described, the increase in the cysteine content of the fish would be in the range of 4–8%.

Table II. Cysteine Recovered in 100 ml of Extracting Solution Containing 0.2% Cysteine·HCl·H_2O after Each Extraction Stage[a]

Extraction stage	mg of Cysteine[b] in extractant	% of the total added cysteine
1	137	68
2	44	22
3	4	2
Total	185	92

[a] Mean value of three determinations. [b] Calculated as cysteine·HCl·H_2O.

Table III. Organoleptic Evaluations of Fish Portions Prepared from Modified Fish Blocks of Comminuted Halibut Extracted with 0.1 *M* NaCl and with 0.1 *M* NaCl Containing 0.2% Cysteine

	Extracted with 0.1 *M* NaCl		Extracted with 0.1 *M* NaCl containing 0.2% cysteine	
Test interval	Flavor	Texture	Flavor	Texture
Initial	4.5	4.7	4.3	4.3
2 months	4.6	4.8	4.0	4.3

NOTE: Flavor and texture scores are given as the mean value of 12 separate tests.

Utilization of Comminuted and Extracted Fish Tissue. Recent developments have shown that deboned comminuted fish flesh can be formed into fish blocks of high quality for further processing into fish sticks and portions (King and Carver, 1966; Miyauchi and Steinberg, 1970). Current work by Miyauchi (1972) shows that extraction of at least some of the sarcoplasmic proteins with water prior to formulating the block retards the development of rancid flavors during frozen storage. The feasibility of using cysteine-NaCl-extracted tissue for use in comminuted fish blocks was evaluated in this study.

Organoleptic Evaluation of Portions Prepared from Fish Blocks. Fish blocks were prepared from comminuted halibut tissue that had been extracted with NaCl containing 0.2% cysteine. The blocks were prepared as described by Teeny and Miyauchi (1972). They were frozen and held at −20°F prior to organoleptic evaluation. After 0 and 2 months of storage, portions were cut from the prepared blocks, deep-fat fried, and evaluated. The results given in Table III show that no significant differences could be detected between control and cysteine-treated samples on the initial evaluation. After 2 months of storage, the cysteine-treated samples received lower test scores than the untreated samples (95% level of significance). Questioning of the panel judges revealed that they gave lower flavor scores to the cysteine-treated samples because of lower flavor intensity rather than to the development of an "off" flavor. They felt that slight texture difference between the samples was related to their flavor reaction. It is possible that some of the added cysteine could react with proteins to provide S–S linkages, thus changing the textural characteristics of the protein. Although further research is indicated in this area, the formation of S–S linkages with oxidizing agents in fish meat to increase the jell strength of kamaboko was demonstrated in 1961 by Okada and Nakayama.

Reduction of Mercury in Fish Protein Concentrate (FPC). Although most of the commercial catch of fish falls below the current guideline of 0.5 ppm of Hg, the utilization of cysteine in removing Hg from fish suggests that much of the fish that exceeds the guideline could be processed into useful food. The preparation of FPC offers considerable promise in processing fish of low commercial value into protein supplements of relatively high commercial value (Finch, 1970). An evaluation was made therefore to assess the effectiveness of cysteine in reducing the

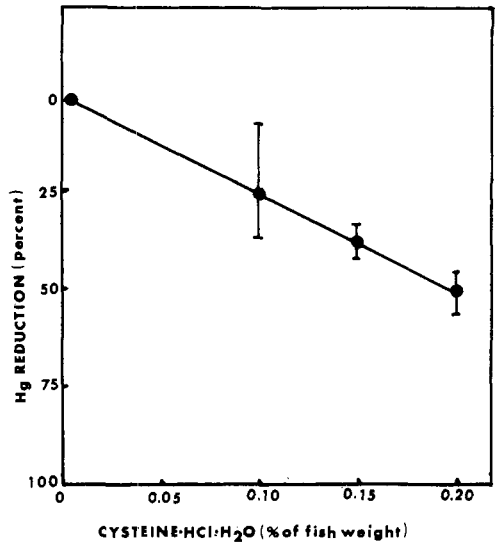

Figure 4. Relation of Hg reduction to cysteine concentration in laboratory preparation of FPC.

Hg content of FPC made by the isopropyl alcohol extraction process.

Effect of Cysteine Concentrations. FPC was made in the laboratory to determine the amount of Hg that could be removed in a countercurrent extraction system when the cysteine concentration in the process was varied. Figure 4 shows the range and mean % reduction of Hg when the cysteine content of the isopropyl alcohol extractant was varied between 0 and 0.2%. Mean reduction values of 25, 38, and 50%, respectively, were achieved when 0.1, 0.15, and 0.2% cysteine was added in the process. Table IV shows the amount of Hg in each of four batches of FPC produced in a full four-stage countercurrent process. The distribution of Hg reduction values between the first and fourth batches indicated that Hg reduction was proceeding at a fairly uniform rate after the system had been brought to a full countercurrent operation. This observation was confirmed when the FPC was made at the EDP

Table IV. Reduction of Mercury in Each Batch of FPC Prepared by a Four-Stage Countercurrent Extraction Procedure

% cysteine added based on weight of wet fish	Batch number	ppm of Hg found in FPC	% Hg reduction[a]
0	1	0.44	
	2	0.45	
	3	0.44	
	4	0.42	
		0.44[b]	
0.10	1	0.28	36
	2	0.36	18
	3	0.32	27
	4	0.28	36
0.15	1	0.20	55
	2	0.21	52
	3	0.25	43
	4	0.24	45
0.20	1	0.20	55
	2	0.20	55
	3	0.24	45
	4	0.24	45

[a] % Hg reduction was calculated by subtracting the Hg found in the FPC in each batch from the mean Hg value in the FPC prepared without cysteine. [b] Mean value.

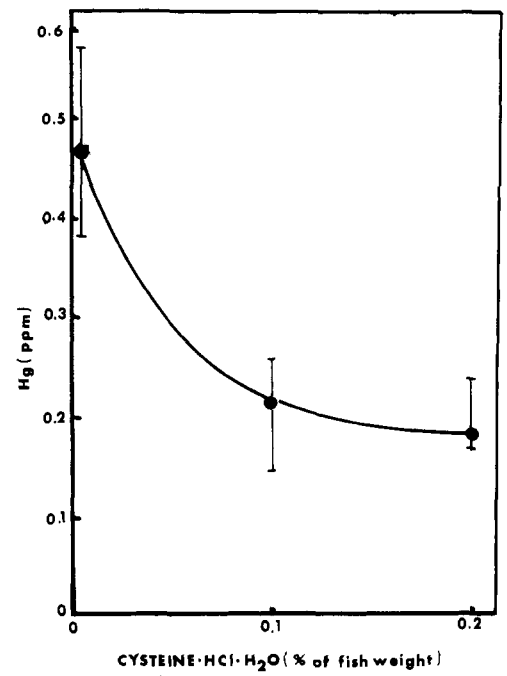

Figure 5. Relation of Hg reduction to cysteine concentration in FPC prepared at the Experimental Demonstration Plant in Aberdeen, Wash.

located in Aberdeen, Wash. Twenty-eight batches of FPC using 0.1% cysteine in the system and 20 batches using 0.2% cysteine were prepared. All batches were analyzed for Hg and the results are summarized in Figure 5. These data show a mean reduction of 55% when 0.1% cysteine was used and 59% when 0.2% cysteine was used.

Other than the addition of the cysteine to the first extraction stage, no change in processing techniques was required. Some of the plant personnel said that they could detect a slight "sulfury" odor when cysteine was being used in the process. No measurement, however, was made for H_2S or the mercaptan content of the plant atmosphere. Organoleptic evaluation of the FPC made at the EDP showed no detectable differences between that made with no cysteine and that made with cysteine in the system. When prepared in the laboratory, we occasionally observed that at the higher level of cysteine (0.2%) the FPC was slightly darker than that prepared without cysteine.

Analysis of Still Bottoms for Hg. To establish a material balance of the mercury removed during FPC processing with cysteine in the system, the alcohol miscellas were distilled and the still bottoms were analyzed for Hg. During normal plant operations, the miscella is often acidified to prevent amines from codistilling with the alcohol (Ernst, 1971). In the following experiments, the pH of the miscellas was adjusted to 6.5, 4.5, and 3.8 with 1 *M* H_2SO_4 prior to distillation. Results from this work (Table V) show that all of the mercury was accounted for in the combined still bottom fractions. In a countercurrent FPC

Table V. Total Amount of Mercury Recovered in Still Bottoms after Alcohol Was Distilled at pH 6.5, 4.5, and 3.8

pH of miscella	μg Hg per liter M-1	μg Hg per liter M-2	μg Hg per liter M-3	μg Hg per liter M-4	μg total Hg in M-1, 2, 3, and 4	% of total theoretical[a] Hg
6.5	18.2	3.8	1.6	1.0	23.7	101
4.5	18.8	2.0	1.6	3.2	25.6	112
3.8	16.4	2.0	0.8	2.4	21.6	92

[a] Theoretical Hg based on a 59% reduction of Hg in hake containing 0.08 ppm of Hg (23.6 μg). 500 grams of fish were used in each experiment; 0.2% cysteine was used during preparation of the FPC.

Table VI. Cysteine[a] Content of FPC Prepared with 0, 0.1, and 0.2% Cysteine Added in the Process

	Method of preparation					
	With no cysteine		With 0.1% cysteine		With 0.2% cysteine	
Sample number	μM cysteic per mg FPC	Ratio cysteic to alanine	μM cysteic per mg FPC	Ratio cysteic to alanine	μM cysteic per mg FPC	Ratio cysteic to alanine
1	0.075	0.15	0.077	0.15	0.086	0.16
2	0.050	0.13	0.075	0.16	0.086	0.17
3	0.062	0.14			0.089	0.16
1[b]	0.065	0.13	0.062	0.13	0.070	0.15

[a] Determined as cysteic acid. [b] Determination made after the samples had been refluxed for 4 hr with azeotropic isopropyl alcohol.

process, alcohol is recovered from the first miscella (M-1). About 75% of the removed Hg was found in M-1 regardless of the pH at which the alcohol was recovered. These data show that all of the removed Hg can be accounted for and indicate that during alcohol recovery Hg will not contaminate the alcohol.

Cysteic Acid Content of FPC. The cysteic acid content of FPC prepared with 0, 0.1, and 0.2% added cysteine during processing was determined and the results are given in Table VI. These data show the μM of cysteic acid per mg of FPC increased from a mean value of 0.062 for the FPC prepared with no cysteine during processing to 0.076 and 0.087 for those FPC's prepared with 0.1 and 0.2% added cysteine, respectively, during processing. From these analyses, we calculated that 25% of the added cysteic acid at the 0.1% level of addition and 34% at the 0.2% level of addition remained associated with the FPC. To determine whether the increased cysteic acid content of the FPC had resulted from irreversible binding with fish proteins, aliquots from a set of the FPC's shown in Table VI (sample No. 1) were refluxed with azeotropic IPA and the cysteic acid values were redetermined. The results given in Table VI show that all three FPC samples have practically the same cysteic acid content after refluxing. This would indicate either loose binding of the cysteine with the protein or that the cysteine was oxidized to the relatively insoluble cystine that could either bind to the proteins or be trapped with the fish solids.

CONCLUSIONS

This work demonstrates that with some methods of fish processing, the use of cysteine could prove useful in reducing the Hg content of fish that are over the current 0.5-ppm guideline. The amount of Hg that can be removed from the fish is related to the concentration of cysteine and to the pH of the system. The concentration relation, however, is not linear and reductions of over 50% may be difficult to achieve without modifying the properties of the proteins. The most efficient use of cysteine in reducing the Hg content of comminuted fish was obtained when the cysteine-containing extraction solution was percolated through the fish contained in the column.

When cysteine is used in an aqueous system, only minimal cysteine (4–8%) residues are left in the product, indicating that cysteine does not bind irreversibly with the fish proteins.

Cysteine can also be added to isopropyl alcohol to reduce by at least 50% the Hg content of FPC that is prepared by the isopropyl alcohol extraction process. The removed Hg is soluble in the alcohol miscella, but does not codistill during alcohol recovery.

The cysteine (calculated as cysteic acid) content of the FPC is increased by about 20%. The increased cysteic acid content of the FPC can be reduced to normal levels by refluxing with azeotropic isopropyl alcohol, again indicating that the cysteine or possibly cystine (converted from cysteine during processing) is weakly bound to the FPC.

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