Reduction in Mercury Content of Fish Protein Concentrate by Enzymatic Digestion

The effect of proteolytic digestion on the distribution of mercury in fish protein concentrate (FPC) has been investigated. A two- to sevenfold concentration of mercury in the insoluble FPC fraction and a corresponding reduction of mercury level in the soluble FPC fraction was achieved after proteolysis.

Recently there has been considerable interest and concern about the occurrence of mercury in fish. Most of the mercury is reportedly in the methyl form, probably attached to protein thiol groups (Westoo, 1967). The U. S. Food and Drug Administration's interim guideline for methyl mercury in fish is 0.5 ppm.

During the processing of fish into FPC (fish protein concentrate) by isopropyl alcohol extraction, the protein is concentrated by a factor of 4–6 because of the removal of water and lipids, and there is a corresponding fivefold enhancement of mercury concentration (Gasiewicz and Dinan, 1972). This indicates that little or no mercury is extracted from fish during concentrate manufacture. It would be advantageous to find ways of removing mercury from fish protein in order to lower the amount of mercury in FPC and fish that are found to be above the U. S. Food and Drug Administration's guideline.

Recently two reports showed that significant amounts of mercury can be removed during FPC processing by extracting either with acidified isopropyl alcohol (Regier, 1972) or with a cysteine wash (Spinelli *et al.*, 1973). In this study, we show that the amount of mercury can be reduced in FPC by proteolytic digestion.

MATERIALS AND METHODS

Enzymes. The following commercial enzyme preparations were used: Pancreatin, B grade (porcine pancreas), Calbiochem; Pronase, B grade *(Streptomyces griseus)*, Calbiochem; and Monzyme PA-I *(Bacillus subtilis)*, Monsanto. Mercury levels in the commercial enzyme preparations used were too low to have any significant effect on the results (Pancreatin, 0.2 ppm; Pronase, 2.6 ppm; Monzyme, 0.2 ppm).

Preparation of FPC Samples. FPC samples were prepared from either Pacific hake *(Merluccius productus)* or swordfish *(Xiphias gladius)*. The hake FPC sample was produced by isopropyl alcohol extraction of whole fish in an experiment and demonstration plant as described by Ernst (1971). The swordfish FPC sample was produced in the laboratory by a cross-current isopropyl alcohol extraction method (Brown and Miller, 1969). The extracted sample was then freeze-dried and ground.

Enzyme Digestion. 10% slurries of FPC were digested for periods of 1 and 6 hr at 50° with Monzyme at pH 8.8 and with Pronase and Pancreatin at pH 8.5. Enzyme levels were 1% enzyme/FPC (w/w); pH levels were maintained by the addition of 7 *N* ammonium hydroxide. After digestion for 1 or 6 hr, the reaction was stopped by pouring the mixture into two 50-ml centrifuge tubes and spinning down the residue at 4° for 5 min and 5090 × *g*. The residue was suspended in distilled water and recentrifuged. The clear supernatants were combined and freeze-dried after most of the water was removed by flash evaporation at a bath temperature of 40°. The residue was also freeze-dried. The soluble and insoluble fractions were then analyzed for mercury content.

Mercury Analysis. The method used for mercury analysis was based on a modification of the procedure developed by the Canadian Freshwater Institute (Armstrong and Uthe, 1971; Uthe *et al.*, 1970).

Triplicate freeze-dried samples weighing 0.1–0.3 g were placed on 125-ml glass-stoppered Erlenmeyer flasks and digested overnight with 7 ml of concentrated sulfuric acid in a 55° water bath. Standard solutions of mercuric chloride (Johnson Matthey Spec. pure) containing 200, 400, and 600 ng of mercury or blanks consisting of 1 ml of distilled water were treated in a similar manner. After cooling in an ice bath, samples were oxidized with 20 ml of freshly prepared filtered 6% potassium permanganate. Samples were allowed to stand with occasional shaking for a minimum of 1 hr. Excess permanganate was destroyed by the dropwise addition of 30% hydrogen peroxide and the sample was diluted to 35 ml with freshly redistilled water.

By use of a Technicon sampler II, operating at 20 samples/hr with a 1:6 sample to wash (1 *N* H_2SO_4) ratio, 4-ml sample cups and a Technicon proportioning pump, the mercury in a 3.4-ml subsample was reduced with an equal volume of reductant (1% hydroxylamine sulfate, 2% stannous sulfate, and 0.5% sodium chloride solution) in a 14-turn mixing coil. The resulting mercury vapor was equilibrated with air from a Metaframe (aquarium) pump, forced through a 28-turn mixing coil, and measured by flameless atomic absorption at 2537 Å in a 250 × 15 mm heated (heat tape 35–40°) flow-through cell (Techtron). The atomic absorption spectrometer was a Varian Techtron AA5 unit equipped with a mercury hollow cathode tube, 100-μ slit, and R106 photomultiplier tube.

The concentration of mercury in samples was calculated from the corresponding standard curve (standards treated with 7 ml of H_2SO_4 and 20 ml of $KMnO_4$).

RESULTS AND DISCUSSION

The effect of proteolytic digestion on the mercury content of the water-soluble and insoluble fractions of FPC is shown in Table I. A two- to sevenfold concentration of mercury in the insoluble FPC fraction and corresponding reduction in mercury level in the soluble FPC fraction was achieved after proteolysis. For example with Pancreatin, in 1 hr roughly 90% of the mercury remained in the insoluble fraction whereas only 10% of the mercury was in the soluble product. After 6 hr, roughly 80% of the mercury was found in the insoluble fraction and 20% in the soluble product. The results with Pronase and Monzyme were similar to those obtained with Pancreatin, although the 6-hr level of mercury in the soluble fraction of Monzyme-treated hake FPC was extremely low. We consider the absolute mercury concentrations to be less significant than the fact that in every case the mercury level in the soluble fraction was much lower than that of the insoluble fraction. Of equal significance is the fact that FPC prepared from swordfish behaved in an identical fashion to that prepared from hake.

The concentration of mercury in the enzyme-resistant fraction may have important implications for the toxicological interpretation of mercury levels in FPC or other highly denatured protein concentrates. On the basis of results with Pancreatin, the biological availability of mercury is only about 20% of the total mercury content if we assume that the insoluble fraction would pass through the

Table I. Effect of Enzyme Treatment on Mercury Content of Soluble and Insoluble Fractions of FPC Made from Pacific Hake and Swordfish

Enzyme	Digestion time, hr	% FPC in		Mercury content, ppm		% recovery	
		Soluble fraction	Insoluble fraction	Soluble fraction	Insoluble fraction	FPC	Hg
		Pacific hake, 0.49 ± 0.03 ppm of Hg					
Pancreatin	1	63.3	34.4	0.08 ± 0.02	1.33 ± 0.06	97.7	104
	6	84.3	16.8	0.12 ± 0.01	2.49 ± 0.10	101.1	106
Pronase	1	57.6	41.3	0.14 ± 0.01	1.13 ± 0.07	98.9	112
	6	89.6	11.8	0.15 ± 0.02	3.71 ± 0.15	101.7	116
Monzyme	1	49.3	47.6	0.19 ± 0.04	0.84 ± 0.04	96.9	100
	6	77.7	23.4	0.05 ± 0.01	2.05 ± 0.07	101.1	106
		Swordfish, 6.39 ± 0.51 ppm of Hg					
Monzyme	1	50.3	43.8	0.64 ± 0.01	13.63 ± 0.92	94.1	99
	6	77.9	15.5	1.04 ± 0.01	31.94 ± 0.63	93.4	90

gut unabsorbed. Either the mercury is bound to an extremely enzyme-resistant fraction or the presence of mercury in the protein lends the property of enzyme resistance.

FPC has poor functional properties for incorporation into food systems. Enzymatic solubilization has been suggested as a method for improving its functionality (Archer *et al.*, 1973; Cheftel *et al.*, 1971). We have shown that an additional benefit gained by enzyme digestion is a significant reduction in mercury levels in the soluble product.

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Long-Term Preservation of Carotene in Alfalfa Meal

Alfalfa stems and leaves were steam-blanched, dried, and ground to a meal containing 374 ppm of carotene. Samples stored *in vacuo* in darkness at 90° for 27, 35, and 55 weeks retained 98% of the carotene. A final sample, kept 27 years at room temperature, retained 95.5% carotene. Isomerization of carotene was negligible in all samples. Possible importance for space trips is suggested.

It is well established that molecular O_2 is responsible for most loss of carotene in the preparation of alfalfa meals (Griffith and Thompson, 1949) and other dried plant products (Booth, 1957; Seshan *et al.*, 1942). Enzymic oxidation is very important, hence the need for blanching soon after harvest (Goodwin, 1954; Zscheile *et al.*, 1943). Zscheile and Whitmore (1947) found no appreciable loss in dried alfalfa meal during 4 months of storage in bottles at −18°.

EXPERIMENTAL SECTION

Soon after the development of a reliable and simple method for determination of carotene in alfalfa (Zscheile and Whitmore, 1947) samples of first-cutting alfalfa were prepared for accelerated storage tests at the Department of Botany, University of Chicago. Stems with leaves were harvested in mid-afternoon of a bright sunny day (May 8, 1945) and blanched with steam for 3 min at 15 lb within 10 min of cutting. After drying in a circulating hot air