MFR PAPER 1034

An enzymatic FPC process could make profitable use of some of our underutilized or latent resources.

Using Enzymes to Make Fish Protein Concentrates

MALCOLM B. HALE

ABSTRACT

Fish protein concentrates having desirable functional properties can be prepared by using selected enzymes to solubilize protein and release lipids. An enzymatic FPC process could make profitable use of some of our underutilized or latent resources.

The relative activities of 23 commercially available proteolytic enzyme preparations acting on a fish protein substrate were measured. Pancreatin, pepsin, and papain had highest activity per unit cost of enzyme.

Soluble hydrolysates were prepared from red hake (Urophycis chuss) *using a variety of enzyme and digestion conditions. Concentrations of the amino acids tryptophan and histidine in the soluble products were critical nutritionally and varied with the pH of hydrolysis. A soluble FPC having a protein efficiency ratio equal to that of casein was prepared with an alkaline bacterial enzyme at pH 8.5.*

Production costs have been estimated for a soluble product prepared from whole fish and a partially soluble product prepared from presscake through the use of enzymes.

INTRODUCTION

Fish protein concentrates (FPC's) are made by processes which concentrate fish protein into a more stable form. As a form of animal protein, FPC's can supplement vegetable proteins very effectively. Added in low concentrations they can markedly improve the nutritive value of bread and many other common foods by supplying certain essential amino acids deficient in vegetable proteins. Produced from species of fish not normally used as food, they are an inexpensive source of animal protein and could provide a profitable use for some of our presently underutilized or latent resources.

FPC's may be produced by various processes to give products with different costs and properties, thus different applications. Most of these processes are described in a recently compiled bibliography published by the Library of Congress (1970). Most of the processing methods can be classified as chemical (solvent extraction) or biological (enzymatic and microbial) procedures. During recent years most efforts have involved the use of solvents, usually isopropyl alcohol, and several pilot plants and a few full-scale industrial plants have been constructed (Ernst, 1971). By solvent extraction FPC can be produced that is a bland, nearly odorless, lightly-colored, water-insoluble, but highly nutritive powder. FPC's prepared by biological procedures are usually more flavorful and may have desirable functional properties. In general the biological procedures have not advanced beyond the laboratory or small pilot plant stage.

Malcolm B. Hale is a member of the staff of the College Park Fishery Products Technology Laboratory, National Marine Fisheries Service, NOAA, College Park, MD 20740.

THE BIOLOGICAL METHOD

The work described in this paper has been called the "Biological Method" within the fish protein concentrate (FPC) program of the National Marine Fisheries Service. Biological processing studies at the College Park Fishery Products Technology Laboratory have been concentrated on the use of proteolytic enzymes to prepare soluble hydrolysates of fish protein.

Fish proteins are complex molecules consisting of chains of simpler molecules called amino acids linked together by peptide bonds. Proteolytic enzymes are also proteins and are structured in such a way that they act as catalysts in the breakage of peptide bonds by a process called hydrolysis. Most of the shorter amino acid chains broken off from the fish protein are water-soluble. Fish lipids (fat) are also released from the tissue during hydrolysis and can be physically separated by centrifugation.

The enzyme systems employed in fish protein hydrolysis may be either the natural enzymes contained in the fish (autolysis), purified commercial preparations from various biological sources (vegetable, animal, or microbial), or the enzymes supplied by living cultures of microorganisms.

Development of a biological process was pursued because (1) the product can have special properties (e.g., solubility) which make it more suitable for certain applications than is the solvent extracted FPC, (2) it may be possible to produce an acceptable protein concentrate more cheaply

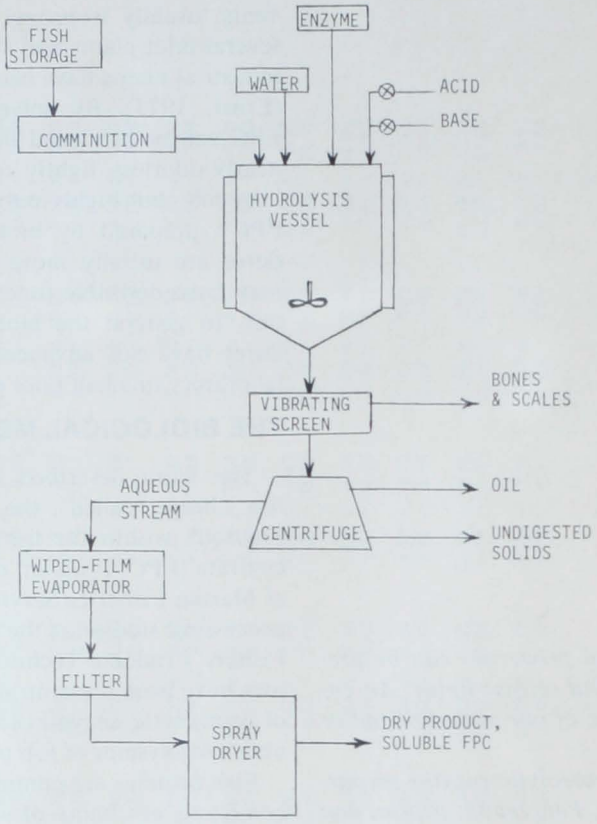

Figure 1.—Basic process outline for enzymatic hydrolysis of fish.

through biological means than through solvent extraction, and (3) a biological method with proper controls would be more suitable for use on shipboard or for use in remote areas.

A process has been developed for the preparation of a nutritious, totally water-soluble concentrate derived from fish protein which may be used to advantage in foods such as soups and beverages. Although processing costs have been considered throughout the work, the major concern has been for improvement of the amino acid pattern and nutritive value of the soluble product.

The basic biological process is depicted in Figure 1. Enzymatic digestion of a whole fish slurry with control of pH and temperature is followed by screening to remove bones and scales. Insoluble solids are separated by centrifugation and a clarified hydrolysate is spray-dried (Figure 2) to yield a soluble product consisting of peptides, polypeptides, and some free amino acids. An alternate product, easily dispersible but only partially soluble, is prepared by eliminating the centrifugation step. Either product could be sold as a concentrated paste with elimination of the drying step.

SELECTION OF ENZYMES

In early research the relative proteolytic activities of 23 commercially available enzyme preparations were compared. A specially prepared fish protein substrate was used in standard hydrolysis tests of 1-hour and 24-hour durations. Ficin was most active for a 1-hour period. Over the longer hydrolysis period the enzymes pepsin, papain, and pancreatin ranked highest on the basis of activity per unit cost. These results were detailed in a previous publication (Hale, 1969).

When whole red hake *(Urophycis chuss)* was used the relative effectiveness of the various enzymes changed because of the added effect of native fish enzymes. The relative effectiveness of pepsin, for instance, was low with whole fish because the native enzymes are not active at pH 2 which is optimum for pepsin. Over a period of time, many products were prepared in 5-liter batches (Figure 3) using different enzymes and processing conditions. Average yields of dry, soluble product with each type of enzyme are listed in Table 1. Amino acid analyses and

Table 1.—Average yields of dry solids from red hake solubilized by proteolytic enzymes.

Enzyme(s)	Number of runs	Yield, dry solubles/wet fish, % ± std. deviation
Autolysis	5	10.0 ± 0.4
Pepsin	1	10.9
Papain	5	11.3 ± 0.8
BPN	4	11.7 ± 0.10
Bromelin, Ficin	3	12.3 ± 0.3
Rhozyme P-11	3	12.5 ± 0.75
Pancreatin	7	13.8 ± 1.3
Alkaline proteases	5	14.3 ± 1.0

limited feeding studies indicated that hydrolysis at pH 8.5 with an alkaline protease of *Bacillus subtilis* resulted in the best nutritive value as well as the highest yield of product.

HYDROLYSIS CONDITIONS AND AMINO ACIDS

Raw fish is hydrolyzed so that the activity of native enzymes is combined with that of added enzymes. In initial runs the fish was cooked as a control measure prior to hydrolysis. Both yields and nutritive value were poor. An experiment showed that the degree of solubilization achieved with raw hake could be obtained with cooked hake only if six times as much commercial enzyme were added.

Soluble hydrolysates prepared from cooked hake had low nutritive values as the sole source of protein in feeding studies with rats. The concentration of the essential amino acid tryptophan was quite low in these products. Treating raw hake under slightly alkaline conditions gave a good yield of product containing adequate tryptophan. The positive correlation between the level of tryptophan and the yield of

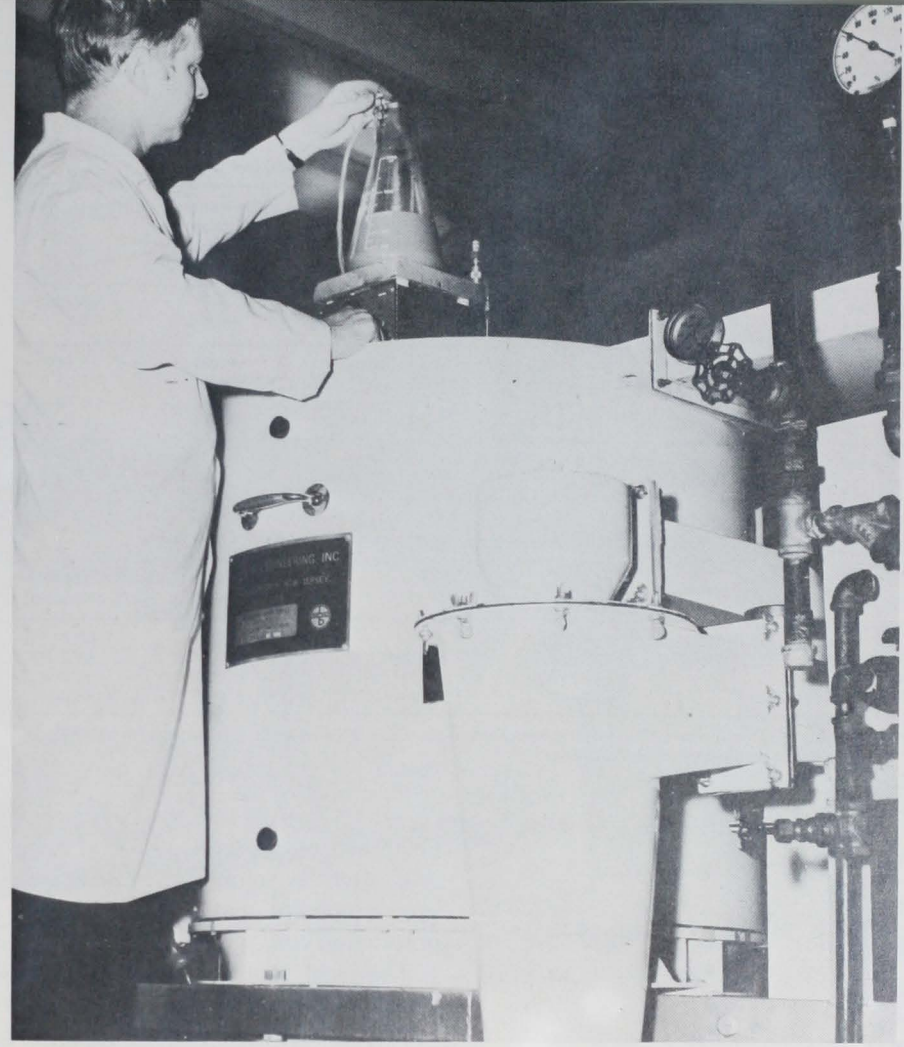

Figure 2.—Spray drying a partially soluble hydrolysate.

soluble solids is shown in Figure 4. The required yield of 13 percent (of fish weight) can only be obtained with added enzyme, not by autolysis alone.

Hydrolysis with pancreatin at pH 8 gave good yields of product containing adequate levels of tryptophan but the nutritive value was unsatisfactory. This corresponded with a low level of histidine, an amino acid which is essential for infants (or young rats) although not for adults. When an alkaline protease derived from the bacteria *B. subtilis* was acquired and tested at pH 8.5, both the histidine level and nutritive value of the product were significantly improved. Pancreatin also gave better results at pH 8.5 than at pH 8.

EVALUATION RUNS

Three enzymatic processes were evaluated in replicate runs using red hake. Hydrolyses with pancreatin, Alcalase[1], and by autolysis were tested by three 5-liter (1.3 gallon) batches plus a 20-gallon batch for each process. Three processes were also tested in runs with the fatty fish alewife *(Alosa pseudoharengus)* using autolysis and the alkaline protease Alcalase. Average yields and chemical compositions of raw fish and of the hydrolysates prepared from hake and alewife by each of two processes are listed in Table 2.

Protein efficiency ratios (PER's) were determined by rat feeding trials with the hydrolysates of hake and alewife and are listed in Table 3. The products prepared from hake were inferior to casein as a sole source of protein, but were statistically equivalent to casein as a supplement to wheat flour. All of the products prepared from alewife were statistically equivalent to casein as a sole source of protein. Thus, we obtained a totally soluble hydrolysate from alewife with

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

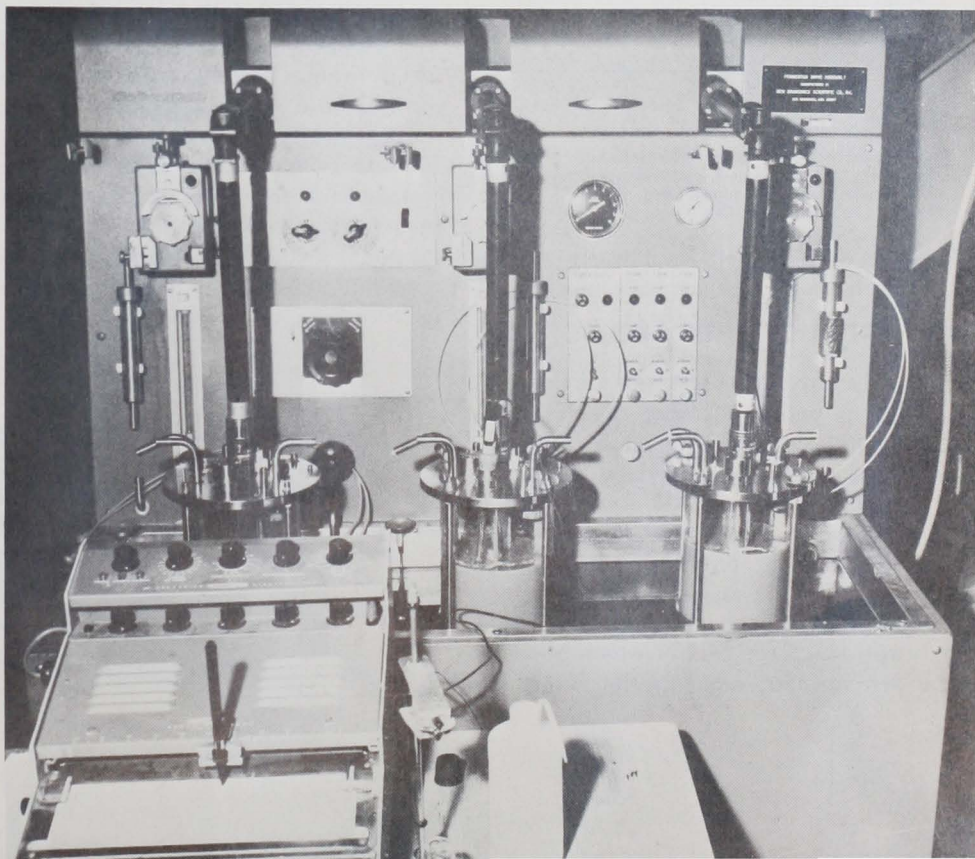

Figure 3.—Experimental system used for fish protein hydrolysis in 5-liter batch.

Table 2.—Yields and composition of hydrolysates and raw fish.

Item	Red hake hydrolysates		Raw hake	Alewife hydrolysates		Raw alewife
Enzyme	Pancreatin	Autolysis		Alcalase	Alcalase	—
Concentration	0.5%	—	—	0.5%	0.3%	—
Type of product	Soluble	Whole slurry	—	Soluble	Whole slurry[1]	—
Number of runs	4	4	8 samples	3	3	3 samples
Average yield[2]	14.3%	17.9%	—	13.0%	18.7%	—
Avg. % Protein	77.23	77.85	15.89	76.01	65.24	17.16
% Moisture	4.44	4.02	79.09	4.89	4.21	71.07
% Ash	16.02	6.73	2.73	16.52	9.32	2.74
% Fat[3]	0.16	12.41	3.06	0.21	25.07	8.35

[1] Prepared from alewife presscake.
[2] Dry product as weight percent of raw fish.
[3] Total lipids by the SAK method (Smith, Ambrose and Knobl, 1964).

Table 3.—Nutritive quality of enzymatic hydrolysates of red hake and alewife.[1]

Material hydrolyzed	Enzyme added	Product solubility	Protein efficiency ratio Actual	Protein efficiency ratio % of casein
Raw hake	Pancreatin	Total	[2]2.89 ± 0.07	82.2
Raw hake	(Autolysis)	Partial	2.87 ± 0.07	81.7
Raw alewife	Alcalase	Total	3.44 ± 0.06	97.2
Alewife presscake	Alcalase	Partial	3.40 ± 0.08	96.0

[1] Each feeding sample was a blend of the spray-dried products from three 5-liter hydrolysis batches.
[2] Average plus standard error of the mean.

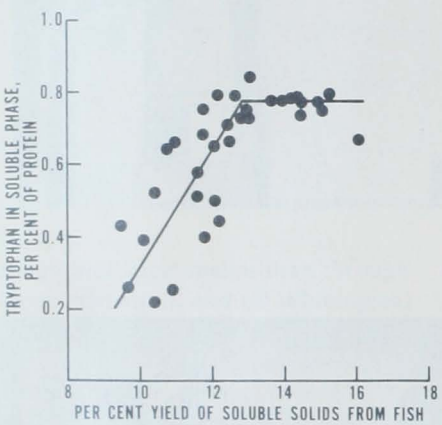

Figure 4.—Tryptophan concentration in soluble solids versus percent yield of solubles from enzymatic hydrolysis of red hake.

the desired PER level which we had been unable to reach with hake.

PRODUCT USES AND COSTS

Limited food research studies have been carried out with the enzymatic hydrolysates of whole fish. For most food applications a bland product is desirable and a good soluble protein for use in carbonated beverages would command a premium price. Flavor is probably the major unsolved problem but FPC's from whole fish could be of real value for uses in which good nutritive and supplemental value are combined either with a special property, such as solubility, or with process simplicity and a relatively low production cost.

The most promising immediate application for biological FPC's is as a partial replacement for milk in the diets of weanling calves. The combination of good nutritive value and dispersibility make fish protein hydrolysates well suited for this use. Fish autolysates have been used as milk replacers in France. The presscake hydrolysis process also shows promise of economic feasibility.

Process flowsheets have been developed for enzymatic processes using raw whole fish and fish presscake. Material balances obtained experimentally were used to estimate production costs for the two types of products through use of a recently developed computer program (Almenas et al., 1972). Assuming a plant processing 250 tons per day of fish costing 1.5¢/lb and operating for 200 days/year, the estimated production costs are 17¢/lb for a totally soluble FPC and 12¢/lb for the partially soluble presscake product. For a 20 percent profit after taxes on total capital investment, the estimated selling prices are 38.4¢/lb of soluble FPC and 26.7¢/lb of presscake product.

NOAA TECHNICAL REPORT

The research summarized in this paper has been described in more detail in a technical report which also includes a literature survey of biological methods for FPC production (Hale, 1972).

LITERATURE CITED

Almenas, K. K., L. C. Durilla, R. C. Ernst, J. W. Gentry, M. B. Hale, and J. M. Marchello. 1972. Engineering economic model for fish protein concentration processes. U.S. Dep. Commer., NOAA Tech. Rep. NMFS CIRC 367, 175 p.

Ernst, R. C., Jr. 1971. FPC: the NMFS experiment & demonstration plant process. Commer. Fish. Rev. 33(2):22-28.

Hale, M. B. 1969. Relative activities of commercially-available enzymes in the hydrolysis of fish protein. Food Technol. 23(1): 107-110.

1972. Making fish protein concentrates by enzymatic hydrolysis. A status report on research and some processes and products studied by NMFS. U.S. Dep. Commer., NOAA Tech. Rep. NMFS SSRF 657, 32 p.

Smith, P., Jr., M. E. Ambrose, and G. M. Knobl, Jr. 1964. Improved rapid method for determining total lipids in fish meal. Commer. Fish. Rev. 26(7):1-5.

U.S. Library of Congress. 1970. Fish protein concentrate: A comprehensive bibliography. U.S. Libr. Congr., Sci. Technol. Div., Spec. Biliogr. Sect., 77 p.

MFR Paper 1034. From Marine Fisheries Review, *Vol. 36, No. 2, February 1974. Copies of this paper, in limited numbers, are available from D83, Technical Information Division, Environmental Science Information Center, NOAA, Washington, DC 20235.*