PROTEIN AUTOLYSIS RATES AT VARIOUS pH'S AND TEMPERATURES IN HAKE, *Merluccius productus*, AND PACIFIC HERRING, *Clupea harengus pallasi*, AND THEIR EFFECT ON YIELD IN THE PREPARATION OF FISH PROTEIN CONCENTRATE

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ABSTRACT

The rate of protein autolysis at temperatures ranging from 30° to 80° C and at pH's ranging from 3.0 to 7.0 were determined on hake and Pacific herring. Autolysis rates were generally greatest at acidic pH's and began to decrease after temperatures exceeded 50° C. Autolysis rates were much greater in hake than in Pacific herring. The yield of fish protein concentrate prepared from hake showed a close inverse correlation to the degree of autolysis.

The production of fish protein concentrate (FPC) requires a process that efficiently removes oil and water from the fish and provides high yields of protein. Although FPC can be prepared by several different methods (Knobl, 1967), the most effective procedures developed to date are based on systems in which comminuted fish is successively extracted with a suitable solvent system.

Dambergs (1969), in studying the extracting efficiency of isopropyl alcohol (IPA)-water mixtures in producing FPC from herring, found that low molecular weight compounds were readily removed with these solvent systems. Fish flesh and viscera contain highly active catheptic enzymes which utilize fish tissue as a substrate, forming low molecular weight protein degradation products (Siebert, 1962; Ting, Montgomery, and Anglemeier, 1968). Normal autolysis of fish tissue during storage has been related to decreased yields in FPC production. Dubrow, Brown, Pariser, Miller, Sidwell, and Ambrose (1971) found that when ice-stored fish was used to make FPC, the yield was significantly lower than the yield obtained when FPC was prepared from freshly caught fish. In another study by Dubrow and Hammerle (1969), in which FPC was prepared from samples of comminuted fish held in 91 % IPA for various lengths of time, similar results were obtained.

Preliminary studies in this laboratory, on the development of an aqueous process for FPC production, indicated that under certain conditions significant protein losses may also occur during actual processing procedures as a result of enzymatic hydrolysis.

In solvent extraction procedures for FPC production, the yield of product (excluding physical losses) is dependent upon the amount of proteinaceous material soluble in the extracting solution. While losses can be controlled to some extent by the choice of solvent systems, the possibility of losses due to enzymatic degradation of protein must also be considered.

The purposes of this study were as follows:

1. To determine the effect of pH, time, and temperature on the rate of protein autolysis in two species of fish that are considered for use in the production of FPC.

2. To determine the effect of autolytic activity on FPC yields.

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PROTEOLYTIC ENZYME ACTIVITY IN WHOLE AND EVISCERATED FISH

Presented in this experiment are data on the effect of time, temperature, and pH on the rate of proteolytic enzyme activity in samples of whole and eviscerated comminuted fish.

MATERIALS AND METHODS

Fish

Hake, *Merluccius productus*, and Pacific herring, *Clupea harengus pallasi*, were used in this study. Hake samples were obtained from both coastal waters (outside hake) and Puget Sound (inside hake). Herring were obtained from Bellingham Bay. All samples were obtained fresh and were immediately frozen and held at —20° C.

Preparation of Samples

To insure sample homogeneity, lots of fish were partially thawed and then ground in a Hobart grinder[2] through a 1/8-inch plate. The ground fish was mixed well and divided into portions of approximately 50 g each. These were frozen to —40° C in a plate freezer and stored at —20° C.

Sufficient comminuted fish was prepared to permit running all pH and temperature variables on subsamples from the same lot. Samples of herring and outside hake were prepared from both whole and eviscerated fish. With inside hake, autolytic rates were measured only on whole fish.

METHODS

Fifty grams of comminuted fish were allowed to thaw at room temperature and then were mixed well with 100 ml of H_2O to form a slurry. The pH of the slurry was adjusted to the desired level with 0.1 M HCl and then sufficient water was added to give a final volume of 250 ml. After dilution, the sample was placed in a water bath.

[2] The use of trade names is merely to facilitate descriptions; no endorsement is implied.

Aliquots of the slurry were taken (1) immediately after the final dilution was made, (2) when the sample reached the desired temperature, and (3) at intervals of 10, 20, 30, and 60 minutes after the sample reached the desired temperature. These aliquots were mixed immediately with an equal volume of 10% TCA, allowed to stand for 30 min, and then filtered. The nonprotein nitrogen (NPN) content of the filtrates was determined by the procedure of Lowry, Rosebrough, Farr, and Randall (1951) and is presented in terms of optical density (OD) measurements at 500 mμ.

Rates of proteolytic enzyme activity at pH 6.0, 5.0, 4.5, 4.0, and 3.0 were measured at temperatures of 30°, 40°, 50°, 60°, 70°, and 80° C. Controls consisted of samples in which no pH adjustment had been made.

RESULTS AND DISCUSSION

The results of the above experiments are shown in Figures 1 through 5.

In whole herring (Fig. 1), no significant degree of proteolytic enzyme activity was observed at pH levels higher than pH 5.0 over the temperature range studied. At pH 4.0-4.5, maximum activity was found at 40° C, and at pH 3.0, maximum activity was found at 30° C.

Under these experimental conditions, only negligible proteolytic enzyme activity was observed in eviscerated herring samples (Fig. 2) indicating that in herring proteolytic enzymes are essentially of visceral origin.

Figures 3 and 4 show the results of the experiments using whole inside and outside hake. At 30° C, the rate of proteolytic activity in both control samples was negligible. As the pH was lowered, the rate of activity increased. At 40° C, the rate increased at all pH levels, and these rates remained elevated until the temperature exceeded 60° C. In these two samples, differences were observed in the pH of optimum activity at various temperatures. These variations were attributed to differences in the age, feeding habits, etc., of the two populations.

The effects of pH and temperature on the rate of enzyme activity in eviscerated outside hake

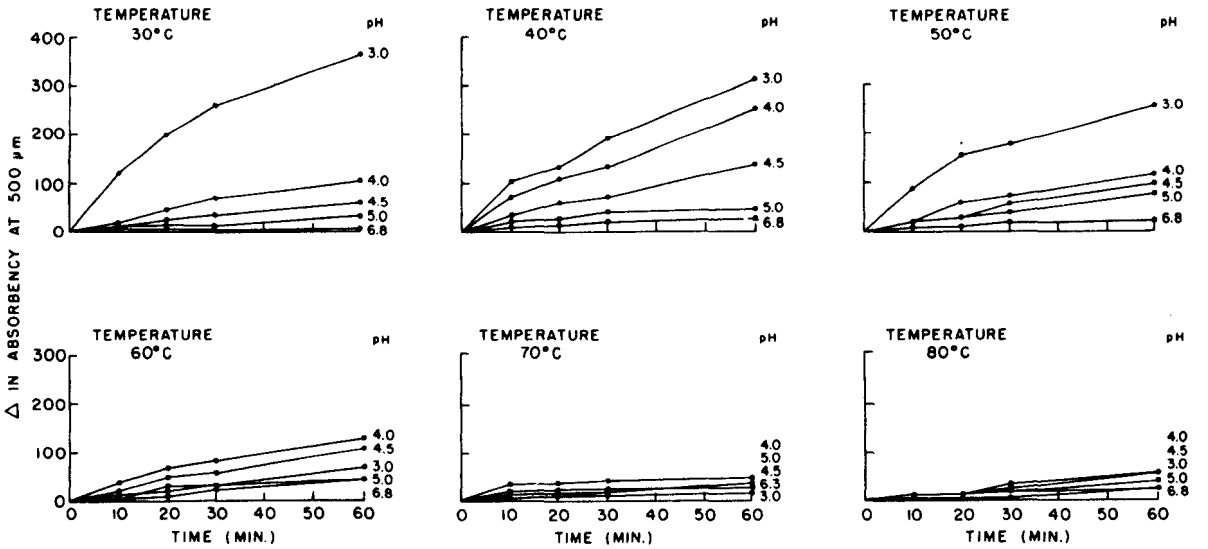

FIGURE 1.—Protein autolysis rates of whole herring homogenates held at various pH's and temperatures.

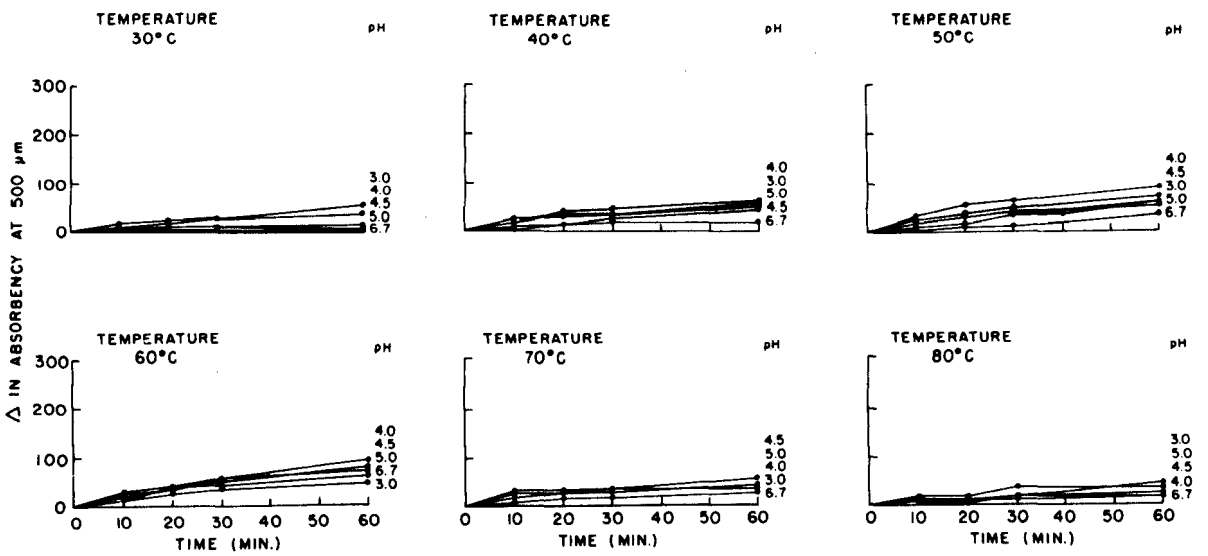

FIGURE 2.—Protein autolysis rates of eviscerated herring homogenates held at various pH's and temperatures.

are shown in Figure 5. The pattern is similar to that of whole hake, both in magnitude of activity and the effects of pH and temperature. Previous investigations in this laboratory (Dassow, Patashnik, and Koury, 1970) have shown that hake is often infected with the parasite, myxosporidian. The degree of this infestation is much greater in the outside than in the inside hake (Patashnik, personal communication, 1970). These investigators reported that infested hake autolyze rapidly during storage, making them unsuitable for processing into blocks or portions. No detailed study relating to parasites was made in this study, but the similar autolytic rates between whole and eviscerated outside hake shows that the main source of pro-

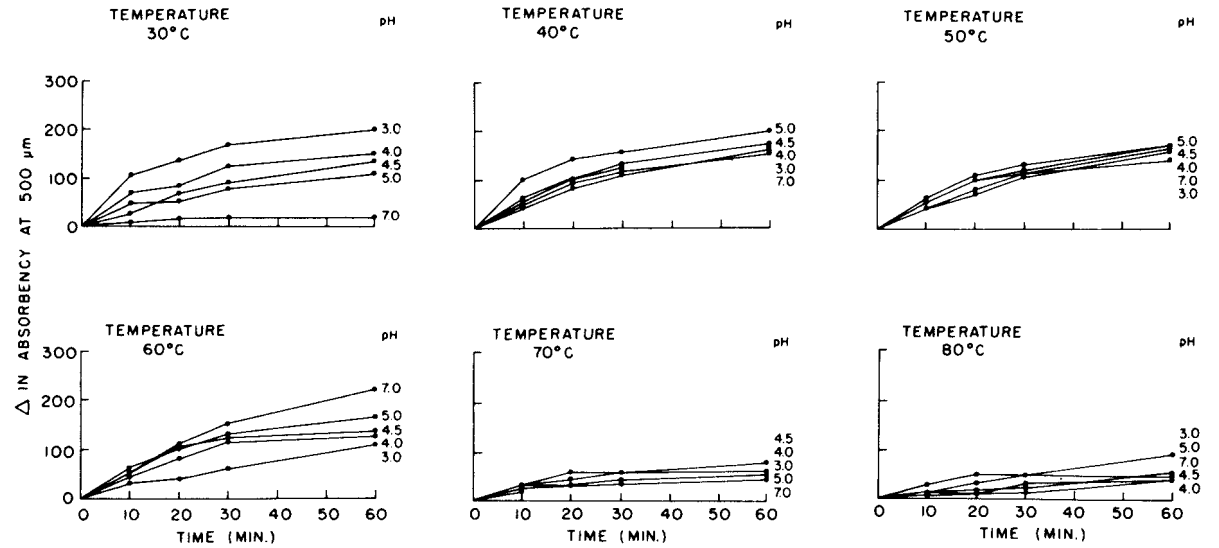

FIGURE 3.—Protein autolysis rates of whole hake (outside) homogenates held at various pH's and temperatures.

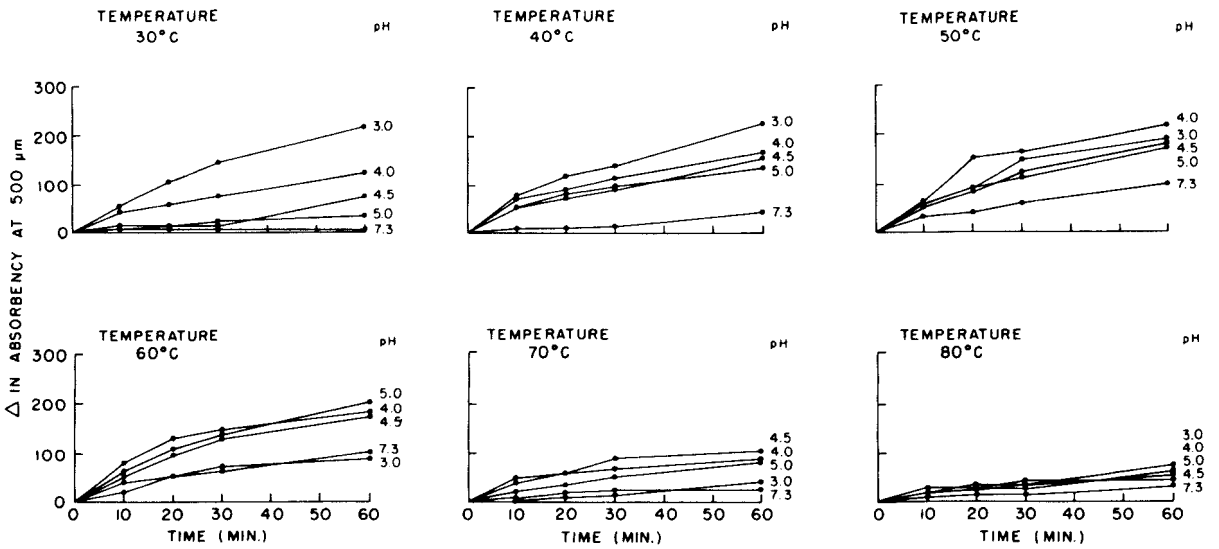

FIGURE 4.—Protein autolysis rates of whole hake (inside) homogenates held at various pH's and temperatures.

teolytic enzyme in the outside hake taken for this study resides in the tissue.

EFFECT OF AUTOLYTIC ACTIVITY ON YIELD OF FPC

MATERIALS AND METHODS

Whole inside hake were used throughout this experiment. For the preparation of FPC, whole hake was comminuted by passing through a Hobart grinder (equipped with a 1/8-inch plate). Aliquots of the comminuted fish were acidified with 0.1 M HCl to pH 5.5 and 4.5. The original pH of the fish was 6.9. The treated fish was allowed to autolyze at 50° C for 0, 20, 40, and 60 min. After each time interval, FPC was prepared by isopropanol (IPA) extraction as follows: The fish was successively extracted (4

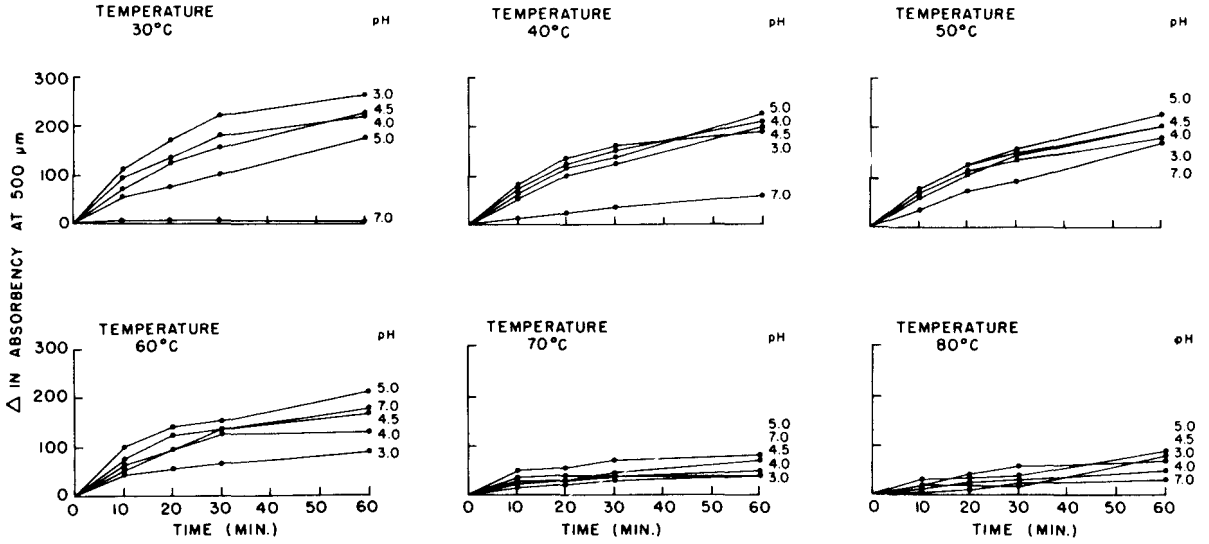

FIGURE 5.—Protein autolysis rates of eviscerated hake (inside) homogenates held at various pH's and temperatures.

times) with azeotropic IPA at a ratio of 2 parts IPA to 1 part fish. The first extraction was carried out at ambient temperature, and the final three extractions were carried aut at 70° C. Yields were calculated after drying the fish solids for 16 hr at 70° C at 25 inches of vacuum.

RESULTS AND DISCUSSION

The effect of autolytic activity on the subsequent yield of FPC is shown in Figure 6. It can be seen that while the acidified samples showed the greatest loss of yield with time of autolysis, the largest yield losses occurred during the first 20 min. The pH values, time, and temperature were arbitrarily chosen to simply demonstrate the effect of autolysis with respect to yield. They do, however, show a close correlation with the autolysis data shown in the previous experiment. For example, in referring to Figures 1 through 5, it can be seen that the rate of NPN formation is greatest during the first 20 min regardless of pH and temperature. Several investigators (Whaley, 1966; Sen, Sayanarayana Rao, Kadkol, Krishnaswamy, Venkata Rao, and Lahiry, 1969) have proposed acidifying comminuted fish prior to processing without taking into account the effect of protein losses due to autolysis. The combined experiments presented here clearly demonstrate the need to control temperature and pH, both before and during processing.

SUMMARY AND CONCLUSIONS

The above study shows that the endogenous proteolytic enzymes in fish hydrolyze the proteins into subunits that are not coagulable by

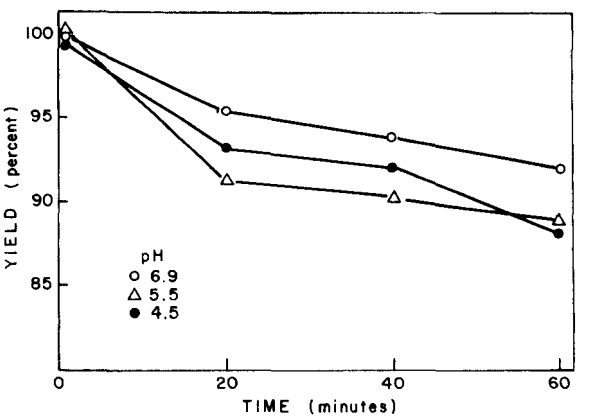

FIGURE 6.—Effect of protein autolysis on the yield of PFC made from inside hake. Autolysis was allowed to proceed for 0, 20, 40, and 60 min at 50° C prior to preparation of FPC.

the isopropanol concentration normally used in the preparation of FPC. The preparation of FPC made from autolyzing hake showed that the yield bore a close inverse relation to the degree of autolysis.

The autolysis rates in Pacific herring and hake were related to pH and temperature, the rates showing an increase with increasing temperature and a decreasing pH. Maximum activity was reached at about 50° C. Inactivation of the enzymic systems occurred when temperatures exceeded 70° C.

Since the economic success of any method that is used for the preparation of FPC is largely dependent on the yield of finished product obtained from a given amount of raw material, autolysis rates are a process parameter that should be closely controlled.

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