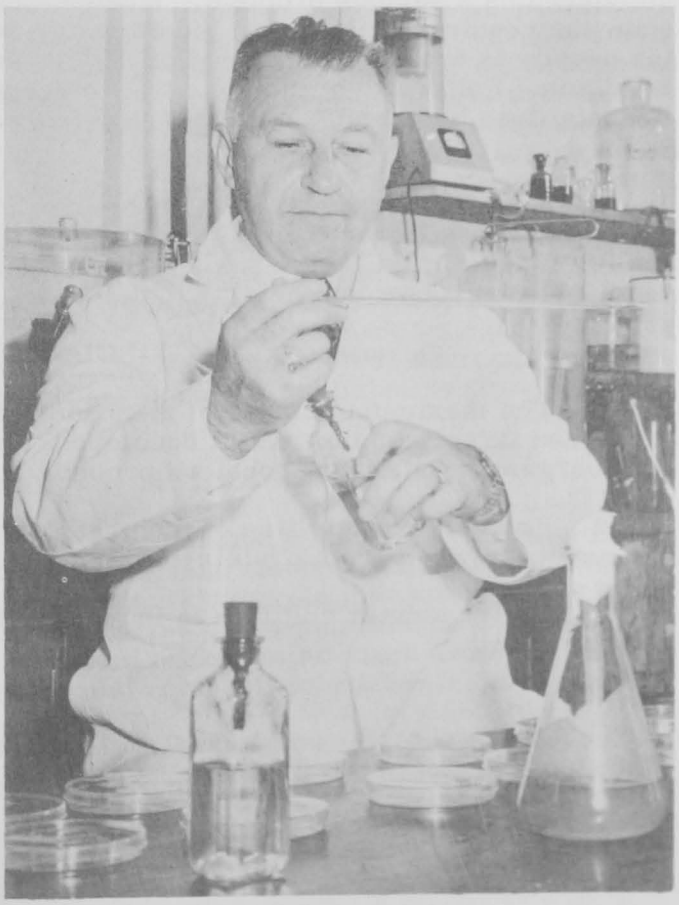
# RESEARCH
## IN SERVICE LABORATORIES

## TECHNICAL NOTE NO. 37 - USE OF BACTERIAL CULTURE TO AID SEPARATION OF MENHADEN OIL IN GRAVITY TANKS

ABSTRACT

Use of a starter culture to speed "break-out" is recommended in the gravity separation of menhaden oil. The bacterium involved is probably a member of the genus Clostridium.

Microscopic examination of press liquor obtained in the processing of menhaden has indicated that certain types of bacteria must be present for the optimum separation of oil from stickwater in gravity separation. The hot liquor pressed from the cooked fish--a complex mixture of oil, water, soluble fish-tissue compounds, and fine material escaping the screening process--is transferred to large tanks of approximately 5,000-gallon capacity. The oil separates from the press liquor and rises to the surface, producing a barrier that prevents air from getting into the lower level.



TAKING OF DILUTION BOTTLE SAMPLE OF STICKWATER FOR PLATING OF BACTERIA.

Semi-anaerobic conditions are produced as a consequence of the initial temperature of the press liquor (approximately 70° C.), the subsequent release of some of the dissolved air from the lower level, and the lack of further extensive aeration. During the following 8 to 10 hours, a thick layer of foam is formed over the surface of the oil, indicating that a proper "break-out" of oil has occurred. Water is introduced into the bottom of the tank, which raises the layer of oil to a trough by which the oil is transferred to another tank.

Samples for bacterial study of the press liquor or stickwater were obtained from a tank at the completion of filling, 1 hour after filling, and at the completion of the process during the flooding operation. Large loopsfull were smeared on glass slides, air dried, and fixed by heating in a gas flame. The preparations were stained for 3 to 5 minutes with crystal-violet, blotted, and viewed

under the oil-immersion objective lens of the microscope. Duplicate smears were stained by the conventional Gram stain procedure.

Examination of the stain preparations revealed a relatively pure culture of large gram positive bacilli, occurring singly and containing a central oval spore located terminally. Distention of the cell around the spore was evident. Preliminary investigation indicates that the organism is a member of the genus Clostridium The organism is probably a proteolytic species, since there is little carbohydrate available in menhaden press liquor. This inference is supported by the fact that ammonia, liberated during active protein degradation, is detected in the tanks. In addition, hydrogen sulfide, methane, hydrogen, and other gases resulting from the anaerobic decomposition of protein often are evolved.

In this gravity separation method, initial separation of oil from press liquor is accomplished by taking advantage of the differences in the specific gravities of the two fluids. Large quantities of oil are held in the aqueous layer, however, by particles of insoluble protein, by emulsion, and by other physical and physico-chemical activity and complete separation of the oil from the press liquor is not attained. The importance of the microbiological activity is clearly evident, since a complete break out of the oil does not occur in the absence of the proteolytic decomposition.

Processing menhaden into meal, condensed solubles, and oil is always a rapid operation carried out for meal on a tonnage, and for oil and solubles on a tank-car scale. Any interruption in the smooth flow of materials through the plant immediately creates a serious problem. When a tank of press liquor fails properly to show the required activity and the emulsion accordingly does not break, the operator usually is forced to take what oil he can and to discard the remaining intractable emulsion. Failure to break the emulsion results therefore not only in a substantial financial loss but creates a further problem in how to dispose of the emulsion as well.

In such cases of delay, it is recommended that the operator add 200 and 300 gallons of press liquor from a tank that has undergone a typical proteolytic activity. This added liquor will serve as a starter culture to promote the desired break out of oil in the inactive tank.

## CONCLUSIONS

1. The maximum release of menhaden oil during gravity separation processing is due largely to a bacterial decomposition of the suspended protein. The active microorganism is an anaerobe and probably a member of the genus Clostridium.

2. Proteolytic activities of the organism appear responsible for more complete release of oil from protein-oil emulsions and other non-specific complexes.

3. Use of starter cultures to insure typical effective "break-out" of oil is recommended.

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## PROGRESS ON FISH MEAL NUTRITIVE VALUE STUDIES

The term "fish meal" does not refer to a specific substance, for it applies to any dried material prepared from fish or from any parts of fish, such as fillet waste. When the different constituents of the fish--such as skin, muscle, bones, and organs, which may make up the raw material for fish meal--are considered, it can be seen that any one fish meal, by virtue of the raw material variability alone, is very complex. When this complexity of fish parts is compounded by the different species of fish used for fish meal being manufactured, the number of possibilities for variation becomes enormous. The amazing thing, therefore, is not that differences in nutritive value are found among the commercial fish meals, but rather that these differences are not larger than have been reported.

Since these differences do occur and since a standard product is desirable, it is important to learn the factors that affect the nutritive value of fish meal. The Technological Section of the Service's Branch of Commercial Fisheries, in cooperation with a number of collaborators, has undertaken such a study. This study is being made on the effects of (1) the raw material used and its condition; (2) processing conditions employed; (3) storage conditions for the meal; (4) amount and availability of protein and amino acids in the meal; and (5) type, amount, and degree of oxidation of the oil in the meal. These variables are being studied through use of chick-growth tests that measure the effects of these variables on the unknown growth factors and the protein quality of the meals, as they may relate to the nutritive value of these meals. This approach can best be illustrated by describing for one particular sample of menhaden meal the studies now being made. Work on this sample has not been completed, so results cannot be reported fully at this time, but enough has been accomplished to provide the desired example.

PREPARATION OF EXPERIMENTAL DIET TO STUDY PROTEIN QUALITY OF MENHADEN MEAL.

### PREPARATION OF THE MEAL

PROCESSING: Menhaden press cake was obtained by staff members of the Technological Laboratory, College Park, Md., and shipped to Seattle, where the press cake was dried in a small steam-jacketed drier. In this work, press cake sufficient to supply 100 pounds of meal was dried in a single batch, ground, and thoroughly mixed. Part of the meal was then packed in nitrogen and sent to the various collaborators for animal-feeding and composition studies.

STORAGE CONDITIONS: The remainder of the meal was divided and stored both at room temperature and at -20° F. in atmospheres both of air and of nitrogen.[1/] These samples will be tested by the following procedures after storage periods of 6 months, 12 months, and longer--if little or no change occurs within the 12-month period.

1/ NITROGEN WAS PACKED WITH THE MEAL TO EXCLUDE AIR. THE NITROGEN IS INERT AND DOES NOT REACT CHEMICALLY, WHEREAS OXYGEN IN THE AIR DOES REACT, ESPECIALLY WITH THE OIL IN THE MEAL.

## ANIMAL-FEEDING STUDIES

UNKNOWN GROWTH FACTORS: Dr. H. R. Bird, of the Poultry Husbandry Department at the University of Wisconsin, is testing the meal for effects of unknown growth factors, according to the method described by Barnett and Bird (1956). In this chick assay, the growth response induced by the sample under test is compared with that of a sample of fish solubles that serves as a reference standard. Tests on the freshly-prepared meal now have been completed. The tests show that the relative growth response of the chicks fed this meal is excellent.

PROTEIN EVALUATION: Dr. C. R. Grau, of the University of California at Davis, is evaluating the protein in the meal. The procedure used is that of Grau and Williams (1955) and modified as described by Grau, Barnes, Karrick, and McKee (1956). The values obtained by feeding the freshly prepared meal to chicks indicated that the protein was of good quality.

## COMPOSITION STUDIES

PROTEIN, MOISTURE, AND ASH CONTENT: The composition of the meal was as follows: protein, 59.3 percent; moisture, 9.2 percent; and ash, 20.5 percent.

OIL CONTENT: The apparent oil content of the meal varied with the kind of solvent used to extract the oil. (This problem of oil extraction is being studied in connection with another project. In this other work, it has been found also that the amount of oil extracted decreases with the length of time the fish meal has been in storage.) The initial values obtained with the present menhaden meal sample were 9.5 percent oil extracted with ethyl ether and 12.7 percent oil extracted with acetone. The amount of oil measured by the use of these solvents will be determined periodically as the meal ages. Any changes in the solubility of the oil with increased age of the meal will be checked by comparison with chick-growth studies to find out whether such changes are reflected in the nutritive value of the meal.

AMINO ACID CONTENT: The amount and availability of amino acids in the meal undoubtedly affect the quality of the protein. The question is whether in vitro (test-tube) assays of the individual amino acids give results that can be correlated with those of in vivo (in living tissue) assays of the protein.

Microbiological assays (in vitro) for amino acids are being made at the Wisconsin Alumni Foundation. A sample of the freshly prepared meal was analyzed for the total amount of 13 amino acids and for the proportion of these that was available to micro-organisms. This analysis will be repeated if the stored meal shows a deterioration in the quality of the protein as measured by the chick-growth tests (in vivo).

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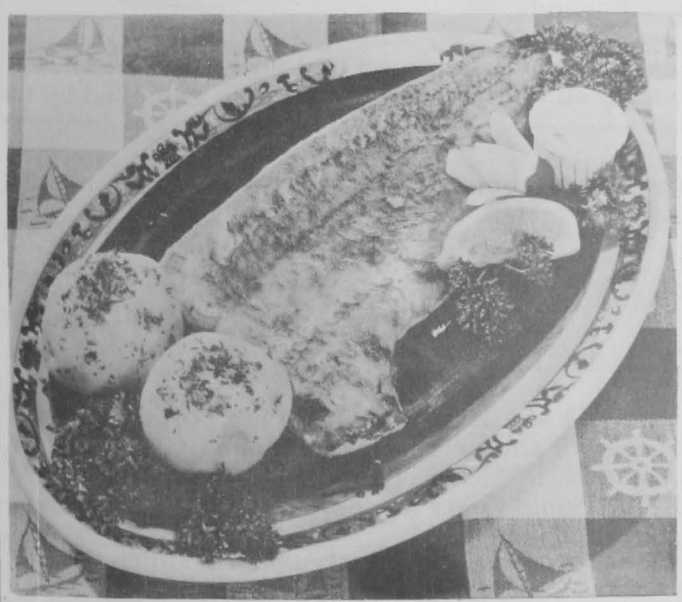
## IRON SULFIDE DISCOLORATION OF TUNA CANS

Certain batches of tuna, when canned, cause an iron sulfide deposit to form on the can area adjacent to the headspace. The deposit is caused by a reaction between sulfide from the fish and iron in the can. In a study jointly sponsored by the Continental Can Company and the U. S. Fish and Wildlife Service, investigations were made on the effects of retorting and cooking on the formation of black ferrous sulfide discoloration in canned tuna. Free sulfide was not found in the unprocessed fish but appeared in all canned tuna after processing. The amount of free sulfide was found to increase with longer retorting periods. Free sulfide did not form a black precipitate of ferrous sulfide unless the free iron in the ferrous state was available. Discoloration occurred in the cans during the cooling period and was greater in cans held at elevated temperatures while being cooled. Thus, one of the important considerations in the prevention of iron sulfide discoloration of tuna cans is a quick cooling period.

### BAKED SHAD FILLETS

The annual cherry blossom festival in the Nation's capital and the appearance of an abundance of shad on the market heralds spring.



Regular as a clock each spring the shad migrate from the ocean to our coastal rivers to spawn above tidewater. They are found in the Atlantic from Maine to Florida and and in the Pacific from Washington to California.

The shad range in size from $1\frac{1}{2}$ to 7 pounds and are most commonly sold as roe or buck shad. The meat from both is tender and white, with a distinctive flavor. The roe, from the roe shad, is considered a great delicacy.

Shad may be purchased whole, drawn, or as fillets. The fillets require no preparation for cooking as they are the sides of fish cut lengthwise away from the backbone. Shad fillets are delicious when prepared by any of the basic cooking methods such as baking, broiling, or frying. The home economists of the United States Fish and Wildlife Service suggest that you serve "Baked Shad Fillets" to your family to celebrate the arrival of spring.

BAKED SHAD FILLETS

2 POUNDS SHAD FILLETS
1 TEASPOON SALT
DASH PEPPER
1 TEASPOON PAPRIKA
2 TABLESPOONS LEMON JUICE
1 TEASPOON GRATED ONION
$\frac{1}{4}$ CUP BUTTER OR OTHER FAT, MELTED

Cut fillets into serving-size portions. Place in a single layer, skin side down, in a well-greased baking pan. Combine remaining ingredients and pour over fish. Bake in a moderate oven, 350° F., for 20 to 25 minutes or until fish flakes easily when tested with a fork. Serves 6.