DYE-BINDING CHARACTERISTICS OF FISH-MEAL PROTEIN

Part 1 - Some Preliminary Findings as to Suitable Dyes

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ABSTRACT

THERE ARE REPORTS IN THE SCIENTIFIC LITERATURE THAT THE QUALITY OF A VEGETABLE PROTEIN CAN BE DETERMINED BY ITS DYE-BINDING CHARACTERISTICS. IN AN INVESTIGATION TO FIND IF A SIMILAR RELATIONSHIP EXISTS BETWEEN DYES AND THE PROTEIN IN FISH MEAL, MORE THAN 100 DYES WERE SCREENED AS TO THEIR SUITABILITY. EIGHT DYES WERE FOUND TO HAVE GOOD BINDING PROPERTIES. SIX OF THEM--ACID FUCHSIN, ANILINE BLUE, BROMOCRESOL GREEN, ALIZARIN RED S, ORANGE II, AND ORANGE G--WERE ACID DYES; AND TWO OF THEM--CONGO RED AND TETRABROMOPHENOLBLUE--WERE BASIC DYES. IN THE USE OF THESE, FISH MEALS EXHIBITED A WIDE VARIATION IN THE EXTENT OF DYE BINDING. SUFFICIENT DATA, HOWEVER, ARE NOT AVAILABLE AS YET TO DETERMINE THE RELATIONSHIP OF THE DYE-BINDING CHARACTERISTICS TO THE NUTRITIVE VALUE OF FISH-MEAL PROTEIN.

INTRODUCTION

Several of the investigations reported in the scientific literature indicate that the quality of a vegetable protein can be determined by its dye-binding characteristic. Loeb (1922), studying the process of digestion, stated that pepsin is an anion and that it combines with cations. Chapman, Greenberg, and Schmidt (1927) showed, by reactions of several acid dyes with various protein solutions, that the amount of dye that was bound was proportional to the number of basic groups in the protein. Rawlins and Schmidt (1929) extended the investigation to include basic dyes and obtained similar results; they later (1930) used acid dyes with gelatin granules and gelatin solutions and verified their previous conclusions. Fraenkel-Conrat and Cooper (1944) found that dyes could be used to determine the number of acidic and basic groups in a protein. Udy (1954)--working with vegetable proteins, chiefly wheat--found that the quality of the protein could be determined from its dye-binding characteristics.

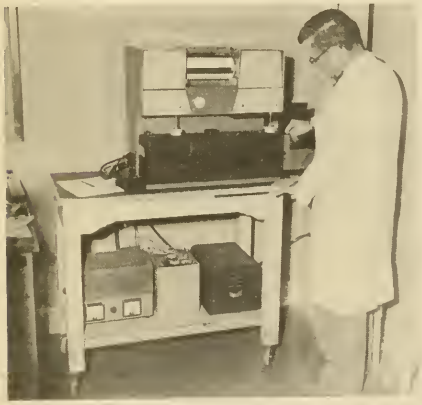

FIG. 1 - DETERMINING LIGHT TRANSMISSION OF DYE SOLUTIONS WITH A RECORDING SPECTROPHOTOMETER.

If a similar relationship exists between dyes and the proteins in fish meal, the nutritive value of the proteins might be determined by a chemical index, in hours, rather than in 1 to 3 weeks as is now required when a feeding test is used. An investigation of the dye-binding characteristics of the protein in fish meal therefore has been undertaken at the Seattle Technological Laboratory in order to determine whether there is any correlation between the nutritive value of the meal, as determined by chick-feeding tests, and the extent of binding of the dye. The specific objectives of the work reported in the present paper were to determine (1) what dyes will bind the proteins of fish meal and (2) what are the optimum conditions in the use of these dyes.

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EXPERIMENTAL

More than 100 dyes, including all of the major types of chemical structures listed in the Color Index (Rowe 1924) have been screened. Most of these dyes had to be discarded for one or more of the following reasons:

1. The dye was insoluble in the buffer solution used.
2. Solutions of maximum solubility failed to give adequate depth of color.
3. The dye adhered to the spectrophotometer cells to such an extent that the readings were valueless, and cleaning was unduly tedious.
4. The color faded on standing or on exposure to light.
5. The pH of the solution changed greatly on standing.
6. The color of the dye was discharged by the sample without apparent binding.
7. The dye was not appreciably taken up by the meal.

DISCUSSION

SUITABLE DYES: On the basis of the above screening tests, eight dyes exhibited satisfactory dye-binding characteristics. Six of them--acid fuchsin, aniline blue, bromocresol green, alizarin red S, orange II and orange G--were found to be satisfactory in acidic solution (pH 2.5), and two of them--congo red and tetrabromophenolblue--were found to be satisfactory in alkaline solution (pH 11).

Summary of Experimental Results

Satisfactory acid dyes (pH 2.5):	Acid fuchsin Aniline blue Bromocresol green Alizarin red S Orange II Orange G
Satisfactory basic dyes (pH 11):	Congo red Tetrabromophenolblue
Optimum concentration of dye:	0.0001 to 0.003 percent
Optimum particle size of meal:	60 mesh
Optimum meal-to-dye ratio:	1 milligram of meal to 1 milliliter of dye solution
Optimum time of shaking:	$4\frac{1}{2}$ hours
Amount of dye bound by meal:	25 to 40 percent of basic dye 60 to 90 percent of acid dye

OPTIMUM CONDITIONS: Dye concentrations from 0.0001 percent to 0.003 percent gave transmission ranges from 90 to 10 percent in the spectrophotometer. Meal samples varying from 8 to 80 mesh were used, with 60 mesh giving the best results. The meal samples were employed in amounts varying from 5 to 25 milligrams in 10 to 50 milliliters of dye solution. The ratios of meal to dye solution that gave the best results were of the order of 1 milligram of meal to 1 milliliter of solution. The mixtures of meal and dye were shaken in a mechanical shaker for 2 to 24 hours, but 4 to 5 hours of shaking gave the maximum binding. Under these conditions, the meals bound 25 to 40 percent of the basic dyes and 60 to 90 percent of the acid dyes.

PRELIMINARY RESULTS: Preliminary work on the application of these dyes has been carried out on meal samples that have been used in feeding tests and for which the nutritive value of the protein is known. A wide variation in the extent of

dye binding was observed, but sufficient data are not available as yet to determine the relationship of the dye-binding characteristics to the nutritive value of the protein in the meal.

CONCLUSIONS

Eight dyes have been selected, from a group of approximately 100 tested, which showed satisfactory protein-binding characteristics. The dyes will be used to study the possibility of relating the dye-binding characteristics of the protein of various fish meals to the known nutritive value of the respective meals.

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"DRIP" IN FROZEN FISH

Drip is the term applied to the clear or sometimes slightly cloudy fluid that is not reabsorbed by the fish tissue when frozen fish thaws. The fluid consists of water with dissolved protein, other nitrogenous constituents, and minerals. The quantity of drip from frozen fish depends upon many factors, including the kind of fish involved and the length and temperature of storage prior to thawing. Drip may be less than one percent or more than 20 percent of the weight of the fish.

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