

# Methods of Measuring the Effect of Processing on the Nutritive Value of Fish Proteins<sup>a</sup>

251-9

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## A REVIEW

The effect of processing on the nutritive value of proteins is reviewed on the basis of nitrogen distribution, quality indexes, chemical scores, efficiency ratios (enzyme activity), and amino acid content. It is generally agreed that amino acid composition does not reflect changes in nutritive value effected by heat, storage, dehydration, unless some measure of decomposition has taken place. Rate of liberation of essential amino acids on *in vitro* hydrolysis has given sensitive and reproducible tests of protein changes. The need of measuring nutrients as they are modified by processing is discussed.

Papers entitled "nutritive value . . ." have been published on a great many organic substances used for human and animal diets. Results are usually expressed as protein quality index (1), chemical score (2), digestion index (6), biological value (2), protein efficiency ratio (2), apparent digestibility (12). They represent chemical and *in vitro* tests on food products, chemical, morphological, growth, maintenance, production studies on experimental animals.

The purpose of this paper is to review briefly laboratory measures of nutritive value. To properly process, store, and utilize fishery products, the fisheries technologist must have accurate, reproducible, sensitive tests, applicable to laboratory control of plant production. They must not only detect changes in nutritive value but also provide an insight into the reasons for these changes. The time may come when the nutritive value of canned and frozen fish has as much economic importance to the processor as the mechanics of processing. Fish meals are ready now to make the transition from the status of reduction products to chemical compounds. Yet before the market reflects exact values of nutrients, we must be able to express them as they are modified by mechanical and chemical treatments.

We know that chemical analysis of nitrogen does not measure a difference in quality (7). Only in chronic inanition is there a measurable difference (decrease) in total nitrogen or non-protein nitrogen of experimental animals (15). Nitrogen distribution has been suggested as an index of nutritive value of fishery products (8). Straight chemical tests of nitrogen fractions are correlated more with spoilage than with nutritive value. Lassen (8), studying storage of fish press liquor before condensation to solubles, found that as true-protein nitrogen decreased, non-protein and ammonical nitrogen increased. The growth-promoting ability of the product and also the amino acid content showed corresponding

decreases with loss of true protein nitrogen. Total amino nitrogen by formol titration increased with decrease of true protein and decrease in amino acid content.

For years we have been unable to arrive at an objective test for spoilage on this basis, due largely to different proportions of nitrogen fractions in different fish, different bacterial flora and different initial conditions of environment. Possibly if absolute values for initial nitrogen fractions were established on a single product prior to change, and the rate of change followed, spoilage might be measured.

Almquist (1) expanded the nitrogen distribution by chemical tests into *in vitro* tests for indigestible nitrogen. This factor, brought into an equation of nitrogen fractions, has made the "protein quality index" a measure of digestibility, hydrolysis, autolysis and natural distribution. Indexes based on analytical tests for nitrogen fractions can be correlated with nutritive value only by biological verification and there is still no explanation of what happens when a change is noted.

Quantitative proportions of amino acids occupy the attention of many research and quality control workers today. While these determinations are imperfect, extensive checks between laboratories show an average coefficient of variation of 8.9 percent (5, 11). Acceptable assay procedures which include strong acid and alkali hydrolysis show no significant lowering of amino acid content of fish products after canning. It seems likely that some measure of decomposition must take place before the percentage of amino acids reflects this change. In the case of tryptophan, which occurs in fish muscle slightly over 1 percent of the total protein, the coefficient of variation found in this work (11) was 17.5 percent which means that occasionally a single laboratory check on a processed product could be 0.65 percent or 1.35 percent and still not be significantly different from an initial value of 1.0 percent.

Block and Mitchell (2) have used amino acid composition to arrive at a "chemical score" in an attempt to correlate chemical composition with nutritive value. The score is based on the percentage of a limiting amino acid compared with the composition of whole egg protein taken as an optimum standard. An example of this measure is wheat protein which contains 63 percent less lysine than whole egg protein and this value subtracted from 100 gives a chemical score of 37. The work shows excellent agreement in most cases with protein efficiency ratios and biological values by the nitrogen balance method. Comparisons of chemical score with bioassays show a correlation coefficient of about 0.83. This means that seven out of ten changes

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in chemical score are due to variations in biological value but that three are not, due to error in measurements or the fact that no correlation exists.

The authors explain that, especially as influenced by heat, proteins may undergo changes, that impair their nutritive value without disturbing the amino acid composition as ordinarily determined. The same conclusions were reached by Clandinin (3) who found little difference in total amino acids after acid hydrolysis in five fish meals processed quite differently. On the basis of growth tests it would appear that these values do not predict nutritive value. Amino acids liberated by enzyme hydrolysis (hog intestinal mucosa for three days digestion) were depressed from 21.53 percent in the case of stack temperatures (flame drying method) of 185° F. (85° C.) to 2.99 percent at 220° F. (105° C.). Fish meals showing damage by growth tests and decreases in enzymatically available amino acids were supplemented with the suggested limiting amino acid with only partial success in restoring nutritive value.

Previous work of this same nature (13) brings out the fact that the total amino acid content of soy bean oil meals is not changed from the raw to the properly heated product and that in the case of overheating, only three essential amino acids, lysine, arginine and tryptophan, show a loss. In contrast, each essential amino acid showed increase by proper heat treatment and decrease by excessive heat when aliquots for the assay were taken from pancreatin digestions. Hydrolysis was carried to five days and the alpha amino nitrogen in the digested fraction (undigested proteins were cleared by precipitation with glacial acetic acid at the iso-electric point) was measured by the nitrous acid micro-method of Van Slyke.

Melnick and Oser (10) are also of the opinion that products may be improved or impaired in value although showing no change in amino acid composition. They propose a method of *in vitro* digestibility for following nutritive values in food processing. The method consists of digestion under toluene of a sample with suboptimum levels of pancreatin at the optimum pH with periodic formol titrations on aliquots. There is some question as to what is measured in this titration, but under the conditions of this work, starting at a pH of 7.0, adding formalin and carrying the titration to pH 9.0, it is a measure of free amino groups.

Proponents of the *in vitro* enzyme hydrolysis method as a measure of nutritive value contend that more important than amino acid composition or liberation of amino acids under optimum conditions of enzyme hydrolysis is a measure of the rate of liberation. Sensitive tests for protein denaturation can apparently be worked out by formol titrations showing percent digestion against time. In the work cited (10), two samples of dried skim milk, one freshly made and one after a year's storage, showed the same amino acid composition and coefficient of digestibility, yet an appreciable difference in biological value and rate of *in vitro* hydrolysis. For factory control of processing, this test, standardized to an enzyme and substrate level for each product, may have a distinct use. True, it measures

only susceptibility of a material to pancreatin digestion and does not elucidate the problem of what happened if a change is noted. Accompanying amino acid assays could be run from aliquots and compared with initial content to study rates of amino acid liberation. For example, microbiologically active lysine was liberated from heat-treated casein at a much slower rate by this method of pancreatin digestion than from unheated casein. It was pointed out that the rate of release of an essential amino acid may be so slow as to limit its use in the gastrointestinal tract. If the *in vitro* rate of hydrolysis can predict this action it is more important than a measure of amino acids by acid hydrolysis or continued enzyme action, which may show optimum amounts available.

Another report along the same thought is by March, Stupich and Biely (9) suggesting a measure of nutritive value from the amino acid content based on total (acid) hydrolysis, corrected for indigestible protein (residue from pepsin digestion, factor "B" in Almquist's protein quality index) and non-protein fractions. Here, however, neither total nor available amino acid levels of different fish meals gave a satisfactory correlation with growth response in chicks. Good correlations were found between available tryptophan times digestible protein-nitrogen, and chick growth, and between available methionine plus cystine times digestible protein-nitrogen, and chick growth.

Freed, Brenner and Fevold (6), using *in vitro* pancreatin hydrolysis of protein as a digestibility measure, have eliminated the variability in expressing results by rate of digestion or titration values. They find a constant index regardless of sample weight or time of digestion in a formula "percent hydrolysis/gram<sup>1/2</sup> protein x days<sup>1/2</sup>." They emphasize the fact that this digestion index is not necessarily analogous to biological evaluation.

We do not know from this technic whether different values mean a destruction of amino acids, decomposition of the product, changes in solubility or actual changes in susceptibility to enzyme action. A process may destroy an essential amino acid and at the same time permit a better rate of hydrolysis in the remaining protein.

Rizzo, Davis and Smith (4, 14), have shown that lactalbumin goes through a deleterious change when autoclaved at 248° F. (120° C.). They found decreased digestibility (nitrogen excreted per gram food consumed), decreased formol titration values (nitrogen in supernatant fluid after *in vitro* digestion) and decreased biological values (change in weight per gram food eaten) and concluded that this change is due to a lowered susceptibility to enzymatic hydrolysis. Formol titration on pancreatin hydrolysates showed very nicely that this change is more rapid when the protein is autoclaved than when baked in a dry oven.

Enzyme activity has been suggested as a measure of general protein metabolism or possibly amino acid availability. Williams and Elvehjem (16) working with experimental animals and cognizant of the fact that gross body changes are not usually sensitive enough to reflect small differences in protein metabolism, have

measured the relation of liver xanthine oxidase activity to dietary protein. Xanthine oxidase is a flavo-protein, the prosthetic group of which consists in part of riboflavin-adenine dinucleotide. Its decreased activity may indicate incomplete assimilation of amino acids or incomplete digestion of a protein. Using adult male rats of uniform strain and measuring enzyme activity in  $\mu\text{l O}_2$  per gram liver protein, a 14.6 percent casein ration showed less than normal activity. The addition of 0.25 percent methionine to the basal casein ration, an isonitrogenous level of acid-hydrolyzed casein adjusted for tryptophan, a purified amino acid mixture corresponding to the basic ration and 40 percent casein, produced normal enzyme activity. The conclusions were that xanthine oxidase activity was subnormal on a 14.6 percent casein diet because the animals were unable to utilize all the methionine and that this was due to the incomplete digestion of the protein.

Research in the writer's laboratory on the effects of processing on fish protein as measured by amino acid content, digestibility by formal titration, rate of liberation of essential amino acids by pancreatin and chick growth, will be presented in a later paper. Results so far indicate that where chick growth and feed efficiency comparisons are taken as accurate expressions of nutritive value, the essential amino acid composition expressed as a chemical score and re-expressed as a biological value by regression equations, accurately reflects changes in fish protein due to processing. It does not reflect the comparative value of casein or casein supplemented with cystine as related to fish protein. Neither rate of essential amino acid liberation in pancreatin digests nor digestibility by formal titration indicates essential amino acid deficiencies which were reflected in the chemical score and chick assay.

With pressure to develop new processes from the "customer acceptance" and the "economics of processing" standpoints, it seems essential in the interests of science that food technologists have methods of following the nutritive value as an aid to the utilization of a product. We know that there is some change in proteins when enzymes are inactivated, when bacteria lose their power to reproduce, in short, when preservation is effected. We want to produce processed foods of as high or higher nutritive value, if possible, than they had in the original condition. There is evidence that some means of preservation makes proteins more susceptible to enzyme action than they are in the native

form. The volume of recent literature on nutritive values of foods as influenced by processing shows that the importance of this subject is recognized by food technologists.

#### LITERATURE CITED

1. ALMQUIST, H. J. Chemical estimation of quality in animal protein concentrates. *J. Nutrition*, 21, 347 (1941).
2. BLOCK, R. J., AND MITCHELL, H. H. Correlation of the amino acid composition of proteins with their nutritive value. *Nutr. Abstracts and Rev.*, 16, 249 (1946).
3. CLANDININ, D. R. Effects of methods of processing on the nutritive value of herring meals. *Poultry Sci.*, 28, 128 (1949).
4. DAVIS, R. M., RIZZO, C., AND SMITH, A. H. Effect of heat on the nutritive value of lactalbumin. *J. Nutrition*, 37, 115 (1949).
5. DUNN, M. S., CAMIEN, M. N., EIDUSON, S., AND MALIN, R. B. The nutritive value of canned foods. I. Amino acid content of fish and meat products. *J. Nutrition*, 39, 177 (1949).
6. FREED, M., BRESSER, S., AND FEVOLD, H. L. Studies on the rate of "in vitro" pancreatic hydrolysis of protein. *Food Technol.*, 3, 170 (1949).
7. GEIGER, E. Biochemistry of fish proteins. *Fortschr. Chem. org. Naturstoffe*, 5, 267 (1948).
8. LASSEN, S., BACON, E. K., AND DUNN, H. J. Relationship of nutritive value of condensed fish solubles to quality of raw material. *Poultry Sci.*, 28, 134 (1949).
9. MARCH, B. E., STUPICH, D., AND BIELY, J. The evaluation of the nutritional value of fish meals and meat meals. *Poultry Sci.*, 28, 718 (1949).
10. MELNICK, D., AND OSER, B. L. The influence of heat processing on the functional and nutritive properties of protein. *Food Technol.*, 3, 57 (1949).
11. NEILANDS, J. B., SIRNY, R. J., SOHLJELL, I., STRONG, F. M., AND ELVEHJEM, C. A. The nutritive value of canned foods. II. Amino acid content of fish and meat products. *J. Nutrition*, 39, 187 (1949).
12. NILSON, H. W., MARTINEK, W. A., AND JACOBS, B. Nutritive value for growth of some fish proteins. U. S. Dept. Int. FWS sep. 178 *Com. Fish Rev.*, 9, (7) (1947).
13. RIESEN, W. H., CLANDININ, D. R., ELVEHJEM, C. A., AND CRAVENS, W. W. Liberation of essential amino acids from raw, properly heated and overheated soy bean oil meal. *J. Biol. Chem.*, 167, 143 (1947).
14. RIZZO, P., DAVIS, R. M., AND SMITH, A. H. Effect of heat on the nutritive value of lactalbumin (chemical and morphologic changes). *Arch. Path.*, 47, 464 (1949).
15. SEIFTER, S., HARKNESS, D. M., RUBIN, L., AND MUNTWYLER, E. The nicotinic acid, riboflavin, d-amino acid oxidase and arginase levels of the livers of rats on a protein-free diet. *J. Biol. Chem.*, 176, 1371 (1948).
16. WILLIAMS, J. N., JR., AND ELVEHJEM, C. A. The relation of amino acid availability in dietary protein to liver enzyme activity. *J. Biol. Chem.*, 181, 559 (1949).