PROTEIN ANALYSIS OF SHRIMP-WASTE MEAL

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ABSTRACT

A method for the analysis of chitin nitrogen in shrimp meals and a procedure for correcting the crude protein fraction for the apparent protein contributed by chitin nitrogen is given.

INTRODUCTION

Fish meal is characterized for animal-feeding purposes by a proximate analysis for protein, oil, moisture, and ash. In shellfish meals, this analysis gives erroneously high results if no correction is made for the apparent protein contributed by chitin, an N-acetylated glucosamine polysaccharide. This shortcoming has been recognized by South African workers (Black and Schwartz 1950), who have devised a method for the estimation of chitin and chitin nitrogen.

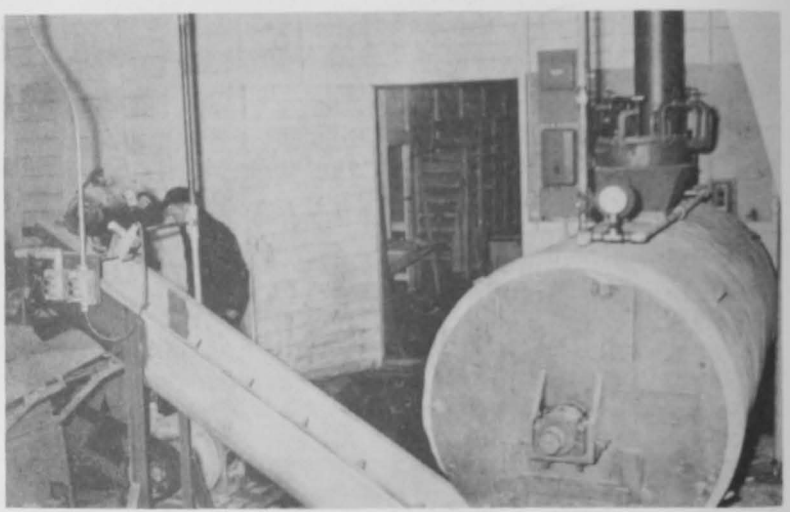

Fig 1 - A rotary steam-jacketed batch drier used for the preparation of meal from shrimp waste. The drier is fitted with a water-injection vacuum pump (not shown).

Since the protein fraction is of great interest to both the buyer and the producer of shrimp-waste meal, a more reliable estimate of the protein was needed. In the use of the Kjeldahl method for determining the protein fraction, a figure is obtained that includes not only protein nitrogen but also chitin nitrogen. Attempting to solve this problem, we turned to the South African work. Upon investigation, we found that this method involved filtration and that, possibly owing to fats in the meal, the filtration was very laborious and sometimes impossible to carry out. A variety of filtration techniques such as using different grades of filter paper, filtercel, butcher's linen, and filter-paper pulp were studied, but all proved to be impractical. A review of the literature therefore was undertaken, aimed at finding a more satisfactory analysis for chitin nitrogen.

Table 1 - Crude Protein in Shrimp-Waste Meal Corrected for Apparent Protein Contributed by Chitin

Sample	Protein as Calculated from Kjeldahl Nitrogen	Apparent Crude Protein Contributed by Chitin Nitrogen			Average	Corrected Crude Protein
		Replicate				
		1	2	3		
	(Percent)					
Meal A	45.70	3.34	3.33	3.27	3.31	42.39
Meal B	53.85	3.24	3.30	3.21	3.25	50.60
Meal C	54.85	3.14	3.06	3.07	3.09	51.76
Meal D	51.79	3.21	3.20	3.23	3.21	48.58
Meal E	53.58	3.55	3.49	3.51	3.52	50.06

The literature indicated that in almost all cases, the major problem involved in this analysis was filtration. An investigation of a procedure for the isolation of

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chitin (Horowitz, Roseman, and Blumenthal 1957) showed that if the formic acid digestion used in this method was preceded by a modified acetone extraction (Dam-

Table 2 - Proximate Analysis of Shrimp-Waste Meals

Sample	Corrected Crude Protein	Oil	Moisture	Ash	Part of Meal Not Accounted For
	(Percent)				
Meal A	42.39	8.83	19.56	20.80	8.42
Meal B	50.60	15.33	5.54	20.66	7.87
Meal C	51.76	13.09	5.21	21.15	8.79
Meal D	48.58	13.15	8.07	21.99	8.21
Meal E	50.06	14.20	2.28	23.52	9.94

bergs 1956) and that if it then was combined with the caustic digestion used by Black and Schwartz, filtration could be replaced, for the most part, by centrifugation. This combination finally was found to be satisfactory.

The principle of this method, in brief, is as follows: (1) acetone extracts the fat, (2) formic acid decalcifies the meal and extracts colored impurities and part of the protein, and (3) sodium hydroxide extracts the remainder of the protein, leaving only chitin and any silica present in the sample.

PURPOSE

The purpose of this paper is to describe this procedure and to report the results obtained by use of it.

PROCEDURE

The determination of the amount of crude protein in shrimp meal, corrected for the amount of apparent protein contributed by chitin nitrogen, is as follows:

1. Weigh a 5-gram sample of meal into a 250-milliliter centrifuge bottle fitted with a reflux condenser.

2. Add 100 milliliters of acetone, reflux for 45 minutes using a boiling water bath, centrifuge for 20 minutes at 2,000 r.p.m., and discard the supernatant.

3. Add 100 milliliters of a 70-percent acetone-water mixture, shake well, centrifuge for 20 minutes at 2,000 r.p.m., and discard the supernatant.

4. Add 100 milliliters of 90-percent formic acid, stopper, shake for 18 hours, centrifuge for 15 minutes at 2,000 r.p.m., and discard the supernatant.

5. Wash the residue with acetone, centrifuge for 20 minutes at 2,000 r.p.m., and discard the supernatant.

6. Repeat step 5, substituting 70-percent acetone-water for the acetone.

7. Add 100 milliliters of 5-percent (w/v) sodium hydroxide, reflux for 90 minutes on a steam bath, filter with suction through a sintered glass funnel (coarse porosity), and discard the filtrate.

8. Wash the residue with boiling water once, discard the filtrate, transfer the residue quantitatively to a 250 milliliter Kjeldahl flask with the smallest possible amount of water, evaporate until less than 5 milliliters of water remains, and proceed with the Kjeldahl determination in the usual manner, but digesting for 6 hours.

9. Calculate the percent protein from the amount of nitrogen determined, employing the usual factor of 6.25. (The figure obtained by this method is the percentage of apparent protein contributed by chitin nitrogen.)

10. Subtract the figure obtained in step 9 from the percent of crude protein obtained by the regular Kjeldahl determination.

RESULTS AND DISCUSSION

Samples of commercial shrimp-waste meals were obtained, and by the use of the procedure just described, the true amount of crude protein in the meals was estimated, with results reported in table 1. The data for the proximate analyses, using these estimated values, are given in table 2.

Chitin isolated by this method was dried and added to a commercial meal to test the recovery. These results indicated a 109-percent recovery of chitin.

The 8 to 10 percent of the meal left unaccounted for by the proximate analysis is probably due, for the most part, to the chitin fraction itself. Although a method for estimating this fraction might be derived, no attempts were made to do so because some deacetylation of the N-acetylglucosamine fragments reportedly occurs (Horowitz, Roseman, and Blumenthal 1957) during the digestion steps.

SUMMARY AND CONCLUSIONS

As the result of production of commercial shrimp-waste meal in Alaska, a method of analysis for chitin nitrogen was needed that would permit a reliable estimate of the true crude protein fraction of such meals.

The present paper reports a method involving (1) extraction with acetone to remove fats, (2) digestion with formic acid to decalcify the meal and extract colored impurities and part of the protein, (3) digestion with sodium hydroxide to extract the remainder of the protein, and (4) determination of Kjeldahl nitrogen. Use of centrifugation eliminates many of the troublesome filtrations required in earlier methods.

The results obtained by the present method are sufficiently reproducible to favor its adoption for use in proximate analyses of shrimp-waste meal.

LITERATURE CITED

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