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Low Molecular Weight Enzymatic Fish Protein Hydrolysates: Chemical Composition and Nutritive Value

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Low molecular weight enzymatic fish protein hydrolysates were produced from deboned cod filleting offal (DCO) and from functional fish protein (FFP, produced by low-temperature isopropanol extraction of DCO), by treatment with Alcalase followed by pancreatine. The yields of soluble nitrogen were between 85–90% and the hydrolysates had a balanced amino acid composition. The hydrolysates had an average molecular weight of less than 1000 and a free amino acid content between 15–45%. Treatment of DCO with only Alcalase gave a high percentage of free amino acids (30%), owing to the presence of exopeptidases in the raw material. Nutritional evaluations of hydrolysates in nitrogen balance experiments and growth experiments on rats showed the hydrolysates to have a high nutritional value. Taste evaluations showed that treatment with Alcalase followed by pancreatine gave bitterfree hydrolysates.

Elemental diets are increasingly used when there is a need for a diet which can be rapidly absorbed because of malfunction of the digestive system. Many elemental diets are made up of synthetic free amino acids, low molecular weight carbohydrates, vitamins, minerals, and minimal amounts of fat. These diets can be absorbed even in the absence of digestive enzymes and after extensive resection of the intestine. Due to the high degree of absorbtion, they give a very low fecal output, which is sometimes of value in pre- as well as postoperative nutrition (Spiller et al., 1975). A further advantage is that the composition can be clearly defined and adjusted to specific requirements.

Elemental diets based on free amino acids have, however, some disadvantages. The cost is relatively high (Woolfsson et al., 1976), mainly owing to the high prices of the amino acids. The palatability is often poor, due to bitterness of some free amino acids (Solms, 1969). One physiological problem is diarrhea, owing to the high osmolality (Lancet, 1975). The demonstration of a peptide transport system which is distinct from the amino acid transport and the finding that dietary protein is absorbed in the form of oligopeptides as well as free amino acids have also suggested that a protein hydrolysate might have some physiological advantages over a mixture of free amino acids (Matthews, 1971; Imondi and Stradely, 1974; Silk, 1974).

Protein hydrolysates as a source of readily assimilable protein have been prepared from, e.g., casein (Clegg et al., 1974). However, the preparation of protein hydrolysates will also sometimes give bitter-tasting products, depending on the protein raw material and enzymes used for proteolysis. For example, casein and soy protein are known to give bitter protein hydrolysates. Particularly, exhaustive protein hydrolysis with endopeptidases produces bitter-tasting peptides which make the end product unpalatable.

These peptides have a predominantly hydrophobic amino acid composition and are more bitter than their free corresponding amino acids (Fujimaki et al., 1971). Arai et al. (1970) have found that if a bitter-tasting soy hydrolysate is treated with acid carboxypeptidase from *Aspergillus* or with carboxypeptidase A (exopeptidases), it reduces the bitterness with formation of significant amounts of free amino acids, especially hydrophobic amino acids.

Fish protein has in several studies been tested as raw material for production of protein hydrolysates. Hale, in an extensive paper (1972), reported the effects of various processing conditions and commercially available proteolytic enzymes on yield and composition of water-soluble fish protein hydrolysates. He concluded that the hydrolysis of raw fish with protease of *Bacillus subtilis* at pH 8.5 or above gave the best balance of essential amino acids and a high yield of soluble product. Quite recently, Yánez et al. (1976) reported production of a fish protein hydrolysate from hake for supplementation of cereal protein. The present experiments were undertaken to study the possibility of making an extensively hydrolyzed but still bland-tasting protein hydrolysate from fish protein for use in elemental diets. Fish protein was used as raw material because of its well-balanced amino acid composition. The nutritional value of the fish protein hydrolysates was also tested in rat experiments and compared with a diet based on free amino acids with the same amino acid composition. Also, commercially available elemental diet and casein were used as reference diets.

MATERIALS AND METHODS

Substrates and Enzymes. The following substrates were used: deboned cod filleting offal (DCO) (17.5% dry substance; analytical data of freeze-dried sample: 86.3%

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Table I. Amino Acid (AA) Compositions of FFP, Alcalase Hydrolysate from FFP, and Free Amino Acid (FAA) after Continued Hydrolysis with Pancreatine[a]

Amino acid	g/16 g of N							
	A	B	C	D	E	F	G	H
Asp	10.75	10.20	0.25	0.44	0.71	0.40	0.38	0.49
Thr	5.13	4.50	0.11	0.49	0.33	0.30	0.34	1.06
Ser	5.43	4.68	0.37	0.30	0.70	0.92	0.71	0.33
Glu	16.05	16.35	0.54	0.67	1.20	0.82	0.83	0.84
Pro	4.70	4.46	trace	trace	trace	trace	trace	trace
Gly	7.53	6.81	0.06	0.11	0.15	0.17	0.12	0.12
Ala	6.90	6.36	0.25	0.44	0.60	0.46	0.51	0.75
Val	5.68	4.53	0.79	0.60	1.11	0.89	0.90	0.97
Met	3.77	3.14	0.49	0.91	0.79	0.71	0.38	0.96
Ile	5.08	4.23	0.27	0.60	0.75	0.68	0.72	0.91
Leu	8.61	7.34	2.45	2.97	3.22	2.79	3.04	4.35
Tyr	4.22	3.48	1.38	1.92	2.20	1.86	1.96	2.30
Phe	4.65	3.80	1.38	2.01	2.36	1.93	1.70	2.30
Lys	8.45	8.42	3.19	3.62	4.60	3.60	3.52	4.88
His	2.68	2.13	0.72	0.79	0.67	0.84	0.79	0.91
Arg	6.86	6.70	3.25	4.25	4.55	4.16	3.98	4.50
Cys	1.40	1.02	trace	trace	trace	trace	trace	trace
Trp	1.20	1.06	0.44	0.62	0.63	0.56	0.45	0.55
Total FAA			15.9	20.7	24.6	21.1	20.3	26.2
$\frac{\text{Ess. AA}}{\text{Tot. AA}} \times 100$	39.0	37.3	57.2	57.1	56.1	54.3	54.3	60.9

[a] A, FFP (functional fish protein); B, Alcalase hydrolysate of FFP; C-E, free amino acids after 4, 8, and 24 h pancreatine (1.25%) hydrolysis of B; F-H, free amino acids after 4, 8, and 24 h pancreatine (2.5%) hydrolysis of B.

protein (N × 6.25), 3.6% fat, 9.0% ash, and 5.6% water).

Functional fish protein (FFP) produced by low-temperature isopropanol extraction of DCO, chemical composition 89.1% protein (N × 6.25), 0.18% fat, 9,3% ash, 5.8% moisture. The amino acid composition of FFP is shown in Table IA. DCO has a similar amino acid composition.

Enzymes used were Alcalase, a nonspecific bacterial endopeptidase from *Bacillus licheniformis* having a specific activity of 6 Anson units/g (Novo Industri A/S, Denmark), and pancreatine, having a specific activity of 6 N × F (Scientific Protein Laboratories, USA).

Preparation of Protein Hydrolysates. Preparation of protein hydrolysates followed the following general pattern. The protein hydrolysis was carried out in two separate hydrolytic steps, the first with Alcalase and the second with pancreatine. Both FFP and DCO are mainly water-insoluble substrates. The Alcalase was used to bring the protein in the solution, and the exopeptidase activity of the pancreatin has been used to reduce the average molecular weight of the peptides and simultaneously debitter the bitter-tasting hydrolysates which are obtained with Alcalase. Amounts of enzyme are expressed as percent of the amount of protein supplied by the raw material.

Step I. FFP Hydrolysis with Alcalase. One hundred grams of FFP was suspended with 1.9 L of water (5% w/w). The pH was adjusted to 8.5 with 5 M KOH and was kept at this value for 15 min at 50 °C. After that 0.5 g (0.62% w/w) of Alcalase was added. The hydrolysis was followed by automatic titration by means of a pH stat (Radiometer TTT 60, DHM 62, Copenhagen, Denmark). The hydrolysis continued for 3 h. pH was kept constant at 8.5 with 1 M NAOH-ammonia (1:1). After hydrolysis with Alcalase the pH was reduced to 7.7 with 5 M HCl and the solution was centrifuged to remove insoluble material. Part of the supernatant was freeze-dried.

Step II. Continued Hydrolysis of FFP with Pancreatin. The supernatant was cooled down to 37 °C and 1 g (1.25% w/w) of pancreatin was added. To prevent bacterial growth 0.2% v/v chloroform was added. After 4, 8, and 24 h hydrolysis aliquots of peptide hydrolysate were taken for analysis and freeze-dried. The enzymes were inactivated by heating the solution to 80 °C.

The experiment described above was repeated under identical conditions but with 2 g (2.5% w/w) of pancreatin.

Hydrolysis of DCO. One kilogram of frozen and thawed DCO was suspended in 1 L of water. The hydrolysis was carried out under the same conditions as described above. The protein was hydrolyzed first with 1.00 g (0.66% w/w) of Alcalase for 3 h, then with 1.5 g (1.0% w/w) and 3.0 g (2.0% w/w) of pancreatin for 4, 8, and 24 h, respectively. Aliquots of peptide hydrolysate were freeze-dried.

Sephadex Chromatography. The molecular weight of the hydrolysates was investigated by means of Sephadex chromatography. A glass column (bed dimensions, 57 × 0.87 cm) was packed with Sephadex G:10 (Pharmacia Fine Chemicals, Uppsala, Sweden) and equilibrated with 50% (v/v) analytical grade acetic acid in water. Samples (30 mg) of hydrolysates were dissolved in 0.5 mL of the elution medium (50% acetic acid). The elution was carried out at room temperature and the eluent collected in 2.5 mL. The flow rate was 5 mL/min. The peptide distribution in the eluate was determined by measuring the absorbance at 280 nm and by carrying out the ninhydrin reaction (Yemm and Cocking, 1955). The column was calibrated with a number of reference substances of known molecular weight dissolved in 50% acetic acid.

Nitrogen content in fish protein products and in samples of urine and feces from animal experiments was determined by the Kjeldahl method with the use of the Kjell Foss automatic apparatus (A/SN Foss Electric, Denmark).

Ash content was determined according to the AOAC method (AOAC, 1970).

Lipid content in the fish protein products was determined by extraction with chloroform–methanol 2:1 v/v.

Amino acid composition in the fish protein products was determined by hydrolysis in 6 N HCl under vacuum at 110 °C for 22 h, followed by ion-exchange chromatography essentially according to the method of Stein and Moore (1954). The free amino acid composition was determined in the same matter but without HCl treatment. Small peptides interfere with the determination of free amino

acids and presented figures therefore must be considered as estimates.

Cysteine–cystine was determined as cysteic acid and "total methionine" as methionine sulfone after oxidation with performic acid as described by Moore (1963).

Tryptophan was determined according to the method of Spies and Chambers (1948 and 1949).

Biological Evaluations. Biological evaluations were performed on rats as nitrogen balance and growth experiments.

For all animal experiments, male rats of the Spraque Dawley strain (Anticimex, Stockholm) were used. The animals were housed in individual cages in a room with constant humidity and temperature (50% RH and 21 °C) and with a 12-h light and darkness cycle. Food and water were offered ad libitum.

Diet Composition. The diets were formulated to imitate the composition of Flexical (product from Mead Johnson, Evansville, Ind.), a commercially available elemental diet based on casein hydrolysate supplemented with amino acids.

This gave the following diet composition: protein, 9.28% (as protein hydrolysates, free amino acids or casein, N × 6.25); fat, 15% (corrected for fat contribution from test protein sample, soy oil 77.6%, medium chain triglycerides 19.42%, lecithin 2.92%); mineral mixture, 5% (corrected for the contribution of ash from test protein sample); vitamin mixture, 2%; carbohydrates up to 100% (sucrose 51.58%, corn syrup solids 42.16%, arrow root starch 6.26%).

Nitrogen Balance Procedure. The starting weight of the animals was 70–75 g. Seven rats were randomly assigned to each test diet. After a 3-day adaptation period on the test diet a 4-day experimental period followed. During the experimental period weight gain and food intake were registered and urine and feces were collected separately for nitrogen determination. Digestibility (D), biological value (BV), and net protein utilization (NPU) were calculated after correction for endogenous loss of nitrogen on a protein-free diet.

Growth Experiments. The starting weight of the animals was 56–62 g. Ten rats were randomly assigned to each test diet. During a 4-week period food intake and weight gain were registered. The protein efficiency ratio (PER) was calculated as g weight gain/g consumed protein.

Blood Sampling. Blood samples were taken from anesthetized animals from the aorta and collected in heparinized tubes. Blood samples for determination of urea were taken after 6 h withdrawal of food.

Urea concentration in plasma and urine was analyzed with the Boehringer test kit 15930 THAC.

Experimental Outline. Since all diets had the same composition with the exception of the protein part, the differences in amino acid composition and the relationship between free amino acids and peptides will be the only difference between different diets.

The experimental diets had the following nitrogen source. N-balance experiments: diet 1: casein; diet 2: flexical (contains a casein hydrolysate supplemented with methionine, tryptophan, and tyrosine); diet 3: fish protein hydrolysate from FFP, hydrolyzed for 3 h with 0.62% w/w Alcalase and for 24 h with 1.25% w/w pancreatine; diet 4: functional fish protein (FFP, raw material to the fish protein hydrolysate); diet 5: free amino acids with the same composition as diet 3. Growth experiments: diet 6: casein; diet 7: fish protein hydrolysate from FFP, hydrolyzed for 3 h with 0.62% w/w Alcalase and for 6 h with 2.5% w/w pancreatine; diet 8: same as diet 7 but supplemented with 0.1% tryptophan, calculated on the whole diet.

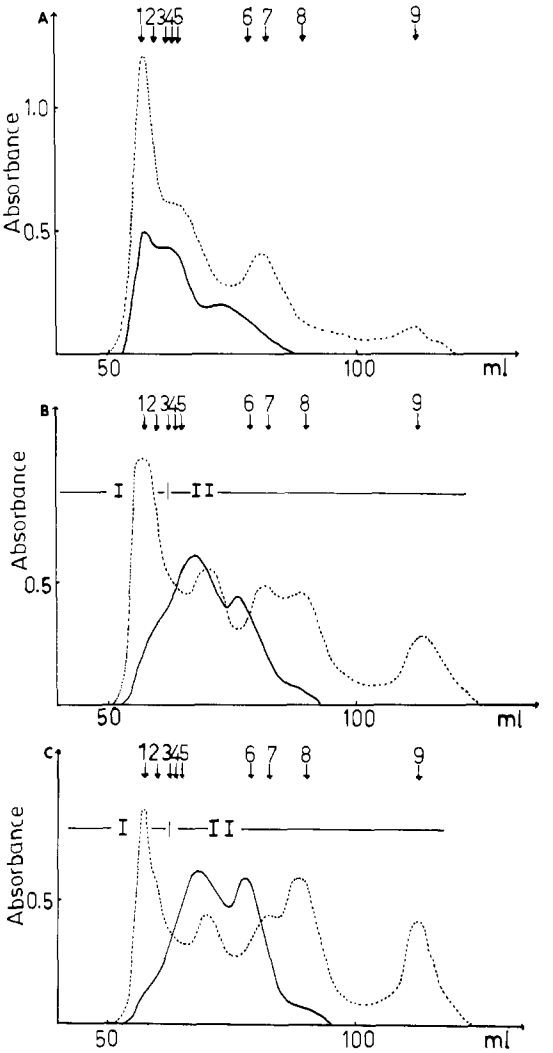

Figure 1. (A) Chromatography on Sephadex G:10 of 30 mg of hydrolysate from FFP (functional fish protein), hydrolyzed with 0.56% Alcalase for 3 h. Solid lines: the material distribution in the eluate estimated by measuring the ninhydrin colour at 570 nm. Dotted lines: the material distribution in the eluate estimated by measuring the absorbance at 280 nm. The numbers denote elution positions of the following compounds: (1) insulin (mol wt 5733) and β-chain of insulin (mol wt 3300); (2) H-Phe-Phe-Val-Arg-O-isopropyl-2HCl; (3) H-Phe-Val-Arg-O-Met-2HCl; (4) H-Phe-Arg-NH_2-2HCl; (5) lysine, arginine, and histidine; (6) amino acid mixture except basic and aromatic amino acids; (7) phenylalanine; (8) tyrosine; (9) tryptophan. (B) Sephadex chromatography of 30 mg of hydrolysate from FFP (functional fish protein), first hydrolyzed with Alcalase and then with 1.25% pancreatine for 4 h. Other symbols and experimental conditions as in Figure 1A. (C) Sephadex chromatography of 30 mg of hydrolysate from FFP (functional fish protein), first hydrolyzed with Alcalase and then with 1.25% pancreatine for 24 h. Other symbols and experimental conditions as in Figure 1A.

At the end of the growth experiment, the following analyses were performed on all animals in each test group: hematologi (packed cell volume and hemoglobin), serum analysis (GOT, GPT, alkaline phosphatase), (phosphate, calcium, bilirubin, creatinine, protein, potassium, and sodium), urinanalysis (pH, protein, glucose, ketone bodies, and blood), fresh organ weights (liver, kidneys and spleen).

(1) Composition of mineral mixture (USP salt mixture XIV supplemented with trace minerals): calcium carbonate 68.3 g, calcium citrate 308.3 g, calcium diphosphate 112.8 g, magnesium carbonate 35.2 g, magnesium sulfate

Table II. Amino Acids (AA) Compositions of Alcalase Hydrolysate from DCO and Free Amino Acid (FAA) Fractions after Continued Hydrolysis with Pancreatine[a]

Amino acid	g/16 g of N						
	A	B	C	D	E	F	G
Asp	11.07	1.24	1.60	1.67	2.01	1.64	1.69
Thr	4.79	1.32	1.58	1.38	1.64	1.46	1.79
Ser	4.57	2.15	2.22	2.24	2.66	2.50	2.61
Glu	17.21	1.78	1.85	2.06	2.49	2.12	2.42
Pro	5.04	0.28	0.12	0.13	0.20	0.29	0.21
Gly	7.89	0.56	0.62	0.68	0.83	0.59	0.72
Ala	6.96	2.46	2.31	2.65	3.09	2.66	2.68
Val	5.82	2.08	2.16	2.38	2.93	2.49	2.52
Met	3.38	2.02	2.22	2.33	2.46	2.77	2.22
Ile	4.51	1.91	2.10	2.15	2.58	2.52	2.28
Leu	8.21	4.23	4.94	5.31	6.00	5.51	5.84
Tyr	3.57	1.65	2.45	2.75	2.85	2.66	2.56
Phe	4.01	2.21	2.56	2.73	3.02	2.74	2.59
Lys	8.00	2.36	4.56	4.82	5.71	4.82	5.22
His	2.63	1.03	2.05	1.84	2.22	1.84	1.96
Arg	6.60	2.01	4.36	4.63	5.39	4.89	5.28
Cys	1.04	trace	trace	trace	trace	trace	trace
Trp	0.92	0.48	0.87	0.63	0.91	0.90	0.94
Total FAA		29.8	38.6	40.4	47.1	42.7	43.5
$\frac{\text{Ess. AA}}{\text{Tot. AA}} \times 100$	37.3	55.8	54.4	53.7	53.6	54.3	53.7

[a] A, Alcalase hydrolysis of DCO (deboned cod offal); B, free amino acids in A; C-E, free amino acids after 4, 8, and 24 h pancreatine (1%) hydrolysis of A; F-G, free amino acids after 4 and 8 h pancreatine (2%) hydrolysis of A.

38.3 g, potassium chloride 124.7 g, dibasic potassium phosphate 218.8 g, sodium chloride 77.1 g, ferric ammonium citrate 15.281 g, cupric sulfate 0.077 g, manganese sulfate 4.200 g, ammonium aluminium sulfate 0.092 g, zinc sulfate 0.500 g, potassium iodine 0.740 g, sodium fluoride 0.507 g, cobalt chloride 0.023 g, sodium arsenite 0.009 g, sodium borate 0.022 g, sodium molybdate 0.003 g.

(2) Vitamin mixture in dextrose with following supply of vitamins when used in 2% of the diet (all values per kg diet): Retinol 19.800 IE, cholecalciferol 2.000 IE, ascorbic acid 0.991 g, α-tocopherol 0.110 g, myoinositol 0.110 g, choline chloride 1.651 g, menaphthone 0.049 g, *p*-aminobenzoic acid 0.110 g, nicotinic acid 0.099 g, riboflavin 0.022 g, pyridoxine HCl 0.022 g, thiamine HCl 0.022 g, calcium pantothenate 0.066 g, biotin 0.440 mg, pteroylmonoglutamic acid 1.982 mg, hydroxocobalamin 0.029 mg.

RESULTS

FFP Hydrolysis with Alcalase. The yield of soluble nitrogen was 86%. The hydrolysate contained 87.6% protein (nitrogen × 6.25), 9.5% ash, and 6% moisture. Its amino acid composition and the molecular weight distribution are shown in Table IB and Figure IA, respectively. The amino acid composition was well balanced and almost the same as in the raw material (FFP). The hydrolysate had almost no free amino acids and had a weak bitter taste at 5% w/w protein concentration.

Continued Hydrolysis of FFP with Pancreatine. After 4 h hydrolysis with 1.25% pancreatine, the peptide solution, at 5% w/w, still had a weak bitter aftertaste. Hydrolysates recovered after 8 and 24 h treatment were free from bitterness at 5% w/w concentration. After 4, 8, and 24 h free amino acids accounted for about 16, 21, and 25% of the total nitrogen, respectively (Table IC,D,E). The free amino acids contained a high level of essential amino acids. The molecular weight distribution in 4- and 24-h samples are shown in Figures 1B and 1C.

When the amount of pacreatine was increased to 2.5% there was an increased liberation of free amino acids. As shown in Table I (F, G, H), after 4, 8, and 24 h treatment about 21, 20, and 26%, respectively, of the total nitrogen was present as free amino acids. At 5% protein concentration all the hydrolysates were free from bitterness. Their molecular weight distributions were similar to the one presented in Figure 1C.

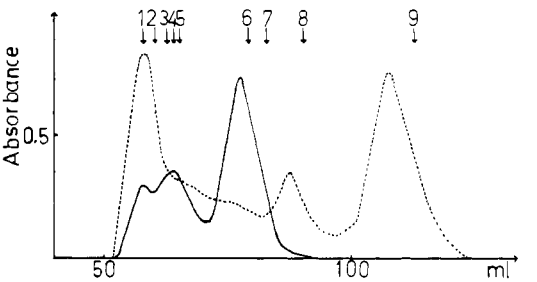

Figure 2. Sephadex chromatography of 30 mg of hydrolysate from DCO (deboned cod offal), hydrolyzed with 0.64% Alcalase for 3 h. Other symbols as in Figure 1A.

DCO Hydrolysis with Alcalase. The yield of soluble nitrogen was about 89%. The hydrolysate contained 90.5% protein (nitrogen × 6.25), 8.1% ash, 0.3% fat, and 4.3% moisture. Its amino acid composition and the molecular weight distribution are shown in Table IIA and Figure 2, respectively. The amino acid composition was well balanced and similar to that of the FFP hydrolysate. The peptide solution from DCO, in contrast to that from FFP, had no bitter taste. It had a high content of free amino acids, about 30% of the total nitrogen (Table IIB). These were evidently formed because of the exopeptidase activity present in the fish raw material.

Continued Hydrolysis of DCO with Pancreatine. Hydrolysis with pancreatine, 1.0% for 4, 8, and 24 h further increased the amounts of free amino acids to about 39, 40, and 47%, respectively, of the total nitrogen (Table IIC,D,E). Hydrolysis with 2% pancreatine for 4 and 8 h resulted in very high levels of free amino acids, about 43 and 47%, respectively, of the total nitrogen (Table IIF,G). At 5% w/w protein concentration all the hydrolysates were free from bitterness and their molecular weight distributions were similar to the one presented in Figure 2.

Amino Acid Composition in Different Fractions of Hydrolysates. Amino acid analyses of low and high molecular fractions were performed. The low molecular

Table III. Amino Acid Composition of Different Fractions of FFP Hydrolysate (mg)

Amino acid	A	B	C	D
Asp	1.04	1.32	0.83	1.39
Thr	0.34	0.67	0.32	0.64
Ser	0.40	0.69	0.51	0.74
Glu	1.43	2.48	1.04	2.73
Pro	0.63	0.33	0.70	0.42
Gly	0.95	0.76	0.92	0.99
Ala	0.46	1.01	0.45	1.09
Val	0.53	0.75	0.35	0.77
Met	0.05	0.21	0.05	0.15
Ile	0.28	0.58	0.22	0.65
Leu	0.36	1.48	0.24	1.37
Tyr	0.14	0.29	0.06	0.40
Phe	0.14	0.83	0.09	0.78
Lys	1.91	1.03	0.67	2.08
His	0.45	0.25	0.25	0.26
Arg	1.74	1.28	1.23	1.00
Cys	0.15	0.10	0.11	0.13
Trp[b]				
Total	11.00	14.06	8.04	15.59
	25.06		23.63	

[a] A, fraction I after hydrolysis with Alcalase followed by 4 h with 1.25% pancreatine; B, fraction II after hydrolysis with Alcalase followed by 4 h with 1.25% pancreatine; C, fraction I after hydrolysis with Alcalase followed by 24 h with 1.25% pancreatine; D, fraction II after hydrolysis with Alcalase followed by 24 h with 1.25% pancreatine. [b] Not determined.

fraction (II) and high molecular fraction (I) were defined as material eluted after and before, respectively, of reference substance number 3. Results from analysis of 25 mg (N × 6.25) hydrolysates of FFP (Alcalase followed by 4- and 24-h treatment with pancreatine) are presented in Table III. Results show a higher level of essential amino acids in the low molecular fractions after both 4- and 24-h treatment with pancreatine. Proline was mainly found in the high molecular fraction.

Influence of Hydrolysis on Allergenic Properties. The protein in cod responsible for allergic reaction against cod has been isolated and characterized by Aas (1967). Aas and Elsayed (1969) also showed the allergenic protein to be sensitive for treatment with proteases. Analyses performed by Aas (unpublished) showed that treatment of DCO with alcalase and pancreatine, as described in the Materials and Methods section, almost completely destroyed the allergic activity.

Biological Evaluation of Hydrolysates. Results from nitrogen balance experiments are presented in Table IV. All diets showed a high digestibility. The biological value (BV), which is the relationship between retained nitrogen and absorbed nitrogen, shows significantly ($B < 0.01$) lower values for the amino acid based diet (diet 5). This was an effect of relatively high excretion of nitrogen in urine. The 24-h pancreatine hydrolysate of FFP (diet 3) has a BV below that for the corresponding raw material (diet 4) and casein (diet 1), but comparable to that of Flexical. Plasma urea N and the ratio of urine urea N to total urine N, indicators of protein quality, showed the lowest values for FFP (diet 4).

Results from growth experiments are presented in Table V. It was found that a protein hydrolysate, produced from FFP with Alcalase, followed by 6-h treatment with pancreatine, has a PER value equivalent to that of casein. Supplementation with tryptophan gave a nonsignificant increase in the PER value.

The results from chemical analyses of blood, serum, and urine and fresh organ weights showed some significantly different values. However, the values were not considered as pathological but rather as the result of adaptation to the different diets.

DISCUSSION

In our experiments the hydrolysis was performed at low protein concentration (less than 10%), which gave a high yield of soluble nitrogen, 85–90%, and balanced amino acid composition. This is in accordance with data from Cheftel et al. (1971). When FFP was used as the raw material, treatment with pancreatine was necessary to yield a product with 15–25% free amino acids. Due to active endogenous exopeptidases in fresh or frozen cod offal, hydrolysis of this material with Alcalase gave values for free amino acids of around 30%. Pancreatine treatment increased this further to about 40–45%. As pointed out in the Materials and Methods section, values for free amino

Table IV. Nitrogen Balance Experiments on Rats

	Diet 1[b]	Diet 2[c]	Diet 3[d]	Diet 4[e]	Diet 5[f]
Weight gain, g	15.6 ± 2.4[a]	14.3 ± 1.9	11.6 ± 1.7	10.4 ± 2.0	7.1 ± 0.9
Diet intake, g	41.7 ± 2.3	37.4 ± 2.5	37.3 ± 1.3	38.4 ± 2.3	32.1 ± 1.4
Plasma urea N, mg/100 mL	10.5 ± 1.3	5.9 ± 1.2	6.1 ± 1.0	5.4 ± 1.2	6.7 ± 0.3
$\frac{\text{Urine urea N}}{\text{Total urine N}} \times 100$	70.5 ± 4.0	73.2 ± 3.2	80.9 ± 4.1	57.4 ± 5.5	83.2 ± 2.1
D	94.3 ± 0.5	96.9 ± 0.9	95.0 ± 0.8	94.6 ± 0.6	96.5 ± 0.4
BV	85.7 ± 1.0	81.9 ± 2.7	80.0 ± 1.9	86.2 ± 1.9	71.1 ± 0.7
NPU	80.8 ± 1.1	79.4 ± 3.0	76.0 ± 1.8	81.6 ± 1.7	68.6 ± 0.7

[a] Average ± SEM. N = 7. [b] Diet 1, casein. [c] Diet 2, Flexical. [d] Diet 3, fish protein hydrolysate from FFP. Hydrolyzed 3 h with Alcalase followed by 24 h with pancreatine. [e] Diet 4, FFP (raw material to diet 3). [f] Diet 5, free amino acid mixture with the same composition as diet 3.

Table V. Results from Growth Experiments on Rats

	Diet 6[b]	Diet 7[c]	Diet 8[d]
Weight gain, g	99.6 ± 6.8[a]	108.6 ± 8.8	110.7 ± 8.0
Diet intake, g	293.6 ± 12.7	315.3 ± 13.8	301.8 ± 17.7
PER (Protein Efficiency Ratio)	3.36 ± 0.09	3.40 ± 0.16	3.64 ± 0.10

[a] Average ±SEM. N = 10. [b] Diet 6, casein. [c] Diet 7, fish protein hydrolysate from FFP. Hydrolyzed 3 h with Alcalase followed by 6 h with pancreatine. [d] Diet 8. As diet 7 but supplemented with 0.1% L-tryptophan (calculated on the weight of the whole diet).

acids are determined by direct ion-exchange chromatography of the hydrolysates and some interference from small peptides must be expected.

The results from the nutritional evaluation of the fish protein hydrolysates show that the biological value (BV) as well as the PER values are high. The biological value for the fish protein hydrolysate is higher than the biological value for a diet composed of free amino acids with the same amino acid composition. The reason for the higher biological value might possibly partly be differences in the course of events during the absorption as shown by Matthews (1971), Imondi and Stradely (1974), and Silk (1974). Judged from the supplementary effect on tryptophan in the growth experiment, tryptophan might not be the first limiting amino acid in this fish protein hydrolysate. However, other experiments (unpublished) in our laboratory with similar hydrolysates have shown a supplementary effect with tryptophan.

Data from clinical chemical analyses and determination of fresh organ weights showed no negative effects of the protein hydrolysate.

As regards the taste of fish protein hydrolysates, it is our experience that treatment with the endopeptidase, Alcalase, gives hydrolysates which have a weak bitter taste. If the hydrolysate is further treated with pancreatine, the bitter taste is eliminated. During hydrolysis of deboned cod filleting offal, exopeptidases present in the raw material evidently eliminated the bitterness of the hydrolysate. This effect of exopeptidases is in accordance with results reported by Fujimaki et al. (1971) and Arai et al. (1970). The effect was explained by the liberation of free hydrophobic amino acids from bitter-tasting hydrophobic peptides. In our experiments with fish protein hydrolysates the bitterness had disappeared (tested at 5% w/w concentration) when about 20% or more of total nitrogen was recovered as free amino acids. Hydrophobic amino acids (leucine, isoleucine, phenylalanine, tryptophan, and valine) represented a relatively large part of this free amino acid fraction. The hydrophobic amino acids are less bitter as free amino acids than when they are incorporated in some peptides. Our results support this hypothesis. In general, hydrolysates from FFP had an almost bland taste and odor. Hydrolysates from DCO had a faint marine taste and odor.

Pronase, a proteolytic enzyme derived from *Streptomyces griseus*, which contains both endo- and exopeptidases, has also been tried with positive results for debittering purposes (Cheftel, 1971).

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