THE UTILIZATION OF FISH PROTEIN AND OIL FROM ANCHOVY (*Engraulis japonicus*) FOR HUMAN CONSUMPTION

by

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ABSTRACT

The comprehensive utilization of fish protein and oil from anchovy (*Engraulis japonicus*) for human consumption was studied. Proteolytic enzymes were used to hydrolyze anchovy protein and the bitter taste of the hydrolysate was eliminated by active carbon and beta-cyclodextrin. Another non-bitter fish protein hydrolysate also could be prepared by limited hydrolysis with proteolytic enzymes. The final protein product from anchovy can dissolve in water and form a transparent solution, which is rich in peptides and free amino acids with an appropriate essential amino acid ratio. A highly polyunsaturated fatty acids (PUFA) product was prepared from anchovy oil by molecular distillation. The contents of EPA/DHA in oil products with light yellow color from anchovy can reach 70%. The wastes from anchovy after protein and oil recovery were converted to fish meal. Growth and metabolic experiments with young male rats indicated that the efficiency of digestion and utilization of the protein from the anchovy hydrolysate were very close to that of milk protein. The product can be used as a protein supplement for babies and patients, or as raw materials for seafood flavorings.

INTRODUCTION

The underutilized fish anchovy *Engraulis japonicus* is one of the important resources in China for manufacturing fish meal and fish oil. It is rich in fish protein with a well-balanced amino acid composition, and the lipid is rich in highly polyunsaturated fatty acids (PUFA). Because of its rapid spoilage, it is difficult to process for human food.

The trend in the utilization of underutilized fishery resources is toward direct consumption as human food, such as food products and food supplements used in different nutrition programmes (Lalasidis *et al.*; 1978, Mackie, 1982; Yanez *et al.*, 1976). The usual method is hydrolysis where enzymes are used to produce protein rich food for human consumption. Lack of functional properties and a bitter taste have, however, inhibited the use of hydrolysates as human food (Hevia *et al.*, 1977; Lalasidis *et al.*, 1978; Vega *et al.*, 1988).

Fish oil products derived from omega-3 fatty acids have been suggested as beneficial in the prevention and/or treatment of major cardiovascular diseases affecting human health (Gordon *et al.*, 1992). The present experiments were undertaken to convert anchovy protein into a water-soluble protein product suitable for direct addition to food systems, and to prepare anchovy oil as a PUFA concentrate for use in medicine and health foods. The comprehensive utilization process from anchovy is also discussed.

MATERIALS AND METHODS

RAW MATERIALS

Anchovy, caught in the Yellow Sea in February 1990, was supplied by the Qingdao Marine Fisheries Co., China, and was frozen until required. The crude composition of raw material was as follows: 16.70%

protein (N x 6.25), 10.82% fat, 1.40% ash and 69.75% water. The neutral proteolytic enzyme from *Bacillus subtilis* was purchased from Wuxi Enzyme Products Factory, China.

PREPARATION OF PROTEIN HYDROLYSATES

1. Exhaustive hydrolysis for enzymatic anchovy protein hydrolysates (EAPH).

A homogenate was prepared from frozen anchovy by thawing, mincing and suspending in the same volume water. The pH was adjusted to 7.0 with NaOH and kept at this value at 50°C. After that 0.7% (w/w) of neutral proteinase was added. The hydrolysis continued for 12 h. After hydrolysis the enzyme was inactivated by heating the homogenate to 85°C for 10 min and the suspension was centrifuged to remove the insoluble materials. Then the supernatant was treated by absorption with active carbon and beta-cyclodextrin to remove the bitterness (Helbig *et al.*, 1980; Lalasidis *et al.*, 1978). The clear aliquots of hydrolysate were spray dried for analysis and preparation of food supplements.

2. Partial Hydrolysis for EAPH.

The process of partial hydrolysis is similar to that of exhaustive hydrolysis. The anchovy homogenate was hydrolyzed with 0.1% (w/w) neutral proteolytic enzyme at 50°C, pH 7.0 for 1.5 h. The hydrolysate was treated with 1% w/v active carbon. The clear aliquots of hydrolysate were analyzed and spray-dried.

SENSORY TEST ON BITTERNESS

All the taste evaluations were carried out according to the methods of Helbig *et al.*(1980) and Lalasidis *et al.*(1978). The bitterness scores of hydrolysates were graded on a five point scale (0 = no bitterness; 1 = weak aftertaste; 2 = weak bitter taste; 3 = bitter; 4 = strong bitter taste; 5 = extremely bitter) by a panel of 10 trained members.

PREPARATION OF PUFA CONCENTRATES

Refined anchovy oil was prepared from crude fish oil, which was obtained from exhaustive hydrolysis process, by a general oil refining process such as degumming, deacidification, decolorization and deodorization. PUFA concentrates were prepared by urea addition and molecular distillation (Sumerwell, 1957; Ackman *et al.*,1973). Before PUFA concentration fish oil needed to be converted to the ethyl ester by transesterification with a catalyst. In the urea addition method, the amounts of urea used in three experiments were 1.5,2.0,and 2.5 times the of ethyl ester of fish oil. The ester mixture was distilled in two stages on a SIBATA MS-300 molecular still.

BIOLOGICAL EVALUATION OF EAPH

Biological evaluation was performed according to McLaughlan (1980) and Pellett *et al.* (1980). Thirty male Wistar rats weighing 57±1 g were randomly divided into five matched groups. The experimental feeds had the following nitrogen sources: A, nitrogen-free; B, milk powder protein; C, EAPH (from exhaustive hydrolysis); D, wheat protein; E, wheat protein supplemented with EAPH (12% protein in the feed was from EAPH). The feed compositions were as follows: 10±0.12% protein; 10±0.36% fat; 5% mineral mixture; 2% vitamin mixture; 5% nitrogen-free cellulose; and up to 100% corn starch. Feed and water were offered *ad libitum*. During the 4-d nitrogen balance experimental periods, weight gain and feed intake were measured, and urine and feces were collected separately for nitrogen determination. During the 4-week growth experiment, feed intake and weight gain were measured. At the end of the experiments, all rats were put to death, and analyses of fresh organs (liver, kidney, lung and spleen) were performed.

RESULTS

EAPH FROM EXHAUSTIVE HYDROLYSIS

The degree of exhaustive hydrolysis was 68.66%. The hydrolysate prepared from exhaustive hydrolysis had a strong bitter taste (bitterness score 4.5). The hydrolysate can be debittered by absorbing the bitter peptides with active carbon and masking with beta-cyclodextrin. The end product EAPH was free from bitterness (bitterness score 0.5). The yield of soluble nitrogen in EAPH was 89.80%. The spray dried product from the pilot plant production was a fine white powder with a pleasant odor containing 88.2% protein, 6.89% ash, 0.38% fat and 3.82% moisture. The amino acid composition was well balanced, and the content of essential amino acids was higher than that of enzymatic fish protein hydrolysate (EFPH) used as a reference from Yanez *et al.* (1976). EAPH had a high content of free amino acids (62.70% of total amino acids) (Table 1). Synthetic milk-like beverages were prepared with EAPH, and stored at room and refrigerated temperatures for several weeks with no evidence of de-emulsification. As a protein supplement in cake formulations, the product could replace wheat flour with no change in cake volume and texture. Therefore EAPH may be used as a food ingredient or additive.

EAPH FROM PARTIAL HYDROLYSIS

The degree of partial hydrolysis was 31.86%. The hydrolysate prepared by partial hydrolysis had no bitterness (bitterness score 0.5). The yield of soluble nitrogen in EAPH prepared by partial hydrolysis was 41.17%. The spray dried product was a powder with a seafood flavour containing 93.10% protein, 2.28% ash, 0.52% fat and 2.17% moisture. The amino acid composition was also well balanced. The amount of essential amino acids was also higher than that of EFPH (Table 1). It can also be used as a seafood flavoring or a seafood additive.

Table 1. Amino acid composition of EAPH (g/100g protein).

Amino Acids	**Raw anchovy**	**EAPH**			**EFPH**
		a	**b**	**c**	
Asparagine	9.21	8.63	3.41	8.66	13.9
Threonine	4.11	3.73	0.25	3.62	3.7
Serine	3.97	3.30	1.48	3.08	3.5
Glutamic acid	13.62	19.30	8.87	19.03	17.7
Glycine	5.47	4.87	2.31	4.88	3.9
Alanine	6.51	7.57	5.85	7.45	5.7
Cysteine	0.35	0.25	0.54	0.25	0.8
Methionine	3.65	3.18	2.68	3.48	3.4
Valine	6.60	6.58	5.36	6.48	Trace
Isoleucine	3.75	5.58	5.05	4.58	4.4
Leucine	7.51	9.93	8.88	9.80	9.5
Tyrosine	2.94	2.47	2.48	2.50	3.4
Phenylalanine	3.97	3.37	Trace	3.18	3.2
Lysine	7.53	10.05	6.24	10.09	11.8
Histidine	9.15	3.38	2.66	3.20	1.4
Arginine	8.47	6.66	5.76	6.62	6.5
Tryptophan	1.46	1.19	0.88	1.19	1.3
Proline	1.23	Trace	Trace	1.90	Trace
T	99.50	100.00	62.70	99.99	94.1
E/T	38.77	43.61	46.79	42.42	39.6

a: from exhaustive hydrolysis; b: free amino acids from exhaustive hydrolysis; c: from partial hydrolysis; EFPH: enzymatic fish protein hydrolysate; T: total amino acids; E/T: Essential amino acids/total amino acids(%).

HUFA CONCENTRATES

The concentration of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in HUFA concentrates prepared by urea addition method increased with the amount of urea used in the process. In the concentrate obtained by using 2.5 times of urea, the content of EPA and DHA was up to 70.0% (EPA 30.7%, DHA 39.3%). In the molecular distillation method, the content of EPA and DHA in HUFA concentrates fraction obtained in the first stage was 48.1% and in the second stage, up to 70.1% (EPA 15.8%, DHA 54.3%). The analysis of iodine values indicated that the degree of unsaturated fatty acids in HUFA concentrate was high. The colour values showed that the products were yellow and transparent(Table 2).

Table 2. PUFA concentrates from anchovy fish oil.

Fatty acids	Raw Oil	Urea addition			Molecular distillation		
C14:0	7.1	0.8	0.7	0.9	15.5	/	/
C16:0	18.6	/	/	/	31.4	1.5	1.2
C16:1	6.9	8.2	6.3	0.8	12.1	0.7	0.5
C18:0	3.7	/	/	/	3.5	2.3	2.0
C18:1	12.3	14.6	3.8	0.8	12.0	5.9	6.0
C18:2	3.6	10.6	8.1	1.9	3.5	1.6	1.6
C18:3	1.7	4.8	3.8	1.5	1.5	1.6	1.0
C20:1	2.3	0.8	/	/	1.0	4.9	4.4
C20:4	1.1	2.0	3.0	3.2	0.5	1.2	1.6
C20:5	9.5	16.7	26.4	30.7	4.2	9.8	15.8
C22:1	/	/	/	/	0.3	10.6	10.4
C22:6	17.2	18.4	34.6	39.3	3.5	38.3	54.3
Other	16.0	23.3	13.3	20.9	11.0	21.6	1.2
EPA+DHA	26.7	35.1	61.0	70.0	7.7	48.1	70.1
Yield	100	25.2	22.1	19.0	53.3	45.3	20.5
Iodine value	120	160	280	320	86	220	320
Color L value	38.2	69.2	71.3	70.6	85.0	68.7	72.0
A value	8.9	8.6	9.3	9.5	14.1	10.1	9.4
B value	3.2	7.5	7.3	8.1	5.6	6.4	7.9

a, b, c: from process using 1.5,2.0,2.5 times of urea, respectively. A: saturated fatty acid fraction from the first stage; B: concentrated HUFA from the first stage; C: concentrated HUFA fraction from the second stage.

BIOLOGICAL EVALUATION OF EAPH

The nitrogen balance experiments indicated that the biologic value (BV), true digestibility (TD) and net protein utilization (NPU) were significantly higher for the EAPH-based feed (Table 3). Growth experiments showed that the growth rate and protein efficiency ratio (PER) of rats fed with EAPH-based feed was also higher than that of rats fed with milk protein-based feed. Supplement with EAPH gave a significant increase in the PER value of the wheat protein. The results from analysis of fresh organ weights showed no significantly different values among the tested rats. Histological examination of the livers, lungs, adrenals and kidneys revealed no differences between control and experiment groups.

Table 3. Nitrogen balance and growth experiments on rats fed with different feeds.

Nitrogen Balance Experiments	A	B	C	D	E
Feces nitrogen (g)	0.03	0.11	0.08	0.11	0.08
Urine nitrogen (g)	0.06	0.16	0.15	0.23	0.16
Nitrogen intake	Trace	0.66	0.69	046	0.52
Absorbed nitrogen (g)	Trace	0.58	0.64	0.38	0.47
Retained nitrogen (g)	Trace	0.48	0.55	0.21	0.37
True digestibility(TD)	87.88	92.75	82.26	90.38	
Biological value(BV)	82.76	85.94	55.26	78.72	
Net protein utilization(NPU)	72.73	79.71	45.65	71.15	
GrowthExperiments					
Weight gained	-31.32	103.38	106.27	25.46	70.52
Growth rate	-54.04	176.37	184.56	44.13	122.73
Diet intake(g)	347.71	339.65	335.68	386.78	358.62
Protein intake(g)	trace	34.30	33.90	37.40	31.60
Protein efficiency ratio(PER)	3.01	3.13	0.68	2.23	

A: nitrogen-free; B: milk powder protein; C: EAPH(from exhaustive hydrolysis);
D: wheat protein; E: wheat protein supplemented with EAPH.

DISCUSSION

In the exhaustive hydrolysis of anchovy protein, some factors such as different proteolytic enzymes, the hydrolysis temperature, the hydrolysis time and the amounts of enzyme addition should be carefully considered. To compare with specific sulfhydryl group proteolytic enzyme such as papain and bromelin, neutral proteinase No. 1398 has higher hydrolysis efficiency. High yield of soluble nitrogen, well balanced amino acid composition and the high contents of free amino acids. The hydrolysate had a strong bitter taste which was eliminated by using active carbon and beta-cyclodextrin. Bitterness of the fish hydrolysate comes from short peptides with hydrophobic groups such as Leu (Gly)-ASP-Lys. Different sizes of active carbon, the amount of its addition, beta-cyclodextrin addition, the combination of the two substances also affected debittering activities in fish hydrolysate preparation.

The final product had a faint marine taste and odour. In partial hydrolysis, the fish protein was not fully hydrolyzed and little bitter-taste peptides were less produced, but the yield of soluble nitrogen was much lower than that of exhaustive hydrolysis. The exhaustive hydrolysis processes from under utilized fish have been proven practicable in the pilot-plant production and large scale industrial production. In the nutritional evaluation experiments, all feeds had the same composition except for the amino acid composition. EAPH showed the high biological value (BV) as well as the PER value. It had a high nutritive value and was easily digested, adsorbed and utilized and can be used as a protein supplement in a variety of foods, especially cereal.

Anchovy oil contains high contents of HUFA and HUFA concentrates can be prepared by several methods. Among them the urea addition method and molecular distillation method have been used for industrial production in China. The molecular distillation is carried on under a high vacuum condition and by two to five stages, 70% or more of EPA and DHA concentrates can be prepared.

Anchovy fish is rich in the East Sea of China, the Yellow Sea, and other parts of the West Pacific. The resources have not been utilized and developed. The idea of the comprehensive utilization of under utilized fish such as anchovy has been pointed out. The preparation of four products are EAPH for use as a protein supplement; fish oil for animal feed additives and for HUPA production; HUFA concentrates with different contents of EPA and DHA and the residue from EAPH to make fish meal.

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