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Utilization of Fish Protein and Oil from the Anchovy *Engraulis japonicus*

C. WANG, C. XUE, Y. LIU, W. LOU and X. CHEN

Fisheries School
Ocean University of Qingdao
5, Yushan Road
Qingdao 266003, China

Abstract

Anchovy *Engraulis japonicus*, a small unutilized fish, is a rich resource of fish protein and oil. The utilization of anchovy protein and oil was studied. A non-bitter fish protein hydrolysate was prepared by exhaustive hydrolysis with proteolytic enzyme. The end product gained by spray dry was a protein-rich white powder with the appropriate ratio of essential amino acids. The results of a biological evaluation on rats indicated that the hydrolysate had a high nutritive value. The product may be used as a protein supplement. Another non-bitter fish protein hydrolysate was prepared by partial hydrolysis with proteolytic enzyme. The spray-dried product with a seafood flavor may be used as seafood flavoring. Polyunsaturated fatty acid concentrates were prepared from anchovy oil by using the urea addition and molecular distillation methods.

Introduction

Anchovy *Engraulis japonicus* is a small, unutilized fatty fish. It is a rich resource of fish protein with a well-balanced amino acid composition, and fish lipid high in polyunsaturated fatty acids (PUFA). It, however, falls easily into decay, and is difficult to process for human food.

The trend in the utilization of unutilized fishery resources is toward direct consumption as human food. Unutilized fish as well as fish processing wastes can be used as base material for food products and food supplements used in different nutrition programs (Yanez et al. 1976; Lalasidis et al. 1978; Mackie 1982). The usual method is hydrolysis by which enzymes are added to produce protein-rich food for human consumption. Lack of functional properties and a bitter taste have, however, inhibited the use of the hydrolysates as human food (Hevia and Olcott 1977; Lalasidis and Sjoberg 1978; Vega and Brennan 1988). Lipid mediators derived from omega-3 fatty acids have been suggested as beneficial in the prevention and/or treatment of major diseases affecting human health (Gordon and Ratliff 1992). The present experiments were undertaken to convert anchovy protein into protein products suitable for direct addition to food systems, and to convert anchovy oil into PUFA

concentrate for use in medicine and health foods. The comprehensive utilization process for anchovy protein and oil is also discussed.

Materials and Methods

Raw Material and Enzyme

E. japonicus, caught from the Yellow Sea in February 1990, was supplied by the Qingdao Marine Fisheries Co., China, and was frozen at -18°C until required. The analytical data of the raw material is as follows: 16.70% protein (N x 6.25), 10.82% fat, 1.31% carbohydrate, 1.40% ash and 69.75% water. The enzyme used, purchased from Wuxi Enzyme Products Factory, China, was neutral proteolytic enzyme from *Bacillus subtilis* (AS1.398).

Exhaustive Hydrolysis for EAPH

The processes of Mackie (1982) and Vega and Brennan (1988) were the bases for the preparation of enzymatic anchovy protein hydrolysates (EAPH). A homogenate was prepared from frozen anchovy by thawing, mincing and suspending (in 1 volume water) successively. The homogenate was hydrolyzed by 0.7% w/w of enzyme (based on the amount of protein of the raw material) at 50°C, pH 7.0 for 12 h. After the enzyme was inactivated, the solution was treated by adsorption method with 40% w/w (as above) active carbon and masking method with 2% w/w (as above) β-cyclodextrin successively to remove the bitterness (Lalasidis and Sjoberg 1978; Helbig et al. 1980). The clear aliquots of hydrolysate were analyzed. In the biological evaluation experiments, the EAPH used in the tested feeds was produced by spray-drying with a Wuxi QZ-5 spray drier (China) in a pilot-plant production.

Partial Hydrolysis for EAPH

The process of partial hydrolysis is similar to that of exhaustive hydrolysis. The anchovy homogenate was limited hydrolyzed with 0.1% w/w (as above) enzyme at 40°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 Lalasidis and Sjoberg (1978) and Helbig et al. (1980). The bitterness scores of hydrolysates were graded on a five-point scale (0 = no bitterness; 1 = weak bitter aftertaste; 2 = weak bitter taste; 3 = bitter; 4 = strong bitter taste; 5 = extremely bitter) by a panel of 10 trained members.

Biological Evaluations of EAPH

Biological evaluations were performed according to McLanghlan (1980) and Pellett and Young (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% (A<0.10%) protein; 10±0.36% fat; 5% mineral mixture; 2% vitamin mixture; 5% nitrogen-free cellulose; and up to 100% cornstarch. Feed and water were offered *ad libitum*. During the 4-d nitrogen balance experimental period, 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.

Preparation of PUFA Concentrate

Refined oil was obtained from crude fish oil, which was gained from exhaustive hydrolysis process, by being degummed, deacided, washed and dehydrated. PUFA concentrates were prepared by the urea addition and molecular distillation methods (Sumerwell 1957; Ackman et al. 1973). In the urea addition method, the amounts of urea used in three aliquots were 1.5, 2.0 and 2.5 volumes. In the molecular distillation method, the ethyl ester mixture was distilled in two stages on a SIBATA MS-300 molecular distillator (Japan).

Methods of Analysis

Nitrogen content was determined by Kjeldahl method. Lipid content was determined by extraction with chloroform-methanol 2:1 v/v. Ash content and iodine value were determined according to the AOAC method (AOAC 1970). Amino acid composition was determined by hydrolysis in 6 N HCl under vacuum at 110°C for 22 h, followed by ion-exchange chromatography on a Hitachi 835-50 amino acid analyzer (Japan). The free amino acid composition was determined in the same manner but without the HCl treatment. Color was tested on a Model-1001 DP colorimeter (China). The fatty acid content was determined on an HP5890 gas chromatograph (USA).

Results

EAPH from Exhaustive Hydrolysis

The degree of exhaustive hydrolysis was 68.66%. The hydrolysate gained by exhaustive hydrolysis had a strong bitter taste (bitterness score 4.5). Organoleptic stability could best be assured by absorbing the bitter peptides with ac-

tive carbon and masking with β-cyclodextrin. The end product was free from bitterness (bitterness score 0.5). The yield of soluble nitrogen was 89.80%. The spray-dried product from the pilot-plant production was a fine white powder with a pleasant odor containing 88.26% 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 EFPA used as a reference from Yanez et al. (1976). The product had a high content of free amino acids (62.70% of total amino acids) (Table 1). Synthetic milk-like beverages were prepared with the product, 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. EAPH may be used as a food ingredient or additive.

Table 1. Amino acid composition (mg•100 mg^{-1} 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: from exhaustive hydrolysis (free amino acids); c: from partial hydrolysis; EFPH: enzymatic fish protein hydrolysate, from Yanez et al. (1976). T, Total amino acids; E/T, Essential amino acids/Total amino acids (%).

EAPH from Partial Hydrolysis

The degree of partial hydrolysis was 31.86%. The hydrolysate gained by partial hydrolysis had no bitterness (bitterness score 0.5). The yield of soluble nitrogen was 41.17%. The spray-dried product was a powder with a seafood flavor containing 93.10% protein, 0.52% fat, 2.28% ash 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 (Yanez et al. 1976) (Table 1). It can be used as a seafood flavoring or a seafood additive.

Biological Evaluation of EAPH

Results from the nitrogen balance experiments indicated that the biological value (BV), true digestibility (TD) and net protein utilization (NPU) showed significantly higher value for the EAPH-based feed (Table 2). 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 wheat protein (Table 2). 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 2. Results from nitrogen balance and growth experiments on rats.

		A	B	C	D	E
Nitrogen balance experiments	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	0.46	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
Growth experiments	Weight gained (g)	-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.

PUFA Concentrate

The content of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in PUFA concentrates gained by the urea addition method increased with the amount of urea used in the process. In the concentrate obtained by using 2.5 volumes 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 PUFA concentrate 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 PUFA concentrate was high. The color values showed that the products were yellow and transparent (Table 3).

Discussion

In the present experiment, the exhaustive hydrolysis of anchovy protein gave a high yield of soluble nitrogen, a well balanced amino acid composition, and a high value of free amino acids. The hydrolysate had a strong bitter taste which was eliminated by using active carbon and β-cyclodextrin. The end product had a faint marine taste and odor. In partial hydrolysis, the protein was not fully hydrolyzed to avoid the release of bitter-tasting peptides, but the yield of soluble nitrogen was lower than that of exhaustive hydrolysis. The processes, especially the exhaustive hydrolysis process, which have been proven practicable in the pilot-plant production, can be used in largescale industrial production. In the nutritional evaluation experiments, all feeds had the same composition except for the amino acid composition. Results from the animal experiments indicate that EAPH with a high nutritive value was easily digested, absorbed and utilized; and that it had a supplementary effect on wheat protein. With these properties, EAPH can be used as a protein supplement in a variety of foods, especially cereal. The molecular distillation process is suggested for the production of PUFA concentrate which is not easily oxidized in a vacuum, and production cost is lower. More studies are needed to assess the pharmacological effects of PUFA concentrates on humans.

Table 3. Analytical results of PUFA concentrates (%).

		Raw oil	Urea addition			Molecular distillation		
			a	b	c	A	B	C
Fatty acids	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	23.1	13.3	20.9	11.0	21.6	1.2
EPA and DHA		26.7	35.1	61.0	70.0	7.7	48.1	70.1
Yield			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 processes using 1.5, 2.0, 2.5 urea, respectively. A, Saturated fatty acid fraction from first stage; B, Concentrated PUFA fraction from first stage; C, Concentrated PUFA fraction from second stage.

The reserve of anchovy is rich in the East Sea of China, the Yellow Sea, and other parts of the West Pacific. It is estimated that the anchovy catch in these areas can reach up to 500,000 tonnes•year^{-1}. This resource has, however, not been fully developed and utilized. The processes proposed in this paper for preparing EAPH and PUFA concentrate are practicable: anchovy protein can be processed into EAPH for use as a protein supplement; anchovy oil can be produced into PUFA concentrate for use in medicines and health foods; protein residue and fish bone can be used as raw materials for fish meal; and oil residue can be used as a fish oil product. In this way, the anchovy resource can be fully utilized (Fig. 1).

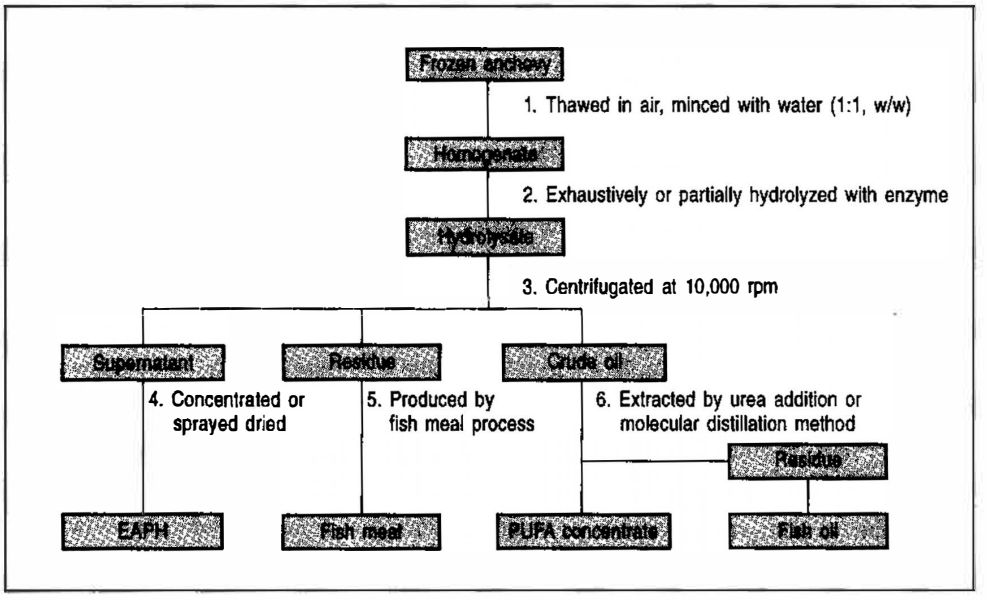

Fig. 1. Flow diagram of utilization of anchovy.

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