STUDIES ON THE PREPARATION OF FISH PROTEIN CONCENTRATE

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The paper deals with the method of preparation of an edible fish protein concentrate from cheap miscellaneous fish. The method consists in cooking the fish with 0.5% glacial acetic acid, and extracting batch-wise, using ethyl alcohol followed by an azeotropic mixture of hexane and alcohol (B. Pt. 58 68°C). The product is finally vacuum dried during which the residual solvent is also removed. The concentrate prepared by this method contains 85% protein of which 96% is pepsin digestible. The product is practically odourless and almost white in colour.

INTRODUCTION

Protein malnutrition as is being experienced all over the world has been attracting the attention of nutritionists and others since quite sometime and much effort has already been expended in this field. Production of inexpensive and at the same time nutritious food can do much to tackle this problem and free the millions out of this grave situation. In the opinion of experts fish is considered to be the ultimate answer to this burning problem mainly because of its abundance in nature, cheapness and high nutritive value. The main consideration is how to make fish which is highly perishable useful for the purpose. The importance this problem has attained is evident from the numerous publications on the subject and the interest shown by world organisations.

Of the annual fish landings in India, considerable amount is constituted by cheap variety of fishes of miscellaneous types, which find no ready response from consumers. In a country like ours, where the problem of protein malnutrition is really serious, conversion of the above fish to edible flour will be of much use. Work done in India on utilization of surplus fish has mainly been confined to production of fish meal. The main attempts made to prepare edible flour from fish consisted in the works of Pillai (1956 and 1957), Bhatia, Moorjani, Iyengar and Visweswaraiah (Bhatia et. al, 1955), Moorjani and Lahiri (Moorjani et. al, 1961), Moorjani, Bala-

krishnan Nair, Krishnaswamy and Lahiry (Moorjani et. al, 1962), Kadkol and Lahiry (Kadkol et. al, 1962) and Revankar, Khabade and Suryanarayana Rao (Revankar et. al, 1965).

The methods practised elsewhere based on solvent extration e. g. French Process, Viobin Process, Canadian Process etc. are not free from draw backs. In extraction by acetone, difficulty is experienced to remove traces of acetone even under reduced pressure. Moreover acetone is less efficient in lipid liberation and will extract substances other than lipids (Moorjani and Lahiry 1962). In Viobin process, which employs the principle of azeotropic extraction with ethylene dichloride, Morrison and Munro (Morrison et.al, 1965) showed that ethylene dichloride destroys cystein, histidine and interfere with the release of cystein, histidine and methionine by pancreatic digestion. Another important process employed for the preparation of edible fish flour is the Canadian process which employs isopropyl alcohol for the extraction; but the flour retains a solvent taint even after prolonged steam stripping under vacuum (Moorjani etal 1962). The process was later modified and extended to the preparation of edible fish protein concentrate from whole fish by the Bureau of Commercial Fisheries U. S. A. (1966). MIT-UNICEF process described by Allen (1963) employs hexane and alcohol for the extraction of fat.

The present paper describes a modification of the MIT-UNICEF process and establishes the advantages of using an azeotrope of hexane and alcohol for the extraction of fat.

Materials and methods

Fish for the experimental work were collected from the catches of trawlers operated off Cochin by the Institute and kept iced till used for preparation of Fish Protein Concentrate. The fish after washing in potable water were processed in four different ways.

(1) Flesh alone taken after removal of head, viscera, scales bones etc. from individual species.

(2) Discarding only head, viscera, and scales and retaining the bones from individual species.

(3) Employing a mixture of different trash fishes comprising mainly of jew fish (*Pseudosciaena Spp*) arana (*Saurida tumbil*), silver belly (*Leiognathus Spp.*) and malabar sole (*Cyanoglossus Spp.*) after dressing as in (2).

(4) Employing a mixture of different trash fishes comprising the same species as in (3), without dressing.

Analytical methods

The composition of fish flour as regards the moisture, fat, nitrogen, ash and pepsin digestibility were determined by the methods of AOAC (1960) and the available lysine was estimated by Carpenter's method (1960).

Preparation of the flour

The fish after mincing is suspended in an equal amount of water to which has been added 0.5% acetic acid and kept at 70—80°C. for 30 minutes under constant stirring. The slurry is filtered and pressed in a screw press so that a good amount of liquid is removed. The press cake will contain nearly 60—62% moisture. It is then dried in a tunnel dryer at a temperature range of 75—80°C. to various levels of moisture. The dry matter (moisture content below 10%) is coarsely ground in an ordinary milling machine and used for extraction of fat.

Extraction

The material is charged in to the extra-

ction drum having arrangements for stirring and refluxing. The solvent, azeotropic mixture of hexane and ethyl alcohol (33.2 mole per cent alcohol b. p. 58.68°C) is added in two parts by volume to one part by weight of material and kept at the boiling temperature of the solvent for 30 minutes, under constant stirring. The solvent is drained off and fresh solvent added in 1.5 parts by volume to one part by weight of original material and refluxed under constant stirring for 30 minutes. After draining off the solvent the process is repeated once again. After final draining the adhering solvent is distilled off and the last traces removed by steam stripping under reduced pressure. The dried matter is pulverized to fine mesh size using a milling machine.

In the course of the experiments data were also gathered on the effect of various concentrations of acid added to the minced fish during the pre-cook stage on the final product, on the relative rates of extraction of fat by different solvent systems and on the effect of temperature and mode of extraction on rate of defatting and deodourization by the azeotropic mixture of hexane and alcohol.

Results and discussion

In all the trials on acidification, acetic acid was used although polyphosphoric acid (Power 1962; and Guttmann et. al, 1957) and hydrochloric acid (Pariser et. al, 1959) have been mentioned by other workers. The effect of different concentrations upto 1.5% on the minced fish is given in Table I. It is seen that acidification beyond a concentration of 0.5% (W/V) produces an adverse effect on the digestibility of the protein. Although the odour of the product is considerably reduced in the acid treated press cake, which is in agreement with the observations of Guttmann and Vandenheuvel (1957) the product acquires a deeper colour with increasing concentration of acid beyond 0.5%.

In their efficiency for removal of fat, all the solvents except ethyl alcohol are more or less equally effective (Table II) and all of them bring down the fat level below 0.75%, which is in conformity with the standards prescribed by FAO (1962). Acetone and isopropyl alcohol extraction is found to result in a slightly higher fat content compared to other solvents used in the experiments. Hexane alone is not effective in deodourizing the flour as it is a poor extractant for bound lipid fraction which comprises largely of phospholipids (Moorjani et. al. 1962), although it is found to be the most efficient single solvent for fat extraction and alcohol alone is insufficient for the removal of fat but is the most efficient solvent for liberating and extracting bound lipids (Moorjani et. al. 1962). The azeotropic mixture however, is found to satisfy both needs, almost complete removal of odour and bringing down the fat content to around 0.3%.

The ratio of the solvent to the dry material, extent of extraction of fat at 29°C., 45°C. and at the boiling point at intervals of 30 minutes during continuous and batch extraction are given in Table III. The extraction rate increases with increase in temperature and period, the extraction being more in batch process by changing the solvent after every 30 minutes, the residual fat content after 1½ hours extraction being only around 0.25% whereas 3.68% fat is retained in continuous extraction for the same period.

Azeotropic mixture of the two solvents - hexane and alcohol - has been observed to have many advantages. It boils at a temperature lower than the boiling point of either hexane or alcohol.

It is found to be miscible only at the boiling point and the mixture after extraction and draining can be distilled off completely free from fat, which on cooling separates into two layers, making the further purification of alcohol easy. Alcohol which will contain the odouriferous matter can be purified either by refluxing with acid and alkali respectively followed by distillation or by treatment with activated carbon.

In order to find out the possibility of subjecting the press cake after acid treatment for direct extraction with azeotropic mixture at different moisture levels of press cake was studied and the data are presented in Table IV. It is evident from the table that there is no difference in the extent of extractability at 67% and 20% moisture, difference becoming significant only at 7% moisture level. This indicates that partial drying has no special advantage. This led to a possible prediction that pess cake can be extracted even before drying, which was confirmed in actual trials carried out (Table IV). By avoiding drying of the press cake the colour of the finished product also was found to be improved, which is considered to be a definite advantage apart from the savings in labour and time. This procedure might also probably remove the possibility of secondary oxidation products of fat and formation of polymerisation products which are known to be toxic, (Privett 1959, Venolia et. al, 1958, Kaunitz et. al, 1956) the former of which can destroy vitamins and in the case of the latter it has been reported that formation of co-polymers with protein may effect reduction in the content of available lysine (Lea et. al, 1960). The data presented in the table also show that alcohol alone does not efficiently remove fat in 30 minutes extraction. However, when this was followed by an extraction with zeotropic mixture for 1½ hours the effect is very significant, the fat content being brought down to 0.53% as against a fat content of 1.01% in a 2 hour extraction with azeotropic mixture alone.

From the data presented in Table VI it is evident that apart from the difference in the protein and ash contents which could be accounted for by the presence of bones in the sample, there is no significant difference between samples prepared from individual type of fish used in the form of flesh alone or from dressed fish or from whole fish regarding digestibility and available lysine content. All the samples were free from any objectionable odour and taste but the colour of the latter was found to be deeper yellow.

Summary

Possibility of preparation of edible fish flour from the commercially available and cheap varieties of fish is dicussed. Effect of different solvents on the extraction of fat from press cake after drying and also modification of MIT-UNICEF method using azeotropic extraction of fat has been worked out. Possibility of using wet press cake directly for extraction by modification of this using extraction by alcohol prior to extraction by azeotropic mixture has been discussed. A comparison has been made of the fish flour prepared from fish muscle alone, dressed fish (of the same species), mixed dressed fish and from whole fish.

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TABLE I EFFECT OF ACID ON THE QUALITY OF FLOUR.

Analytical Characteristics %	Percentage of acid used			
	0.00%	0.5%	1%	1.5%
Moisture	7.81	7.75	6.74	6.56
Ash	21.87	21.13	20.25	17.57
Fat	9.14	9.52	10.77	11.63
Protein	64.44	62.94	61.37	59.87
Pepsin Digestibility	96.08	96 12	93.80	91.20

TABLE II EFFECT OF DIFFERENT SOLVENTS ON EXTRACTABILITY OF FAT.*

Time in min.	Wet press cake		Dried powder		
	Isoproponal	Acetone	Alcohol	Hexane	Hex-Alcohol
30	7.44	8.53	8.63	7.51	5.75
60	4.24	4.14	6.65	4.12	1.49
90	2.89	2.84	5.02	0.81	0.43
120	0.44	0.71	4.82	0.12	0.30

* The figures represent the residual fat (%) in the finished flour.

TABLE III EFFECT OF TEMPERATURE AND MODE OF EXTRACTION OF FAT CONTENT

Solvent Ratio	Mode of Extraction	Time in min	Residual fat content at intervals with temperature (%)			Recovery of solvent with temperature (%)		
			29°C	45°C	58 68 °C (Boiling point)	29°C	45°C	58.7°C
1:2	Continuous	30	7.31	6.37	5.06			
		60	4.62	5.77	3.95			
		90	4.41	4.75	3.68	92.8	91.1	93
		120	4.16	4.20	3.22			
1:2 in the first extraction and 1:1.5 in subsequent extraction	Batch-wise	30	5.75	3.96	2.89			
		60	1.49	0.67	0.40			
		90	0.43	0.28	0.26	93.7	92	91.8
		120	0.30	0.16	0.08			

TABLE IV EFFECT OF MOISTURE CONTENT OF PRESS CAKE ON FAT EXTRACTION

(Temp. 58.68°C. Batchwise, (1.2 in the first extraction, 1:1.5 in subsequent)

Moisture %	Time in min.	Residual fat content (%)	Solvent recovery %
67	30	4.74	86.4
	60	4.54	
	90	1.09	
	120	1.01	
47	30	4.63	90
	60	4.17	
	90	1.56	
	120	0.83	
20	30	4.59	90
	60	4.09	
	90	1.22	
	120	0.41	
7	30	2.89	91
	60	0.67	
	90	0.26	
	120	0.08	

TABLE V COMPARISON OF EXTRACTION BY VARIOUS SOLVENTS ON WET PRESS CAKE

Time in min.	Percentage of residual fat (dry basis)		
	Alcohol Hex.	Alcohol Azeotrop.	Alcohol followed by Azeotrop
30	8.63	4.74	2.08
60	6.65	3.54	1.00
90	5.02	1.09	0.54

TABLE VI ANALYTICAL CHARACTERISTICS OF THE DIFEERENT SAMPLES

Description of the material	Moisture %	Ash %	Fat residual %	Protein %	Pepsin digesti-bility %	Available lysine gm/16gm of nitrogen
Flour from the muscle alone	7.16	6.48	0.26	84.87	96.6	8.73
Flour from dressed fish	7.15	21.03	0.38	72.64	94.1	7.81
Flour from dressed mis-cellaneous fish	7.50	18.11	0.24	73.17	94.5	7.84
Flour from mix. fish muscle alone wet extraction	6.94	4.78	0.54	87.50	95.9	8.12
Wet press cake misc. dressed fish	6.40	14.36	0.59	79.69	93.8	7.71
Flour from whole fish wet extraction	7.22	23.11	0.51	68.24	93.4	7.51

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