Technology Section

Pakistan J. Sci. Ind. Res., Vol. 17, Nos. 4-5, August-October 1974

EFFECT OF HEAT ON THE IN VITRO DIGESTIBILITY OF FISH PROTEIN

ABDUS-SALAM SHEIKH and F.H. SHAH

PCSIR Laboratories, Lahore 16

(Received May 7, 1974; revised July 10, 1974)

Abstract. The enzymic digestion of raw as well as heated fish protein prepared from fatty and non-fatty samples was carried out with pepsin, trypsin and the enzymes present in ox-pancreas. The effect of lipid oxidation products, produced during heating of fish protein, on the proteolytic enzymes was determined by estimating free amino acids of whole and defatted samples after 3, 6, 9 and 24 hr. The digestibility of fish protein varied with the variety of fish, nature of enzyme and the type of heat treatment. Extraction of lipids with chloroform–methanol raised the digestibility of all the samples.

Despite the fact that fish is a high calorie diet and is comparatively cheaper, it is being neglected because of its poor shelf-life. To increase the shelf-life, it is necessary to reduce the moisture and lipid contents of the product. Removal of water by heating increases the shelf-life but affects the quality of the product.[1–2] This has been attributed to denaturation of proteins and destruction of heat-labile amino acids. Moreover, oxidation products of the lipids present in fish meat have been reported to be toxic to proteolytic enzymes.[3]

The present work was carried out to study the effect of lipid-oxidation products, produced during heating of fatty (*Wallogo attu*, i.e. malhi) and non-fatty (*Labeo rohita*, i.e. rahu) fish, on the proteolytic enzymes and to determine the extent of free amino acids.

Experimental

Preparation of Fish Protein. Fresh malhi or rahu fish was minced after removal of fins, mid-ribs, head and viscera and stored at–10°C in polythene bags.

Drying of Fish Samples. About 500 g lots of the homogenised product were dried at 60, 80 or 100°C as well as roller-dried. Samples to be dried were evenly spread to form 1-cm thick layer in steel trays. The trays were placed in a forced draught oven and the protein layer was turned after every 1/2 hr. This was continued for 3 hr. Dried samples were ground to 16 mesh and stored at—10°C.

Removal of Lipids. Fifty g lots of the protein samples (on dry matter basis) were defatted by the method of Folch *et al.*[4] and all the lipids present in the chloroform layer were estimated by evaporating it at 55°C under reduced pressure. The defatted protein was heated at 40°C under vacuum to remove the solvents present in it.

Determination of Dry Matter and Nitrogen Content. Dry matter was determined by heating the homogenised protein at 100–105°C in an oven for 8-12 hr.[5]

Nitrogen contents were determined by a microkjeldahl procedure using copper–selenium catalyst.[6] Proteinous nitrogen (PN) and nonproteinous nitrogen (NPN) were estimated as trichloroacetic-acid (TCA)-insoluble and TCA-soluble N respectively. The protein contents were calculated as protein $N \times 6.25$.[7]

Determination of Digestibility. Trypsin and pepsin (E. Merck) and the enzymes present in the ox-pancreas (trypsin, chymotrypsin and carboxy peptidase) were used for the determination of digestibility of the protein samples. Samples containing 25 mg proteinous nitrogen were suspended in 20 ml buffer of optimum pH. These samples were then incubated at optimum temperature after adding 1 ml either pepsin (2.5%), trypsin (2.5%) or the aqueous extract of ox-pancreas.[8]

Discussion

The digestibility of samples was determined by the rate of conversion of proteinous nitrogen (PN) into nonproteinous nitrogen (NPN). The composition of fish meals is given in Table 1.

Malhi showed minimum peptic digestibility and 18% protein present in the fresh samples was hydrolysed in 24 hr (Table 2). Drying at 60°C slightly improved the digestibility to 20%. Drying at 80°C adversely affected the digestibility of malhi only to a small extent, however, drying at 100°C and roller-drying lowered the digestibility by 4 and 3% respectively. The tryptic digestibility was found to be comparatively higher for all samples of malhi (Table 2). The digestibility was adversely affected by heating to various temperatures and on roller-drying. Again the loss in the digestibility was maximum in case of samples dried at 100°C. Pancreatic extract showed maximum rate of digestion and 61% proteins present in malhi were digested in only 9 hr (Table 2). Heating at 60 and 80°C slightly raised the pancreatic digestibility of malhi, whereas samples dried at 100°C and those which

TABLE 1. COMPOSITION OF FISH MEAL.

Fish sample	Treatment	Time drying (hr)	Dry matter (%)	Proteins* (% PN×6.25)	Lipids* (%)
(a) Malhi (whole)	Fresh	—	22.31	68.66	8.01
	Heated 60°C	3	88.23	71.31	
	Heated 80°C	3	95.81	71.81	
	Heated 100°C	3	97.59	73.75	
	Roller-dried	—	91.39	76.50	2.37
(b) Malhi (defatted)	Fresh	—	89.08	99.56	
	Heated 60°C	3	92.21	90.25	
	Heated 80°C	3	93.33	94.13	
	Heated 100°C	3	93.79	93.31	
	Roller-dried	—	91.37	94.63	
(c) Rahu (whole)	Fresh	—	18.61	66.06	3.54
	Heated 60°C	3	88.96	82.69	
	Heated 80°C	3	98.30	83.69	
	Heated 100°C	3	99.25	86.38	
	Roller-dried	—	91.65	82.69	1.46
(d) Rahu (defatted)	Fresh	—	88.72	96.63	
	Heated 60°C	3	90.35	85.56	
	Heated 80°C	3	94.65	89.38	
	Heated 100°C	3	94.29	92.88	
	Roller-dried	—	92.75	89.44	

*Calculated on dry matter basis.

TABLE 2. DIGESTIBILITY OF MALHI.

Enzyme	Time (hr)	Digestibility (%)		Digestibility dried at 60°C(%)		Digestibility dried at 80°C (%)		Digestibility dried at 100°C(%)		Digestibility roller-dried(%)	
		Whole	Defatted	Whole	Defatted	Whole	Defatted	Whole	Defatted	Whole	Defatted
(a) Pepsin (25 mg)	3	5.9	7.3	12.5	9.1	4.6	8.7	6.0	8.7	4.6	6.3
	6	11.3	11.5	14.0	14.9	11.0	10.2	6.1	11.3	8.3	8.9
	9	14.5	14.5	14.5	15.5	11.3	13.3	9.9	14.0	10.9	13.2
	24	17.9	27.6	20.1	25.9	16.5	18.4	14.2	19.7	15.0	16.9
(b) Trypsin (25 mg)	3	21.5	26.3	28.0	26.9	23.6	22.7	21.0	22.1	24.8	28.9
	6	32.2	33.6	33.5	32.2	28.4	25.6	26.0	24.8	27.2	34.4
	9	40.0	46.4	35.9	34.7	33.1	28.6	31.4	31.7	33.8	38.6
	24	39.9	47.8	29.7	39.3	32.6	38.0	31.2	39.5	32.9	38.5
(c) Pancreatic extract (1 ml)	3	33.4	66.1	56.8	49.0	54.3	28.7	25.1	30.4	42.7	39.3
	6	60.6	75.4	63.7	66.8	64.3	41.3	40.9	43.5	44.1	51.8
	9	60.9	74.2	63.4	66.9	66.4	59.8	46.7	46.8	55.5	59.1
	24	38.4	72.7	33.8	68.2	35.0	70.0	37.0	59.1	39.1	64.5

TABLE 3. DIGESTIBILITY OF RAHU.

Enzyme	Time (hr)	Digestibility (%)		Digestibility dried at 60°C(%)		Digestibility dried at 80°C (%)		Digestibility dried at 100°C(%)		Digestibility roller-dried(%)	
		Whole	Defatted	Whole	Defatted	Whole	Defatted	Whole	Defatted	Whole	Defatted
(a) Pepsin (25 mg)	3	9.8	6.6	7.1	9.9	8.0	10.8	3.9	9.4	3.0	9.2
	6	14.6	14.0	8.5	13.5	9.1	12.2	6.6	11.3	4.9	14.9
	9	16.9	19.7	13.3	16.2	11.5	14.7	8.4	13.3	8.6	15.8
	24	19.6	30.2	19.9	28.8	12.9	26.9	12.0	16.3	16.6	21.4
(b) Trypsin (25 mg)	3	20.9	32.9	13.8	27.3	14.3	17.7	7.8	14.1	17.4	37.0
	6	28.5	38.2	18.3	32.1	17.9	22.2	11.6	21.1	20.6	41.5
	9	33.5	43.7	20.8	37.7	22.8	33.9	12.7	25.8	24.3	46.6
	24	28.2	59.4	15.9	48.8	20.1	46.6	9.4	28.7	26.8	52.4
(c) Pancreatic extract (1 ml)	3	14.1	72.6	32.3	59.7	22.9	50.4	4.2	31.5	38.5	59.8
	6	39.0	74.2	59.3	71.1	30.1	58.5	17.2	39.4	51.4	66.3
	9	56.7	77.6	64.3	74.4	48.0	65.0	23.0	42.2	62.9	68.3
	24	56.3	75.8	64.0	78.3	44.6	68.6	22.9	50.0	64.2	74.9

were roller-dried lost their digestibility by 14 and 5% respectively.

Twenty per cent proteins present in the fresh rahu sample were hydrolysed after 24 hr incubation with pepsin (Table 3). Drying at 60°C also slightly improved the digestibility of rahu protein as in case of malhi. Drying at 80 and 100°C lowered the digestibility by 7 and 8% respectively. However, the loss on roller-drying was only 3%. The tryptic digestibility of rahu was found to be comparatively higher and 34% proteins were digested in 9 hr (Table 3). Rahu showed a marked decrease in the tryptic digestibility of heating to various temperatures. However, the loss on roller-drying was minimum. Pancreatic extract showed maximum rate of digestion and 57% proteins present in rahu were digested in 9 hr (Table 3). Drying at 80 and 100°C adversely affected the digestibility, however, digestibility was raised at 60°C and in roller-drying. The difference in the digestibility of malhi and rahu fish proteins was understandable due to the marked difference in their lipid contents. Moreover, the difference in digestibility of fish proteins by these enzymes was expected due to the specificity of the enzymes. Pepsin attacks peptide bonds at the amino side of tyrosine or phenyl alanine; trypsin attacks at the carboxyl side of the peptide bonds containing lysine or arginine and chymotrypsin attacks peptide bonds at the carboxyl side of tyrosine or phenylalanine.[9] Carboxypeptidase attacks many peptides that contain different terminal amino acids splitting off the amino acid at the carboxyl end of the chain.[9]

The digestibility of all these samples was adversely affected by the denaturation of proteins, production of protein complexes with lipids[10] and carbohydrates[11] and the presence of lipids and their oxidation products. However, the simultaneous production of simple peptides preferably attackable by a particular enzyme tend to increase the digestibility. Overall digestibility was the resultant of these opposing factors. Heating of both of these varieties at 60°C produced simple peptides easily attackable by pepsin and enzymes present in the pancreatic extract. So, on heat treatment at 60°C, the digestibility of these varieties increased with pepsin or pancreatic extract but decreased when trypsin was used.

Fish samples containing lipids showed a decrease in NPN beyond 9 hr of incubation with trypsin. This has been attributed to oxypolymerization of unsaturated lipids in the presence of proteins. Amino acids then copolymerize with the lipids or become occluded in oxypolymerized lipids.[12] Venolia and Tappel observed that this process is favoured at a pH towards alkalinity.[12] Hence the decrease in NPN was only observed in cases where proteolysis was carried out at pH 7.0. The decrease in the amount of NPN beyond 9 hr of incubation was more conspicuous in case of malhi as it had higher lipid content. This was expected as the decrease would naturally depend on the amount and the nature of lipids and amino acids present. Further, no such decrease in NPN was observed in case of defatted samples. This suggested that decrease in NPN was due to lipids and their oxypolymerized products.

Extraction of lipids and their oxidation products with chloroform–methanol raised the digestibility of all the samples. Again the extent of increase in the digestibility varied. The increase was maximum in case of fresh samples as these proteins had no heat treatment and were not denatured. Polar solvents, chloroform–methanol, extracted lipids and their oxypolymers and thus increased the digestibility of fish proteins by removing these components. But, at the same time, simple peptides produced during the heat treatment, which could have been easily attacked by the enzyme, were removed.

The digestibility pattern of malhi and rahu with various enzymes was different throughout the investigations. This was expected because several workers have shown a marked variation in the nutritional quality of proteins prepared from different samples of fish.[13–14]

In view of the above discussion it may be concluded that the presence of lipids and their oxidation products adversely affect the *in vitro* digestibility by trypsin, pepsin and the enzymes present in the pancreatic extract. This effect depends upon the nature and amount of lipids, the temperature and manner of heat treatment, the nature of the protein present in fish and the enzyme used. The effect was most drastic at 100°C. Heating at 60°C had practically no adverse effect on *in vitro* digestibility of fish protein. The loss due to denaturation of proteins and the presence of lipids and their oxidation products may well be compensated by the simultaneous production of simple peptides. Defatting not only improved the keeping quality of fish but also improved the digestibility.

References

1. K.J. Carpenter, B.E. March, C.K. Milner and R.C. Campbell, Brit. J. Nutr., **17,** 309 (1963).
2. E.L. Miller, K.J. Carpenter and C.K. Milner, Brit. J. Nutr., **19,** 547 (1965).
3. E.D. Wills, Biochem. Pharmacol., **7,** 7 (1961).
4. J. Folch, M. Lees and G.H.S. Stanley, J. Biol. Chem., **226,** 497 (1957).
5. M.B. Jacobs, *The Chemical Analysis of Foods and Food Products* (Von Nostrand, New York, 1942), second edition, p. 21.
6. R. Markham, Biochem. J., **36,** 790 (1942).
7. J.R. Nicholls, *Aids to Analysis of Food and Drugs* (Bailliere, Tindall and Cox, London, 1952), p.217
8. A. Salam and F.H. Shah, Pakistan J. Sci. Ind. Res., **10,** 181 (1967).
9. M.E. Rafelson Jr. and S.B. Binkley, *Basic Biochemistry* (MacMillan, New York, 1965), first edition, p. 115
10. K.A. Narayan and F.A. Kummerow, J. Am. Oil Chem. Soc., **35,** 52 (1958).
11. K.J. Carpenter, C.B. Morgan, C.H. Lea and L.J. Parr, Brit. J. Nutr., **16,** 451 (1962).
12. A.W. Venolia and A.L. Tappel, J. Am. Oil Chem. Soc., **35,** 135 (1958).
13. A. B. Morrison and J. A. Campbell, Can. J. Biochem. Physiol., **38,** 467 (1960).
14. B. Sure, J. Nutr., **61,** 547 (1957).