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Biochemical and Nutritional Changes in Fish Proteins during Drying

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Abstract: Biochemical and nutritional changes in the muscle proteins of a lean marine fish *Nempiterus japonicus* during drying at 50, 60 and 70°C were investigated. Solubility of proteins in water, 0·6 M NaCl, 1·5 M urea, 8 M urea and 10 g litre^{-1} sodium dodecyl sulphate (SDS) decreased as drying progressed at all three temperatures; most of the decrease occurring in the initial 4 h of drying. SDS–polyacrylamide gel electrophoresis of 1·5 M urea, 8 M urea and SDS extracts showed that higher molecular weight (MW) protein fractions were more sensitive to drying and disappeared much earlier from electropherograms than the lower MW protein fractions. Residual solubility of proteins near the pH range of 4–6 was found to increase during drying, but solubility at acid and alkaline pH was adversely affected. Decrease of solubility by drying was more affected at acid pH, especially at higher temperatures than at alkaline pH. Sulphydryl groups registered a regular and sharp decrease with drying except at 50°C, where initially an increase was observed. Apart from disulphide and hydrophobic bonds, free amino groups also appear to be involved in denaturation reactions during drying. Pepsin digestibility of fish muscle decreased slightly during drying but a clear relationship with drying temperature was not evident. Highly significant differences in proteins between protein efficiency ratio (PER), net protein utilisation (NPU) and biological value were observed between the drying temperatures. The PER and NPU of fish dried at 60°C were significantly higher than those dried at 50 or 70°C.

Key words: fish drying, protein changes

INTRODUCTION

Considerable quantities of dried fish are consumed in developing countries while in advanced countries fish meal manufacture is a major industry. In both these processes fish protein is subjected to heating and dehydration, though the actual conditions of processing vary widely. While the biochemical and nutritional changes occurring in fish muscle proteins during heating at high temperature (100°C and higher) have been extensively studied (Bjarnarson and Carpenter 1970; Howgate and Ahmed 1972; Opstevdt *et al* 1984; Opstevdt 1988), similar changes taking place in fish proteins during drying and dehydration at much lower temperatures of 40–60°C have not received much attention. Drying fish for human consumption is usually done at these lower temperatures, except when smoked. Recent studies have shown that deteriorative changes in proteins occur also during the storage of freeze-dried or sun-dried fish, lipid oxidation products playing an important part in the latter (Lee *et al* 1986; Smith and Hole 1991).

Heating of fish brings about complex changes in muscle proteins like denaturation involving their molecular aggregation, formation of disulphide (S–S) linkages and the reaction of e–NH_2 groups of lysine with lipid oxidation products/amide groups. All these changes have either been predicted or shown to occur by various workers (Bjarnarson and Carpenter 1970; Gardener 1979; Ledward 1979; Opstevdt *et al* 1984; Kunimoto *et al* 1985; Synowiecki and Shahidi 1991). Whether the

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course of such changes in proteins is altered by the additional influence of dehydration is not fully known. In this paper we attempt to monitor the biochemical and nutritional changes in proteins of a lean fish, Japanese threadfin bream (*Nemipterus japonicus*) during drying at three temperatures: 50, 60 and 70°C.

MATERIALS AND METHODS

Japanese threadfin bream (*Nemipterus japonicus*) was caught by demersal trawling at 13°N 73°E during January 1992. The fish were washed and block frozen immediately at −40°C in an on-board blast freezer, packed in 200 gauge high-density polythene sacks and stored at −20°C till use. Fishes (12–18 cm) thawed overnight at 4°C, were dressed by removal of fins, scales and viscera and were split open from the ventral side for drying. After washing and draining, the fish were dried at 50, 60 or 70°C in a cross flow dryer on trays lined with 100 gauge low-density polyethylene film (tray loading 6·57 kg m^{-2}, air velocity 54 m min^{-1}/3·24 km h^{-1}). During drying, the fishes were turned over once at 10 h to expose both sides for drying. Samples for analysis were drawn at 0, 2, 4, 6, 8, 12 and 24 h of drying; minced or powdered, and stored in 200 gauge polypropylene pouches at −20°C pending analysis.

The samples collected at various stages of drying were analysed for moisture (AOAC 1975). Total sulphydryl groups in the samples were determined using Ellman's reagent (Sedlak and Lindsay 1968). Solubility of proteins in water, 0·6 M NaCl, 1·5 M urea, 8 M urea and 10 g $litre^{-1}$ sodium dodecyl sulphate (SDS) were determined by homogenising the sample in 20 volumes of the solvent at maximum speed in a Silverson omnimixer for 30 s. After 30 min at 0°C the samples were filtered through Whatman no 42 paper and protein in the filtrate was determined with the Lowry Folin–phenol reagent (Scopes 1982) against appropriate blanks. Bovine serum albumin was used as the standard. Filtrate from water, 1·5 M urea and 10 g $litre^{-1}$ SDS homogenates were taken for characterization by SDS–polyacrylamide gel electrophoresis (SDS–PAGE), performed according to Laemmli (1970) with minor modifications. Protein bands stained by Coomassie brilliant blue were scanned at 545 nm using a gel scanner attachment to a Hitachi double-beam spectrophotometer.

Protein solubility at different pH was determined by suspending 1 g of sample in 80 ml water and adjusting the pH with 0·2 M HCl/NaOH while stirring. After adjustment of pH, indicated by a constant pH for 2 min, the volumes were made up to 100 ml with water and centrifuged after 30 min (20 000 × *g* for 15 min). Protein in the supernate was determined as before. For in-vitro digestibility studies the sample was homogenised with 24 volumes of 0·3 M HCl and after temperature equilibration to 37°C, six volumes of a pepsi solution (1·51 units ml^{-1} min^{-1}, assayed as per Ry (1970) were added and incubated for 1 h with inte mittent shaking. Following termination of the hydr lysis with trichloroacetic acid (final concentratio 50 g $litre^{-1}$) the sample was kept in a boiling wat bath for 2 min and TCA soluble nitrogen was dete mined by the semi-micro Kjeldahl procedure (AAC 1976). Controls with enzyme and substrate alone wer also run.

Nutritional studies were done with 24 h drie samples only using albino rats. Dried fish samples wer powdered and incorporated in the diet at 100 g kg^{-} protein (TN × 6·25) levels. Fat, minerals, vitamins, fibr etc, were adjusted as recommended (Oser 1976). Si 3-week-old male albino rats (Wistar strain) each weigh ing *c* 30 g were housed individually in metabolic cage and were given the test feed and water *ad libidun* Protein efficiency ratio (PER), net protein utilizatio (NPU) and biological value (BV) were determined a per Oser (1976) and the results were analysed by *F* an *t* tests (Snedecor and Cochran 1961).

RESULTS AND DISCUSSION

The drying curves of the fish at the three temperature are given in Fig 1. Drying curves were similar for all th three temperatures for the first 2 h after which dryin was faster at 70°C. Drying rates at 50 and 60°C wer similar until 8 h, beyond which the fish dried mor rapidly at 60°C. These drying curves are similar t those reported earlier (Myklestad 1973).

The changes in sulphydryl (–SH) groups of the pro teins during drying are presented in Table 1. Except i

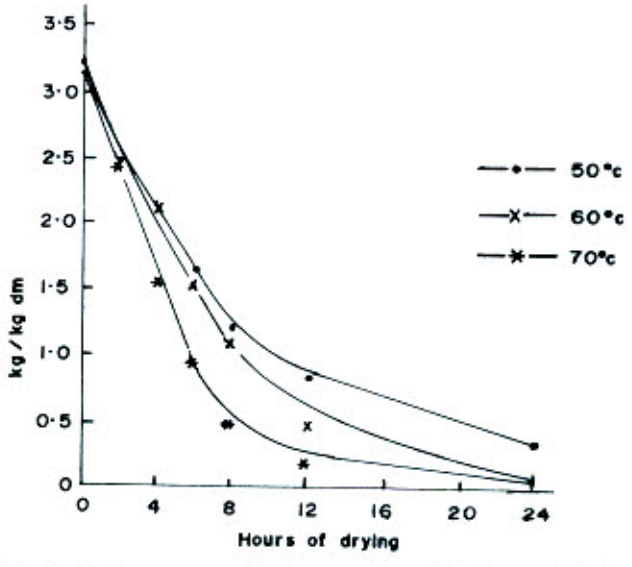

Fig 1. Drying curves of Japanese threadfin bream dried at various temperatures in a cross flow dryer.

TABLE 1
Changes in total sulphydryl groups of fish muscle during drying at different temperatures

Duration of drying (h)	*Total sulphydryl groups at different drying temperatures (as μmols g^{-1} dry matter^{-1})*		
	50°C	*60°C*	*70°C*
0	27·30	27·30	27·30
2	28·20	27·76	13·70
4	29·40	25·45	10·25
6	24·28	17·26	10·30
8	24·28	12·69	7·03
12	20·26	11·01	5·19
24	13·62	4·76	3·50

the case of 50°C, where a small increase was noted initially, –SH groups showed a regular decline with drying and reached minimal values by the end of the drying period. The rate of decrease was maximum in the first 2–4 h, and also proportional to the temperature of drying. The final –SH contents were also inversely proportional to the drying temperatures. The initial increase in –SH levels at 50°C can be attributed to the unfolding of protein chains exposing the 'buried' –SH groups which subsequently become oxidised. Such increases have been noted in solutions of carp actomyosin heated at 40, 60 and 80°C (Itoh *et al* 1979) and in mackerel and pollock muscle heated at 50°C (Opstevdt *et al* 1984). The initial rise in –SH groups is perhaps masked by their immediate oxidation at the higher temperatures. Freeze-drying and cooking do not appear to adversely affect the –SH content of fish except when heated at high temperatures of over 100°C, but drying in a stream of hot air as in this experiment, appears to result in their rapid loss as well as a very low level of –SH groups in the final dried product.

Changes in the solubility of proteins in various solvents during drying at different temperatures are shown in Figs 2, 3 and 4. Solubility in all the solvents decreased with the progress of drying. In general, maximum protein solubility of both fresh and dried fish was in 10 g litre^{-1} SDS, followed by 8 M urea, 1·5 M urea, 0·6 M NaCl and water, in that order. The rates of decrease in solubility in 1·5 M urea, 8 M urea and SDS were generally higher in the initial period (0–4 h) than the corresponding rates in the other two solvents. Most of the loss in solubility however, occurred in the initial period of drying for all the solvents. From the lower solubilisation of denatured/dried proteins by water, 0.6 M NaCl and 1·5 M urea it would appear that hydrogen and ionic bonding do not contribute as much towards denaturation by drying as do hydrophobic bonds which appear to play a major role. However, the decreased solubility of proteins, even in 8 M urea and SDS as the drying progressed, suggest the formation of more stable bonds than hydrophobic, ionic or hydrogen in protein denaturation. Formation of covalent disulphide linkages (Table 1) and the reaction of e–NH_2 groups of lysine with lipid oxidation products or amide groups (Bjarnarson and Carpenter 1970; Kunimoto *et al* 1985; Smith and Hole 1991) could be implicated in these denaturation processes. The effect of increasing the drying temperatures on solubility was not very clearly evident and could be seen to a limited extent in the decreasing solubility of 24 h dried fish in 0·6 M NaCl and SDS.

Examination of the electrophoretic patterns by SDS–PAGE, of the proteins solubilised by water, 1·5 M urea and 10 g litre^{-1} SDS revealed that various protein fractions differed greatly in their sensitivity towards denaturation by drying (Figs 5–7). With the increase in drying temperature, protein bands disappeared earlier. In general, higher molecular weight (HMW) protein fractions were found to be more sensitive and these

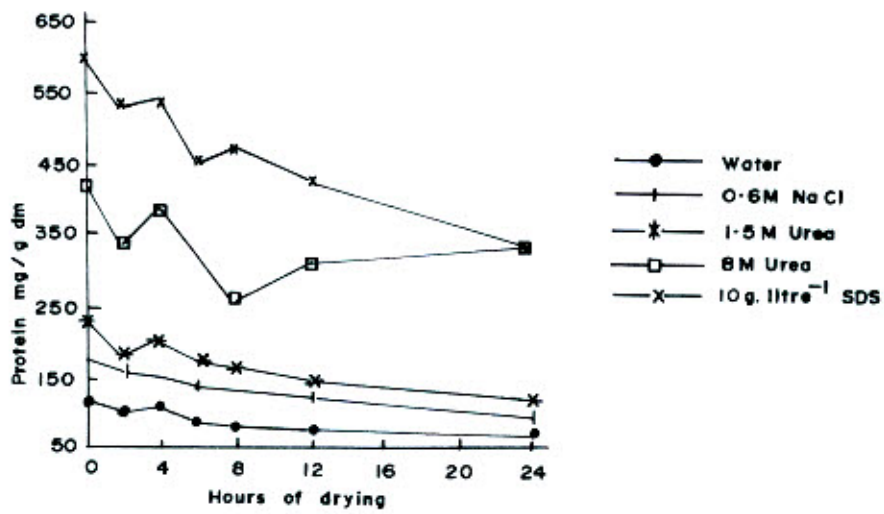

Fig 2. Changes in the solubility of proteins in various solvents during drying at 50°C.

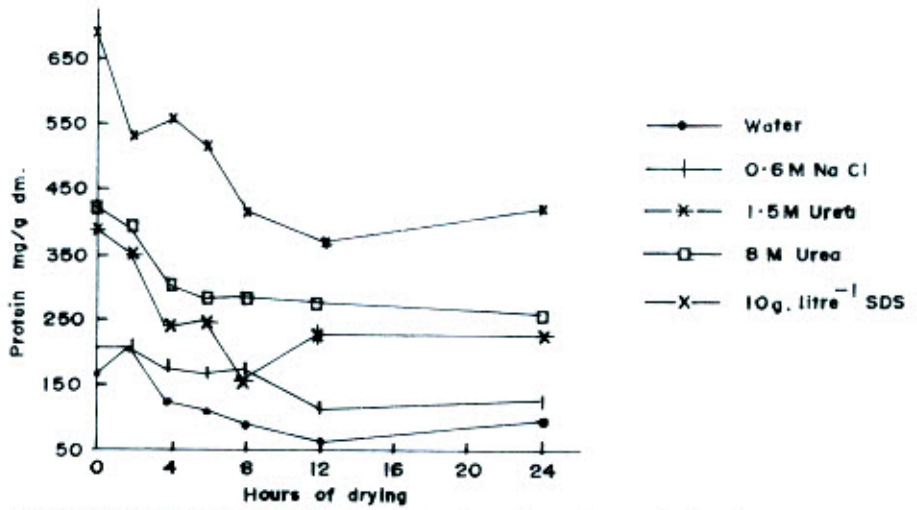

Fig 3. Changes in the solubility of proteins in various solvents during drying at 60°C.

bands disappeared earlier during drying than the lower molecular weight (LMW) protein bands. The LMW proteins did not seem to be affected much by drying, except at 70°C, where the intensity of their bands decreased during drying. A few protein fractions, however, were more easily denatured by drying than their HMW counterparts, and disappeared earlier. A greater number of protein fractions were present in 1·5 M urea and SDS extracts, but the pattern of disappearance during drying was similar. Meat myosin has been observed to be sensitive to heating while F-actin was quite stable (Kako 1968a, b). Disappearance of HMW proteins from SDS–PAGE electropherograms of heated meat proteins has been noted earlier (Caldironi and Bazan 1980). Fish actomyosin undergoes transition to β-structure upon hot air drying (Niwa *et al* 1975), but whether this leads to its disappearance from electrophoregrams, ie its non-extractability in the early periods of drying, is doubtful. As the HMW proteins disappeared even from the SDS extracted electrophoregram it is likely that stainability by Coomassie brilliant blu (CBB) is lost upon denaturation by drying. Poor stain ing of 8 M urea extracted fish meal proteins has bee recorded earlier (Moodie and Eva 1974). The CBB dy anion reacts with the NH_3^+ groups of proteins for stain ing (Sedmak and Grossberg 1977); loss of stainabilit would indicate modification of NH_3^+ groups in denatur ation reactions during drying and thus, mostly basi amino acids by implication. Data from the followin analysis also supports the participation of basic amin acids.

The changes in the water solubility of proteins a various pH during drying are shown in Figs 8–10. The changes are represented as percentage deviations in solubility from those for fresh muscle. At all drying temperatures, solubility at the acid and alkaline pH extremes were greatly decreased as the drying progressed, while solubility near the pH range of 4–6

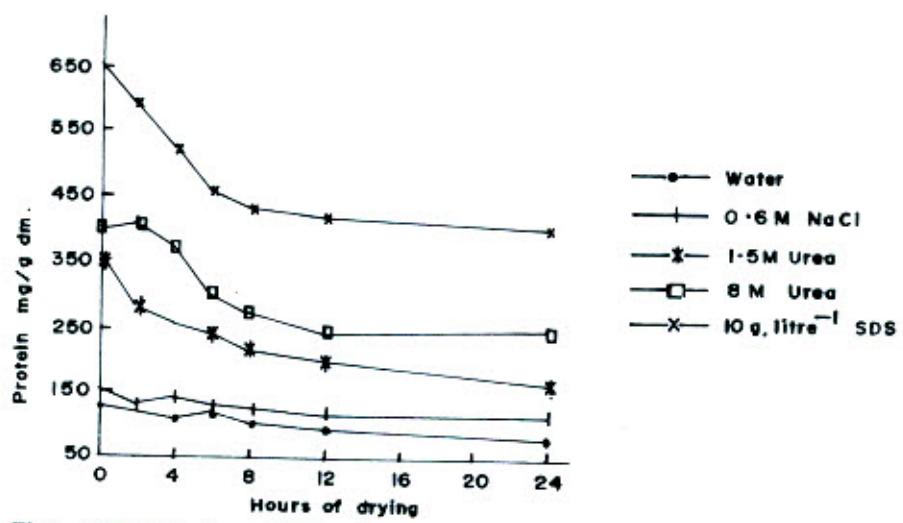

Fig 4. Changes in the solubility of proteins in various solvents during drying at 70°C.

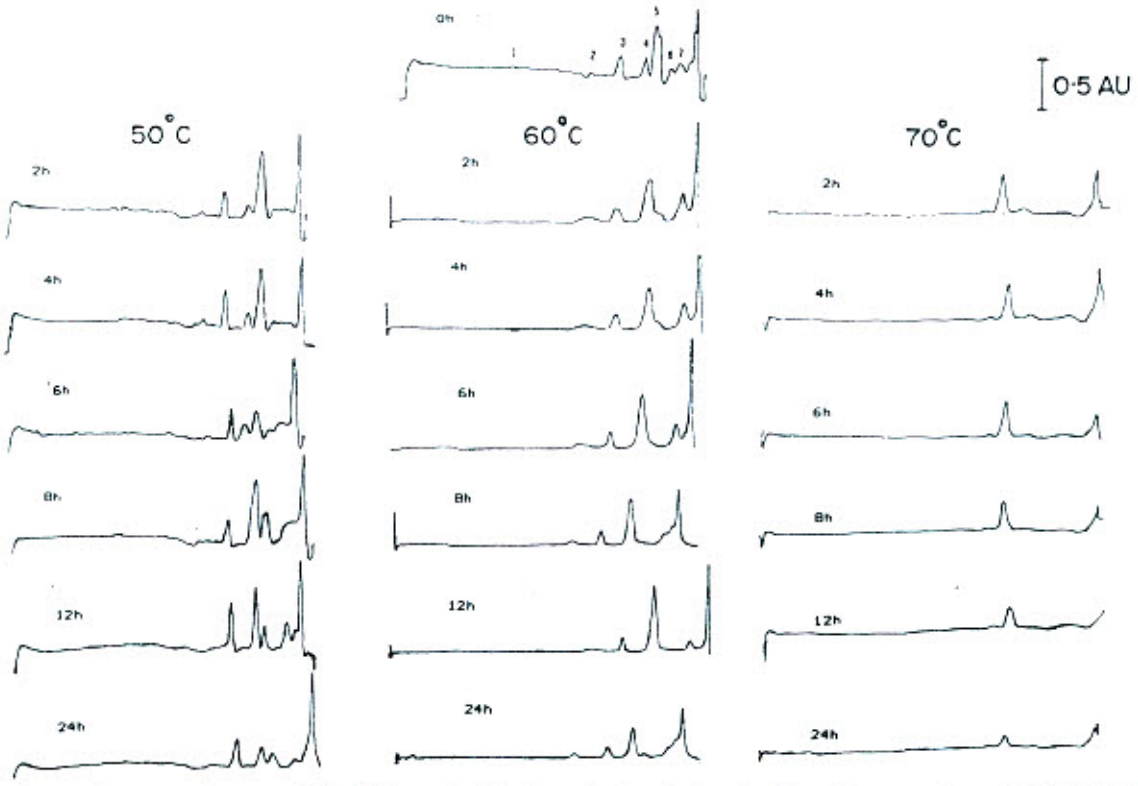

Fig 5. Densitometric scans of water soluble fish protein fractions during drying at different temperatures. SDS–PAGE (Laemmli 1970) was performed on 6% running gels (5%C) without stacking gels. Twenty μl of prepared samples were loaded on each tube which were run at 3 mA per tube. Protein concentrations at each temperature were equalised by diluting with sample buffer.

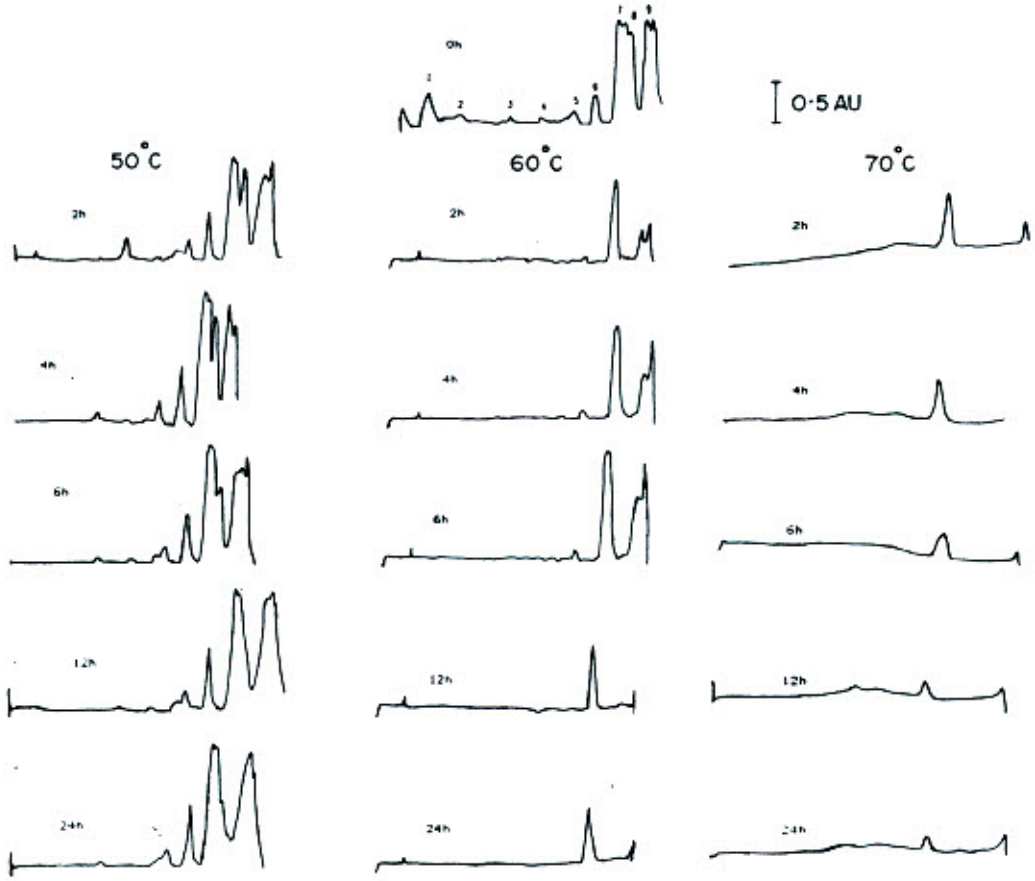

Fig 6. Densitometric scans of 1·5 M urea soluble fish protein fractions during drying at different temperatures. Experimental conditions as detailed in Fig 5.

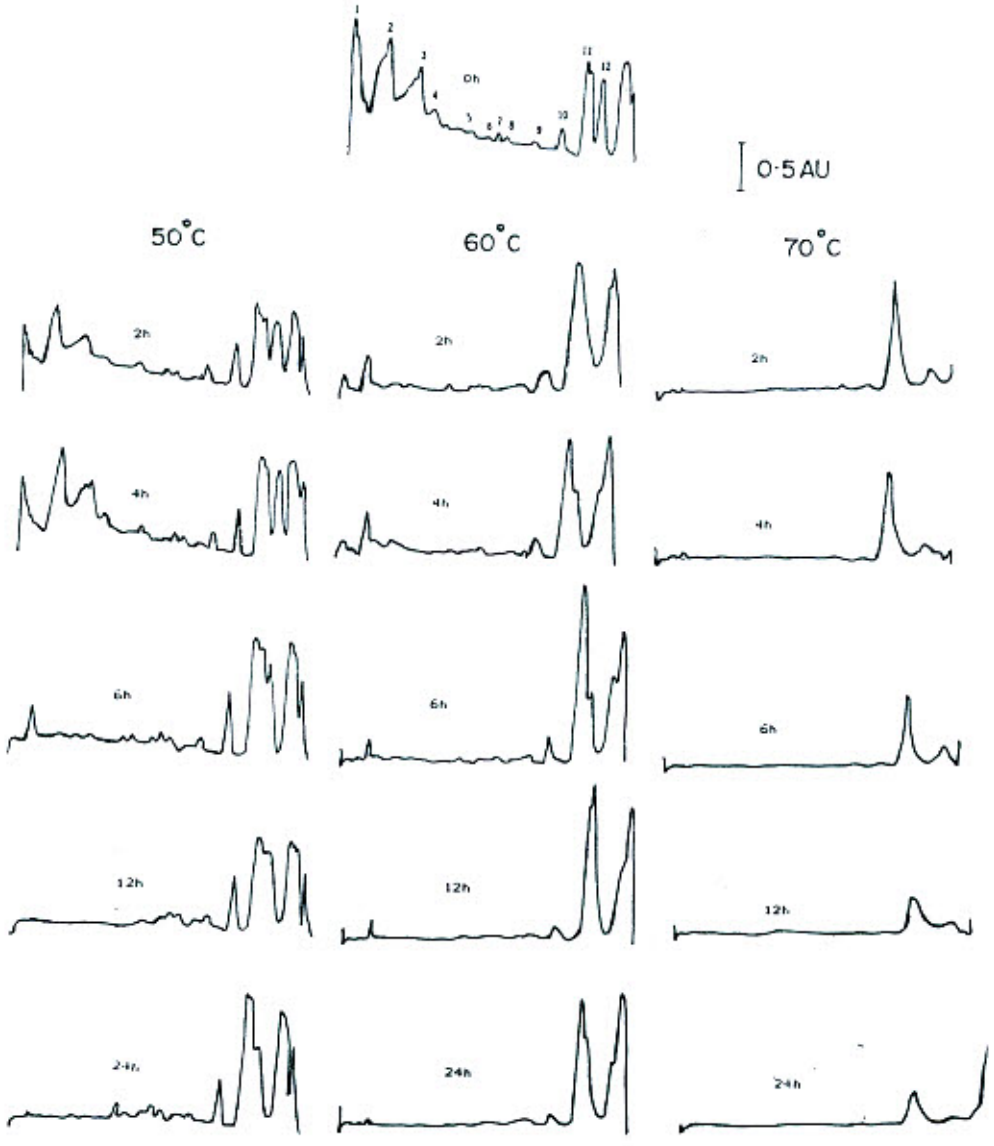

Fig 7. Densitometric scans of 10 g litre^{-1} SDS soluble fish protein fractions during drying at different temperatures. Experimen conditions as detailed in Fig 5.

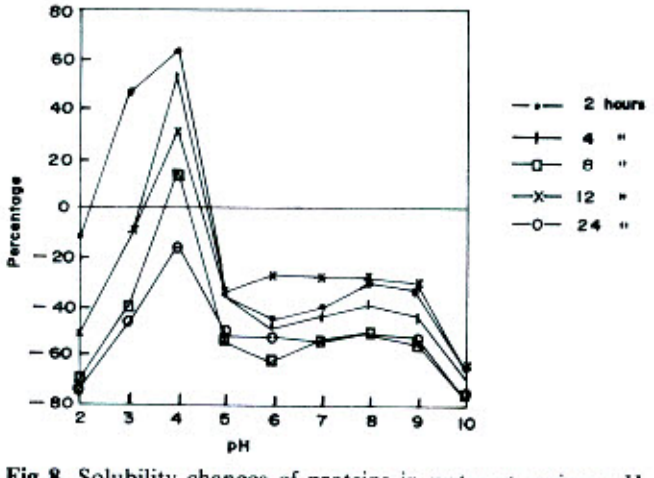

Fig 8. Solubility changes of proteins in water at various pH during drying at 50°C, as deviations from that of fresh fish proteins.

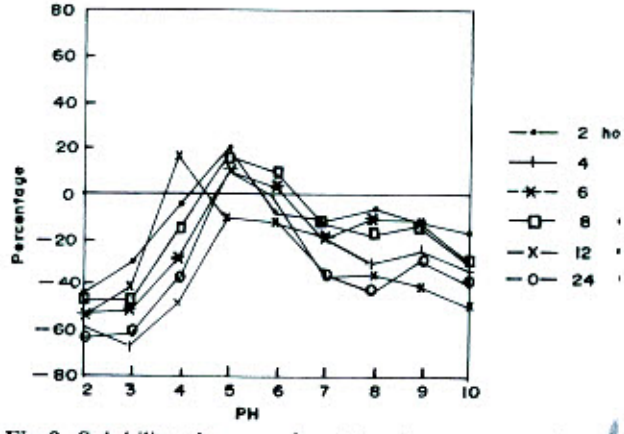

Fig 9. Solubility changes of proteins in water at various during drying at 60°C, as deviations from that of fresh fi proteins.

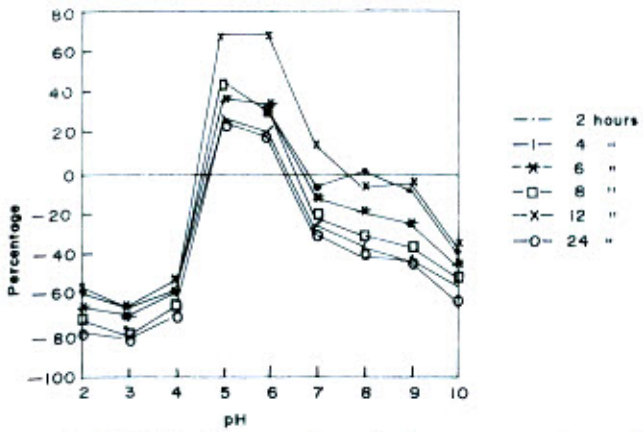

Fig 10. Solubility changes of protein in water at various pH during drying at 70°C, as deviations from that of fresh fish proteins.

increased. At 50°C the decrease in solubility at both acid and alkaline pH extremes were about equal, but at 60°C and more prominently at 70°C, solubility at acid pH was more affected than at the alkaline pH extreme. There was, however, no shift in the isoelectric pH as a result of drying (data not shown). Since ionisation of the side-chain groups of basic amino acids contribute to the higher solubility of proteins at acid pH, it would appear that these side-chains are more involved in new bond formations during drying than the side-chains of acidic amino acids. Thus, reactions involving free amino groups of basic amino acids seem to play an important role in denaturation of proteins during drying. As our data suggest, the new bonds formed are likely to be covalent and may involve reactions with lipid oxidation products and/or isopeptide bond formations. Decreased staining of protein bands in electrophoretograms with increase in drying temperature also suggest damage to the free amino groups of proteins.

The changes in pepsin digestibility of fish muscle during drying are shown in Table 2. There was a marginal increase in digestibility after 2 h of drying at 60 and 70°C, which was not seen at 50°C. Digestibility at later stages of drying decreased progressively in comparison with fresh fish muscle. Sheikh and Shah (1974) and Lee and Ryu (1987) have reported decreased digestibilities of fish dried in hot air. However, a clear relationship between drying temperatures and decreased in-vitro digestibility as observed by Sheikh and Shah (1974) was not evident in this study. This is perhaps because of the lower temperatures used in our experiments. Protein digestibility according to some reports was unaffected when proteins were heated at less than 100°C (Opstvedt 1988).

The nutritional quality of fish as assessed by animal feeding is summarised in Table 3. Highly significant differences were observed between the samples dried at different temperatures as assessed by PER, NPU and BV. Fish dried at 60°C was significantly better than those dried at 50 or 70°C as per PER and NPU evaluations, but its BV was not significantly higher than the 50°C dried fish. Earlier reports have not found much deterioration in the quality of fish proteins dried at tempratures below 100°C (Opstvedt 1988). The difference observed in this experiment could be due to the different fish species used and/or the use of whole fish with bones for feeding rather than flesh alone in our experiments. The exact reason for the superior nutritional quality of fish dried at 60°C is unclear, but 60°C could be considered as ideal temperature for maximum unfolding of polypeptide chains by denaturation, making them more amenable to attack by digestive enzymes.

TABLE 2
In-vitro digestibility of fish muscle during drying at different temperatures

Duration of drying (h)	*In-vitro digestibility at different drying temperatures (as % of total nitrogen)*		
	50°C	*60°C*	*70°C*
0	75·61	75·61	75·61
2	74·38	78·01	77·86
6	72·27	78·95	71·14
12	71·14	68·48	72·25
24	67·85	70·06	64·88

TABLE 3
Nutritional quality of fish dried at different temperatures[a]

Protein quality parameter	*Temperature of drying (°C)*			*Least significant difference at 5%*
	50	*60*	*70*	
PER[b]	2·9	3·65[c]	2·69	0·391
NPU	67·3	77·60[c]	61·00	7·470
BV	84·1	87·00	73·80	3·810

[a] Degrees of freedom: F test, 2 and 15; t test, 15.
[b] PER for casein: 2·23.
[c] Significant at 5%.

REFERENCES

AACC 1976 *Approved Methods of AACC*. American Association of Cereal Chemists, St Paul, MN, USA, method 46-12.

AOAC 1975 *Official methods of analysis*. Association of Official Analytical Chemists, Washington, DC, USA, method 24.003.

Bjarnarson J, Carpenter K J 1970 Mechanisms of heat damage in proteins 2. Chemical changes in pure proteins. *Br J Nutr* **24** 313–329.

Caldironi H A, Bazan N G 1980 Quantitative determination of low salt soluble protein patterns of bovine muscles

cooked at different temperatures by SDS-PAGE. *J Food Sci* **45** 901–904.

Gardener H W 1979 Lipid hydroperoxide reactivity with proteins and amino acids. A review. *J Agric Food Chem* **27** 220–229.

Howgate P F, Ahmed S F 1972 Chemical and bacteriological changes in fish muscle during heating and drying at 30°C. *J Sci Food Agric* **23** 615–629.

Itoh Y, Yoshinaka R, Ikeda S 1979 Behaviour of the sulfhydryl groups of carp actomyosin by heating. *Bull Jap Soc Scient Fish* **45**(8) 1019–1022.

Kako Y 1968a Studies on muscle proteins I. Behaviour of muscle proteins before and after the heat coagulation in the 7M urea containing buffer solution. *Mem Fac Agric Kagoshima Univ* **6(2)** 161–174.

Kako Y 1968b Studies of muscle proteins II. Changes of beef, pork and chicken proteins during the meat products manufacturing process. *Mem Fac Agric Kagoshima Univ* **6**(2) 175–186.

Kunimoto M, Matsumoto K, Zama K 1985 The interaction of fish proteins and lipids during freeze drying and storage—I. The interaction of carp myofibrils and fish oil. *Bull Fac Fish Hokkaido Univ* **36**(1) 50–56.

Laemmli U K 1970 Cleavage of structural proteins during the assembly of head of bacteriophage T4. *Nature* **227** 680–682.

Ledward D A 1979 Proteins. In: *Effects of Heating on Food Stuffs*, ed Priestly R J. Applied Science Publishers, London, UK, pp 1–34.

Lee K H, Ryu H S 1987 Evaluation of seafood protein quality as predicted by C-PER assays. In: *Seafood Quality Determination*, eds Kramer D E & Liston J. Elsevier Science Publishers, Amsterdam, The Netherlands, pp 473–485.

Lee K H, Jung W J, Sah J S, Jeong J H, Kim C B 1986 Protein crosslinking in freeze dried fish meat. *Bull Kor Fish Soc* **19**(2) 100–108

Moodie I M, Eva A 1974 Electrophoresis of fish muscle protein. *FIRI Annual Rep* **28** 57–61.

Myklestad O 1973 Physical aspects of drying of fish meals *Sci Food Agric* **24** 1209–1215.

Niwa E, Nakayama T, Hamada I 1975 Mechanism of tra sition of actomyosin to B structure upon hot air dryir *Bull Jap Soc Scient Fish* **41**(10) 1075–1078.

Opstvedt J 1988 Influence of drying and smoking on prote quality. In: *Fish Drying and Smoking*, ed Burt J R. Elsevi Applied Science, Essex, UK, pp 23–36.

Opstvedt J, Miller, R. Hardy R W, Spinelli J 1984 He induced changes in sulfhydryl groups and disulfide bonds fish protein and their effect on protein and amino ac digestibility in rainbow trout. *J Agric Food Chem* **32** 92 935.

Oser B L (ed) 1976 *Hawk's Physiological Chemistry*. Ta McGraw Hill Publishing Company, New Delhi, India, 858–859.

Ryle A P 1970 The porcine pepsins and pepsinogens. I *Methods in Enzymology* (Vol 19), ed Perlmann G E ar Lorand L. Academic Press, New York, USA, pp 316–318.

Scopes R K 1982 *Protein Purification: Principles and Practic* Springer-Verlag, New York, USA, Appendix-B.

Sedlak J, Lindsay R H 1968 Estimation of total protein bour and non-protein sulfhydryl groups in tissue with Ellman reagent. *Anal Biochem* **25** 192–205.

Sedmak J J, Grossberg S E 1977 A rapid sensitive and vers tile assay for protein using coomassie brilliant blue G-25 *Anal Biochem* **79** 544–552.

Sheikh A S, Shah F H 1974 Effect of heat on the in vitr digestibility of fish protein. *Pakistan J Sci Ind Res* **17** 136 138.

Smith G, Hole M 1991 Browning of salted sundried fish. *J S Food Agric* **55**(2) 291–301.

Snedecor G W, Cochran W G 1961 *Statistical Methods*. Th Iowa State College Press, Ames, IA, USA.

Synowiecki J, Shahidi F 1991 Heat induced changes in sul fhydryl groups of harp seal muscle proteins. *J. Agric Foo Chem* **39** 2006–2009.