Tenderised, Dehydrated Steaks of Freshwater Fish, Rohu: Preparation and Storage Characteristics

K. Smruti, S. Rai, V. Yardi, S.B. Warrier* and V. Venugopal

Food Technology Division, Bhabha Atomic Research Centre, Trombay, Mumbai - 400 085

A process to prepare tenderised, dried steaks of freshwater fish, rohu (*Labeo rohita*) is reported. Steaks of fresh rohu were treated with papain for tenderisation, followed by dip treatment in sodium chloride solution. The brined steaks were dried either in a solar or tunnel dryer at a maximum temperature of 60°C. Although solar drying required a longer drying time than tunnel drying, the products dried by both ways, had comparable proximate composition, with moisture content in the range of 15 to 16%, 59% protein and 5.7% crude fat. The aerobically packaged product, which had a water activity as low as 0.66, did not favour microbial growth when stored at ambient temperature. Tenderisation reduced hardness of dried product as observed by lower breaking force as well as sensory evaluation. The treatment also enhanced rehydration capacity of the dried steaks. Tenderisation followed by solar drying is suggested as a cost-effective process for value addition of rohu.

Key words: Rohu, aquaculture, dehydration, tenderisation, storage characteristics, value addition

India is the second-largest producer of farmed fish in the world, and carp contributes the most of the country's aquaculture production. The three major carp species indigenous to the rivers of the Indian subcontinent are Rohu (Labeo rohita). Catla (Catla catla) and Mrigal (Cirrhinus mrigala). The current annual production of these species is about 2 million tons and the average annual production of Rohu is 60,000 tons. Rohu is currently consumed mostly in the fresh form, especially in the eastern parts of India. While value addition of marine fishery products has received attention, freshwater fish species are generally neglected in this respect (Bawa and Jayathilakan, 2002). Processing of rohu can help to enhance its marketability and availability to consumers far away from the production centres. Of the different methods available for value addition, dehydration is the cost effective method for preservation of small or medium sized low cost fish such as pelagic species (Venugopal and Shahidi, 1998; Horner, 1992).

Dehydration of large fish, their meat or steaks has been rarely attempted essentially because the process has limited applicability for these fish items, most of which have high commercial value. In addition, the hard texture of the dried fish meat attracts poor consumer response. Scope of dehydration technology for these fish items could be enhanced provided the finished products are acceptable in terms of sensory qualities, particularly with respect to their hardness.

Red meat items, which have unacceptable hard texture have been successfully tenderised to soften their texture. The process involves treatment of the meat by proteolytic enzymes such as papain, bromelin or ficin, which loosens the structural integrity of myofibrillar and stromal or connective tissue proteins imparting softening of the texture (Dransfield and Etherington, 1981). Tenderisation has not been found generally necessary for fish because of their soft texture, with exceptions such as squid. The tough skin of squid has been softened by

^{*} Corresponding Author

treatment with papain from papaya latex in presence of salt (Borresen, 1992). Meleno (1998; 1997) improved texture of squid meat by treatment with commercial bromelin and a crude lysosomal extract from bovine spleen. Although toughening of fish muscle during dehydration is a general phenomenon, tenderisation has not been attempted to improve their textural properties. Dehydrated rohu steaks had exceptionally hard texture, limiting their acceptability. In this paper, we describe a process for development of soft, dehydrated rohu steaks by tenderisation using papain. Further, the dehydration was carried out using natural solar dryer instead of expensive, artificial tunnel dryer, with a view to make the process commercially viable.

Materials and Methods

Fresh rohu (*Labeo rohita*) samples were purchased from the local market and transported to the laboratory in ice during October and November. The average length and weight of the fish were 75 cm and 700 g, respectively. The fish were washed with cold water, cut into steaks of about 1 cm thickness, each weighing approximately 50 g. The steaks were washed in chilled water and drained.

The steaks (2.4 Kg) were held in equal amount of potable water containing 0.01 to 0.03% (w/v) papain (Sigma Chemical Co., USA, specific activity, 2.5 units/mg) at ambient temperature (23-24°C) for 3 hrs. After the treatment, the steaks were drained off the solution. The tenderised steaks were transferred to equal amount of 10% (w/v) aqueous solution of sodium chloride and 0.5% butyl hydroxyanisole (BHA) (Sigma, IL. USA). After treatment for 1 hr, the brined steaks were drained.

The treated fish were divided into two equal portions for dehydration by tunnel and solar dryers. One portion (1.2 kg) was spread on perforated aluminium trays and held in a mechanical air dryer for 11 hrs until the moisture content was reduced to about 15%.

The air temperature in the dryer was maintained at 60°C at a relative humidity of 12%. The other half of the brined steaks (1.2 kg) was subjected to solar drying in a BARC model solar dryer at a maximum temperature of 60°C. The solar dryer, having a total height of 150cm, had a rectangular base (30x90x45cm) made of aluminium sheet, blackened to absorb heat. The fish items were kept on stainless steel trays above the rectangular box. A tent made of 500 gauge polyethylene sheet, with a slit on the top for passage of air was fixed on the base. The tent protected the food items during drying. The air heated at the blackened aluminium base moves upward and passes through the slit. The temperature profiles outside and inside the solar dryer are given in Fig. 1. Drying rate was determined by noting the weight of the steaks in the dryer at known intervals.

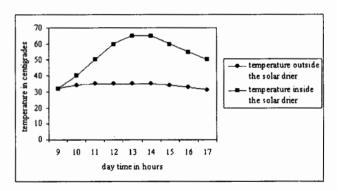


Fig. 1. Performance characteristics of solar dryer

After 10 hrs of drying in both the dryers, the steaks were taken out and were subjected to pressing for 5 min between two metal plates of a screw press in order to remove a portion of moisture and to get uniform flat shape (Jeevanandam *et al.*, 2001). After pressing, drying of both the set of samples was continued in the respective dryers till the moisture content was reduced to about 15%.

The dried fish laminates were either vacuum or air packaged in a 500-gauge polyethylene pouches. The packages were stored at ambient temperature (23-25°C). At periodic intervals samples were withdrawn for analyses.

Extent of tenderisation was determined by measuring the soluble nitrogen content in trichloro acetic acid (TCA) extract of the sample, after the enzyme treatment. An amount of 10g meat from the steaks was homogenized in 20 mL of 10% (w/v) aqueous solution of TCA. The homogenate after standing for 1 hr was filtered through Whatman No. 1 filter paper, and the TCA soluble protein content of the filtrate was determined (Miller, 1959). Extent of tenderisation was expressed as mg of TCA soluble proteins per gram of the sample.

The dried fish was powdered in a kitchen homogenizer and about 5 g of the powder was taken for measurement. The water activity of the dehydrated sample was estimated using a water activity meter (Aqua lab, Cx2T, Decagon Devices Inc, USA).

Visual appearance of the dehydrated product was measured using Minolta CR-14 colourimeter (Jay Instruments, Mumbai). The equipment was calibrated using a white background. Average of six readings was noted.

Hardness of the dehydrated steak was measured in terms of shear force by penetrating it to a breaking point with a knife attached to Universal Testing Machine (Table model, TM Instron Corporation, Canton, MA) using a load cell size CCTM. The scale was calibrated to 5 Kg. Cross head speed was 10 mm per min. Shear force (Kg) was taken as the force required to break the steak and was considered as the maximum height of the peak. All determinations were carried out in quadruplicate.

The dehydrated laminated product was powdered and an accurately weighed sample was kept for three days in desiccators containing saturated aqueous solutions of salts, namely, LiCl MgCl₂, K₂CO₃, NaCl, and KCl, which gave equilibrium relative humidities of 11.30, 32.78, 43.16, 75.29, and 93.58, respectively, at 25°C. After equilibration, the moisture contents of the samples were determined.

Rehydration capacity was estimated by determining the percent of water taken up by holding known amount of the dried sample in boiling water for 15 min.

Rancidity was determined in terms of 2-thiobarbituric acid (TBA) values as described by Ghadi and Venugopal (1991). The values were expressed as mg malonaldehyde per g dry fish.

The dehydrated steaks were boiled for 15 min in aqueous solution of 3% (w/w) sodium chloride. After draining, surface of the rehydrated steaks was smeared with refined groundnut oil and was subjected to heating in a microwave for a period of two min. The cooked sample was served hot to a panel of 6 staff members for sensory evaluation. The texture of the product was evaluated on a ten point scale, where a score of '10' denoted product of highest quality in terms of texture, flavour and appearance characteristic of rohu, and '1' denoted hard textured, off-flavoured product having least qualities of rohu. The mean scores for each parameter were noted as mean deviation.

Moisture and protein were determined as described by A.O.A.C. (1990). Crude lipid contents of the fresh and dehydrated rohu were determined, as described by Bligh and Dyer (1959). Wherever applicable, the data were analyzed as per standard statistical methods.

Results and Discussion

The yield of the steaks was about 80% of the weight of whole fish. The proximate compositions of fresh rohu and dehydrated steaks are given in Table 1. The fresh fish muscle had moisture, protein and fat contents of 77.2%, 17.0% and 1.7%, respectively; while the corresponding values for tunnel dried product were 15%, 58.8% and 5.7%. The solar dried products had slightly higher moisture content of 16% however; the other parameters were similar to that of tunnel dried products.

Table 1. Proximate composition of fresh and dehydrated rohu steaks

Parameters	Fresh	Dehydrated product
Moisture %	77.2	15.1
Protein %	17	58.8
Crude lipid %	1.7	5.7
Ash %	1.1	12.4
Salt (sodium chloride) %	_	7.7

Dried in tunnel dryer. Average of two independent experiments.

The extent of tenderisation could be measured in terms of soluble nitrogen, which was higher in the case of the enzyme treated steaks, as shown in Fig. 2. Incubation of the steaks in presence of 0.01% enzyme for 1 h ambient temperature significantly tenderised the sample, as shown by the increase in TCA soluble digested protein. Higher enzyme concentrations and prolonged incubation resulted in further increase in soluble protein. Based on sensory data of the dehydrated product, a treatment time of 3 hr at ambient temperature in presence of 0.02% enzyme was considered ideal for optimal tenderisation of the steaks. The effect of papain in tenderisation of the enzyme could be presumably because of its broad specificity (Ashie et al, 2002). Alternately, papaya latex could be a cost-effective agent to tenderise the steaks. Instead of enzyme treatment, heating at 60°C for 10 min, however, was not successful for tenderisation, due to disintegration of the meat.

Fig. 3 shows drying curve of tenderised and non-tenderised steaks when subjected to dehydration in tunnel and solar dryers. Drying rate was higher in the tunnel dryer,

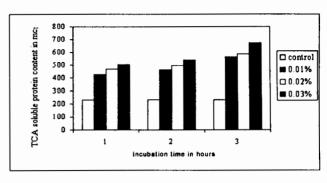
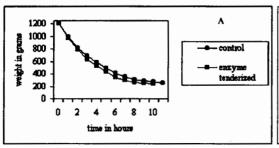


Fig. 2. Extent of tenderisation of rohu after treatment with papain at varying concentrations. The treatment conditions are described in Materials and Methods.

as compared with solar dryer, essentially due to the constant drying temperature of 60°C of the former, unlike in solar dryer, which had variable internal temperatures due to outside temperature fluctuations. Dehydration in solar dryer took 16 hrs as compared to 11 hrs in tunnel dryer to give a product having 15% moisture content. Pressing the partially dried steaks (after 10 hrs drying) in between two metal plates of a screw press increased surface area, helped even rate of drying and better product appearance, as also observed by Jeevanandam et al (2001). Kochi et al (1993) observed that drying of eviscerated fish without lamination such as herring often resulted in uneven rate of dehydration between the skin and the muscle, with higher rates of water loss and lipid oxidation occurring in the skin.

Figure 4 depicts the equilibrium moisture contents of the dried steaks as a function of relative humidities. The products were not hygroscopic, as indicated by the lower equilibrium moisture contents at higher RH values, in the case of both non-tenderised and tenderised steaks.



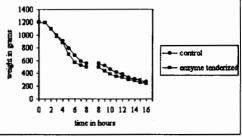


Fig. 3. Drying rate of Rohu in tunnel dryer (A) and in solar dryer (B)

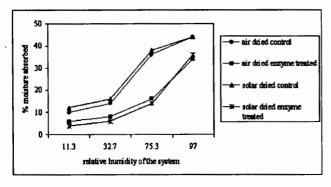


Fig. 4. Equilibrium moisture content of dehydrated steaks

The products obtained after dehydration in both the dryers were, however, comparable, with their moisture contents ranging from 15 to 16. The rehydration capacities of freshly prepared non-tenderised and tenderised steaks dried in tunnel dryer were 42% and 61.7%, respectively. The corresponding values for solar dried samples were 39.8 and 55.8, respectively (Table 2). The effect of tenderisation on the texture of the steaks was also evident by hardness index, measured in terms to break the steaks, as determined by Instron measurement. The tenderised steaks required significantly lesser (p= .001) breaking force, as compared with non-tenderised, dried steaks (Table 2). The Instron data are also comparable with those of Ashie et al (2002), who observed 20% reduction in Warner-Bratzler shear of

Table 2. Properties of fresh dehydrated rohu steaks

Properties	Tunnel dried steaks		Solar dried steaks	
	Non- tenderised	Tenderised	Non- tenderised	Tenderised
Hardness				
(Breaking force)	0.6	0.2	0.7	0.3
Rehydration capacity	38.8	55.3	25.0	43.0
Whiteness index, fresh samples	36.6	41.00	36.5	40.5
Total plate count, 1 month stored	24	ND	17	ND
Total fungal coun 1 month stored	t, ND	ND	ND	ND
Water activity	0.66	0.67	0.65	0.66

The dehydrated samples were aerobically packaged and stored under ambient conditions. Rehydration capacity, extent of tenderisation, whiteness index, hardness and water activity were measured as described in Materials and Methods. ND: not detected. Values are average of three independent experiments

enzyme-treated beef. The dehydrated steaks had negligible total bacterial counts immediately after preparation. After storage for one month at ambient temperature, tunnel and solar dried control samples had a count of 24 and 17 cfu/g, respectively, while tenderised samples did not show any microorganisms after storage for the same period. The bacterial counts did not change significantly nor any fungal growth was detected even after 3 months of storage. The low bacterial counts in the product could be due to their salt content as well as low water activity of 0.66, which did not favour microbial growth (Table 2). Dehydration by both methods gave a whiteness index of about 36 for the non-tenderised steaks, whereas, tenderisation resulted in slightly higher whiteness index, in the range of 40 to 41, in the case of samples dehydrated by both solar as well as tunnel dryers.

Table 3. Influence of papain treatment on sensory score of freshly prepared, solar dried rohu steaks.

Papain concentration	Score for texture	Overall acceptability
Nil	5.1±1.3	5.0±2.0
0.01%	6.3±0.5	6.3±0.8
0.02%	7.4 ± 0.4	7.8 ± 0.4
0.03%	6.5 ± 0.1	6.5±0.3

Sensory evaluation was conducted as described in Materials and Methods.

Data on the sensory evaluation of the steaks are given in Table 3. The tenderised steaks, upon rehydration, and microwave cooking gave products, which were soft, unlike non-tenderised steaks, which were hard to bite. Panellists, thus, gave higher sensory scores for texture of tenderised steaks, giving maximum for the samples treated with 0.02% enzyme. At higher enzyme concentration, the score was lower. The steaks dried by both tunnel as well as solar dryers were acceptable to the panel. The overall acceptability of tenderised steaks was also higher than the non-tenderised rohu steaks. The enhanced whiteness of the product as a result of the treatment (as also observed by visual colour measurement, as mentioned earlier) could have contributed to higher overall acceptability of the product.

Fig. 5 depicts the rehydration capacities of dried rohu steaks during the course of ambient temperature storage. The non-tenderised steaks, dried by both tunnel and solar dryers had lower rehydration capacities, throughout the storage period. On the other hand, tenderised, dried steaks had significantly higher rehydration capacities. However, during the course of storage, there was small decrease in rehydration capacities in all the samples.

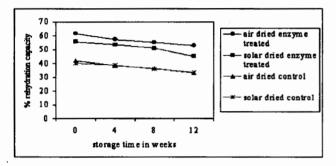


Fig. 5. Rehydration capacity of fresh dehydrated steaks

The extent of lipid oxidation in the dried steaks, in terms of TBA values, is given in Table 4. There was no significant difference in TBA values of fresh steaks, irrespective of tenderisation and different drying conditions. However, after two months of storage at ambient temperature, there was slight increase in TBA values in all the samples. The increase in extent of lipid oxidation is a general phenomenon of aerobically stored, dehydrated fishery products (Horner, 1992; Jeevanandam et al, 2001). Vacuum packaging although is known to reduce rancidity development during storage, the process was not successful in the present product because of the sharp edges of the dried steaks, which pierced the packaging material and released vacuum.

The hard texture of the non-tenderised steaks could be ascribed to loss of bound water from the matrix of structural proteins during dehydration, interaction of lipids with the proteins and formation of eglutamyl lysine cross links in the proteins (Ailken and Connell, 1979; Arai, 1980;

Table 4. TBA value of dehydrated Rohu steaks during storage

Storage period at ambient temperature	TBA value (µg malonaldehyde/g fish) Tunnel dried Rohu Solar dried Rohu				
(weeks)	Control	Enzyme tenderised	Control	Enzyme tenderised	
0	13.0	13.0	11.0	12.0	
4	14.0	13.0	14.0	12.5	
8	17.5	14.3	15.5	13.0	
12	18.0	16.5	18.0	14.0	

Values are average of three independent experiments

Ikegami et al., 1971). Significant formation of e-glutamyl lysine cross-links has been observed in mackerel during drying resulting in hard texture of the product (Kumazawa et al., 1993; Maruf et al., 1990). Papain treatment caused softening of the steaks by hydrolysing myofibrillar, stromal and connective tissue proteins of the steaks (Ashie et al, 2002). This could be due to increased liberation of amino acids during enzyme hydrolysis, which could undergo Maillard type reactions with carbonyl groups to give brown pigments (Bligh et al, 1988; Olley et al, 1988). In addition, the release of polar sites in the tenderised samples is available for binding of water, enhancing rehydration capacities.

Solar drying of fishery products, especially in tropical countries including India is economically viable. The present studies on value addition of rohu, involving tenderisation by papain followed by solar drying offer a cost-effective method for value addition of rohu fish. The process appears to have potential for adoption by fishermen and entrepreneurs in food processing industry. The dehydrated rohu fish can play as a cheap source of nutritive and digestible protein, due to partial hydrolysis of the structural proteins during the tenderisation process.

The authors thank Dr. A.K. Sharma, Head, Food Technology Division, BARC for valuable encouragement. Thanks are also due to Dr. Brij Bhushan for texture measurement using Instron and Dr. (Ms). S.V. Sherekar for visual colour measurements. The technical assistance of Ms. V. Mahale is gratefully acknowledged.

References

- A.O.A.C. (1990) Methods of Analysis. Association of Official Agricultural Chemists 15th Ed., Washington, D.C.
- Ailken, A. and Connell, J.J. (1979) In: Effects of Heating on Foodstuffs. R.J. Priestley (Ed.), Appl. Sci. Publ., London, pp. 219-254
- Ashie, I.N.A., Sorensen, T.L. and Nielsen, P.M. (2002) Effects of papain and a microbial enzyme on meat proteins and beef tenderness. *J. Food Sci.* **67**, pp 2138-2142
- Bawa, A.S. and Jayathilakan, K. (2002) Fresh water fish processing—A review. *Indian Food Industry.* **21**, pp 34-40
- Bligh, E.G. and Dyer, W.J. (1959) A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37**, pp 911-917
- Bligh, E.G., Shaw, S.J. and Woyewoda, A.D. (1988) *Effects of drying and smoking on lipids of fish*. In: Fish Smoking and Drying. Elsevier Appl. Sci., pp. 41-52
- Borresen, T. (1992) *Biotechnology, by-products* and aquaculture. In: Seafood Science and Technology, Fishing News Books, pp. 278-287, Blackwell Publications, UK.
- Dransfield, E. and Etherington, D. (1981)

 Enzymes in the tenderisation of meat In:
 Enzymes and Food Processing, Birch, G.
 G., Blakebrough, N. and Parker, K.J. Eds.
 pp. 177-194 Appl. Sci. Publ, London.
- Foegiding, E.A. and Larick, D.K. (1986) Tenderisation of beef with bacterial collagenase. *Meat Sci.* **18**, pp 201-214
- Ghadi, S.V., and Venugopal, V. (1991) Influence of gamma irradiation and ice storage on fat oxidation in three Indian fish. *Int. J. Food Sci. Technol.* **26**, pp 397-401
- Horner, W.F.A. (1992) Preservation of fish by curing (drying, salting and smoking). In Fish Processing Technology, Blackie Academic & Professional, pp 31-72, London
- Ikegami, Y., Takai, Y. and Shibuya, K. (1971) Nutritive value of frozen food. VII.

- Nutritive value of frozen squid and mackerel. Report 73-78 p Inst. Nutr.
- Jeevanandam, K., Venugopal, V., Doke, S.N., Rao, B.Y.K. and Bongirwar, D.R. (2001) Preparation and storage characteristics of ribbon fish laminates. *J. Aquatic Food Prod. Technol.* **10**, pp 77-86
- Kochi, M., Hamada, M., Kunimoto, M., Hatate, H., Kaneniwa, M., Ishizaki, S., Fujimoto, M. and Nakemura, M. (1993) Change during the production process in fish meat composition and quality of the maruboshi product of mildly salted and semi dried round herring. *J. Shimonoseki Univ. of Fish.* 41: pp 217-228
- Kumazawa, Y., Seguro, K., Takemura, M. and Motoki, M. (1993) Formation of e-(gamma-glutamyl) lysine cross link in cured horse mackerel induced by drying. *J. Food Sci.* **58**, pp 1062-1064
- Maruf, F.W., Ledward, D.A., Neale, R.J. and Poulter, R.G. (1990) Chemical and nutritional quality of Indonesian dried salted mackerel (*Rastrelliger kanagurta*). *Int. J. Food Sci. Technol.* **25**, pp 66-77
- Melendo, J.A., Beltran Jose, A. and Pedro, R. (1997) Tenderisation of squid (*Loligo vulgarus* and *Illex coindetii*) with bromelain and a bovine spleen lysosomal-enriched extract. *Food Res. Int.* **30**, pp 335-341
- Melendo, J.A., Beltran Jose, A. and Roncalesa, P. (1998) Characterization of acrude lysosomal extract from bovine spleen for its use in the processing of muscle foods. *Food Biotechnol.* **12**, pp 239-262
- Miller, G.L. (1959) Protein determination for large number of samples. *Anal. Chem.* **31**, pp 964
- Olley, J., Doe, P.E. and Heruwati, E.S. (1988) The influence of drying and smoking on the nutritional properties of fish: an introductory overview. In: Fish smoking and drying Elsevier Appl. Sci., New York, pp. 1-22
- Venugopal, V. and Shahidi, F. (1998) Traditional methods to process underutilized fish for human consumption. *Food Res. Int.* **14**, pp 35-97