J Food Sci Technol (Sept–Oct 2010) 47(5):534–540
DOI 10.1007/s13197-010-0092-3

ORIGINAL ARTICLE

Improvement of cooking quality and gel formation capacity of Bombay duck (*Harpodon nehereus*) fish meat

Chakrabarti Rupsankar

Revised: 9 April 2010 / Accepted: 15 April 2010 / Published online: 9 October 2010

Abstract High moisture content (89%) along with high enzymatic and bacteriological activity in Bombay duck (*Harpodon nehereus*) meat are responsible for short shelf life and disintegration of meat in cooking. Minimum solubility was at pH 5 (iso-electric point) of muscle protein. Citric acid- sodium citrate buffer (pH 5) with 0.2% potassium sorbate was very effective in reducing moisture in dressed fish and in increasing shelf life up to 4 days at ambient temperature (25–28 °C). Reduction in moisture in meat improved its cooking quality and gel formation capacity with increased protein content. Fish meat contained 1.0–1.5% NaCl and produced stronger gel by using 2% NaCl than conventionally prepared gel with 4% NaCl. Washing fish mince with cold water followed by pressing at pH 5, gave fish cake with more salt soluble protein and better gel strength (>500 gcm) than the same operation done at ambient temperature.

Keywords Bombay duck meat · *Harpodon nehereus* Cooking quality · Iso-electric pH · Gel strength

Introduction

Bombay duck (*Harpodon nehereus*) is an abundant marine species in North West coast of India. It contributes about 147,000 tons per year in West coast marine landing (FAO 2003). Fishing season is July to January mainly by dol nets operated by mechanized or country boats. High moisture content (89%) along with high enzymatic and bacteriological activity in Bombay duck (*Harpodon nehereus*) meat are responsible for short shelf life and disintegration of meat during cooking (Warrier et al 1985). Traditionally the demand of fresh fish is limited and major portion of landings are converted to rope dried product. The glut catch in rainy season rarely finds its way to market. Several workers attempted for better utilization of this species by freezing (Radhakrishna et al. 1973) and by preparation of value added products such as laminated and sized dried product (Kondoran et al 1969) and acid induced gel (Kakatkar et al 2003). The recent survey has shown that the processors at the landing sites still indulge to traditional method, which gives dried product foul smell. The product is also amenable to fungal and occasional insect infestation. The increased operational cost to produce dried products and the poor return necessitate fish processors to search for high valued products from Bombay duck. Improvement in cooking quality and storage characteristics will increase the consumption of fresh Bombay duck. The production of fabricated analogue product from white meat is gaining momentum world wide. Fish meat with good gel forming capacity is one of the pre-requisites in the production of fabricated product. The present work was undertaken to study the gel forming capacity of Bombay duck meat and to find out suitable method to improve its capacity of gel formation.

Materials and methods

Bombay duck *(Harpodon nehereus)* of 100–150 g each caught off Mumbai coast by dol nets operated by mechanized/country boats were brought with little flake ice to the laboratory within 2 h from landing centres. The fish were kept moist at ambient temperature (25–28 °C) in

C. Rupsankar (✉)
Central Institute of Fisheries Technology,
Mumbai Research Centre,
Sector-1, Vashi,
Navi Mumbai 400703, India
e-mail: crupsankar@yahoo.com

tray by partly covering with another tray and periodic sprinkling of water on exposed surface, if necessary. Overall quality (OQ) of fish on the basis of change in texture, eye, gill, belly wall, appearance and odour during storage, was examined and rated on 5-point scale (1 to 5 was referred to as poor, slightly poor, fair or acceptable, fair to good or average fresh and good or very fresh, respectively) by 5 trained panelists (Shewan et al 1953). Fish stored in laboratory at ambient temperature were analyzed at an interval of 2 h. Fish were also kept (inverted position) in wide mouth funnel at ambient temperature; drip was collected in a measuring cylinder and the drip was analysed for its components.

Determination of protein solubility at different pH Five grams of finely ground homogenized fish meat sample was added in 100 ml 0.2 M phosphate citrate buffer at pH ranging from 3 to 9 (with 0.5 pH difference) separately and stirred continuously using magnetic stirrer at low controlled speed to avoid foam; supernatant was collected at each pH by centrifugation (5000×*g*; 15 min). Protein content in centrifugate was measured by Lowry et al (1951) method.

Treatment with potassium sorbate solution Fresh fish was degutted, washed, drained and then divided into two parts. First part was dipped in 0.2% potassium sorbate solution (fish to solution ratio 1:2) and pH of the solution was maintained at 4.8–5.2 by intermittent addition of 10% phosphoric acid. Second part was dipped in 0.1 M sodium citrate- citric acid buffer (pH 5) containing 0.2% potassium sorbate solution (fish to buffer ratio 1:2) and pH of the solution was maintained at 4.8–5.2 by intermittent addition of 20% citric acid solution. Fish stored in solution/buffer at ambient conditions (25–28 °C, 48–65% RH), were analysed at interval of 24 h. Used solution/ buffer was boiled for 10 min, cooled, filtered through Whatman 41, pH adjusted to 5 and reused.

Preparation of salt soluble extract Salt soluble extract was prepared by the method of Dyer et al (1950). Five grams muscle of dorsal part of a Bombay duck was ground and then homogenized in pre-cooled (1–5 °C) 5 volumes 5% NaCl solution (pH 7.4) containing 0.02 M sodium bicarbonate. After collecting the supernatant by decantation, the washed ground meat was homogenized in pre-cooled (1–5 °C) 8 volumes of the same NaCl solution. The supernatant was collected and the process was repeated for third time. The collected supernatant was centrifuged (Remi CPR 30 model; R238, Mumbai, India) at 5000×*g* for 10 min at 0–5 °C. Protein in the centrifugate was measured by Lowry et al (1951) method and also estimated by measuring absorbance at 280 nm (Walk and Walk 1969). The extracted protein was recorded as 5% salt soluble protein. The method was repeated by using 2% NaCl solution (pH 7.4) containing 0.02 M sodium bicarbonate; protein soluble in this solution was recorded as 2% salt soluble protein.

Table 1 Composition of fresh meat of Bombay duck

Moisture	% 88.5±1.5
Protein	% 9.1±0.4
Fat	% 0.65±0.1
Ash	% 1.0±0.24
Chlorides	% 1.2±0.3
Ca	mg/100 g 180±20
P	mg/100 g 225±15
Fe	mg/100 g 1.6±0.3
Cu	ppm 1.2±0.4

(*n*=5)

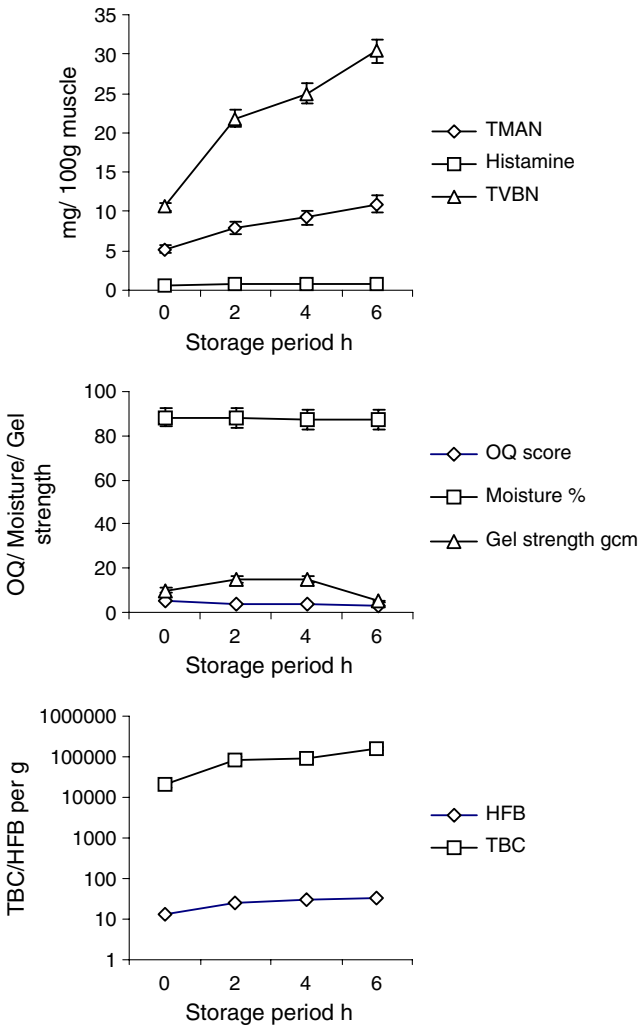

Fig. 1 Changes in Trimethylamine nitrogen (TMAN), histamine, Total volatile base nitrogen (TVBN), moisture, overall quality (OQ), gel strength, total bacterial count (TBC) and histamine forming bacterial (HFB) count in muscle of Bombay duck during storage at ambient condition (25–28 °C; 48–65% RH) (*n*=3)

Preparation of water soluble extract Water soluble extract was prepared by the method of Winton and Winton (1958). Five grams muscle from dorsal part of a Bombay duck was ground, suspended in pre-cooled (1–5 °C) 5 volumes water containing 0.02 M sodium bicarbonate and homogenized. Supernatant was collected by decantation. The washed meat was again homogenized in 8 volumes of water. Supernatant was collected. This process was repeated again. The supernatants were collected and centrifuged (Remi CPR 30 model) at 5,000×*g* for 10 min at 0–5 °C. Protein in the centrifugate was measured by Lowry et al (1951) method and also estimated by measuring absorbance at 280 nm (Walk and Walk 1969). The extracted protein was recorded as water soluble protein. The precipitate after extraction of water soluble extract and the residue remaining after centrifugation were suspended and homogenized in pre-cooled (1–5 °C) 100 ml 5% NaCl solution (pH 7.4) containing 0.02 M sodium bicarbonate (Dyer et al 1950). The supernatants were collected and centrifuged at 5000×*g* for 10 min at 0–5 °C. Protein in the centrifugate was measured by Lowry et al (1951) method and also estimated by measuring absorbance at 280 nm (Walk and Walk 1969). The extracted protein was recorded as 5% salt soluble protein by successive method.

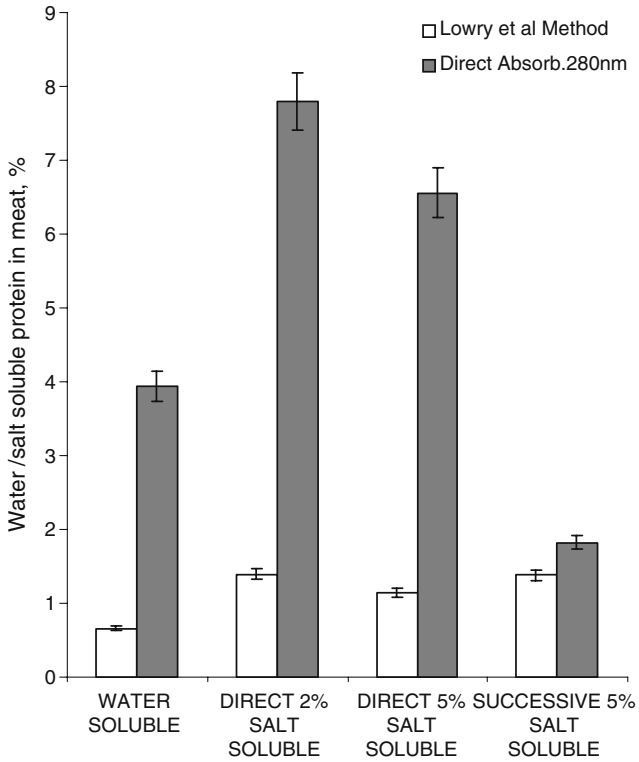

Fig. 2 Difference between two methods of protein analysis; n=3 and standard error ±5%

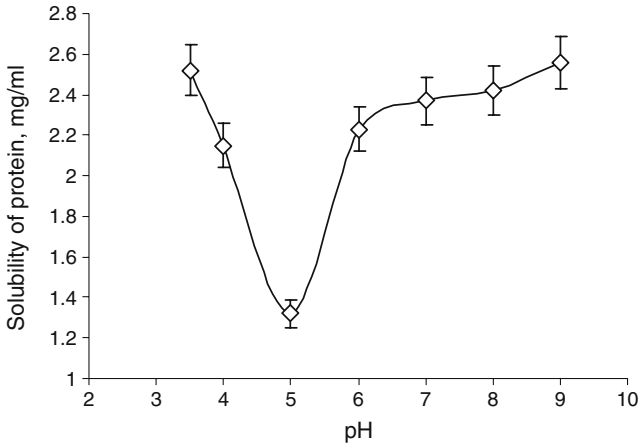

Fig. 3 Solubility of fish protein at different pH; n=3 and standard error ±5%

Preparation of gel Manually picked white meat from the fillets of fresh fish was divided into 5 parts and mixed with 0.0%, 1.0%, 2.0%, 3.0% and 4% NaCl separately to get a smooth paste. Each type of paste was filled in heat stable 2 mm thick plastic casing (3.0 cm length × 2.5 cm diameter), heated in open steam for 40 min and then cooled to ambient temperature. The steamed cakes were taken out from the plastic casing and then cut into 1 cm thick test pieces with 2.5 cm diameter. The break point of test pieces was recorded on standard paper chart using a meter based on the principle of Okada gelometer, which measured the vertical weight on the plunger to break 1 cm test piece (Okada 1959). Similarly the gel strength of meat from fish

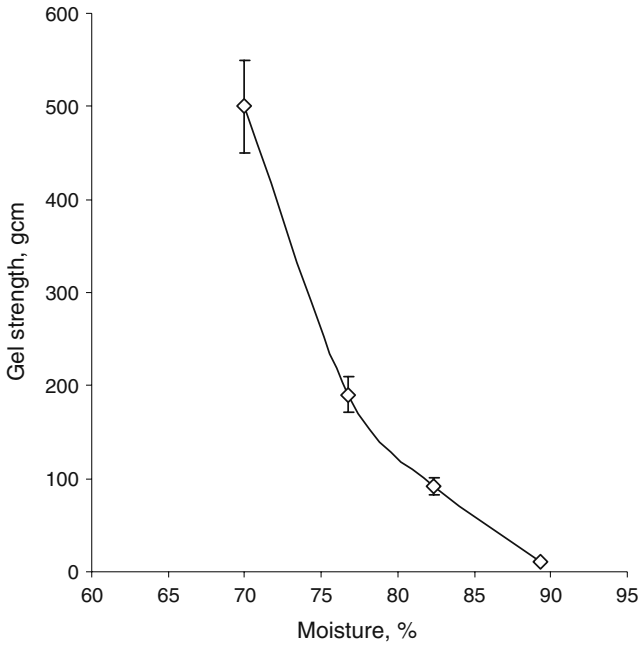

Fig. 4 Change in gel strength with moisture in meat; n=3 and standard error ±10%

stored in potassium sorbate solution was determined after mixing with 4% NaCl (Pan et al 1980). Instead of unwashed fish mince, washed mince was also used in gel preparation; mince was washed with three volume of 0.02 M sodium bicarbonate cold solution (0–5 °C), filtered through filter cloth, re-suspended in cold water (0–5 °C) at pH 5 by addition of 10% phosphoric acid, re-filtered through filter cloth, pressed under 0.064 kg/cm^2 for 30 min in wooden vertical press and used for preparation of gel with 4% NaCl. Similar procedure at ambient temperature was followed using 0.02 M sodium bicarbonate solution and water at pH 5. The pressed meat was used for preparation of gel with 4% NaCl.

Chemical analysis of fresh fish samples Moisture, protein, fat, ash, Ca, Fe, Cu and NaCl contents in fish muscle were determined by AOAC (1975) methods. Phosphorus content was estimated by Fiske and Subbarow (1925) method. Total volatile base nitrogen (TVBN) and trimethyl amine nitrogen (TMAN) contents in muscle were estimated by Conway micro-diffusion method (Conway 1950). Histamine was isolated by AOAC (1975) method and estimated calorimetrically (470 nm) by coupling diazonium salt.

Microbiological analysis of fresh fish Total bacterial count (TBC) in fresh fish muscle was determined by pour plate method using tryptone glucose beef extract agar. The number of histamine forming bacteria (HFB) in fish muscle was determined by spreading 0.2 ml muscle extract on Niven's medium (Niven et al 1981). Dark colonies with change of colour in surrounding were counted as HFB.

Statistical analysis Each experiment was repeated thrice. The data was subjected to analysis of variance by the methods described in Snedecor and Cochran (1967).

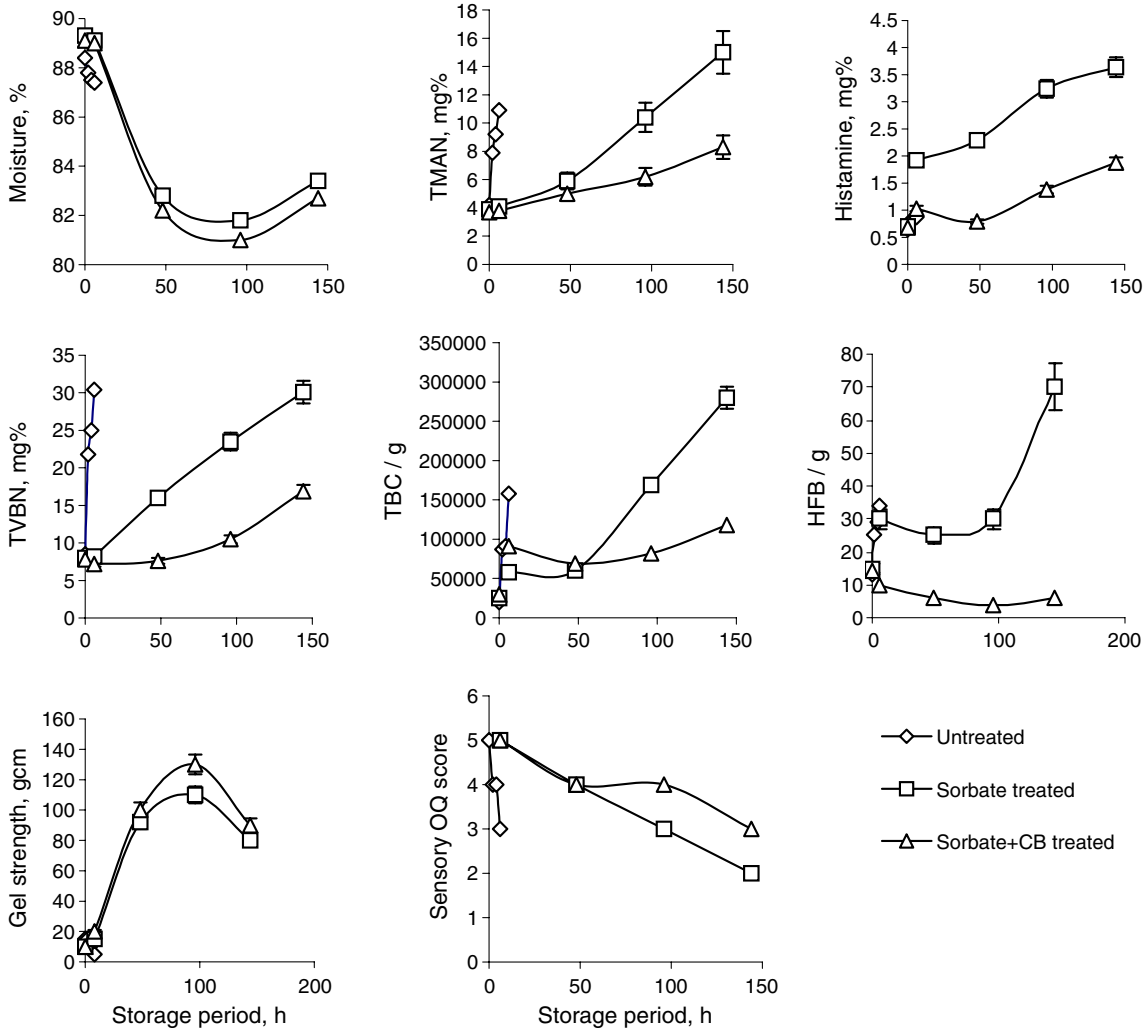

Fig. 5 Effect of sorbate and citrate buffer (CB) on moisture, TMAN, histamine, TVBN content, TBC, HFB count, gel strength and sensory OQ score of muscle of Bombay duck during storage at ambient condition (25–28 °C; 48–65%RH); n=3; TMAN, TVBN, TBC, HFB, OQ: As in Fig. 1

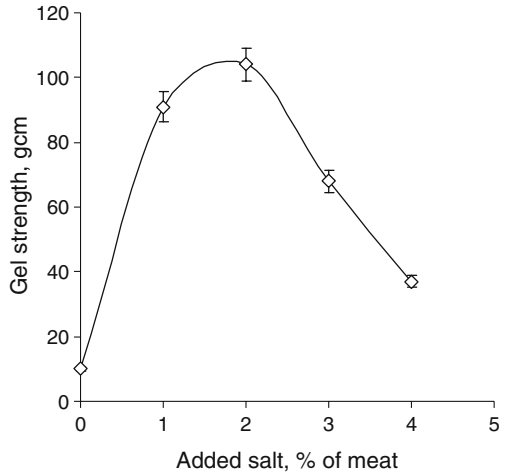

Fig. 6 Change in gel strength with added salt; n=3 and standard error ±5%

Results and discussion

The chemical composition of muscle of Bombay duck is presented in Table 1. High moisture content in meat causes difficulty in frying in oil and cooking in water without disintegration.

Spoilage of Bombay duck at ambient temperature Figure 1 shows that fish were acceptable up to 6 h at ambient temperature. Though histamine level in meat of stored fish was much below the maximum admissible limit of 20 mg/100 g fish flesh (Anon 1990), the presence of TVBN and TMAN in meat was significantly more than that in some food fish. The ratio of TMAN and TVBN was higher than other food fish due to the intrinsic nature of Bombay duck meat. High bacterial load in muscle (Fig. 1) might be responsible for breaking down of tissue protein and release of profuse drip with protein content 487 mg/100 ml, shortly after catch. Radhakrishna et al (1973) reported the presence of 38.8 mg% alpha amino nitrogen in Bombay duck meat. The absorbance at 280 nm in measuring protein in water/salt extract of Bombay duck meat was too high (Fig. 2) and it might be due to release of interfering UV sensitive non-protein substances such as nucleotides and its broken down compounds from rapidly damaged cells. Warrier et al (1985) reported the presence of high concentration of amino acids, UV-sensitive nucleotides and it's broken down compounds in the drip of Bombay duck. They also found that drip was a rich source of tissue breaking enzymes such as lysosomal hydrolases and β-glucuronidase.

Effect of sorbate on gel strength and storage characteristics at ambient temperature Solubility of protein was minimum at iso-electric pH 5 (Fig. 3). Fish mince at pH 5 was pressed under different pressure from 0.032 to 0.064 kg/cm^2 and the pressed meat contained moisture from 68% to 84%. Figure 4 shows that the gel strength of fish meat varied with the moisture content in meat and reduction of moisture improved gel strength. Dressed fish was kept in iso-electric pH solution containing 0.2% potassium sorbate (a) to remove water from meat through thin skin, (b) to facilitate removal of salt from meat by diffusion, (c) to keep minimum loss of protein and (d) to maintain bacterial and fungal load minimum. Figure 5 shows that the moisture content in meat decreased up to 4 days in dressed fish stored in sorbate solution where pH 5 was maintained by using intermittent addition of 10% phosphoric acid. Similar result was obtained with rapidity in storage of dressed fish in sodium citrate and citric acid buffer at pH 5 with intermittent addition of 20% citric acid solution. Increase of gel strength (11–13 fold) of meat with loss of moisture content was observed up to 4 days of storage in iso-electric pH solution at ambient temperature. Sodium citrate (0.1 M) and citric acid buffer at pH 5 caused more release of water due to higher osmotic gradient than that in aqueous sorbate solution. The fish meat in both the solutions were acceptable up to 4–6 days at ambient temperature (Fig. 5) with better texture and less salt (0.3–0.5%) due to bacteriostatic and antimycotic property of sorbic acid and its salts (Doeshurg et al 1969, Chakrabarti and Varma

Table 2 Change in gel strength with temperature

Parameters	Fresh fish Mince	Meat washed at ambient temp. and pressed* at iso-electric pH 5	Meat washed at 0–5 °C and pressed* at iso-electric pH 5
Moisture, %	89.3^{a}±1.5	68.5^{b}±1.1	66.1^{b}±0.9
Water soluble protein, % of total protein	9.2±2.1	–	–
5% salt soluble protein, % of total protein	20.4^{a}±2.5	22.0^{a}±0.5	23.5^{a}±1.5
Total protein, %	9.8^{a}±0.5	26.1^{b}±1.4	28.8^{b}±2.2
Gel strength, gcm	10^{a}±5	452^{b}±35	$>500^{c}$
Loss of protein, %	–	15^{a}±3	11^{a}±2
Yield , % of mince (w/w)	–	37^{a}±2.5	33^{a}±2.0

(n=3), Values in the same row with different superscripts differ significantly (p<0.5); * pressed at 0.064 kg/cm^2

2000). TMAN, TVBN and histamine contents in acceptable meat were below permissible limit of 10, 30 and 20 mg% respectively (Connell 1980, Anon 1990). After initial reduction in TBC and HFB, the increasing trend was noticed with comparatively higher rate in samples in sorbate solution. The loss of sorbate due to interaction with amine, amino acids and protein broken down compounds in stored meat might be responsible for delayed increase of TBC and HFB (Karel 1973).

Effect of NaCl on gel strength Fish meat contained 1–1.5% salt (Table 1). Figure 6 shows that 2% added salt (NaCl) in meat produced strongest gel due to its combined effect with salt present in meat. Pan et al (1980) reported the use of 3–4% salt in minced squid to prepare strong gel with maximum breaking strength. Fish meat contained 180 mg % Ca and 225 mg% P (Table 1). Ca plays a significant role in the formation and stabilization of actomyosin, a key component in the formation of fish gel (Suzuki 1981).

Effect of temperature in dewatering Figure 2 shows that Lowry et al (1951) method recorded almost same value of protein in both direct and successive salt soluble extraction. Successive extraction of water soluble and salt soluble protein from meat showed that their proportion in total protein was 9.2±2.1 and 20.4±2.5%, respectively (Table 2) by Lowry et al. method (1951). Salt soluble protein, mainly myofibril protein, is responsible to good gel forming ability of meat (Suzuki 1981, Chakrabarti and Gupta 2000, Raman and Mathew 2005). Dewatering reduced water soluble protein and increased myofibril protein content in meat; thus good gel strength 450–500 gcm was noticed in pressed fish meat at moisture level 66–68%. Table 2 shows that washing mince at 0–5 °C followed by pressing at iso-electric pH retained more protein with better functional quality (Connell 1968). Thus gel strength of meat increased to 500 gcm and above which was more than the same operation at ambient temperature (25–28 °C). Loss of protein with wash water was comparatively less at 0–5 °C. High bacterial load in muscle (Fig. 1) might be responsible for breaking down of tissue protein to soluble components at faster rate at ambient temperature than that at 0–5 °C.

Conclusion

pH 5 was found to be pH of minimum solubility (iso-electric pH) of Bombay duck protein. Citric acid- sodium citrate buffer (pH 5) was very effective in reducing moisture in dressed fish; addition of 0.2% potassium sorbate enhanced the shelf life up to 4 days at ambient temperature (25–28 °C). Reduction in moisture in meat improved its cooking quality and increased protein content and gel formation capacity. The spent buffer can be reused after filtration and addition of components in proportion. Fish meat produced stronger gel by using 2% NaCl than conventionally prepared gel with 4% NaCl, because of presence of 1.0–1.5% NaCl in fresh muscle. The absorbance at 280 nm in measuring protein in water/salt extract of Bombay duck meat was too high due to release of interfering substances such as nucleotides and their broken down compounds from rapidly damaged cells. Washing of fish mince with cold water, followed by pressing at pH 5 gave fish cake with more salt soluble protein and better gel strength than the same operation done at ambient temperature. Bombay duck meat could be suitably used in preparation of wide range of fish gel products similar to the products from sciaenids, lizard fish and others.

Acknowledgements Author is grateful to Meenakumari B, Director, Central Institute of Fisheries Technology, Kochi for permission to publish this paper. Technical assistance rendered by Gaikwad S, Triveni A, Bait P and Waghmare T is thankfully acknowledged.

References

Anon (1990) Proposal for a council regulation (EEC/C2) laying down the health conditions for the production and the placing on the market of fishery products. Official J EEC / C_2 84:58–70

AOAC (1975) Official methods of analysis, 12th edn. Association of Official Analytical Chemists, Washington DC

Chakrabarti R, Gupta SS (2000) Characteristics of gel from meat of twelve species of fish from Visakhapatnam coast. Fish Technol 37:5–7

Chakrabarti R, Varma PRG (2000) The sensitivity of halotolerant *Aspergillus flavus*, *Aspergillus niger* and *Penicillium sp*. to propionate, sorbate and benzoate. J Food Sci Technol 37:72–74

Connell JJ (1968) The effect of freezing and frozen storage on the proteins of fish muscle. In: Hawthorn J, Rolfe EJ (eds) Low temperature preservation of foodstuffs. Pergamon, Oxford, pp 333–358

Connell JJ (1980) Control of fish quality, 2nd edn. Fishing News Books Ltd, Farnham, pp 137–138

Conway EJ (1950) Microdiffusion analysis and volumetric error, 3rd edn. Crossby Lock Wood and Sons Ltd, London, pp 87–107

Doeshurg UJ, Lamprecht EC, Elliott MC, Reid DA (1969) The use of sorbic acid in salted fish. J Food Technol 4:339–341

Dyer W, French HV, Snow JM (1950) Proteins in fish muscle. I. Extraction of protein fractions in fresh fish. J Fish Res Board Can 7:585–596

Fiske CH, Subbarow Y (1925) The colorimetric determination of phosphorus. J Biol Chem 66:375–400

FAO (2003) Year book of statistics (1992–2001). Food Agriculture Organization, Rome

Karel M (1973) Protein interactions in bio-systems: protein–lipid interaction. J Food Sci 38:756–763

Kakatkar A, Sharma A, Venugopal V (2003) Hydration of muscle proteins of Bombay duck (*H. nehereus)* during acetic acid induced gelation and characteristics of gel dispersion. Food Chem 83:99–106

Kondoran MK, Solanki KK, Venkataraman R (1969) Studies on the storage behavior of laminated and commercial Bombay duck. Fish Technol 6:140–147

Lowry OH, Resebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin Phenol reagent. J Biol Chem 193:265–275

Niven CF Jr, Jeffrey MB, Corlett DA (1981) Differential plating medium for quantitative detection of histamine producing bacteria. Appl Env Microbiol 41:321–322

Okada M (1959) Gelometer. Bull Tokai Reg Fish Lab 24:67–68

Pan BS, Lee DJ, Lin LP (1980) Studies on a minced squid product; effect of raw materials and ingredients on the texture of the product. In: Connell JJ (ed) Advances in fish science and technology. Fishing News Books Ltd, Farnham, pp 434–437

Radhakrishna AG, Solanki KK, Venkataraman R (1973) Preliminary studies on freezing characteristics of Bombay duck (*Harpodon nehereus*). Fish Technol 10:124–130

Raman M, Mathew S (2005) Effect of structural protein on the textural characteristics of rohu and squid at various cooking temperatures. J Food Sci Technol 42:430–433

Shewan JM, Macintosh RG, Tucker CG, Ehenberg ASC (1953) The development of a numerical scoring system for the sensory assessment of spoilage of wet fish stored in ice. J Sci Food Agric 4:283–297

Snedecor GW, Cochran WG (1967) Statistical methods. Oxford and IBH Publ Co, Calcutta, pp 33–65

Suzuki T (1981) Fish and krill protein. Applied Science Publ Ltd, London, pp 62–67

Walk TS, Walk E (1969) Bio-chemistry and molecular biology, vol 1. Academic, New York, pp 365–371

Winton AL, Winton KB (1958) The analysis of foods. Wiley, London, pp 800–801

Warrier SBK, Ninjoor V, Nadkarni GB (1985) Involvement of hydrolytic enzymes in the spoilage of Bombay duck (*Harpodon. nehereus*). In: Proc Sym Harvest and Post harvest Technology of Fish organized by Soc Fish Technol (India), 24–27 November 1982, Kochi, p 470–472