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Effect of Washing on Composition and Properties of Proteins from Tilapia (*Oreochromis mossambicus*) Meat

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The effect of washing on the properties of proteins from tilapia *(Oreochromis mossambicus*) meat has been assessed. The composition of washed meat revealed higher moisture content (86.29%) and lower total protein content (12.80%) compared to unwashed meat. There was reduction in non- protein nitrogen constituents in washed meat. Washing could remove low molecular weight components as revealed by gel filtration profile. The reduced viscosity at 3 mg ml^{-1} of total proteins of washed meat was found to be higher (0.125 ml mg^{-1}) than unwashed meat indicating concentration of myofibrillar proteins. The SDS-PAGE pattern revealed concentration of 205KD protein (Myosin Heavy Chain) upon washing. Higher value of ATPase activity reduced drastically (from 4.1-1.18 μg P_i mg^{-1} of protein min^{-1}) after the removal of sarcoplasmic proteins. High Modori Inducing Proteases (MIPase) activity at 55° C was observed in the muscle extract that might interfere in the gelling ability in unwashed meat but it reduced in washed meat.

Key words : ***Oreochromis mossambicus,*** **washed meat, SDSPAGE, gel strength, dynamic visco-elastic behaviour**

In recent years, production of Surimi and related value-added products for the export market has got a rapid boost with the expansion of technological know-how and greater investment in the seafood sector. In India, mainly fishes of marine origin such as threadfin bream (*Nemipterus japonicus*), ribbon fish (*Trichiurus lepturus*) and lizard fish (*Saurida tumbil*) are being used for surimi production (Muraleedharan *et al*., 1997). Physicochemical and functional properties of the protein of fish intended for surimi production play a pivotal role in the end product quality. Important studies on physicochemical and functional properties of proteins extracted from Indian mackerel, *Rastrelliger kanagurta* (Mohan *et al*., 2008), that of Bigeye snapper, *Priacanthus hamrur* (Binsi *et al*., 2007), influence of pH on the solubility of muscle proteins from mullet, *Mugil cephalus* (Mohan *et al.,* 2007), rheological properties of silver carp actomyosin (Liu *et al*., 2008), changes in textural and rheological properties of gels from Nile tilapia muscle proteins induced by high pressure and setting (Hwang *et al*., 2007) and dynamic viscoelastic properties of grass carp myosin (Tao *et al.,* 2007) have been reported. Condition of the fish species and seasonal variation influence the functional properties of muscle proteins including gel forming ability (Osako *et al*., 2003; Chopin *et al*., 2007).

Freshwater species produced in bulk are being looked upon as substitute species for surimi production. Farmed tilapia, which has greatly influenced the global fish production with rapid expansion of Nile tilapia (*Oreochromis niloticus*) and mossambique tilapia (*Oreochromis mossambicus*) cultured in China, Philippines and Egypt (Hempel, 2002), has offered enough promise for becoming new candidate species for surimi production. Nile tilapia dominates global tilapia culture and its share is about 84% of total tilapia production (FAO, 2009).

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There have been scarce attempts to understand the characteristics of washed mince from *O. mossambicus* (Gopakumar *et al*., 1992; Hassan & Mathew, 1999; Ninan *et al*., 2004). Alteration in functional properties of fish proteins is an area of interest for commercial application (Mohan *et al*., 2007). In this study an attempt was made to investigate the effect of washing on important physico-chemical and functional properties of proteins of mossambique tilapia (*Oreochromis mossambicus*) and its suitability for surimi preparation.

Materials and Methods

Fresh tilapia (*Oreochromis mossambicus*) caught from natural freshwater body near Mysore, India were used for the study. Fish of mean length 21 to 28.5 cm, and mean weight of 150 to 340 g were washed and iced in the ratio of 1:1 (fish: ice) and transported to the laboratory. Head and entrails were removed manually and washed with chilled water (3°C). After washing, the meat was separated using reciprocatory type of meat separating machine (Toyo Seikan kaisha Ltd., Tokyo, Japan). The separated meat was washed in chilled tap water, (fish to water ratio 1:3). The slurry was agitated for five min and allowed to settle. The water was decanted and filtered through double layered muslin cloth. The meat was gently squeezed to remove excess water and washing process was repeated three times. At the time of third wash, sodium chloride at a concentration of 0.01% (w/w) of meat was added. After the final wash, excess water was removed by using basket centrifuge (Remi, India). After dewatering, mincing was carried out using meat mincer (Toyo Seikan kaisha Ltd., Tokyo, Japan) and meat was subjected to further analysis.

Meat was separated manually and macerated well using a pestle and mortar. The macerated meat was used for proximate composition. Moisture, crude protein, fat and ash content in the meat were determined as per AOAC (2000). The pH of washed tilapia meat sample was measured using a pH meter (Systronic 324 pH meter, Ahmedabad, India). Five grams of meat was macerated with 45 ml of distilled water, and the pH was measured. Expressible water content of fresh fish meat was determined by the method of Okada (1963). Non-protein nitrogen (NPN) content of tilapia meat was determined as per Velankar & Govindan (1958). Solubility of protein was studied by extracting the meat in 50 Mm phosphate buffer of pH 7.5 containing 1 M NaCl (1: 10 meat to buffer ratio). The meat was homogenized using an Ultra-Turrax homogenizer (Ultra-Turrax, T25, Janke & Kunkel GMBH & Co., Staufen, Germany) at 9000 rpm for 2 min. Care was taken to keep the temperature below 5°C during the experiment. The homogenate was centrifuged at 9000 x g for 15 min using a refrigerated centrifuge (Intl. equipment Co., IEC, B22, Needham Heights, Mass., U.S.A.) maintained at 4°C. The total nitrogen in supernatant was determined by the Kjeldahl method. The nitrogen value obtained was multiplied by a factor of 6.25 to obtain the protein content and expressed as a percentage of total protein.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed as described by Laemmli (1970). Two microlitre each, of the clear solution was loaded into the wells of the gel and run on a constant current mode (2mA/well) using an electrophoresis power pack (Model PS-3000, Hoefer Pharmacia Biotech Inc., USA) for 90 min. Standard Sigma markers of wide range molecular weight were loaded into wells of the gel. The gels were stained post run in comassie brilliant blue R-250 (0.025% in 40% methanol and 7% acetic acid) overnight. Gels were destained using acetic acid-methanol mixture (7% acetic acid and 2% methanol) repeatedly till protein bands were clearly visible. Molecular weight of the protein bands was calculated by measuring the relative mobility of the standard molecular weight markers.

Apparent reduced viscosity of total proteins extracted in extraction buffer was measured at 25 ± 1°C using an Ostwald's viscometer. Viscosity measurements were carried out using protein solution estimated after determining protein concentration by the Lowry method (Lowry *et al.,* 1951). The

reduced viscosity at different protein concentration was calculated as per Yang (1961). Dynamic Viscoelastic Behaviour (DVB) of tilapia meat in the temperature range of 30 to 90°C was measured using a Carri Med Controlled Stress Rheometer (CSR-500, Carri Med, Surrey, U.K.) under oscillatory mode, using a 4 cm parallel plate measuring geometry. Fish meat devoid of connective tissue, fins, and scales was macerated well using pestle and mortar. About 4 g of macerated tilapia meat was mixed with 2.5% sodium chloride (w/w) and mixed thoroughly to get a fine paste, which was used for for DVB measurement (gap between measuring geometry and peltier plate 2000 μm; 80°C using micrometer; stress, 500 Pa). The linear viscoelastic region was determined by a torque sweep with a frequency of 1 Hz. Measurements were made by applying a small amplitude oscillation (0.0005 rad) with a frequency of 1 Hz. A heating rate of 1°C min^{-1} was achieved through peltier plate of rheometer. Applied stress was compared with the resultant strain. The results of such measurement were expressed as the storage modulus (G′) and loss modulus (G"). Gel filtration profile of total proteins extracted using EB was carried out on a Sepharose 6B gel packed in a column of 1.5 x 80 cm (diameter x height) at ambient temperature (27°C). The eluant used was EB. The total bed volume of the column was 150 ml and the void volume determined by using blue dextran was found to be 50 ml. A protein concentration of 4 mg ml^{-1} was loaded into the column, and elution was carried out at a flow rate of 30 ml h^{-1}. Fractions (3 ml) were collected manually, and the concentration of the eluant was determined by measuring the absorbance at 280 nm using a spectrophotometer (Bausch & Lomb, Model 21-UVD, Austin, Tex, U.S.A.). Calcium-activated ATPase activity was determined according to the method of Noguchi & Matsumoto (1970) and expressed as microgram of inorganic phosphorus (Pi mg^{-1} protein min^{-1} at 27°C). The mixture was filtered through Whatman No. 1 filter paper and the inorganic phosphorus released was estimated as per Taussky & Shorr (1952). Sulphydral group in the tilapia meat was estimated by the Ellman's method (Ellman, 1959). The strength of the prepared gel was measured using Okada gellometer as per Okada & Yamazaki (1957). Modori Inducing Protease (MIP) was according to An *et al*. (1994).

Results and Discussion

The change in proximate composition and properties of proteins from tilapia meat subjected to three washing cycles is given in Table 1. The moisture content of washed meat was 86.29%, which was higher than unwashed meat (81.34%). The higher retention of moisture could be attributed to either hydration of myofibrillar proteins to hydrophilic residues, or to incomplete dehydration procedure. In the present study, the dehydration was achieved by basket centrifuge, which might not have reduced the moisture to the desired level. The final moisture content of commercial surimi is reported to be in the range of 78-80% (Lanier, 1992). Higher moisture content in the surimi, which affects the gelling properties, has a bearing on the textural properties of the products. A

Table 1. Effect of washing on composition and properties of proteins from tilapia meat

Parameters	Unwashed meat	Washed meat
Moisture (%)	81.34 (1.7)	86.29 (0.65)
Total protein (%)	16.72 (0.55)	12.80 (0.01)
Total fat (%)	0.88 (0.02)	0.50 (0.10)
Total ash (%)	0.99 (0.07)	0.33 (0.02)
NPN (mg 100 g^{-1} of meat)	365 (0.51)	255.50 (5.16)
Gel strength (g. cm)	569.39 (79.37)	431.46 (78.16)
Expressible water content (%) of the gel	22.36 (1.23)	23.76 (3.08)
Ca ++ ATPase activity mg pi mg^{-1} of protein min^{-1}	4.10 (0.07)	1.18 (0.06)
Sulfhydral content (mM of – SH g^{-1} of meat)	0.0029 (1.1×10^{-2})	0.00083 (2.8×10^{-6})
Extractability in EB % total protein	86.46 (5.62)	83.37 (0.52)
MIPase activity ΔA_{280}	0.271 (0.06)	0.076 (0.01)

n = 3; Values in parenthesis indicate standard deviation

reduction in protein, fat, ash and NPN content was recorded in the washed meat (Table 1). The reduction in total protein and ash content was substantial, and that of the total crude protein was comparable to other tilapia species and fresh water fishes (Siddaiah *et al*., 2001; Arekere, 1993; Akande, 1989).

The ash content, which is a measure of total mineral in the fish meat, has shown a reduction, (Table 1). This is due to the removal of water soluble mineral constituents in the meat. Similar results were reported with washing of sardine and shark meat (Suvinich *et al*., 2000; Mathew *et al*., 2002). The water washing of tilapia meat has reduced the NPN constituents, mainly, the free amino acids, trimethyl amine-oxide, small peptides, urea and other nitrogenous constituents which are mainly water soluble. The reduction in NPN is reflected in the total crude protein content after water washing of the fish meat. Water washing of fish meat reduces the water soluble protein content and facilitates the concentration of myofibrillar proteins and hence, the washed meat is generally referred to as wet concentrated myofibrillar proteins (Niwa, 1992). The proteins that could be solubilised from washed meat using high ionic strength buffer accounted for 83.37% of total proteins while that from the whole fish accounted for 86.46%. Similar results have been reported in the case of washed meat of *Nemipterus japonicus* (Karthikeyan *et al*., 2006).

The washing of tilapia meat decreased Ca^{++} ATPase activity and sulfhydral content of meat, solubility in extraction buffer and modori inducing protease activity (Table 1). The Ca^{++} ATPase activity of washed meat was reduced considerably. Connell (1960) demonstrated the contribution of sarcoplasm of the muscle cell for ATPase activity of codfish. The free sulphydral group present in the fresh tilapia meat was found to be 2.9 x 10^{-3} mM SH g^{-1} of meat. The data indicated that the free SH group present in tilapia meat was relatively lower as compared to common carp, milk fish and tilapia hybrid which was in the range of 8 x10^{-2} to 5.13x10^{-2} mM g^{-1} of meat (Sompongse *et al*., 1996; Jiang *et al*., 1988; 1989). Water washing of tilapia meat reduced the free Sulphydral (SH) content marginally. The total sulphydral content of salmon myosin was found to be 6.5 mole 10^{-5} g protein (Lin & Park, 1998). It has been shown that the sulphydral content of myosin molecule will not be altered by salt concentration because of the SH group located both on surface and interior (Lin & Park, 1998). The free sulphydral content of washed tilapia meat is considerably lower than that reported for carp myosin (Tsuchiya & Matsumoto, 1975). The reduction in MIPase activity upon washing of tilapia meat clearly indicates localization of MIPase in the sarcoplasm. Yongswatdigul *et al.* (2000) have revealed that the proteolysis of tilapia surimi occurred as the temperature increased and attains highest activity at 65°C. Enzymatic modification responsible for the changes in protein functionality in grass carp (*Ctenopharyngodon idella)* skin was reported by Wasswa *et al*. (2007).

The gel strength and expressed water content of gel was 569.39 (g cm) and 22.36% respectively for unwashed meat. The gel strength of gel prepared from washed meat gave a lower value than that of unwashed meat (Table 1). If the initial moisture content of unwashed and washed meat is taken into account, the reduction of gel strength can be attributed to higher moisture content of washed meat. This was further corroborated by higher expressible water content of gels prepared from washed meat. Increased expressible water content and lower gel strength of washed meat in comparison to unwashed croaker meat was noticed (Basavanagouda, 2001).

The apparent reduced viscosity of total proteins from washed tilapia meat as a function of protein concentration is depicted in Fig. 1. The protein concentration of 3 mg ml^{-1} (derived value) and the $Þ_{red}$ was found to be 0.125 ml mg^{-1}. The decrease in solubility of total proteins of washed tilapia meat in high ionic strength buffer could have different viscosity profile. The reduced viscosity of total protein as a function of protein concentration gave a positive slope. This value was higher than the value obtained for unwashed meat. This is due to concentration of myofibrillar proteins upon washing, which mainly

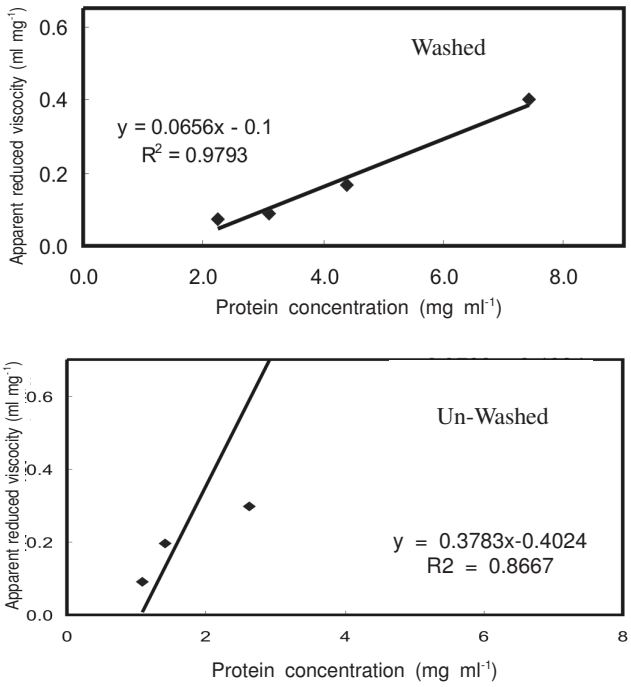

Fig. 1. Apparent reduced viscosity of total proteins from washed and unwashed tilapia meat as a function of protein concentration

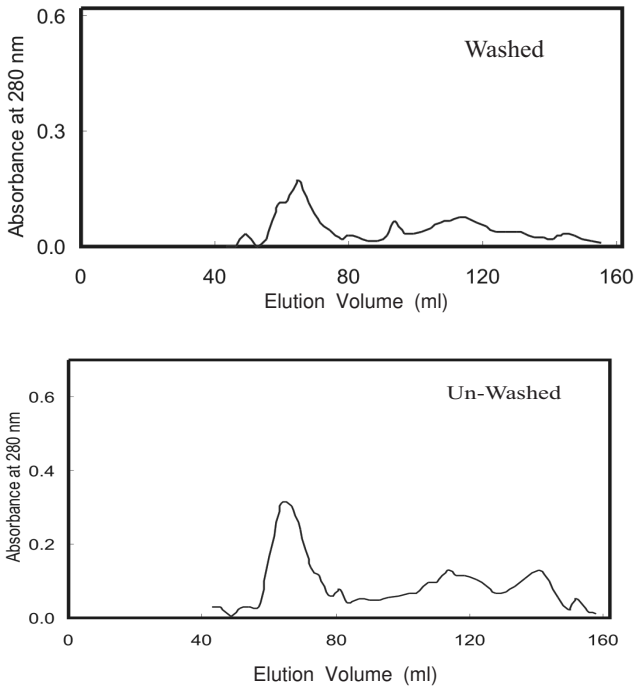

Fig. 2. Gel filtration profile of total proteins from washed and un-washed tilapia meat with as eluant

comprises of rod shaped protein like myosin, which offers more resistance to flow. The gel filtration profile of total proteins from fresh washed tilapia meat (Fig. 2) indicated a high molecular weight component eluting at elution volume of 65.16 ml and a low molecular weight component eluting at elution volume of 113.19 ml. It is clear from the profile that the concentration of low molecular weight component is lower. The total proteins from washed meat comprised of high molecular weight components as revealed by gel filtration profile. The SDS-PAGE pattern of total proteins from washed tilapia meat (Fig. 3) revealed multiple bands in the molecular weight range of 205- 36 KD. The intensity and concentration of bands in washed meat varied when compared to the unwashed fish meat. The thick band in the 205 KD range is myosin heavy chain (Fig. 3) and bands with 97 KD and 84 KD are predominant as compared to other bands. The electrophoretic pattern of washed meat clearly indicated concentration of myosin heavy chain and the concentration increased with the increase in washing cycles. SDS-PAGE pattern of water after first and third

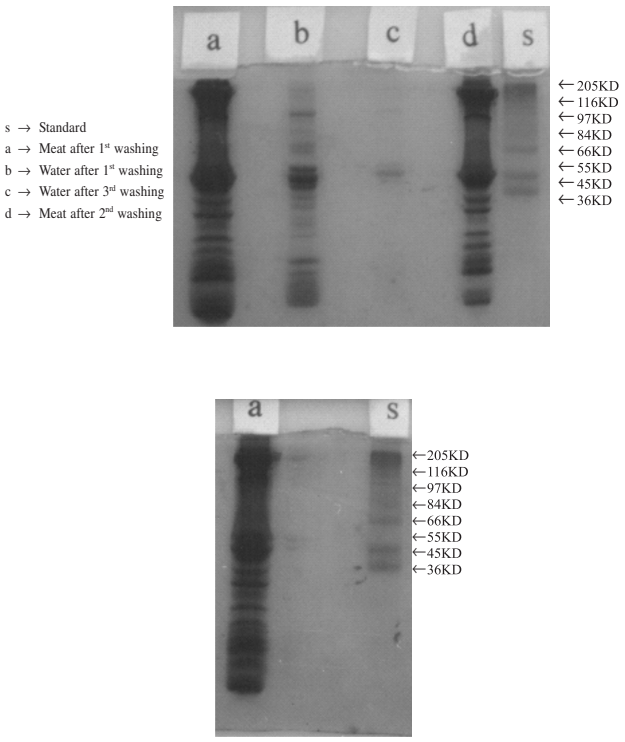

Fig. 3. SDS-PAGE pattern of total proteins from washed and un-washed tilapia meat

wash (Fig. 3, lane b and c) clearly demonstrated the removal of sarcoplasmic proteins as revealed by number of bands in the pattern.

The dynamic viscoelastic behaviour of washed tilapia meat in the temperature range of 30 - 90°C is given in Fig. 4. The storage modulus value (G′) increased with increase in temperature and rate of increase was found to be maximum between 63.3 and 70°C. The maximum loss modulus (G) value of 27.41 K Pa was recorded at 76.6°C. The tan δ values decreased with increase in temperature during heating regime. In the present study, the temperature at which sol-gel transition occurred was not indicated clearly. The dynamic viscoelastic behaviour of washed tilapia meat in the temperature range of 30-90°C indicated a lower G′ value throughout the heating regime in comparison to unwashed meat. The initial tan δ value was higher in the washed meat than that of the unwashed meat. This clearly indicates that the higher moisture content of washed meat is coming in the way of higher

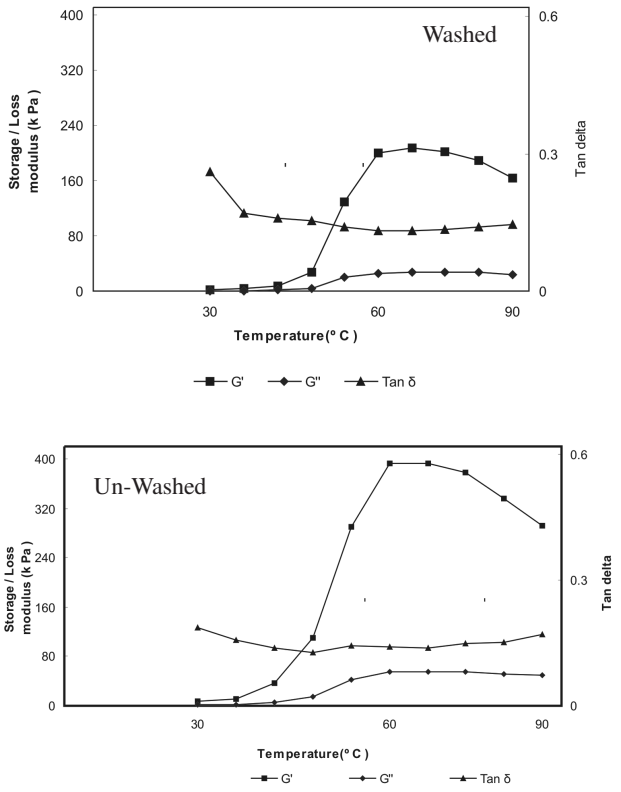

Fig. 4. Dynamic visco-elastic behaviour of washed and un-washed tilapia meat

gelling ability of tilapia meat. Gelation of food protein is dependent on nature of protein, pH, ionic strength, binding agents and rate of heating (Wang & Xiong, 1998).

To summarize, the composition of washed meat revealed higher moisture content (86.29%), lower total protein content (12.80%), fat and ash content and lower gelling ability. It might be due to higher washing cycles which led to extensive hydration of myofibrillar proteins, with guided lower gelling ability of washed meat.

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