Fishery Technology
2002, Vol. 39(2) pp : 114 - 119

Behaviour of Mrigal (*Cirrhinus mrigala*) Meat Components during the Washing process in the Preparation of Surimi

T.V. Sankar and A. Ramachandran*
Central Institute of Fisheries Technology
Cochin - 682 029, India

To elucidate the effect of washing, on flesh components, mrigal flesh was washed through one, two and three washing cycles.. Washing resulted in absorption of water (1-3%) and loss of fat (49%). 35% loss of soluble protein (SP) was noticed in the first washing itself and the loss is almost equally shared by the sarcoplasmic (18% of SP) and the myofibrillar proteins (17% of SP). The subsequent washings removed small portions of water-soluble sarcoplasmic proteins resulting in the concentration of myofibrillar proteins. 73% of the soluble protein was retained in the flesh after three washing cycles. The protein had undergone marginal conformational changes as reflected by the decrease in the actomyosin Ca^{2+} ATPase activity. The rheological properties of the washed flesh were, however, significantly better than that of the unwashed mince.

Key words: Mrigal, washing, flesh composition, protein loss, Ca^{2+} ATPase activity, rheological properties

Surimi, an important intermediate in the production of fabricated seafood products, is usually made from white fleshed and low fat fish species. The versatility of surimi coupled with the shortage of Alaska Pollack (*Theragra chalcogramma*) have prompted processors to explore the suitability of other species for surimi production. (Kim *et al.*, 1996). Threadfin bream (*Nemipterus japonicus*), lizard fish (*Saurida thumbil*) ribbon fish and barracuda (*Sphyraena* sp.) are some of the species from Indian waters used for surimi preparation (Muraleedharan, *et al.*, 1997).

Comminuting the fish damages the tissue structure leading to complex system of changes in the muscle. Mincing accelerates decomposition, aggregation and cross-linking of myofibrillar protein (Laird *et al.*, 1979) with consequent decrease in protein solubility, water holding capacity and other rheological properties. Washing the fish meat in chilled water during surimi preparation, removes solubles which interfere with the storage and rheological characteristics (Lee, 1984) and also inhibits the freeze denaturation of proteins (Suzuki, 1981; Shimizu & Fujita, 1985).

Though, considered as quality fish, fresh water fish species have limited acceptance in India due to its muddy or seaweed odour. Of late freshwater fish farming is getting importance and the increased landings demand alternate processing methods. Sankar & Ramachandran (1998) studied the suitability of the fresh-water carp, *Catla catla* for preparation of surimi. The objective of this study is to investigate the effect of washing on the muscle components of freshwater carp, mrigal (*Cirrhinus mrigala*), in relation to the gel forming ability.

Materials and Methods

Mrigal (*Cirrhinus mrigala*, average weight, 460 g), obtained from a local fish farm, were partially iced and kept for 24 hours to resolve the rigor and the post rigor fish was taken for the study. The fish was

* School of Industrial Fisheries, Cochin University of Science & Technology, Cochin - 682 016, India.

thoroughly washed and the flesh removed using a meat-picking machine (Baadar 694) fitted with a drum of 2 mm perforation. The flesh was subjected to washing once, twice and three times in four volumes of chilled water of less than 5°C (Fig.1). After stirring the suspension for 10 minutes, the flesh was trapped by a nylon screen. The recovered flesh was re-suspended in water for second and third washing. The water for final washing contained 0.1% NaCl and the mince was dehydrated using a manual screw press. The flesh and washed flesh were kept cooled (<5° C) pending analysis.

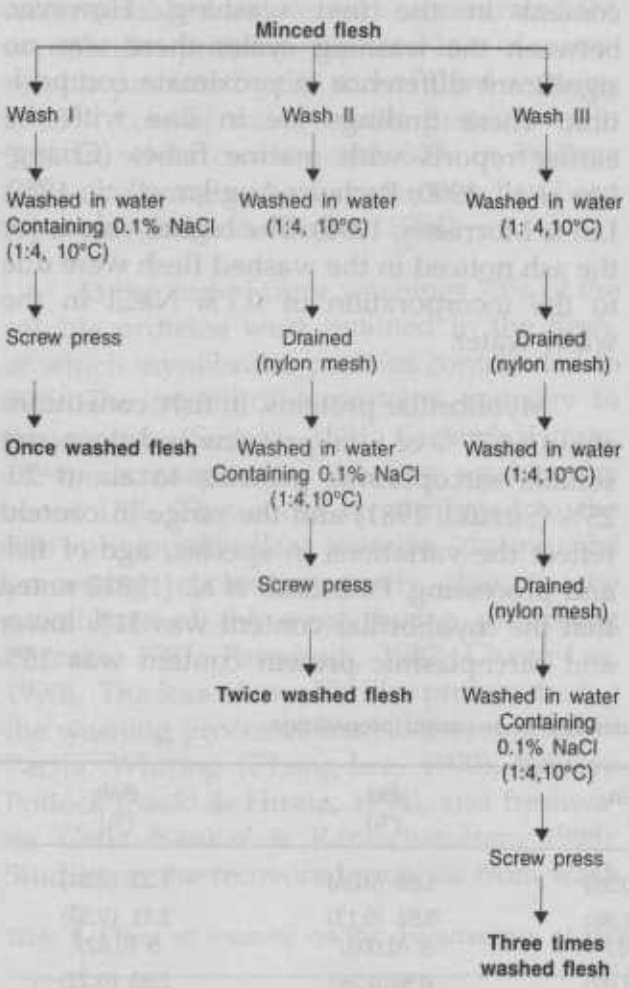

Fig. 1. Washing schedule for fish flesh

Moisture, total nitrogen, lipid and ash, were analysed using AOAC (1990) procedures. The salt soluble proteins were extracted (King & Poulter, 1985) by homogenising the meat in 5% (w/v) NaCl containing 0.02M $NaHCO_3$ (1:20, meat to buffer, pH 7.5) for 60 seconds followed by centrifugation at 7,500 rpm for 20 minutes at 5°C. The supernatant was taken as salt soluble protein. The water-soluble sarcoplasmic proteins were extracted in the same way using 0.02M $NaHCO_3$ (pH 7.5). Protein content was estimated by Biuret method (Gornall *et al.*, 1949). The difference between salt soluble protein and sarcoplasmic protein was taken as myofibrillar protein.

The Ca^{2+} activity of the actomyosin (Jiang *et al*, 1987) was followed to study the extent of denaturation. The Ca^{2+} ATPase assay was performed at 25° C in a mixture containing 1 ml actomyosin (1mg/ml), 0.5 ml 0.5 M Tris maleate buffer (pH 7.0), 0.5 ml of 0.1M $CaCl_2$, 7.5 ml of distilled water and 0.5 ml of 20mM ATP solution. The reaction was stopped 3 minutes after addition of substrate by adding tricholoroacetic acid to a final concentration of 5%. The inorganic phosphate liberated was measured by the method of Fiske and Subbarow (1925).

The heat-induced gels were prepared (Lee, 1984) by grinding the washed mince with 3% NaCl for 3 minutes in a pre-cooled mixer grinder. Care was taken to prevent the increase in temperature beyond 15°C. The paste was filled manually into polypropylene tubing of 6.0 cm diameter, taking care to eliminate the trapped air as much as possible. The ends of the tubes were tied and cooked by immersion in a boiling water bath for 30 minutes. The gels thus formed were cooled at once in ice and then kept at 5°C over night and analysed.

A 3 mm slice of the gel was subjected to folding test (Lee, 1984) by folding between thumb and index finger and depending on the breakage they were graded as AA, A, B, C, etc. The gel strength (force x deformation, g.cm) and compressibility (g) were followed using 'SUN' rheometer using a 2.5 cm long gel.

The data were statistically analysed using the Microsoft Excel software. Data

were analysed for significant difference by using one-way analysis of variance.

Results and Discussion

Fresh mrigal flesh was reported to have a moisture content of 76 -79% (Mukundan, *et al.*,1986; Jose Joseph *et al.*, 1990). The initial higher moisture content of the fish flesh noticed in this case was due to icing of the fish for 24 hours to resolve rigor prior to processing. Similar increase in moisture in deboned fish was reported during the processing of iced pacific herring (ChangLee *et al.*, 1990). The water-soluble sarcoplasmic protein formed about 35% of the total soluble proteins and the myofibrillar protein about 65%. The freshwater fish catla (*catla catla*) had slightly higher soluble proteins and water soluble protein contributed to 50% of the total soluble protein (Sankar & Ramachandran, 1998).

The components of the fish flesh undergo changes (Table 1) during washing. The flesh absorbed water during the process and in the initial washing, only a marginal increase in the water content was noticed. The initial higher water content as a result of icing might have contributed to this. About 3-4% water retention was noticed in the subsequent washings. Removal of soluble components by washing did not really affect the protein content in the first wash, which however, decreased by 4-5% in the subsequent washings. Such loss of crude protein during washing has been reported during the preparation of surimi from Northern Squafish (Lin and Morrissey, 1995). About 49% of the lipids were removed in the first washing itself and the subsequent washings removed additional 3-6% only. However, this is of less significance considering the low fat content of the mrigal meat. The changes in the proximate composition of the washed flesh showed (Table 1) significant difference on comparing with that of unwashed mince except for the moisture content in the first washing. However, between the washing cycles there was no significant difference in proximate composition. These findings are in line with the earlier reports with marine fishes (Chang-Lee *et al.*, 1990; Pacheco-Augilar *et. al.*, 1989; Lin & Morrissey, 1995). The higher values for the ash noticed in the washed flesh were due to the incorporation of 0.1% NaCl in the wash water.

Myofibrillar proteins, in fish, constitutes about 66-77% of total proteins and the water soluble sarcoplasmic proteins to about 20-25% (Suzuki, 1981) and the range in content reflect the variations in species, age of fish and processing. Patashnik *et al.* (19812 noted that the myofibrillar content was 11% lower and sarcoplasmic protein content was 15%

Table 1. Mean proximate composition (% wet wt) of flesh derived from surimi preparation

Samples	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
Unwashed flesh	82.3 (1.14)	16.18 (0.38)	1.06 (0.06)	1.22 (0.24)
Once washed	83.60 (0.87) NS *(0.19)*[a]	15.05 (1.96) S *(0.01)*[a]	0.54 (0.13) S *(0.003)*[a]	2.11 (0.53) S *(0.02)*[a]
Twice washed	86.17 (0.36) S *(0.005)*[b]	12.05 (1.07) S *(0.005)*[b]	0.51(0.26) S *(0.02)*[b]	2.03 (0.22) S *(0.01)*[b]
Thrice washed	85.65 (1.46) S *(0.06)*[c]	12.78 (0.97) S *(0.003)*[c]	0.48 (0.05) S *(0.0001)*[c]	2.28 (0.18) S *(0.004)*[c]

Values are mean of three determinations with standard deviation

The level of significance (a,b,c) calculated against the control unwashed flesh.

NS – not significant; S – significant

The values within the washed groups Protein content were significant (*0.04*). Moisture (*0.05*), fat (*0.91*) and ash (0.42) were not significant.

Bold & italic figures in parenthesis indicate p - value

higher in parasitised Whiting than in uninfected fish flesh. The total soluble proteins in the mrigal flesh accounted to about 76% on dry basis. The water-soluble sarcolplasmic proteins contributed to 35% of total soluble proteins and the myofibrillar proteins 65%. Removing the lipids and water-soluble constituents from the flesh concentrates the myofibrillar protein content. The soluble proteins were reduced by 35% in the first washing itself and the loss was almost equally shared between sarcoplasmic and myofibrillar proteins (Table 2). The subsequent washings removed only the water-soluble fractions and concentrated the myofibrillar proteins. The total loss of water-soluble proteins by the end of third washing is 58%. Loss of water-soluble proteins up to 50% in white croaker and 60% in Walleye Pollock during surimi production has been reported (Saeki & Hirata, 1994).

At the end of three washings 73% of the soluble proteins were retained in the flesh, of which myofibrillar proteins contributed to 59%. The myofibrillar proteins, contrary to the reports (Suzuki, 1981; Saeki & Hirata, 1994) decreased after the first washing by about 17%. This could be attributed to the loss of myofibrillar proteins during the processing cycles or partly due to the proteolysis of the meat during processing (Suzuki, 1981; Patashnik, 1982; Chang-Lee, 1990). The loss of myofibrillar protein during the washing processes have been reported in Pacific Whiting (Chang-Lee, 1990), Walleye Pollock (Saeki & Hirata, 1994), and freshwater Catla (Sankar & Ramachandran, 1998). Studies on the recovered proteins from wash water also revealed the considerable loss of myofibrillar proteins during surimi processing (Lin *et. al.*, 1995).

The myofibrillar ATPase is located in the myosin head region. The ATP hydrolysing activity of myosin is used to explain the conformational changes taking place in the myosin molecule even though it does not necessarily imply the unfolding of protein chain (Chan *et al.*, 1995). The ATPase activity of the actomyosin from washed flesh decreased by 25% during the washing process (Table 2). This could be due to the conformational changes that might have taken place in the myosin molecule as a result of processing. Takashiki *et al.* (1962) found that the helical content myosin molecule decreased by only a few percent even after ATPase activity completely disappeared. Hence, the loss of ATPase does not necessarily relate to the unfolding of myosin molecule but indicate only the conformational changes in the myosin molecule. In this case, even though, a marginal loss in ATPase activity is noticed, the washed flesh still retained its gelling properties.

The unwashed mince itself was found to have good elastic characteristic and gelling properties. The gelling properties such as gel strength and compressibility of the washed flesh, however, increased as a result of washing (Table 3) besides the improvement in colour of the flesh. The statistical analysis of the rheological data (Table 3) showed a significant difference ($P<0.001$) in both gel strength and compressibility when the heat-induced gels from

Table 2. Effect of washing on the characteristics of protein in mrigal flesh

	Water soluble protein* (g/100g flesh)	Salt soluble Protein* (g/100g flesh)	Myofibrillar protein* (g/100g flesh)	ATPase activity u mols Pi/mg/min.
Unwashed flesh	27.23 ± 2.69	76.44 ± 15.9	49.21 ± 13.35	0.41 ± 0.18
Once washed flesh	14.15 ± 1.41	49.57 ± 7.69	35.43 ± 8.02	0.39 ± 0.05
Twice washed flesh	12.11 ± 2.74	57.89 ± 3.44	45.78 ± 1.04	0.34 ± 0.05
Thrice washed flesh	11.29 ± 1.17	56.37 ± 0.80	45.08 ± 1.60	0.32 ± 0.07

Values are mean of three determinations with standard deviation *on dry weight basis

Table 3 Effect of washing on the rheological properties of mrigal flesh

	Unwashed	Washing cycles I	II	III
Folding test[a]	AA	AA	AA	A
Gel strength, (g x cm)	246 ± 5.0	522 ± 12.0 S (P<0.001)	513 ± 7.0 S (P<0.001)	498 ± 9.0 S (P<0.001)
Compressibility, (g)	85 ± 3.0	256 ± 9.0 S (P<0.001)	252 ± 4.0 S (P<0.001)	213 ± 9.0 S (P<0.001)

Value are mean of three determinations with standard deviation.

[a] Folding test grading - AA - no breakage on folding twice; A – breaks on second folding; B - breaks on folding once, C - breaks to finger touch

The level of significance calculated against the control unwashed flesh.

NS – not significant; S – significant

The values within the washed groups compressibility data were significant (P<0.001) and gel strength were not significant (P<0.1)

washed flesh with cryo-protectants were compared with that of unwashed mince. However, there was no significant (P <0.1) difference in gel strength between the washed flesh from different washing cycles but the compressibility data were significantly different (P<0.001). It was stated that the gel degradation was promoted in lizardfish at a higher rate in washed mince rather than in unwashed mince in all seasons (Itoh *et al.*, 1995; Itoh *et al.*, 1997) signifying the characteristic of fish species. The gel characteristics are also related to the water content of the flesh and an inverse relationship was noticed between the gel characteristics and moisture content of surimi from different fishes (Reppond & Babbit, 1997).

In conclusion, there is considerable loss in salt extractable protein, with a marginal loss of myofibrillar protein in the first washing itself. Washing resulted in the conformational changes in fish actomyosin to some extent, as shown by the decrease in the Ca^{2+} ATPase activity. However, the elasticity of the gel, the gel strength and compressibility of the fish flesh showed an increase in the first washing. Subsequent washing, however, did not increase these properties signifying that single washing step with meat to water ratio of 1:4 is ideal for washing original meat for surimi manufacturers.

References

Grantham, G.J. (1981) FAO fisheries technical paper No. 216, FAO, Rome, 72 p.

AOAC (1990) Official *Methods of Analysis*, 15th edn., Association of Official Analytical Chemists, Arlington, Virginia

Chan, J.K., Gill, T.A., Thompson, J.W. & Singer, D.S. (1995) *J. Food Sci.* **60**, 1248.

Chang-Lee, M.V., Lampila, L.E & Crawford, D.L. (1990) *J. Food Sci.* **55**, 83.

Fiske, C.H. & Subbarow, Y. (1925) *J. Biol. Chem.* **66**, 375.

Gornall, A.C., Bardawill, C.J. & David, M.M. (1949) *J. Biol. Chem.* **177**, 75.

Itoh, Y., Maekawa, T., Suwansakornkul, P & Obatake, A. (1995) *Fisheries Science* 61, 942.

Itoh, Y., Maekawa, T., Suwansakornkul, P & Obatake, A. (1997) *Fisheries Science* 63, 286.

Jiang, S., Tsao, C. & Lee, T., (1987) *J. Agric. Food Chem.* **35**, 28.

Jose Joseph, Perigreen, P.A., Surendran, P.K., & Gopakumar, K., (1990) in Proceedings of *Chilling and Freezing of new fish products, International Institute of Refrigeration, 117 – 121, Aberdeen, UK,*.

Kim, J.H., Liu, C.H., Eun, J.B., Park, J.W., Oshim, R., Hayashi, K., Sakine, A.M.,

Hoshikita, Y., Fujimoto, K., Alkawa, T., Welch, C., & Wong, R., (1996) *J. Food Sci.* **61**, 428.

King, D.R., & Poulter, R.G., (1985) Trop. Sci. **25**, 79.

Laird, W.M., Mackie, I.M & Hattula, T. (1979) in *Advances in Fishery Science & Technology*, (Connell, J.J, Ed.), Fishing news books, Farnharm, 428-434.

Lee, C.M. (1984) *Food Technol.* **38**, 69.

Lin, D. & Morrissey, M.T. (1995) *J. Food Sci.* **60**, 1245.

Lin, T.M., Park, J.W., & Morrissey, M.T. (1995) *J. Food Sci*, **60**, 4.

Mukundan, M.K., Radhakrishnan, A.G., Jose Joseph & Antony, P.D, (1986), *Fish. Technol.* **23**. 189.

Muraleedharan, V, Antony, K.P., Perigreen, P.A. & Gopakumar, K. (1997) *Trop. Sci.* **37**, 99.

Pacheco-Aguilar, R., Crawford, D.L., & Lampila, L.E. (1989) *J. Food Sci.* 54, 248.

Patashnik, M., Groninger, H.S., Barnett, Jr. H., Kudo, G., & Koury, B., (1982) *Mar. Fish. Rev*, **41**, 25.

Reppond, K.D. & Babbit, J.K. (1997) *J Food Sci.* **62**, 33.

Saeki, H. & Hirata, F. (1994) *Fisheries Science*, **60**, 335.

Sankar, T.V. & Ramachandran, A. (1998) in Proceedings of the *Asia Pacific Fisheries Commission* (APFIC) symposium, Beijing, People's Republic of China, RAP Publication 1998/24, FAO, 135.

Sano, T., Ohno, T., Otsuka-Fuchino, H., Matsumoto, J.J & Tsuchiya, T. (1994) *J. Food Sci.* 59, 1002.

Shimizu, Y & Fujita, T. (1985) *J. Japan. Soc. Sci. Fish.* 51, 1187.

Suzuki, T. (1981) in *Fish and Krill protein: Processing technology*, Applied science publishers Ltd., London.

Takahashi, K., Yasui, T., Hashimoto, Y., & Tonomura, Y., (1962), *Arch. Biochem. Biophysics*. 99, 45.