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Effect of different washing conditions on the gel forming ability of tilapia (*Oreochromis niloticus*) mince

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Abstract. This study was carried out to observe the effect of washing on tilapia mince. To evaluate the effects of washing, salt-ground (3% NaCl) paste in tubes was incubated in water baths at temperatures of 20, 30, 40, 50, 60, 70and 80°C for 2 hours (one-step heating) followed by 30 min heating at 90°C (two-step heating) to make gel. The resulting suwari gels were subjected to instrumental puncture test. The optimum setting temperature to obtain high quality finished products from surimi was found to be 40°C for one-step heating and 50°C for two-step heating. A two-step heating schedule of incubation at 50°C/2 h and cooking at 90°C/30 min gave high textured good quality suwari gel for both unwashed and washed mince. Washed mince surimi is always superior to unwashed mince surimiin gel quality. In order to find out an efficient washing technique of tilapia mince, a series of experiments on washing solution (0%, 0.05%, 0.1%, 0.15% and 0.2% NaCl), washing period (5, 10 and 15 min.) and washing frequency (1 wash, 2 wash , 3 wash and 4 wash) were conducted. A significantly good textural and sensory quality ($p<0.05$) of gel and a fine grade surimi was obtained from tilapia mince washed with 0.1% NaCl solution. Two times washing gave the best result. Washing time limited within 10 min (6 min agitation, 4 min settling) was found to give good quality surimi.
Key words: Washing effect, Gel forming ability, Tilapia mince

Introduction

The gel forming ability of the fish varies from species to species and within the species depends on the biological conditions of fish. The variation within the species is due to age, season, death condition, freshness and fishing place (Shimizu *et al.* 1981,Kurokawa 1983,Roussel and Cheftel 1988). There are a number of other factors which influence the kamaboko gel forming ability such as high fat content, instability of muscle proteins, and large amount of sarcoplasmic proteins and high proportion of dark to ordinary muscle. High fat content in the muscle weakens the gel forming ability and it is impossible to make surimi from the fishes that are not fresh even if the effective processing technique is applied (Suzuki and Watabe 1987).There is much potentialities lie on tropical freshwater fish species which could successfully be used in time in surimi industry. Freshwater fish are excellent sources of high quality protein since they are well balanced in essential amino acids and highly digestible (Karmas and Lauber 1987). The surimi making ability of many freshwater species could be upgraded by manipulating processing techniques (Onibala *et al.* 1997).

Some investigations have been done on the quality of the mince of freshwater fish for the manufacture of surimi (Kim *et al.* 1996, Onibala *et al.* 1997, Nowsad *et al.* 1999). Onibala *et al.* (1997) studied the gel forming ability and the characteristics of the protein subunit in the heat-induced gel of tilapia. High yielding exotic species has been introduced in our country to remove malnutrition since 1952. Among exotic fishes tilapia (*Oreochromis niloticus*), was first introduced from Thailand in Bangladesh. The production of tilapia is increasing day by day and this abundant production might be utilized as an alternative source of surimi raw material. The muscle of tilapia possesses all the useful qualities to be used for the production of surimi based products. One of the value-added intermediate products particularly promising is washed mince block or surimi. Washing removes water-soluble matter, lipids and blood to improve the color and flavor as well as to increase the gel strength of the surimi. The number of washing cycles and the volume of water vary with fish species, freshness of fish, type of washing unit and the desired quality of the surimi (Hall and Ahmed 1997). It was shown that the gel strength of surimi continued to increase as the number of washing cycles increased. When a longer washing time is allowed and an increased number of washing cycles are practiced, higher dilution factor and more extractable proteins will dissolve in water. Lin and Park (1997) concluded that use of leach water could be reduced by increasing the wash cycles to four and wash time to 10 min. This washing issue sufficient to remove sarcoplasmic proteins but also resulted in higher moisture content. During washing, washing solutions are another important thing. Moisture removal gradually increases when the percent salt concentration of the wash water is increased. The processing methods of some freshwater species have been developed during the last two decades, but rare attempts have been taken for the assessment on thermal gelation properties of tilapia mince and effects of different washing conditions on it. In this study, surimi was prepared from tilapia mince and the effects of different washing conditions on thermal gelation properties of surimi were studied.

Materials and Methods

Collection of experimental fish and sample preparation: Fresh tilapia (*O. niloticus*) (Plate -1 a) used in this study were collected from the local market and brought to laboratory in an insulated ice-box with ice.

Preparation of mince: The fish were weighed and then washed with clean water, beheaded, eviscerated, skinned and washed. The skinned fishes were filleted and deboned manually in iced condition (Plate 1.b) and minced by a mechanical mincer (National Meat Grinder, MK-G3NS, Matsushita Electric Industrial. Co. Ltd., Osaka, Japan) through a 1mm orifice diameter so that all bones were removed from the muscle (Plate 1.c and d). Half of the minced meat was washed with chilled (4°C) washing solution to remove lipid, enzymes, sarcoplasmic protein and other gel inhibitory substances. For washing, the mince was stirred in 5 volumes of the washing solution for 5 min for agitation and settling down before leaching (Plate 1.e).

The meat was drained and pressed in a nylon bag after leaching and excess water was removed. The unwashed and washed minces were ground with 3% NaCl in a mortar for 20 min at <10°C with ice (Plate 1.f) and a viscous paste was formed. The paste was carefully filled into a heat stable polyvinyledene chloride cylinder manually and both ends of the cylinder were wrapped with parafilm (Plate 1.g) and polyethylene sheets before chilling in iced water.

Proximate composition: Three samples from each of the unwashed and washed mince were analyzed for moisture, protein, fat and ash content by the standard procedure of Association of Official Analytical Chemists (AOAC 1990). pH of all the samples was determined from the homogeneous mixtures of sample and distillated water (1:10, w/v) using a digital Mettler Toledo pH meter.

***Sample preparation for investigation on the effects of washing conditions**:* The mince samples were washed with different washing solutions containing 0, 0.05, 0.1, 0.15 and 0.2%NaCl. The mince was stirred in 5 volumes of washing solution with 0.1% NaCl for 5 min(3 min agitation + 2 min settling), 10 min(6 min agitation + 4 min settling) and 15 min(9 min agitation + 6 min settling). The mince was stirred with 0.1% NaCl (washing solution) for 10 min for 1 time, 2 times, 3 times and 4 times. The washed minces were ground with 3% NaCl in a mortar for 20 min at <10°C with ice. Due to grinding, the mince formed a viscous paste which was carefully stuffed into poly*v*inyledene tube (2.8 cm diameter, 12.0 cm long). Both ends of the tube were wrapped by plastic paper before chilling in iced-water (2ºC).

(a)

(b)

(c)

(d)

(e)

(f)

(g)

(h)

(i)

Plate 1. Gel preparation from tilapia mince and measuring of gel strength: a. tilapia fish; b. skinned muscle of tilapia; c. preparation of mince; d. mince separation for washing; e. washing of mince; f. grinding of mince with NaCl; g. paste preparation for heating; h. heating of paste for gel preparation; i. puncture test of gel; j. folding test of gel.

***Preparation of gel**:* The paste in poly*v*inyledene chloride cylinders was heated in triplicates to produce gel. Some samples were heated once only (one-step heating) in well stirred water baths while the rests were heated twice(two-step heating) (Plate 1.h). In one-step heating, both washed and unwashed paste in the tubes were heated for 120 min in water at temperatures 20, 30, 40,50, 60, 70 and 80°C.In case of two-step heating, the first heating of the gels was done for 120 min in water at 20, 30, 40, 50, 60, 70 and 80°C which is treated as pre-heating followed by a second heating for 30minat 90°C. After the heat treatments, the samples were taken out from the water bath, kept in ice water for at least an hour.

Measurement of gel-strength: After removing the gel from the cylinder, the gels were subjected to puncture test, folding test, and teeth cutting test. Puncture test measured the breaking strength of the gel against penetration of a ball type plunger (6 mm diameter). The folding test measured the resistance against breaking along the folds when the sample disc (1mm thickness) was folded into halves and quarters and the teeth cutting test measured the resistance of the disc cutting by the incisors of members of the panel.

Puncture test: The gels were removed from the tube and cut into equal pieces of 2cm. The puncture test was done by measuring the breaking force of the gel against insertion of a ball type spherical plunger (6 mm diameter). The cut gel was placed on the pan of an electronic balance and a spherical plunger was penetrated into it. The force required to break the gel by the plunger was recorded in gram from the balance display window (Plate 1.i).

Folding test: The folding test was conducted by folding a spherical disc of thick gel cut off 1 mm in thickness and placed on the index and middle finger of the right hand. The disc was folded first into halves and then quarter by the help of thumb and forefinger (Plate 1.j). The gel was graded using the scores as described by Poon *et al.* (1981) as presented in Table I.

Table I. Grades used in the folding test of the gel

Grade	Results on folding
AA	No crack visible when disc is folded into quarter.
A	No crack when disc is folded into half but one or more cracks or breaks are visible when folded into quarter.
B	One or more cracks are visible when disc is folded into half.
C	Breaks, but does not split into halves.
D	Splits into halves when folded into half.
O	Sample too soft to evaluate.

Teeth cutting test: The disc gel of same size used in folding test was supplied to the panelists to recognize the taste by cutting it through incisors for teeth cutting test. Gel strength was evaluated by the following numeral scores as suggested by Shimizu *et al.* (1981) are presented in Table II.

Table II. Scores used in the teeth cutting test of the gel

Scores	Characteristics of the gel
0-1	Paste or mud like gel.
2-3	Very frail gel.
4-5	Frail.
6-7	Medium gel strength.
7-8	Strong gel strength.
9-10	Very strong gel.

Statistical analysis: Results of the experiment have been summarized as average ± SD from three replications. By using one-way analysis of variances (ANOVA) the treatment differences were analyzed. Duncan Multiple Range Test (DMRT) was followed to do so. The differences as represented were statistically significant i.e.; $p < 0.05$. A computer package MSTAT was used to analysis all the data.

Results and Discussion

Proximate composition of washed and unwashed mince: Tilapia is a high-moisture but moderate protein and fat content fish. Unwashed mince of tilapia had 78.92% moisture, 16.57% protein, 2.23% lipid and 1.05% ash. After washing the chemical composition was somewhat changed (Table III). Lipid is very important, as far as surimi is concerned, because of its interference with the gel formation. Lipid is at its highest level at peak feeding season and bigger fish contains more lipids (Reppond *et al.* 1995). The significant reduction in lipid content after washing could be explained by the fact that lipid substances were removed effectively by washing. Lipid content was very low (2-4%) in unwashed mince because fatty muscle and external fats were discarded from the meat prior to mincing.

Washing increased the moisture content and decreased the protein and ash contents. It was clearly found that washed muscles have tendency to retain some water during washing even washed with salt water. In this study, the washing water containing 0.1% NaCl effectively removed most of the fats and pigments from the mince. Moderate sized fish of one year or less than one year old were used for the study. Therefore, fishes might have been harvested in the peak feeding period before spawning. Generally, fish harvested during the feeding period produced the highest quality surimi. During this period, fish muscle has the lowest moisture content as well as the highest total protein. The proximate composition could not represent the composition of the whole fish or whole muscle in the present study, because only white muscle was taken for mincing. These results are more or less similar to Hossain *et al*. (2004), where washing of silver carp and pungas fish mince increased the moisture content and slightly protein content.

pH of washed and unwashed mince: The gel forming ability of fresh fish muscle is optimal at neutral pH; it decreases with the decreases in pH. In the present study, pH of the mince was near to neutral because of using pre-rigor prime quality fresh fish. All unwashed and washed mince had pH above 6.0. pH of the mince slightly increased by washing (Table III). Hossain *et al*. (2004) found a slight increase in pH after washing. Good quality gel cannot be formed if the muscle is outside the pH range 6.0-8.0 (Shimizu, 1975). Hashimoto and Arai (1978) reported that the denaturation rate of Pacific mackerel myofibrils at pH 5.8 was twice that measured at pH 6.5.

Table III. Proximate composition and pH of unwashed and washed Tilapia mince

Mince	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)	pH
Unwashed	78.92±1.10	16.57±0.57	2.23±0.09	1.05±0.09	6.52±0.47
Washed	80.08±1.12	14.84±0.28	1.08±0.05	0.56±0.09	6.6±0.12

Effect of washing on one step heating gel: The highest gel-forming ability for both unwashed and washed mince was observed at 40°C for 120 min. The breaking force was 664 g for washed mince and 502 g for unwashed mince (Fig. 1). The folding tests have been judged as grade "AA" for washed and unwashed mince. Teeth cutting score was "7" for both washed and unwashed mince. The results obtained from the present study clearly indicated that the breaking force i.e. gel-forming ability of gel prepared from washed mince was higher than that of unwashed mince. The results obtained from the present study are more or less in agreement with Ahamed (2000) who reported, in case of *O. niloticus*, and in one step heating the gel-strength highest at an incubation temperature of 40° C for 120 min in case of washed and unwashed mince. Khan (1996) also reported that the breaking force of the resulting suwari-gel of *Catla catla* and *Cirrhinus mrigala* was the highest at 40°Cincubation for 180 min and 120 min respectively. Kongpun (1999) reported that the high gel strength and high folding test of unwashed and washed Nile tilapia meat were found at 30°and 40°C of heating.

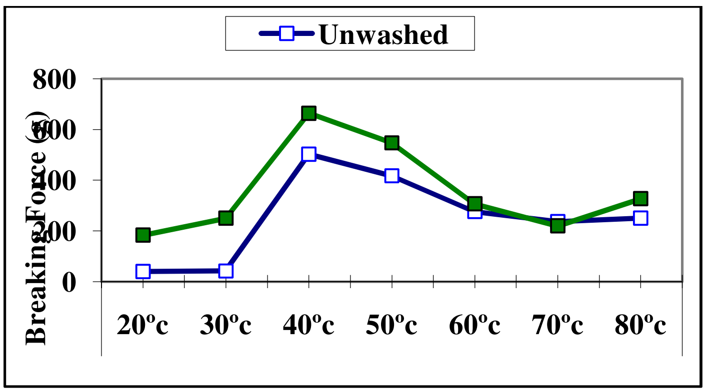

Fig. 1. Gel forming ability of tilapia in one-step heating (heated for 2 hrs)

Effect of washing on two step heating process: In two step heating process, the tubes were cooked at 90°C for 30 min after pre-heating. This cooking makes the myofibrillar proteins more compact and stable; thus whiter-appearing and good quality gel was formed.

The highest gel-forming ability for both unwashed and washed mince was found at 50°C for 120 min and further heating at 90°C for 30 min. The breaking force was 1522g for washed mince and 1321 g for unwashed mince (Fig. 2). The folding tests have been judged as grade "AA" for washed and unwashed mince. Teeth cutting score was "7" for both washed and unwashed mince. The results obtained from the study clearly indicated that the breaking force i.e. gel-forming ability of two-step heating gel prepared from washed mince was higher than that of unwashed mince. The gel-strength was found to be higher in two steps heating than in one step heating and washed mince surimi was always superior to unwashed mince in gel quality (Suwanskornkul *et al*. 1993). The results obtained from the present study are more or less in agreement with Ahamed (2000) who reported, in the case of two step heating in *O. niloticus*, gel strength was highest at 50°C for 120 min both in washed and unwashed mince. Khan (1996) reported that the breaking force of the resulting suwari-gel of *Catla catla* was highest at 50°C incubation of 180 min. The breaking force of the resulting suwari-gel of *Cirrhinus mrigala* was the highest at 50°C incubation temperature for 120 min. Nowsad *et al.,* (1999) observed that the setting ability of wild tilapia and genetically improved farmed tilapia where gels prepared by two steps heating had higher gel strength. Hossain *et al.* (2004) found that the optimum setting temperature for silver carp and pangas to obtain highest gel strength was found at 50°C. A two-step heating process (50°C for 2 hrs prior to heating at 80°C for 30 min) gave better gelling performance than one-step heating process (50°C for 2 hrs).

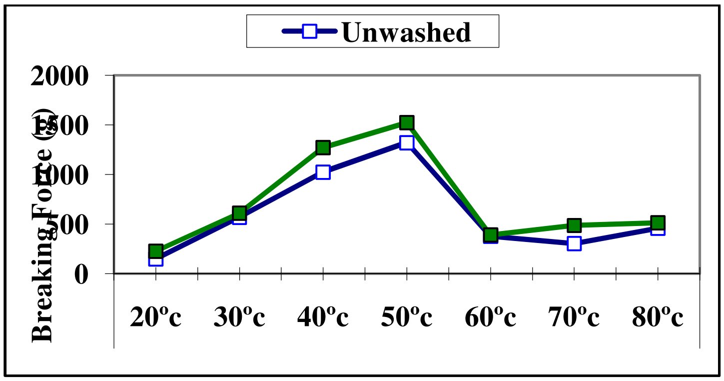

Fig. 2. Gel forming ability of tilapia in two-step heating (after pre-heating for 2 hrs further heating was done at 90°C for 30 min).

Effect of washing solution on gelation: Washing solutions distinctly improved gel quality of tilapia mince. Washing with 0% NaCl showed value of 602 g for tilapia mince. Increase of salt concentration progressed the gel forming ability. Highest gel forming ability was observed when the mince was washed with 0.1% NaCl, showing the value of 718 g. Washing with 0.15 and 0.2% NaCl reduced the gel forming ability (Figure 3). These results suggest addition of more salt during washing might cause a partial unfolding of proteins and increase the sensitivity to denaturation causing weaker gel matrix.

Several studies have been conducted using washing treatment solution to enhance the quality of fish mince. These studies stated that washing solution significantly reduced soluble proteins, pro-oxidative enzymes, lipids and increase gel forming ability and improve color properties of the final product. Wu *et al.*, (1991) showed that the least amount of myofibrillar proteins of red hake mince was solubilized at 0.1% NaCl as well as higher gel forming ability. However, Lin and Park (1996) found that washing with suitable amount of NaCl solution reduced the loss of myofibrillar proteins that ultimately resulted in inferior gel forming ability. Severe loss of MHC (Myosin Heavy Chain) occurred when the fish was washed with higher concentration of NaCl solution (Morrisey *et al*.2000). Hossain *et al*.(2004) investigated the effect of washing solution on the gel properties of silver carp and pangas and showed that to obtain a good quality surimi fish mince required to be washed with 0.1% NaCl.

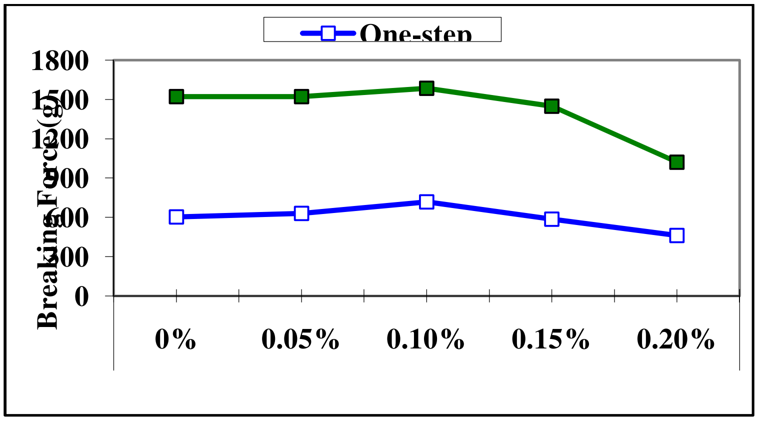

Fig. 3. Effects of washing solution (NaCl) on tilapia minces

Effect of washing period on gelation: Highest gel forming ability was obtained when fish mince was washed for 10 min (6 min agitation + 4 min settling) (Figure 4). The gel texture was severely deteriorated at 15 min or above. This result is more or less similar to Lin and Park (1997), who reported that 10 min washing time gave the highest gel forming ability with sufficient removal of sarcoplasmic proteins. This indicates that a 9-12 min agitation is adequate for washing. Hossain *et al*. (2004) investigated the effect of washing period on the gel properties of silver carp and pangas and reported that to obtain a good quality surimi fish mince washing time should be limited within 10 min (7 min agitation, 3 min settling). If the residence time is unduly prolonged, the fish meat will absorb an excess amount of water and subsequent dewatering becomes difficult. Although the optimal time varies with the freshness of the raw material, water temperature and size of the meat particles, 10-15 min for the entire leaching process is usually regarded as appropriate for a commercial operation. After the sarcoplasmic proteins are completely removed, further washing causes a severe loss of myofibrillar proteins. When a longer washing time is allowed and an increased number of washing cycles are practiced, higher dilution factor and more extractable proteins will dissolve in water.

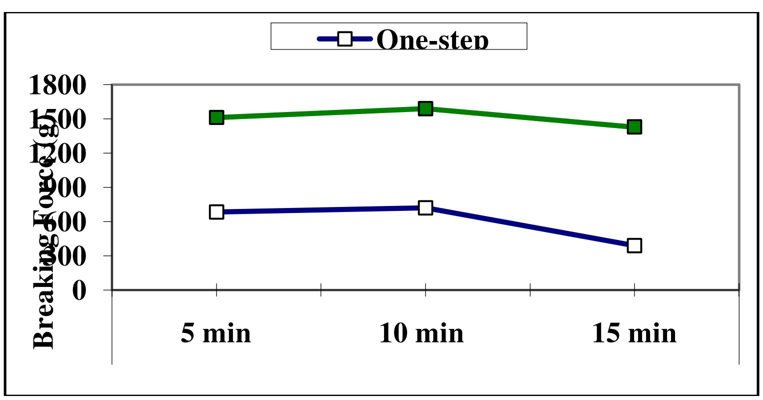

Fig.4. Effects of washing period on tilapia mince

Effect of washing cycle on gelation: We found two times washing better in terms of giving gel performance (Figure 5). Subsequent washing removed the residual sarcoplasmic proteins along with small amount of myofibrillar proteins. The number of washing cycles varies with fish species, freshness of fish, type of washing unit and the desired quality of the surimi (Lee 1984). Earlier studies observed that-the gel strength of surimi continued to increase as the number of washing cycles increased. Generally, with continued washing, the gel strength increases up to two washing cycles and thereafter levels off according to a study conducted at a 3:1 water-to-meat ratio (Nishioka 1984) indicated that the greater number of washing cycles applied to mince produced stronger gel forming ability of surimi. The author also showed that the relative amount of MHC increased as the washing was repeated. Consequently, three and four washing cycles negatively affected the relative amount of MHC in the washed meat. The majority of the soluble components are freely and rapidly removed in the first washing cycle by simple dilution of the free soluble. Lin and Park (1997) concluded that use of leach water could be reduced by increasing the wash cycles to two.

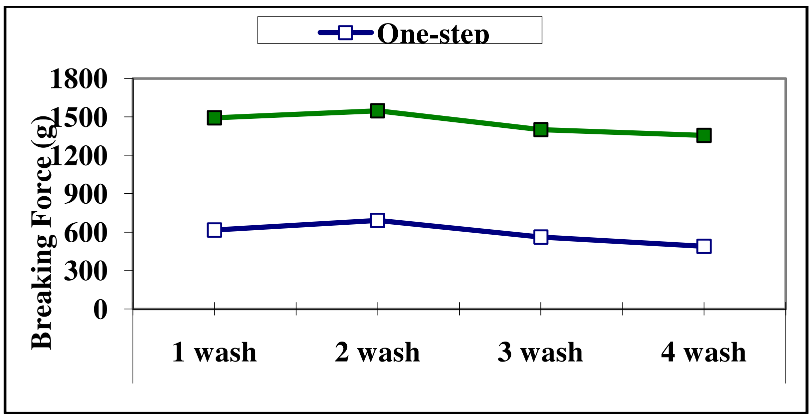

Fig. 5. Effect of washing cycle/ frequency on tilapia mince.

This washing was sufficient to remove sarcoplasmic proteins but also resulted in higher moisture content. However, two washing cycles at a 3:1 water-to-meat ratio failed to remove fish odor and a gray shade completely from the meat when red hake fillet was tested (C.M. Lee, unpublished data).The highest gel-forming ability for both unwashed and washed mince in one-step heating was found at 40°C for 120 min. and 50°C for 120 min. and further heating at 90°C for 30 min. in two-step heating. Washed mince surimi is always superior to unwashed mince surimi in gel quality. A significantly good textural and sensory quality ($p<0.05$) of gel and a fine grade surimi was obtained from tilapia mince washed with 0.1% NaCl solution. Two times washing gave the best result. Washing time limited within 10 min. (6 min. agitation, 4 min. settling) was found to give good quality surimi.

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