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Effect of Different Washing Cycles on the Quality of *Pangasius hypophthalmus* Surimi

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Abstract

Pangasius is a fish with low cost and round the year availability, which can be an alternate raw material to regular marine fish species like pink perch and croaker. Yellow discolouration of meat is the most serious problem in marketing *Pangasius* as fillet. There is a need to develop an alternative ways of utilizing *Pangasius* and making surimi is one of the alternatives. Therefore attempts have been made in the present investigation to optimize the process of surimi preparation from *Pangasius hypophthalmus*. Effect of different washing cycles i.e., one, two, three and four washes with 1: 3 mince to water ratio on the quality of *Pangasius* surimi was investigated. It was found that among the different washing cycles, third and fourth washing cycles have resulted in the highest gel strength and lowest expressible moisture of *Pangasius* surimi as compared with control and other treatments. The sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) study revealed disappearance of myosin heavy chain (MHC) in the surimi gel obtained with third and fourth washing cycle indicating good cross linking. With increasing washing cycle whiteness of surimi was also improved. Sensory evaluation indicated highest overall acceptability for surimi obtained with third and fourth washing. However, the quality of surimi obtained from third and fourth washing was not different. Therefore, it can be concluded that three washing cycles with 1:3 mince: water ration can be optimum for making good quality surimi from *Pangasius.*

Key words: Surimi, washing cycles, gel strength, whiteness and expressible moisture.

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Introduction

Surimi is rich in myofibrillar protein obtained by mechanically deboning and repeatedly washing with chilled water, refined and mixed with cryoprotectants for better-frozen shelf life (Lee 1986; Ofstad et al., 1990). For removing water soluble protein, fat, pigments, blood and odour bearing compound mince washing is an important step. According to Balachandran (2001) for improving gel strength and elasticity of surimi, concentration of myofibrillar proteins is one of the important factors. A reduction in water soluble protein increases the concentration of myofibrillar proteins, thus enhancing the functional properties of surimi (Park & Lin, 2005). The gelling process entails the association of long myofibrillar protein chains which produces a continuous three-dimensional network in which water and other components are trapped. As a result, a visco-elastic gel is obtained (Sánchez-González et al., 2008). Threadfin bream (*Nemipterus japonicus*), ribbon fish (*Trichiurus lepturus*) and lizard fish (*Saurida tumbil*) are mainly used in India for surimi production (Muraleedharan et al., 1997). The marine fish catch is continuously decreasing and there is a scarcity of raw material for surimi preparation. Inland aquaculture production has increased tremendously in last decade. *Pangasius* is one such species which has very good aquaculture potential and availability throughout the year. Orban et al. (2008) said that white or pale pink colour of *Pangasius* flesh and its fillets are characterized by the absence of thin skin, small bones and fishy odour. The cooking process results in delicate and firm texture, allowing a wide range of culinary preparations. These characteristics were together with their availability in the market in standard size, leads preparation of value added sutchi catfish fillets, particularly to meet the consumer demands or preferences of the food industry.

Washing cycle, water to mince ratio and washing time are the most important steps in surimi processing. A huge amount of water is used to remove the water-soluble proteins, blood, fat and other nitrogenous compounds from the minced fish flesh (Hall & Ahmad, 1997). The degree of washing required to produce good quality surimi depends on the type, composition and freshness of the fish. The number of washing cycles and water to mince ratio employed for washing may vary among surimi processors. Texture, colour and odour of the final product are greatly improved when impurities are removed by washing. It was estimated that approximately 50% of the total protein were lost during washing. More mince to water ratio gives a good result for gel strength but, it not only increases the cost of production but also increases the amount of waste water and loss of some amount of myofibrillar protein. Protein solubility was limited to a certain level when the water to mince ratio, washing cycle and washing time were constant (Park & Lin, 2005). There are several reports on the process optimization for surimi preparation from most of the marine and fresh water fishes. However there are no reports on optimization of process for surimi from *Pangasius* fish.

Hence, the present work was undertaken to study the suitability of *Pangasius* sp. as an alternative raw material for surimi preparation and optimization of surimi preparation method, with different mince washing cycles which can be useful for fish processing industries.

Materials and Methods

Sutchi catfish (*Pangasius hypophthalmus*) reared in cages were purchased from Dhasai reservoir, Thane District, Mumbai. Fish was kept in ice with 1:1 ratio and transported to laboratory within 5 h. Mince was separated from fish fillets, using the deboning machine (Baader 694, Lubek, Germany). Prepared mince was further used for surimi preparation.

Separated mince meat was divided into four lots and then used to prepare surimi with different washing cycles (one, two, three and four times). Mince to water ratio of 1:3 was used for repeatedly washing for five minutes, where 3 minutes were used for stirring and 2 minutes for settling. Washing was carried out in Hobbart mixture unit (Hobart AE 200, London, England) with chilled water. After every wash, the fats accumulated at the surface of the water were removed manually. The surimi obtained from these four treatments was then subjected for excess water removal in the basket centrifuge (Centrofix AIM851, Mumbai, India) at 1200 rpm for 2 minutes.

The yield was calculated from the difference between the weight of whole muscle and final mass of surimi.

Yield (%) = (surimi weight/whole muscle weight) x 100

10 g of surimi sample and control were homogenized with 90 ml distilled water in a homogenizer (Polytron system PT 2100, Kinematica, AG, Germany) for the 30s and pH value of homogenate surimi was measured by a digital pH meter (Hanna instruments, HI 2211, USA) standardized by buffer at pH 4.8 and 9.2.

The moisture and ash content of surimi samples was measured, as per A.O.A.C, (2005). The crude protein content was determined by estimating its total nitrogen content by Kjeldahlmethod (A.O.A.C, 2005). A factor of 6.25 was used to convert total nitrogen to crude protein. The crude fat content was measured as per the method given by Folch et al., (1957).

The surimi was smashed with 2.5% salt for 1 minute at 4°C to obtain a homogeneous solution. It was then stuffed into polyvinylidene casing with a diameter of 2.5 cm and both ends of the casing were sealed tightly. Sols were incubated at 40°C for 30 minutes, followed by heating at 90°C for 20 minutes in a water bath (Shanti scientific industries, Mumbai) (Benjakul et al., 2003). All gels were cooled in iced water for 20 minutes and stored overnight at 4°C prior to analyses.

Textural analysis of gels was performed using a texture analyser - Model TA-XT2i (Stable Micro Systems, Surrey, UK). All gels were tested at room temperature. Surimi gels were cut into five pieces of 2.5 cm in length. The breaking force (gel strength) and deformation were measured by keeping the pieces into the texture analyzer equipped with a spherical plunger (5 mm diameter); pre-test speed: 1.0 mm/s; post-test speed: 10.0 mm/s; distance: 4.0 mm; time: 10s with 50 kg load cell. The probe was pressed into the cut surface of gel perpendicularly at a constant speed until puncture occurred. The force in gram (g) required to puncture into the gel and the distance (in mm) at which the probe punctured into the gel (deformation) were recorded.

Gel strength for each surimi gel was calculated from respective breaking force and deformation.

Expressible moisture content was measured according to the method of Benjakul *et al.* (2001) with slight modifications.

Expressible moisture content (%) = (X-Y/X) x 100

Whiteness was measured by using Lab scan XE – Colorimeter (Hunter Lab scan XE, U.S.A.) which gave an acceptable level of surimi sample based on L*, a* and b* values. Whiteness was calculated as per the following formula given by Fujii et al., (1973)

Whiteness = $100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2}$

Protein patterns of surimi and its respective gels were analyzed by SDS–PAGE according to the method of Laemmli (1970).

A 3 mm slice of the gel was subjected to a folding test (Lanier 1992) by folding between the thumb and index finger and depending on the breakage; they were evaluated by a five-stage process.

1 = Breaks by finger pressure.

2 = Cracks immediately when folded half.

3 = Crack occurs gradually when folded half.

4 = No Crack occurs after the folding half.

5 = No crack occurs after folding twice.

Sensory evaluation of surimi sausages was done by 30 untrained panelists, based on the method described by Meilgaard *et al.* (2006) with slight modification. Sausages were evaluated for appearance, colour, taste, texture, odour and overall liking by panelists. A nine-point hedonic scale, in which a score of 1 = not like very much, 5 = neither like nor dislike and 9 = like extremely, was used for evaluation. A high score indicated good quality and vice versa.

Data were subjected to analysis of variance (ANOVA). Comparison of means was carried out by Duncan's multiple-range tests. The analysis was performed using an SPSS package (SPSS 16.0 for Windows, SPSS Inc, Chicago, IL, USA).

Results and Discussions

The yield of *Pangasius* surimi decreased with increasing in a number of washing cycles (Table 1). Chilled water was mainly used to remove the impurities from the mince which otherwise lowered its quality. During processing of surimi filleting, deboning, mincing and washing decrease the percentage of yield from raw material to surimi which ultimately the effect on surimi production (Amiza & Ain, 2012; Jin et al., 2007; Yathavamoorthi et al., 2012). In the present study, removal of sarcoplasmic protein, fat, blood, pigment with increasing washing cycles resulted less yield in surimi. Almost similar trend was observed in surimi obtained with different washing cycles. The yield percentage of surimi obtained with different washing cycles was lower in *Pangasius* surimi in comparison to threadfin bream surimi (Rajalekshmi & Mathew, 2007) and croaker (*Johnius gangeticus*) surimi (Dey & Dora 2011) prepared by the method of Muraleedharan *et al.* (1997). The yield of surimi from *Pangasius* was lower compared to tilapia. The yield obtained from mince to tilapia surimi was 66.46% (Huang et al., 1995). Kaba (2006) measured the yield of raw surimi from anchovy around 22.4%. However, lower mince to surimi yield was obtained by grass carp 56.13%, croaker 43%, hairtail 50% and Atka mackerel 45% (Nopianti et al., 2011).

The pH of mince, mince treated with different washing cycles was significantly different to each other. Increasing washing cycles increased the pH of all samples were also increased significantly ($p<0.05$). According to Benjakul et al. (2002), the increase in pH was postulated to be due to an increase in volatile bases produced by either endogenous or microbial enzymes. The decomposition of nitrogenous compounds causes an increase in pH in fish flesh. The changes in pH also depend on the liberation of inorganic phosphate and ammonia due to the enzymatic degradation of ATP. Alaska pollack surimi showed high pH with respect to the other samples (Jin et al., 2007). The results

Table 1. Yield percentage of surimi prepared from *Pangasius hypophthalmus*.

Treatment washing cycle (WC)	Yield (%, based on initial wt of whole fish)
1 WC	29%
2 WC	22%
3 WC	20.3%
4 WC	18.7%

WC: represents washing cycle.

of the present investigation were in agreement with those findings.

Proximate composition of surimi varies with different environment, culture practices with the different type of fish and size of fish species. Due to hydration effect, moisture percentage of surimi was increased in respect of mince during the different washing cycles (Table 2). According to Tanuja et al. (2014), surimi obtained from *Pangasianodon* with single washing cycle had moisture, crude protein, crude fat and ash content were 83.5%, 14.62%, 2.07% and 2.31% respectively and with double washing cycle had 84.53% moisture, 13.67% crude protein, 1.67% crude fat and 0.76% ash content respectively. In tilapia surimi, moisture content was higher while crude fat, crude protein and ash content were lower (Murthy & Rajanna, 2011). Sánchez-González et al. (2008) reported a moisture and protein content of sol and gels were in agreement with designed formulation with 75.5% and 17% in Alaska Pollock surimi (grade FA). The results obtained in the present investigation were in agreement with these findings. The low fat, protein and ash content in the surimi obtained with increased number of washing cycles might be due to the removal of those components during washing.

Gel strength is one of the prime requisites in deciding the quality and price of surimi. In the present investigation, the gel strength of *Pangasius* surimi increased with increasing number of washing cycles (Table 3). The same was evident with increased myosin heavy chain (MHC) band intensity of surimi obtained with increased number of washing cycles (Fig.1). MHC plays a dominant role in gel formation and more the concentration of MHC, higher will be the gel strength. The gel strength of wash mince was superior to that of unwashed mince because the sarcoplasmic protein in unwashed meat will coagulate during the heat setting of salt added sol and does not participate in the formation of the gel network. With increased number of washing cycles, in the present investigation, the gel strength was also increased. The moisture level was also increased with increased washing cycles. Gel strength was significantly different in surimi obtained with different number of washing cycles. The specific properties of gel strength are due to the three-dimensional network of protein (Zayas, 1997). The gels are formed when partially unfolded proteins develop uncoiled polypeptide segments that interact at specific points forming a three-dimensional cross-linking network. The salt solubilisation of myofibrillar protein concentrate with an adequate amount of water results in the formation of sol, which subsequently turns into an elastic gel upon heating (Lee, 1987). Effect of washing and salt addition on the gel strength of silver catfish increased with increasing washing cycles (Amiza & Ain, 2012). Gel strength of *Pangasius* surimi in the present study was comparatively higher than IMC (Sankar, 2000). Gel strength of tilapia was comparatively higher as compared with *Pangasius* surimi (Ninan et al., 2004).

The expressible moisture values decreased with increase in a number of washing cycles. This was in accordance with increased gel strength of surimi with the increase number of washing cycles. A good surimi gel is three-dimensional networks of myofibrillar protein stabilized by various covalent and non-covalent bonds with entrap water inside it. If the gel is weak, higher expressible moisture will be found and vice-versa. The effect of pressure on binding properties of meat products depends on various factors, such as fish species, type of muscle, pH, ionic strength, the level of fat - protein and

Table 2. Proximate composition and pH of surimi prepared from *Pangasius hypophthalmus.*

Sample	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	pH
Mince	75.27±0.71^{a}	18.90±0.00^{d}	5.2±0.75^{c}	1.50±0.28^{b}	6.33±0.01^{a}
1WC	77.90±0.10^{b}	15.94±0.50^{c}	3.75±0.27^{b}	0.39±0.06^{a}	6.37±0.06^{a,b}
2WC	80.65±0.16^{c}	14.56±0.90^{b,c}	2.59±1.01^{a}	0.31±0.06^{a}	6.4±0.04^{a,b}
3WC	84.26±0.48^{d}	13.10±1.51^{a,b}	2.36±0.08^{a}	0.27±0.05^{a}	6.44±0.02^{b}
4WC	85.05±0.59^{d}	12.93±0.00^{a}	1.77±0.26^{a}	0.20±0.01^{a}	6.53±0.03^{c}

Different letters in the same column indicate the significant differences ($p<0.05$).
Values are mean±SD (n=3). WC: represents washing cycle.

Table 3. Physical properties of surimi prepared from *Pangasius hypophthalmus*

Sample	Gel Strength			Expressible moisture	Folding test*
	Breaking force (g)	Deformation (mm)	Gel strength (g.cm)		
Mince	299.44±6.30^{a}	6.86±2.49^{a}	205.42±4.44^{a}	29.99±1.43^{e}	1.20±0.0.44^{a}
1WC	526.55±0.98^{b}	5.09±0.29^{a}	268.01±5.27^{a}	22.59±2.05^{d}	2.40±0.54^{b}
2WC	608.75±0.98^{b}	5.27±0.03^{a}	320.81±2.27^{ab}	17.85±0.36^{c}	3.00±0.71^{b,c}
3WC	607±11.53^{b}	7.65±2.91^{a}	464.36±10.85^{b}	13.06±1.44^{b}	3.40±.54^{c,d}
4WC	630.57±32.52^{b}	7.58±2.97^{a}	477.97±14.24^{b}	10.50±1.02^{a}	3.80±0.44^{d}

Different letters in the same column indicate the significant differences ($p<0.05$). Values are mean±SD (n=3). WC: represents washing cycle.

*For folding test

1 = Breaks by finger pressure.

2 = Cracks immediately when folded half.

3 = Crack occurs gradually when folded half.

4 = No Crack occurs after folding half.

5 = No crack occurs after folding twice

treatment condition, such as pressure, time and temperature. Increasing number of washing cycles of mince simultaneously decreased the expressible moisture content. Expressible moisture is found to increase as the amount of entrapped water decreased (Ramirez et al., 2007). Lowest percentage of water extracted means highest water holding capacity. Barrera et al. (2002) found expressible moisture percentage of surimi in the range of 19.0 to 26.9. According to Murthy & Rajanna (2011) higher percentage of expressible moisture was found in washed meat of tilapia surimi.

The folding test is a sensory technique to assess the texture of the surimi and carried out by trained panelist. Folding score in the present investigation increased with increasing number of washing cycles (Table 3). This is in accordance with increase gel strength and lowers expressible moisture in the surimi obtained with more number of the washing cycles. This indicates that the quality of surimi gel improved with increasing washing cycles. Folding test is very subjective and can be considered as preliminary test to differentiate high and low-grade surimi but lacks sensitivity to distinguish different functional properties of surimi such as gel strength (Reppond et al., 1987)

Whiteness is another important parameter along with gel strength for deciding the quality of surimi. Whiteness of *Pangasius* in the present investigation increased with increasing number of washing cycles (Table 4). More washing resulted in the removal of most of the pigment, blood and impurities and improve the whiteness of the *Pangasius* surimi. According to Chen (2002) washing is one of the most important factors in deciding quality of surimi. The washing process is necessary for color improvement and gel strength of surimi produced from the mince (Chaijan et al., 2004). Kim et al. (1996) reported that the color of surimi can be improved by increasing the washing cycles and washing time. However this result has shown that the color of surimi was improved by increasing washing cycles. Alaska pollack surimi comparatively had higher whiteness than surimi prepared from *Pangasius* sp. with different washing cycles (Jin et al., 2007) and comparatively similar to big snapper surimi (Julavittayanukul et al., 2006).

The SDS-PAGE pattern of total protein revealed multiple bands with different molecular weights. Thick band with molecular weight of 205kDa was MHC and another prominent band with molecular weight of 44.3kDa is actin. The electrophoretic pattern (Fig. 1a) has shown a clear difference for MHC between unwashed and washed. After washing low molecular weight, proteins were lost. Myosin heavy chain concentration increased with

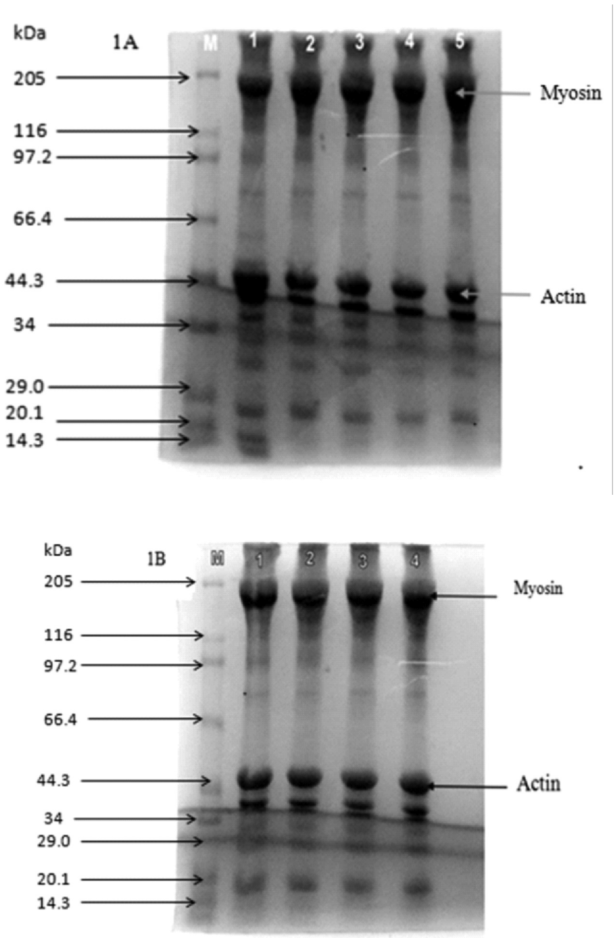

Fig. 1. SDS-PAGE of mince, surimi and gel. (1 A). SDS-PAGE of mince and surimi
Lane M- marker, lane 1- mince and lane 2-5 surimi obtained with different washing cycles (1 B). SDS-PAGE of Gel Lane M- marker, lane 1-4- gel obtained with of different washing cycles.

increasing washing cycles as shown in lane 2-5 (Fig. 1a). In lane 1-4 (Fig. 1b), it was observed that after heating, MHC bands intensity decreased indicating polymerization of MHC to form a good gel. Electrophoresis of the washed mince in major carps showed decreases in the high molecular weight band with the number of washing cycles (Sankar, 2000). The loss of myofibrillar protein increased with increasing in meat to water ratio (Lin & Park, 1996). According to Murthy et al. (2011) intensity and concentration of bands in washed meat varied with unwashed mince and also found that the increasing the washing cycles in surimi preparation increased the concentration of MHC. According to Chin et al. (1998) MHC (myosin heavy chain) 200 kDa protein and 45 kDa protein (actin) decreased after cooking, whereas the tropomyosins (35-36 kDa) remained relatively constant. Acton (1983) observed that the tropomyosin and troponin fractions did not contribute to the gel formation of actomyosin and therefore, the amounts of these proteins were not changed after gel formation. Myofibrillar protein especially myosin and actin, are considered responsible for the viscoelastic texture of processed meats. Asghar et al. (1985) reported that myosin made an excellent gel and that actin served as a synergist for myosin gel formation depending on the weight ratio of myosin/actin or the condition of myosin molecules. Cooking or heating causes meat proteins to unfold and produce a well ordered 3-dimensional matrix linked by hydrophobic and hydrogen bonds (Acton & Dick, 1984). Thus, proteins that might contribute to viscoelastic texture developed during cooking disappear on SDS-gels, and the other proteins probably do not contribute much to the gel matrix during cooking. The higher

Table 4. Whiteness of surimi *Pangasius hypophthalmus* before and after heat setting.

Sample	Whiteness values of surimi before heat setting				Whiteness values of surimi before heat setting			
	L*	a*	b*	Whiteness	L*	a*	b*	Whiteness
Mince	51.19±1.66^{a}	14.37±1.10^{b}	22.43±0.17^{d}	44.39±1.80^{a}	67.42±0.0.23^{a}	4.36±0.16^{c}	19.64±0.15^{c}	61.71±0.16^{a}
1WC	68.69±0.09^{b}	0.17±0.07^{a}	14.14±0.58^{c}	65.64±0.16^{b}	75.40±0.36^{b}	1.89±1.16^{b}	19.15±0.84^{c}	68.75±0.41^{b}
2WC	70.05±0.00^{b}	0.00±0.10^{a}	12.36±0.34^{a}	67.60±0.14^{c}	76.31±0.86^{b}	0.44±0.17^{a}	17.06±0.07^{b}	70.80±0.67^{c}
3WC	74.76±0.58^{c}	-0.28±0.27^{a}	13.33±33^{b}	71.45±0.44^{d}	77.96±0.56^{c}	0.20±0.56^{a}	17.76±0.72^{b}	71.68 ±0.32^{c}
4WC	76.18±0.53^{c}	-0.47±0.01^{a}	11.83±0.09^{a}	73.38±0.44^{e}	78.54±0.87^{c}	-0.06±0.04^{a}	15.39±0.06^{a}	73.59±0.68^{d}

Different letters in the same column indicate the significant differences ($p<0.05$). Values are mean±SD (n=3).WC: represents washing cycle.

Table 5. Sensory evaluation of surimi prepared from *Pangasius hypophthalmus.*

Treatments	Parameters				
	Colour	Appearance	Odour	Texture	Overall
1WC	6.90±1.59^{a}	6.60±2.07^{a}	6.80±1.30^{a}	6.00±2.91^{a}	6.00±2.12^{a}
2WC	6.70±1.20^{a}	6.40±1.14^{a}	7.10±0.89^{a}	8.40±0.89^{c}	7.20±1.03^{b}
3WC	8.00±0.70^{b}	7.40±0.54^{b}	7.50±0.50^{a,b}	6.80±1.64^{b}	7.20±1.73^{b}
4WC	7.8±0.57^{b}	7.20±0.83^{b}	8.40±0.54^{b}	7.80±1.30^{ab}	7.30±0.97^{b}

Different letters in the same column indicate the significant differences ($p<0.05$). Values are mean±SD (n=3).WC : represents washing cycle.

MHC band intensity in surimi obtained with four washing cycles is in accordance with higher gel strength of the same.

Consumer perception of quality is a very important component of marketing in any kind of food products and which can be analyzed by sensory evaluation with the panelists. In the present investigation, it was observed that overall acceptability scores increased in *Pangasius* surimi prepared with the increasing number of washing cycles. Nevertheless, higher scores were recorded for texture attribute in surimi prepared with four washing cycles. The same is evident with higher gel strength whiteness and a folding score of surimi obtained with higher washing cycles. According to Ninan et al. (2004) sensory characteristics of mince improved by washing and by sensory evaluation twice-washed samples were better quality than the single wash.

From the results, it was observed that among the different washing cycles, third and fourth wash were found to be better since it gave highest gel strength and whiteness to the surimi as compared to mince and other treatments. Nevertheless, the yield of surimi was higher in third wash compared with the fourth wash. Therefore, it can be concluded that third washing cycle of mince may be ideal for obtaining a good quality surimi from the *Pangasius hypophthalmus*. This study will help in identifying the suitability of *Pangasius* as an alternative raw material for surimi preparation and thus, will be useful for fish processing and aquaculture industries.

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