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Research Article

Frozen storage stability and gel formation of Nile tilapia (*Oreochromis niloticus*) meat paste and effect of heating time on its gel-forming ability

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A B S T R A C T

Nile tilapia (*Oreochromis niloticus*) is one of the most widely cultured fish species in the world. This study investigated surimi production as a sustainability and profitable method for utilization of this species. The objectives were to evaluate the stability of meat paste prepared from washed and unwashed tilapia muscle during stored frozen and to examine the effects of heating time on gel formation and breakdown in the meat paste. We studied the freeze-thaw cycle of mince and surimi prepared from washed and unwashed muscle to understand the stability of frozen storage. The freeze-thaw cycle of mince and surimi were analyzed to assess frozen storage stability including muscle and drip loss measurements. The results showed that freezing surimi at -25°C for five weeks with cryoprotectants (4% sucrose, 4% sorbitol, and 0.2% polyphosphate) enhanced stability. However, the texture and taste of surimi deteriorated over time. Surimi with cryoprotectants showed significantly lower thaw drip loss ($p < 0.05$) compared to mince without cryoprotectants. Both unwashed and washed minces show the similar result. Further, salt-ground (3% NaCl) paste was incubated in water baths at varying incubation times to evaluate the effects of heating on gel formation and degradation. It was found that gel-forming ability increased with increased heating time at 50°C, whereas extended heating at 60°C led to degradation of meat paste. These findings demonstrated that cryoprotectants are essential additives for maintaining surimi quality while heating time determines the gelation properties of tilapia meat paste.

INTRODUCTION

The relationship between fish, fisheries and the cultural and historical context of Bangladesh is deeply rooted. The country is blessed with a wide array of fisheries resources including various aquatic environments, such as rivers, beels, haors, baors, lakes, ponds, marshlands, inundated agricultural fields, estuaries, and portions of the Bay of Bengal. Fish contribute roughly 60% of the animal protein consumed in the diet of the population in this country (DoF, 2024).

Food preservation through frozen storage of meat paste has been widely implemented by reducing microbial load. Conversely, meat paste becomes unstable during frozen storage and undergoes several changes that determine the end of its storage life. Frozen storage leads to protein aggregation, resulting in the hardening of muscle (Kim & Park, 2000). Similarly, during frozen storage, formaldehyde enhances its interaction with myofibrillar proteins, accelerating their denaturation and aggregation (Sato & Kawabata, 1998). The quality of frozen surimi is significantly influenced by the freezing temperature, the consistency of the storage, and the conditions under which it is thawed (Lanier, 1992). The conditions of frozen storage influence the stability and shelf life of Surimi. The stability of the gel strength of surimi is at -35°C but exhibits reduced stability at -20°C. At -10°C, the gelling quality of surimi significantly deteriorates after 2 months. The shelf life of surimi, similar to other frozen foods, is extended at lower, stable temperatures.

A recommended shelf life was established in 1984 for frozen surimi of 6-12 months at temperatures between -23 and -25°C by the Japanese Association of Refrigeration. The

process of washing is an essential aspect in surimi production, as it plays a vital role in attaining optimal gelling properties while ensuring that the final product remains devoid of color and odor. The washing of minced meat plays a crucial role in significantly diminishing or eradicating numerous concerns associated with color, flavor, and aroma. There are differences in how often and how much water is used for washing depending on a number of factors, such as the type of fish, how fresh it is, the washing unit used, and the quality of surimi that is wanted, as noted by Hall and Ahmed in their 1997 study. The gel strength of surimi was observed to enhance with an increasing number of washing cycles, as noted by Lanier (1992). The duration of heating and the temperature employed are essential elements influencing the gelation process of surimi. When salt-ground surimi paste is preserved at a stable low temperature, specifically below 40ºC, for a certain amount of time, it results in the formation of a semitransparent and elastic gel. The phenomenon of gelation is commonly referred to as setting or "suwari" in Japanese.

Conversely, incubating certain surimi pastes at approximately 60ºC results in the formation of a very brittle heat-set gel. The phenomenon referred to as "modori" (returning) in Japan describes the process by which the formed elastic gel reverts to its original non-elastic state (Sato & Kawab, 1998). The production of Nile tilapia is steadily increasing, prompting considerations for innovative approaches to its management. In the 2023-24 period, total tilapia production reached 3,218,715 tonnes (DOF, 2024). This abundant catch has the potential to function as an alternative source of raw material for Surimi production. The musculature of tilapia illustrates a range of useful attributes that are vital to the formulation of surimi-based products. Over the past two decades, processing methods for certain freshwater species have been established (Lazos, 1997). However, there has been no evaluation of the thermal gelation properties and frozen storage stability of Nile tilapia mince, nor has there been an assessment of the impact of washing on these characteristics. A study conducted by Lizárraga-Mata *et al.* (2016) showed that washing improved mince stability more than cryoprotectant addition. The mince remained microbiologically safe and maintained stable physicochemical and sensory quality for six months, suggesting an extended shelf life, particularly due to washing (Mahawanich *et al*. 2010).

Washed fish mince production can boost Bangladesh's fisheries sector by reducing waste, enhancing tilapia by-products, and improving frozen seafood quality. Cost-effective method for preparation of washed mince might help to extend shelf life, meets international safety standards, and increases export potential. It also supports small-scale fisheries, creates jobs, and aligns with sustainable policies, driving economic growth and market competitiveness. Additionally, it appeals to health-conscious consumers, boosting local and global demand for Bangladesh's seafood. In order to attain optimal capacity utilization and examine the possibilities for the production of a wide variety of products, the processing of underutilized or low-priced fish species, in combination with other promising species, into surimi-based value-added products will yield immediate benefits for the existing fish processing industries within the nation (Nowsad *et al.*, 1994b). The study looked at how to make surimi from tilapia mince, focusing on how stable surimi is when stored frozen and how changing the length of time it is heated affects its ability to gel. The goal was to make tilapia a better source of surimi raw material.

MATERIALS AND METHODS

Sample Collection

Nile tilapia (*Oreochromis niloticus*) utilized in this research were procured from local fishermen at the retail fish market called Mechua Bazar located at Mymensingh City, Mymensingh Sadar in Mymensingh district, Bangladesh. The fish were transported to the Fish Processing and Quality Control Laboratory, located within the Department of Fisheries Technology at the Faculty of Fisheries, Bangladesh Agricultural University in Mymensingh, utilizing an insulated icebox (Cosmos Ltd., Seoul, Korea, with a capacity of 20 kg), ensuring they remained in an adequately iced condition throughout the journey.

Preparation of Mince

The fish performed a series of careful procedures, including weighing, cleansing with fresh water, beheading, evisceration, skinning, and a final washing. In conditions characterized by the presence of ice, the skinned fish went through a manual process of filleting and deboning. After that, the fish was minced using a mechanical mincer (National Meat Grinder, MK-G3NS, Matsushita Electric Industrial Co. Ltd., Osaka, Japan) with a 1 mm orifice diameter, which made sure that all the bones were removed from the muscle tissue. Fatty acids, enzymes, sarcoplasmic proteins, and other substances that stop gels from forming were removed from half of the minced meat by washing it in a cold solution that was kept at 4°C. In order to speed up the settling process before the leaching phase, five volumes of the washing solution were added to the minced material and stirred around for five minutes.

Preparation of Surimi

Using washing solutions at a ratio of 5:1 (wash water to mince), half of the mince completed two washing and dewatering processes, the first wash and the second wash. Stirring the mince-water slurry in a 20-liter plastic bowl with a mixer for a designated period constituted the washing process. Water was removed from the slurry by draining and pressing it with a finely meshed nylon bag during every washing. Combining washed and unwashed mince with cryoprotectants produced surimi. Approximately 4% (w/w) sucrose, 4% (w/w) sorbitol, and 22% (w/l) Na-tripolyphosphate made up the cryoprotectant mix. To guarantee homogenous integration, the components were hand-mixed precisely. Throughout the mixing operation, the temperature was kept below 5ºC. The dough was placed in food grade polyethylene packets after mixing, creating 1 kg per rectangular blocks. Until analysis, the surimi samples were stored at -25ºC. Fig. 1 shows how surimi was prepared.

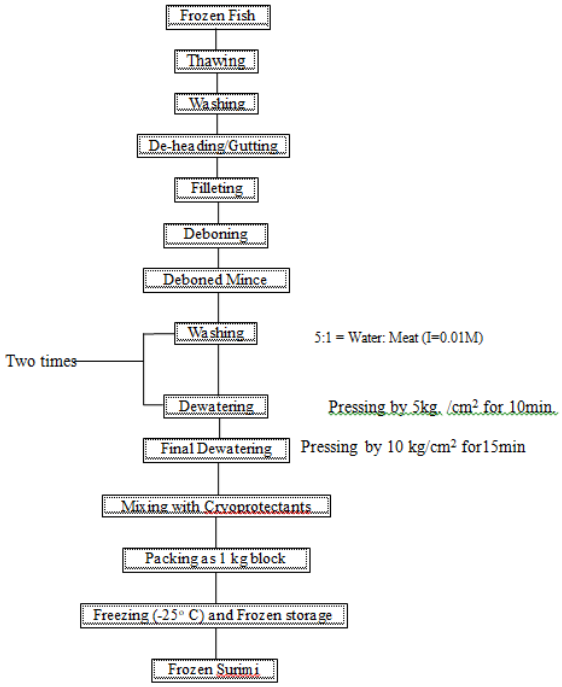

Figure 1: Preparation procedure of surimi

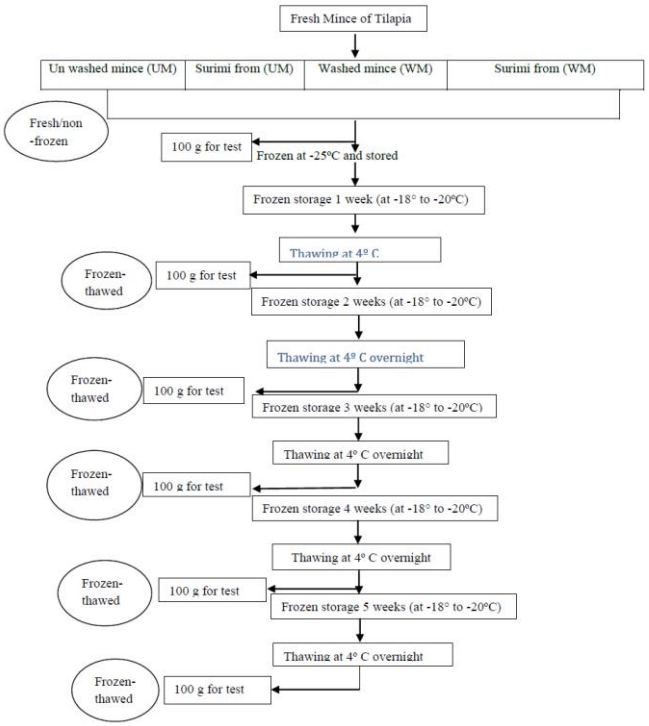

Figure 2: Protocol for Freeze-thaw Stability

Freeze-thaw Stability

Both unwashed and washed minces were systematically packaged in vacuum-sealed containers and subsequently stored in a frozen state for the purpose of conducting a freeze-thaw stability study (Thurber *et al.*, 2022). The minced samples were subjected to a freezing process, both with the incorporation of cryoprotectants (CP) and in the absence of such agents. Five freeze-thaw cycles were conducted on minced samples (Liceaga *et al.*, 2006). Each freeze-thaw cycle was performed at 4°C overnight following a one-week storage duration. Thaw-drip measurements were conducted to evaluate freeze-thaw stability. In order to quantify thaw-drip, frozen mince that had been pre-weighed was subjected to a thawing process, followed by drying with paper towels, after which it was reweighed to determine the amount of liquid lost during thawing (Liceaga *et al.*, 2006). Thaw-drip is characterized as the proportion of weight reduction that transpires subsequent to the thawing process, expressed as a percentage of the original frozen weight. Fig. 2 presents a detailed depiction of the experimental protocol used to conduct the evaluation of freeze-thaw stability.

Preparation of Gel

The paste within polyvinylidene chloride cylinders has been exposed to heating to yield a gel. Some samples were subjected to a single heating in well-stirred water baths, whereas others underwent two heating steps. For ease of reference, the previous method of heating was designated as one-step heating, whereas the subsequent approach is referred to as two-step heating. All heating procedures were conducted in triplicate.

The present study aims to examine the effect of heating duration on the formation of Suwari gel. In this context, both washed and unwashed paste samples contained within tubes were subjected to heating at a constant temperature of 50°C for varying durations, specifically 1 hour, 2 hours, 3 hours, 4 hours, and 5 hours, during a one-step heating process. The preliminary heating phase in the two-step procedure was conducted at a temperature of 50°C for varying durations, specifically 1 hour, 2 hours, 3 hours, 4 hours, and 5 hours. Following the preheating treatment, the samples were immediately exposed to a further heating duration of 30 minutes in water maintained at a temperature of 90°C.

To find out how heating time affected the breaking down of Modori gel, tubes of washed and unwashed paste were heated at 60°C for 1 hour, 2 hours, 3 hours, 4 hours, and 5 hours. The preliminary heating phase in the two-step procedure was carried out at a temperature of 60°C for varying durations of 1 hour, 2 hours, 3 hours, 4 hours, and 5 hours. Following the preheating treatment, the specimens were subsequently exposed to an additional heating duration of 30 minutes in water maintained at a temperature of 90°C. Following the treatments previously described, the samples were extracted from the water bath and subsequently immersed in ice water for a duration of no less than one hour, after which they were subjected to the subsequent testing procedure.

Measurement of Gel-strength

The gels went through a series of evaluations, specifically a puncture test, a folding test, and a teeth cutting test, subsequent to their extraction from the cylinder. The gel's breaking strength was measured by how deep a 6-mm-diameter ball-shaped object could go into it during the puncture test. The teeth cutting test checked how resistant the disc was to breaking when the panel members' incisors were used to cut it. The folding test, on the other hand, checked how resistant the sample disc, which was 1 mm thick, was to breaking along the folds when it was folded into halves and quarters.

Puncture test: The gels were extracted from the tube and subsequently sectioned into uniform segments measuring 2 cm in length. Before the puncture test, the breaking force of the gel was measured when a spherical plunger with a diameter of 6 mm was inserted into it. The gel, which had been sliced into appropriate dimensions, was set on the pan of an electronic balance and subsequently punctured using a spherical plunger. The force required to break the gel utilizing the plunger was quantified in grams through the use of the balance display window.

Folding test: The folding test included the manipulation of a spherical disc of gel, measuring 1 mm in thickness, which was strategically placed on the index and middle digits of the right hand. The disk was first folded into two equal halves and then further divided into quarters through the application of the thumb and fingertips. The gel was evaluated following the scoring system delineated by Poon *et al.* (1997), as presented in Table 1.

Teeth cutting test: The panelists were provided with the disc gel of identical dimensions that was used in the folding test. They were instructed to identify the flavor by slicing it with their incisors during the teeth cutting test. The numerical scores proposed by Shimizu *et al.* (1981) were applied to evaluate gel strength, as illustrated in Table 2.

Table 1: 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.

Table 2: 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

The findings of the experiment were presented in the form of the mean standard deviation derived from three separate replications. The differences in treatment outcomes were examined through the application of one-way analysis of variances (ANOVA). In order to achieve this objective, the Duncan Multiple Range Test (DMRT) was applied. The differences observed were statistically significant, as indicated by a P-value that fell below the minimum value of 0.05. The analysis of the data was conducted utilizing the computer program MSTAT.

RESULTS

Freeze-thaw stability

Nile tilapia surimi was stored at -18° to -20ºC, and its stability during frozen storage was evaluated using freeze-thaw stability analysis. The freeze-thaw stability was assessed through a drip loss study and the results are showed in Table 3 and Figure 3. Thaw-drip was significantly reduced ($P < 0.05$) in surimi with cryoprotectants (CP) prepared from both unwashed and washed minces when compared to the mince without CP. When compared to surimi made from unwashed mince and other types of unwashed and washed mince (excluding CP), surimi made from washed mince had a much lower thaw-drip after one week of freezing. Thaw-drip in all surimi and minces, however, exhibited only a slight decrease ($p > 0.05$) in subsequent freeze-thaw cycles.

Table 3: Freeze-thaw stability of surimi from Tilapia minces

FTC	Thaw-drip			
	UM (%)	**WM** (%)	**S-UM** (%)	**S-WM** (%)
0	-	-	-	-
1	8.85	8.01	2.00	1.12
2	8.85	7.99	1.52	1.05
3	8.81	7.82	1.52	0.89
4	8.79	7.8	1.46	0.87
5	8.76	7.77	1.44	0.87

S.N: FTC= Freeze-thaw cycles; UM = unwashed mince; WM = washed mince; S-UM = surimi prepared from unwashed mince; S-WM = surimi prepared from washed mince

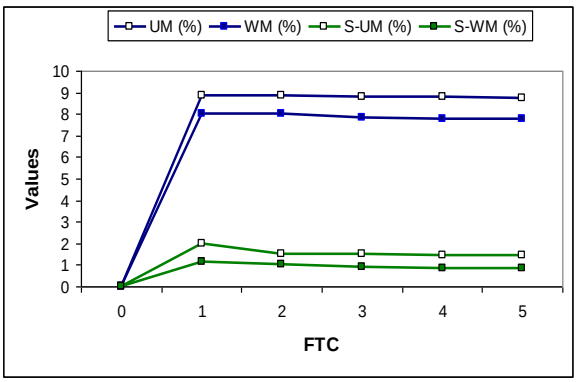

Figure 3: Freeze-thaw stability of surimi from Tilapia minces

Without CP, mince that had been washed or not produced a tougher gel, which was matched by significantly lower sensory scores compared to mince that had CP, irrespective of whether it had been washed or not. It was found by Kimura *et al.* (1991) that adding sorbitol greatly improved the stickiness of fish surimi by changing how proteins cross-link during the setting phase. They showed that the cryoprotectants and washing processes had a big effect on the storage capacity, showing that they kept the Nile tilapia mince and surimi safe during the storage period.

In comparison, premium quality Alaska Pollock surimi was used to produce low fat gels which maintained consistent

stiffness during freeze-thaw cycles 0, 3, 9, or 15. Despite the decreased functional quality of the surimi caused by the freeze-thaw cycles, the stress-to-strain ratio remained unchanged, since both parameters decreased proportionally.

Conversely, when surimi gels are produced in substandard Alaska- Pollock, a significant and long-lasting decrease in stress and strain was exhibited when subjected to 0, 3, 9, or 15 freeze-thaw cycles (Kim *et al*. 1986). Furthermore, when high-quality Pollock surimi gels were put through freeze-thaw cycles (0, 3, 6, and 9), it was found that the gels were damaged the most in the first three cycles due to protein denaturation and structural degradation due to repeated ice crystal formation (Park *et al*., 1997).

Effects of heating time on gel formation (Suwari gel)

We conducted this heating experiment to determine the maximum gelling ability of mince. It was imperative since mince preparation involved a complex protein gelation mechanism induced by heating. The optimal gelation conditions for surimi have been determined for numerous fish species; however, these conditions seem to differ depending on the specific species (Lee, 1984). Table 4 illustrates the effect of heating duration on gel formation in both unwashed and washed mince. The initial gel strength recorded was 480 for unwashed and 543 for washed mince during one-step heating, while for two-step heating, the values were 834 and 964, respectively. After a heating duration of 5 hours, the gel strength increased to 598 for unwashed and 650 for washed mince in one-step heating and to 1302 and 1450, respectively, in two-step heating. The study's results showed that the ability to form gels increased with longer heating times. This was because the myofibrillar protein became less rigid. The breaking strength (BS) of suwari gel is shown in Table 4 and depicted graphically in Fig. 4. The gel strength peaked at 5 hours of heating, exhibiting a significant difference ($P < 0.05$) relative to other heating durations. At that time, the resulting product demonstrated superior performance in the folding test. The findings of this study strongly support Niwa *et al*. (1995) that state that "suwari" gel, made by letting SH-unblocked actomyosin paste harden at 50°C for 3 to 5 hours, had a much higher breaking force than the gel made from SH-blocked paste. A 5-hour pre-cooking period at temperatures below 50°C followed by cooking at temperatures between 80°C and 90°C makes a gel that is stronger than cooking that is done in isolation (Okada, 1963). The process of setting has a crucial role in the systematic alignment of muscle proteins, whereas the cooking process further augments the interactions that were established during the setting phase (Niwa *et al*., 1983).

Table 4: Effects of heating time on gel formation (Suwari gel)

Heating process	Mince	Para-meters	Heating time				
			1 hr	2 hrs	3hrs	4hrs	5hrs
One-step	Unwashed	BF	480±2.2	518±2.3	570±1.2	567±2.3	598±1.2
		FT	AA	AA	AA	AA	AA
		TCT	6	6	7	7	7
	Washed	BF	543±1.9	552±3.2	589±3.1	660±1.9	650±3.3
		FT	AA	AA	AA	AA	AA
		TCT	6	7	7	7	7
Two-step	Unwashed	BF	834±3.3	1050±1.3	1100±5.4	1356±7.2	1302±6.6
		FT	AA	AA	AA	AA	AA
		TCT	7	8	8	8	8
	Washed	BF	964±4.4	1274±8.2	1300±9.3	1290±7.0	1450±4.2
		FT	AA	AA	AA	AA	AA
		TCT	7	8	8	8	8

Values (mean ± SD.) with no common superscript differ significantly ($P < 0.05$).
[ANOVA: Duncans new multiple range test (DMRT)]
Abbreviation used: *BF= Breaking Force, FT= Folding Test, TCT= Teeth Cutting Test

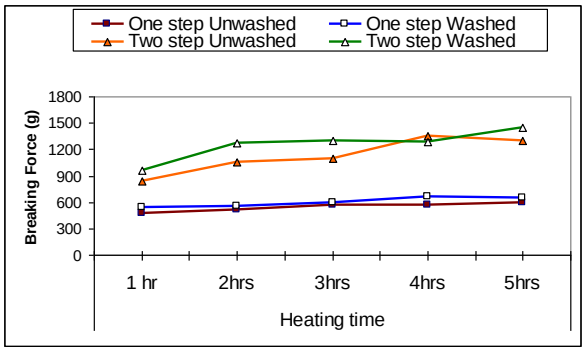

Figure 4: Effects of heating time on gel formation (Suwari gel)

Effects of heating time on gel disintegration (Modori gel)

The breaking strength (BS) of modori gel is presented in Table 5 and is also illustrated graphically in Fig. 5. The gel strength exhibited its lowest value at the 5-hour mark of heating, demonstrating a statistically significant difference ($P < 0.05$) when compared to the gel strengths observed during the other heating durations. During that period, the resultant product exhibited a reduced efficacy in the folding assessment. A study by Tsukamasa and Shimizu in 1991 looked into a specific case of proteinase-independent modori. This is a process that is linked to thermal gel degradation and was seen in sardine meat.

Table 5: Effects of heating time on gel degradation (Modori gel)

Heating process	Mince	Para-meters	Heating time				
			1 hr	1hrs	3hrs	4hrs	5hrs
One-step	Unwashed	BF	273±1.3	236±8.2	209±4.2	218±4	189±2.2
		FT	**A**	**A**	**A**	**A**	**A**
		TCT	4	4	4	4	4
	Washed	BF	387±3.8	350±4.5	350±3.5	300±5	298±1.1
		FT	AA	**AA**	**AA**	**A**	**A**
		TCT	6	6	6	4	4
Two-step	Unwashed	BF	413±2.2	308±3.03	270±2.4	208±2.5	225±2.4
		FT	**AA**	**A**	**A**	**A**	**A**
		TCT	7	5	5	4	4
	Washed	BF	392±3.6	385±2.7	350±2.4	289±1.6	302±1.5
		FT	**A**	**AA**	**AA**	**A**	**A**
		TCT	5	5	5	5	5

Values (mean ± SD.) with no common superscript differ significantly (P <0.05).
[ANOVA: Duncans new multiple range test (DMRT)]
Abbreviation used: *BF= Breaking Force, FT= Folding Test, TCT= Teeth Cutting Test

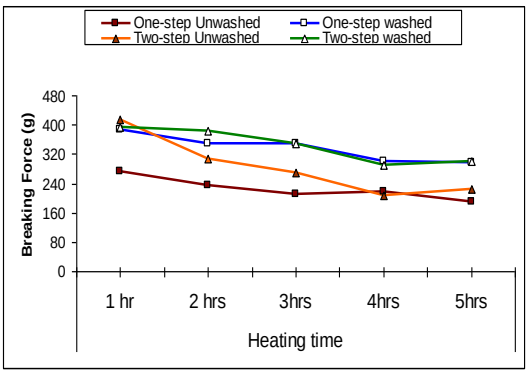

Figure 5: Effects of heating time on gel degradation (Modori gel)

In the case of modori, it was seen that the amount of gel degradation increased as the heating time went from one hour to three hours. When fish mince was heated to 60°C for three hours, its ability to form a gel decreased. This suggests that proteolytic activity or gel degradation took place during that time (Saeki *et al*., 1996). The reduction in gel strength after being heated at temperatures between 50°C and 60°C for 120 minutes (2 hours) is a typical response of myofibrillar proteins found in certain fish species (Shimizu *et al*., 1983), which can be linked to the process of proteolysis. Shimizu *et al*. (1983) further indicated that the rate of gel degradation accelerates with an increase in heating duration. The optimal conditions for the occurrence of Modori gel are achieved at temperatures ranging from 60 to 70ºC, maintained for a duration of 2 hours of heating.

CONCLUSION

The study concluded that Nile tilapia (*Oreochromis niloticus*) is a suitable fish for making surimi. The addition of cryoprotectants significantly improved the stablity of the frozen surimi. Both washed and unwashed mince showed low thaw-drip loss and maintained its texture and taste after being stored for five weeks at temperatures ranging from -18° to -20°C. Another interesting finding was that the process of gel formation was improved with heating times at 50ºC, while the process of gel degradation got worse with prolonged heating at 60ºC. These findings highlight the potential of Nile tilapia surimi for making high-quality foods with longer shelf life under frozen condition.

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