Effect of Phosphate and Freeze-thaw Cycles on Physicochemical and Sensory Properties of Frozen Nile Tilapia (*Oreochromis niloticus*) Fillets

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Abstract

The aim of this study was to investigate the effect of phosphate solution and the different number of freeze-thaw cycles (0, 1, 3 and 5) on frozen Nile tilapia (*Oreochromis niloticus*) fillets. The fish fillets were separated into three groups. First group was immersed in distilled water (control), second group was treated with 1.4% sodium tripolyphosphate solution (STPP) and third group was treated with 1.4% STPP and 2.7% NaCl solution (STPP+NaCl) before subjected to freezing and thawing process. The results obtained that pH, Thiobabutiric acid-reactive substances (TBARS) and Total volatile basic-nitrogen (TVB-N) were increased in all samples and freeze-thaw cycles. TVB-N in treated samples at the 3rd and 5th freeze thaw cycles were significantly lower ($p \leq 0.05$) than those in control sample. Drip loss of both treated samples (STPP or STPP+NaCl) were not significant different when compared with control sample at the beginning and 1st cycle. However, the cooking loss in treated samples was less than control sample as increasing of freeze-thaw cycle. L* value of STPP+NaCl solution was significantly increased ($p \leq 0.05$) than STPP and control sample at 3rd freeze thaw cycle. The STPP+NaCl sample at the 3rd of freeze-thaw cycle was significantly higher ($p \leq 0.05$) in hardness, chewiness and springiness than those in the control and the STPP sample. However, hardness, chewiness and springiness in control sample, sample treated with STPP and STPP+NaCl solution were significantly decreased at the 5th of freeze-thaw cycle. In addition, the raw and cooked samples in this experiment were still accepted after freeze-thaw repeated at the 3rd and 5th of freeze-thaw cycles, respectively.

Keywords: Phosphate, Freezing, Freeze-thaw cycles, Frozen Nile tilapia fillets

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1. Introduction

Nile tilapia (*Oreochromis niloticus*) is a commercially important freshwater aquaculture species throughout Asian Countries for instance Thailand, Vietnam, Indonesia, and China. The trade of tilapia products are growing rapidly, which the major imported regions including the United States, Russia and the European countries. Fishery products made from tilapia are important export food of those countries (Liu *et al*., 2015). In Thailand, whole frozen Nile tilapia was exported about 4,900 tons with value about 300 million baht during January to October in 2016 (Nookrit, 2016). Frozen Nile tilapia is widely used as a raw materials in various food products. Consumers highly expect about the frozen Nile tilapia cloud be applied to maintain the fish qualities after thawing and processing (Cyprian *et al*., 2013).

The demanding high food quality of the consumers is progressively increasing and the product qualities are expected to maintain during production, storage and consumption. However, fresh fishery products are highly perishable. The deterioration is caused by microbiological, chemical and enzymatic activities that are induced by storage temperature (Huss 1995; Simpson *et al*. 2003; Cyprian *et al*., 2013). Freezing process is the effective and most economical method to prolong of fishery products freshness and flavor. Nevertheless, a low temperature used in the freezing process could affect the fish muscle properties resulting in quality changes in the final product. Drip loss affects the quality of frozen fishery products resulting in undesirable sensory properties (Gonçalves and Ribeiro, 2009). Thus raw fishery products have been treated with phosphate compounds before subjected to freezing. The treatment of phosphates in fishery products could improve the qualities by increasing water retention, reducing weight loss from the thawing process, and decreasing cooking loss (Chang and Regenstein, 1997; Masniyom *et al*., 2005). In general, the phosphates (1–6%) are used together with NaCl (2–5%) to improve moisture retention and taste of products (Chang and Regenstein, 1997; Thorarinsdottir *et al*., 2004; Gonçalves and Ribeiro, 2008). Wangtueai *et al*. (2014) report that an application of 2% solutions of sodium acid pyrophosphate (TSPP) or tetrasodium pyrophosphate (STPP) or sodium tripolyphosphate (SHMP) or sodium hexametaphosphate (SHMP) combined with 2.5% NaCl affected the quality of frozen Nile tilapia fillets which overall STPP alone gave the best physiochemical and sensory qualities to frozen Nile tilapia fillets.

Freezing then thawing (freeze-thawing) might be occurred during the storage of frozen fillets and leads to cells breakdown in fish tissue. Moisture migration during freeze thaw cycle can be exhibited the development of ice crystal and possibility effect on protein denaturation and aggregation (Lee *et al*., 2016). Thus the determination of frozen-thawed and fresh fish qualities are important in food industries, especially in case of skinless fish fillets (Diop *et al*.,

2016) are easy to lost protein functional properties such as water holding capacity, solubility and essential nutrient. Frozen fillet from Nile tilapia are higher consumed all around the world. The frozen fillets are thawed and repeat frozen by refrigerator many times before cooking or selling to the consumers. Thus, the physical, chemical, microbiological properties and sensory evaluation of Nile tilapia fillet during repeat freeze-thaw cycle are necessary to be revealed. The industries could be applied information to the Nile tilapia products and suggest to the consumers. The previous study have been reported the quality changes only in specific period, but few information in repeated freezing and thawing process. Thus, the objectives of this study was to investigate changing in physical, chemical properties and sensory evaluation of frozen Nile tilapia fillets as affected by phosphates solution and freeze-thaw treatment.

2. Materials and Methods

2.1 Raw materials

Live Nile tilapia (*Oreochromis niloticus*) samples were purchased from a farm at Nakhon Pathom, Thailand. Fishes were slaughtered, de-scaled, eviscerated, filleted, de-skinned and washed by hand. Individual fillets were in the range of 100–150 g/piece. Nile tilapia fillets were packed approximately 1 kg/bag in polyethylene bags, and placed in ice with a fish/ice ratio of 1:3 (w/w) not longer than 2 h before an experiment. Food grade sodium tripolyphosphate (STPP) was purchased from Haifa Chemicals Ltd. (Bangkok, Thailand). Refined NaCl (99.99%) were obtained from Thai Refined Salt Co., Ltd. (Bangkok, Thailand).

2.2 Frozen fillet processing and freeze-thaw treatment

Nile tilapia fillets were separated in 3 groups. The first group was immersed in distilled water as a control sample. The second group was soaked in 1.4% (w/v) STPP solution and the third group was immersed in the brine solution. The brine solution in the third group was prepared with concentrations of 1.4% STPP and 2.7% NaCl according to previously studied by Wangtueai and Vichasilp (2015). The Nile tilapia fillets in all groups were immersed in the cold solutions (4ºC) with the ratio of fish fillets to solutions 1:5 (w/w) for 115 min. The samples were then drained in a plastic basket for 1 min. The fillets samples in all group were frozen using an air blast freezing machine (Mini Batch Freezer 100, Industrial Gas Co. Ltd., Bangkok, Thailand) at -60ºC for 20 min until the core temperature reached -30ºC. The samples were then glazed using cold water (about 1±1ºC) for 10 s. The obtained one frozen fish fillets were individually packed in polyethylene zip lock bags (Siam Makro Public Co. Ltd., Bangkok, Thailand) and kept at -18 to -20ºC. The frozen fillet sample were thawed by placed at 4ºC for 48 h and repeat frozen at -20ºC for 7 days that was one freeze-thaw cycle. The same procedure of freeze-thaw cycle was repeated for the 3 and 5 (times) freeze-thaw cycles.

2.3 Proximate composition and pH measurement

Moisture, ash, crude protein and fat contents (ether extraction) of Nile tilapia fillets were determined in according to the AOAC standard method 934.01, 942.05, 954.01 and 991.36, respectively (AOAC, 2000). Crude protein of the fish fillets was expressed as 6.25× nitrogen content. The pH measurement, 5 g of chopped fish fillets were blended with 50 mL distilled water and the pH of samples were measured using a pH meter (Metrohm 744, Herisau, Switzerland). All analyses were performed in triplicate.

2.4 Thiobarbituric acid-reactive substances (TBARS) and total volatile basic-nitrogen (TVB-N)

TBARS was determined in triplicate according to the method of Buege and Aust (1978). The 0.5 g of minced fish muscle was placed into a test tube and mixed with 5 mL of the mixture solution containing thiobarbituric acid (0.375 g/100 mL), trichloroacitic acid (15 g/100 mL) and concentrated hydrochloric acid (0.875 mL). The mixture was heated in boiled water (95ºC) for 10 min, followed by cooling with running tap water. The mixture was centrifuged at 3000×g for 15 min (centrifuge Hermle ZK 316, Hermle Labortechnik GmbH, Wehingenand, Germany) and subjected to absorbance measurement at 532 nm using UV/Vis spectrophotometer (Jasco 7800, Tokyo, Japan). TBARS were calculated from the standard curve of malondialdehyde bis (diethyl acetal) (Merck, Germany) and expressed as mg malondialdehyde/kg muscle.

TVB-N in fish muscle were determined in triplicate by using the method of Conway's micro-diffusion (Conway and Byrne, 1933). The 2 g of minced fish fillets were homogenized with 8 ml of 4% Trichloroacetic acid (TCA) and filtered through a Whatman No. 1 filter paper (Whatman International Ltd., Maidstone, UK). The filtrate was subjected to analyses. The edge of Conway's micro-diffusion unit was applied a sealing agent (petroleum jelly, Vaseline, USA). The 1 ml of filtrate was placed into the outer ring of Conway's micro-diffusion unit. The solution of 1% boric acid containing the mix indicator (bromocresol green and methyl red) was then pipetted into the inner ring. To initiate the reaction, 1 mL of saturated K_2CO_3 was pipetted into outer ring and gently mixed with the filtrate and closed the cover of Conway's micro-diffusion unit before incubation at 37ºC for 60 min. The inner ring solution with a green color was then titrated with 0.02 N HCl until the green color turned to be pink. TVB-N was calculated as follows:

TVB-N (mg of Nitrogen/100 g of sample) = [14 × (titration volume of sample – titration volume of blank) × 0.02 × total volume of TCA (mL) before filtration × 100]/ minced fish sample weight (g)

2.5 Drip and cooking loss determination

Drip loss was determined according to Gonçalves and Ribeiro (2009) method. The frozen fish fillets were thawed at 4ºC for 24 h and removed excessive water (drip) from fish fillet surface by using a filter paper absorption. The weight of each fillet was measured before and after thawing. Drip loss was calculated as follows:

Drip loss (%) = [(weight before thawing – weight after thawing)/ weight before thawing] × 100

The cooking loss was determined in according to the method of Rattanasatheirn *et al*. (2008) with slight modifications. The weight of fish fillets after thawing and after cooking by steaming at 95±2ºC for about 15 min until the core temperature reached 70ºC (using a hand-held thermometer for measuring) were carried out. Cooking loss were determined as follows:

Cooking loss (%) = [(weight after thawing – weight after steaming)/ weight after thawing] × 100

2.6 Color measurement

The color parameter of lightness (*L**), redness (*a**), and yellowness (*b**) were measured using a Minolta model CM-3500d colorimeter (Minolta, Osaka, Japan). The upper central line part of the fish fillets (shown in Figure 1) was used for color measurement and six measurement were taken for each fish fillets. The *L** represents on a 0–100 point scale from black to white; *a** is the position between red (+) and green (-); and *b** is the position between yellow (+) and blue (-).

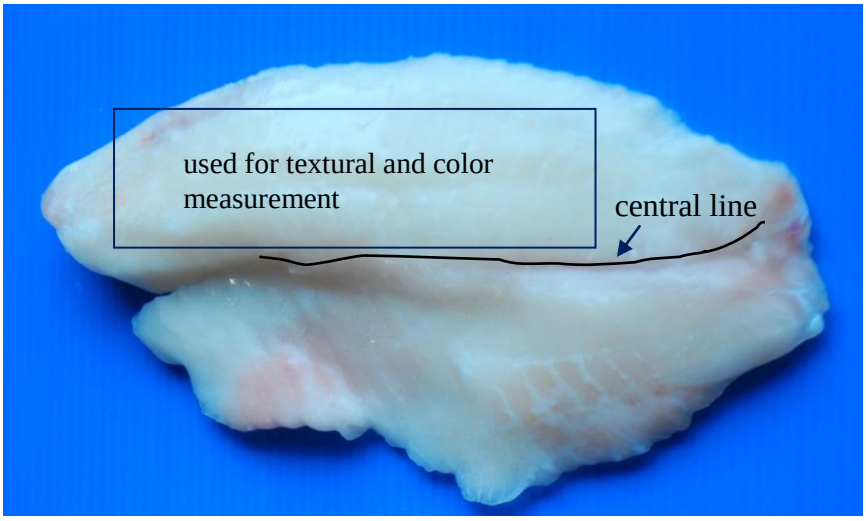

Figure 1 Sampling diagram of frozen Nile tilapia fillet used for color and textural measurements

2.7 Texture measurement

Texture profile analysis (TPA) was evaluated using a Texture analyzer (Stable Micro System, TA-HD, Surrey, UK) with a 25 kg load cell. The upper central line part of the fish fillets (shown in Figure 1) used for TPA determined following the method of Hernández *et al*. (2009).

The raw fillets of each specimen of width × length × high (30×30×20 mm) were oriented with the muscle fibers oriented horizontally for compression using a 35 mm diameter of aluminum cylindrical probe until the deformation reached 25% at a speed of 50 mm/min. The pause between the first and second compressions was 5 s. Three independent measurements were made for each treatment and the force-time curves were obtained to determine the textural parameters including hardness, gumminess, and chewiness.

2.8 Sensory evaluation

Sensory evaluation of whole raw fillets and cube cooked fillets were evaluated with 40 non-trained panelists. The frozen fillet samples were thawed and individual packed into zip lock plastic bag for whole raw fillets evaluation by appearance and texture attributes, while cooked sample was prepared according to the method of Masniyom *et al*. (2005) with slight modification. In briefly, thawed fillets were cut into cubes (30×30×20 mm). The samples were wrapped with aluminum foil and steamed in steaming pot until the core temperature reached 70ºC (measured with a hand-held thermometer) for 15 min. The cooked fillets were evaluated by appearance, odor, taste, and texture. The evaluation of raw and cooked fillets were used a 9-point hedonic scale when 1, extremely dislike; 5, neither like nor dislike; 9, extremely like (Mailgaad *et al*., 1999).

2.9 Statistical analysis

Statistical analysis with ANOVA was performed using IBM SPSS statistics 20 software (IBM Corporation, USA). Completely Randomized Block Design (RCBD) was used for sensory data. Duncan's new multiple range test (DMRT) was used to test for the differences between means. The significance level was at $p \leq 0.05$.

3. Results and Discussions

3.1 Proximate composition, pH, TVB-N and TBARS

The proximate composition of Nile tilapia fillets was 17.3% (db) protein, 2.54% (db) fat, 75.7% (wb) moisture and 0.91% (db) ash. The previous studies have indicated that tilapia fish meat is a high moisture and lower fat content as a lean variety with lower than 2% fat (Subbaiah *et al*., 2015). Liu *et al*. (2015) reported that tilapia fillets in China market had moisture (60.9–80.2%), protein (16.1–22.8%), and fat (1.1–14.4%) that was varied widely depending on source of material.

The pH are presented in Figure 2. The pH of samples varied between 6.12 and 6.80. The initial pH value of treated samples (6.72–6.73) were higher than control sample (6.14) due to affecting of phosphate solution. At the first freeze-thaw cycle, the pH values of control and STPP immersed samples slightly increased, which may be associated with the increasing of

the volatile base nitrogen as obtained also in previous studies of Emire and Gebremarian (2010) and Subbaiah *et al*. (2015). The pH values of sample slightly decreased during the 3th and 5th of freeze-thaw cycles, which was probably caused by increasing concentration of substances in unfrozen remaining water that modified the acid-base equilibrium. This may have made the fish muscle more acid (Rodriguez-Turienzo *et al*., 2011; Soares *et al*. 2015). The treated samples with STPP+NaCl were increased as increasing in freeze-thaw cycle.

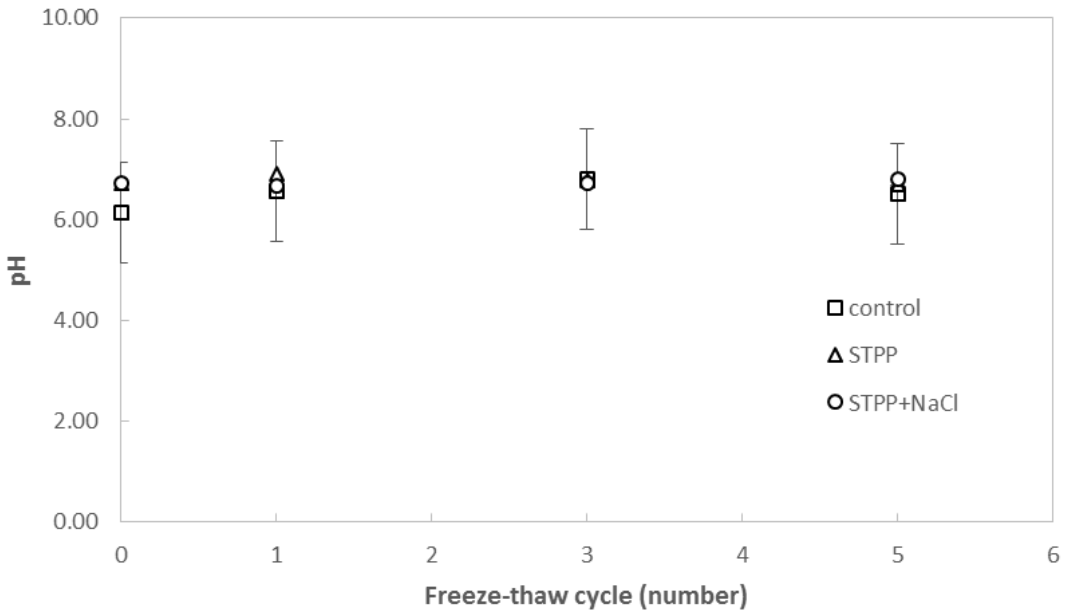

Figure 2 pH of frozen Nile tilapia fillet at 0, 1, 3 and 5 freeze thaw cycles

TVB-N changes are shown in Figure 3. TVB-N values in treated samples were slightly lower than untreated samples ($p \leq 0.05$). This might be due to antimicrobial activity of phosphate compounds, which have been wildly used in fishery products (Masniyom *et al*., 2005). TVB-N in fish species consists mostly of ammonia, dimethylamine (DMA), and trimethylamine (TMA) and is related mostly to number of spoilage microorganisms in fish (Kulawik *et al*., 2013). The TVB-N in treated samples increased significantly ($p<0.05$) compared with control sample at all freeze-thaw cycles. This might be due to washing out of TMA by melting ice crystal during thawing process or the fluctuations in ammonia level, which have been reported during storage of whole Nile tilapia in ice (Kulawik *et al*., 2013). However, TVB-N of Nile tilapia fillets passed 5th freeze-thaw cycle in this study remained (8.51–11.34 mg/100 g) below the upper acceptable limit of 25 mg/100 g (Kulawik *et al*., 2013).

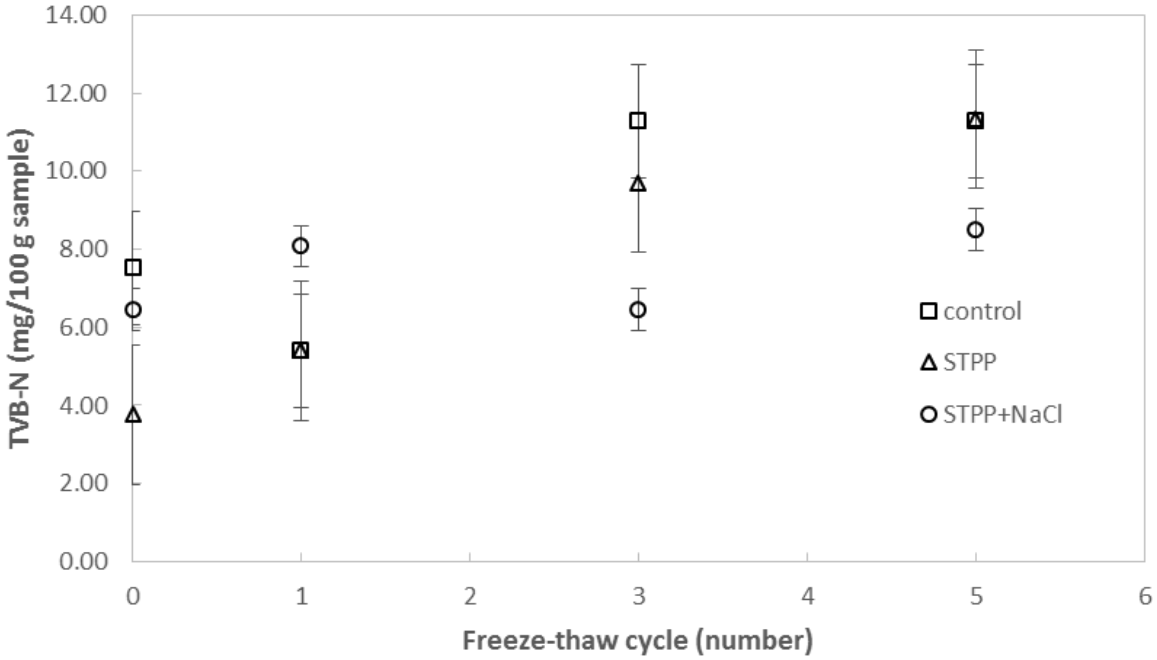

Figure 3 Total volatile base nitrogen (TVB-N) of frozen Nile tilapia fillet at 0, 1, 3 and 5 freeze thaw cycles

The levels of TBARS in fish fillets slightly changed (Figure 4) but were not significant difference (p>0.05) between treated and control samples throughout freeze-thaw cycle. TBARS was in the range of 0.0071–0.0204 mg malondialdehyde/kg.

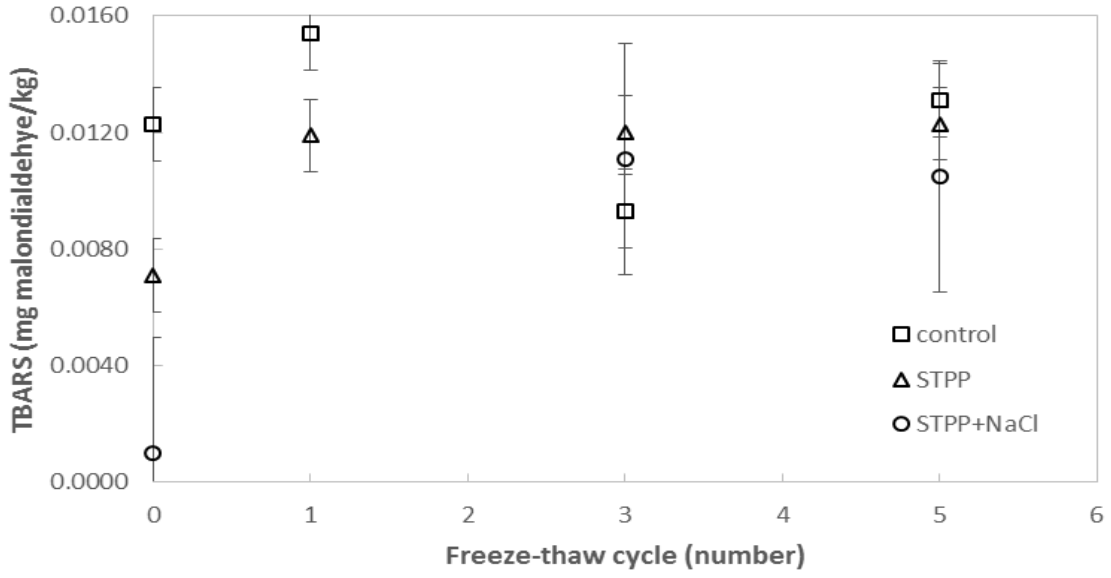

Figure 4 Thiobarbituric acid-reactive substance (TBARS) of frozen Nile tilapia fillet at 0, 1, 3 and 5 freeze thaw cycles

3.2 Drip loss and cooking loss

The drip loss and cooking loss are shown in Table 1. Drip loss in treated samples significantly increased ($p \leq 0.05$) than that in control sample throughout all freeze-thaw cycles. In the other hand, the cooking loss in treated samples with STPP+NaCl decreased with increased in freeze-thaw cycles. The control samples and treated sample with STPP had higher cooking loss than that in treated samples with STPP+NaCl ($p \leq 0.05$). According to Fellow (2000), many factors affected frozen food qualities such as the freezing method, storage temperature, recrystallization, etc. Those factors affect the size of ice crystal within products. The bigger ice crystal deforms and ruptures adjacent cell wall, resulting in increasing leaching out of water-soluble nutrients and increased drip loss. In addition, Subbaiha *et al*. (2015) reported that the ice crystal growth and increased ionic strength during crystallization affected the denaturation of protein, resulting in decreased water holding capacity of fish muscle.

Table 1 Drip loss, cooking loss, and cooking yield of frozen Nile tilapia fillet at 0, 1, 3 and 5 freeze thaw cycles

	Drip loss (%)				Cooking loss (%)		
Cycle	Control^{ns}	STPP	STPP+NaCl^{ns}	Cycle	Control	STPP^{ns}	STPP+NaCl
0^{ns}	8.59±0.92	8.34±4.00^{c}	8.14±5.69	0	6.84±5.39^{Bb}	23.26±3.28^{A}	4.86±1.66^{Bb}
1^{ns}	7.65±6.18	11.40±6.59^{bc}	10.43±3.97	1	5.54±6.24^{Bb}	19.70±9.03^{A}	8.14±1.71^{Ba}
3	10.14±0.97^{C}	14.07±0.61^{Aab}	11.55±0.41^{B}	3	14.25±2.06^{Aa}	15.82±0.76^{A}	7.02±0.69^{Ba}
5	9.32±0.61^{B}	15.78±1.25^{Aa}	14.70±4.37^{A}	5	13.96±0.81^{Aa}	14.78±3.24^{A}	6.96±1.12^{Ba}

Note: control was untreated sample, STPP and STPP+NaCl were treated with phosphate, respectively.

mean±SD (n=5) values with different lowercase letters in the column are significantly different ($p \leq 0.05$) and different capital letters in the row are significantly different ($p \leq 0.05$) in each properties. ^{ns} are not significantly different in the column or row ($p > 0.05$) in each properties.

3.3 Color measurement

Changes in color of fish fillets are shown in Table 2. The L^* values showed a slight increase as a freeze-thaw cycle increased and L^* values of treated sample were higher than untreated samples. This was possibly due to a greater water deposits on the surface of fish fillets during thawing process and washing out the myoglobin (Subbaiha *et al*., 2015). a^* and b^* value of control and treated samples were not different ($p > 0.05$) throughout all freeze thaw cycles. The characteristic of muscle structure and pigment concentration in fish muscle influenced the color, and the color loss in fish fillets during storage might be ascribed to the protein oxidation (Cai *et al*., 2014).

Table 2 Color measurements at 0, 1, 3 and 5 freeze thaw cycles of frozen Nile tilapia fillets

		Mean ± SD		
Color parameter	**cycle**	**control**	**STPP^{ns}**	**STPP+NaCl^{ns}**
L*	0	41.31±1.07^{Ba}	42.25±1.30^{B}	45.29±0.88^{A}
	1	37.32±1.70^{Bb}	44.18±2.48^{A}	45.58±37.32^{A}
	3	41.61±2.14^{Ba}	42.67±1.46^{B}	47.23±2.60^{A}
	5^{ns}	44.12±4.35^{a}	43.72±4.18	45.13±3.32
		control	**STPP**	**STPP+NaCl**
a*	0^{ns}	-3.13±0.33^{ab}	-2.05±1.07^{a}	-2.02±1.01^{a}
	1^{ns}	-3.17±0.46^{ab}	-2.95±0.26^{bc}	-2.89±0.39^{b}
	3	-2.79±0.19^{Aa}	-3.25±0.17^{Bc}	-3.50±0.16^{Cb}
	5	-3.51±0.27^{Bb}	-2.48±0.23^{Aab}	-3.41±0.14^{Bb}
		control^{ns}	**STPP**	**STPP+NaCl^{ns}**
b*	0^{ns}	-3.13±2.06	2.49±1.43^{b}	1.89±1.83
	1^{ns}	3.20±0.91	2.94±1.13^{b}	4.35±1.22
	3	4.35±1.16^{A}	2.18±0.91^{Bb}	3.42±1.12A^{B}
	5	2.91±1.17^{B}	5.88±1.81^{Aa}	2.62±2.15^{B}

Note: control was untreated sample, STPP and STPP+NaCl were treated with phosphate, respectively. mean±SD (n=6) values with different lowercase letters in the column are significantly different ($p \leq 0.05$) and different capital letters in the row are significantly different ($p \leq 0.05$) in each properties.

^{ns} are not significantly different in the column or row ($p > 0.05$) in each properties.

3.4 Texture measurements

The texture profile analysis (TPA) of frozen Nile tilapia fillets is shown in Table 3. Significant variations ($p \leq 0.05$) were observed on the different freeze thaw cycles. The hardness, gumminess and chewiness parameters tended to decreased as increase in freeze thaw cycles, but adhesiveness, cohesiveness and springiness were not significantly different ($p > 0.05$) (data not shown). Subbaiha *et al*. (2015) reported that both ice crystals formation and protein denaturation influenced texture profile of fish during frozen storage. The growth of large ice crystals disrupted fish muscle leading to release of proteases, and ice crystals bind water from the protein resulting in the disruption and weakening of the protein binding system. In addition, texture of fish muscle depends on biological characteristic of fish such as the dense of muscle, fat and collagen composition as well as external factors such as postharvest management contributing to autolysis, microbiological spoilage (Hernandez *et al*., 2009).

Table 3 Instrumental texture analyses at 0, 1, 3 and 5 freeze thaw cycles of frozen Nile tilapia fillets

Parameters	Cycle	Mean ±SD		
		Control	**STPP**	**STPP+NaCl**
Hardness	0^{ns}	1436.75 ± 122.81^{a}	1432.33 ± 95.11^{a}	1657.95 ± 114.79^{a}
	1^{ns}	823.68 ± 315.99^{b}	1020.46 ± 326.59^{b}	1006.48 ± 186.91^{b}
	3	746.48 ± 28.72^{Bb}	816.90 ± 25.99^{Bb}	1241.56 ± 101.33^{Ab}
	5^{ns}	4.60 ± 1.69^{c}	5.53 ± 1.09^{c}	5.13 ± 1.20^{c}
		Control	**STPP**	**STPP+NaCl**
Gumminess	0^{ns}	800.83 ± 78.18^{a}	845.05 ± 61.21^{a}	935.14 ± 40.47^{a}
	1^{ns}	482.45 ± 181.72^{b}	594.83 ± 170.65^{b}	549.18 ± 82.50^{c}
	3	466.04 ± 29.18^{Bb}	489.55 ± 170.65^{Bbc}	720.59 ± 97.69^{Ab}
	5^{ns}	336.16 ± 15.73^{b}	369.47 ± 38.85^{c}	381.44 ± 55.74^{d}
		Control	**STPP**	**STPP+NaCl**
Springiness	0^{ns}	564.55 ± 50.33^{a}	633.18 ± 74.94^{a}	647.67 ± 34.36^{a}
	1^{ns}	307.39 ± 120.25^{b}	411.50 ± 102.10^{b}	290.08 ± 136.30^{c}
	3	326.75 ± 32.00^{Bb}	325.60 ± 9.67^{Bb}	502.03 ± 117.90^{Aab}
	5^{ns}	306.97 ± 7.46^{b}	303.26 ± 38.88^{b}	339.27 ± 58.10^{bc}

Note: control was untreated sample, STPP and STPP+NaCl were treated with phosphate, respectively. mean±SD (n=3) values with different lowercase letters in the column are significantly different ($p\leq0.05$) and different capital letters in the row are significantly different ($p\leq0.05$) in each properties.

^{ns} are not significantly different in the column or row ($p>0.05$) in each properties.

3.5 Sensory evaluation

The acceptability score of appearance and texture of raw fish fillets treated with STTP and STTP+NaCl were significantly higher ($p\leq0.05$) than that in control sample throughout all of the freeze thaw cycles (Table 4). The appearance, odor, flavor and texture scores of cooked treated samples were higher than control ($p\leq0.05$) at all freeze thaw cycles (Table 5). This decreasing score was related with freshness loss during storage. Subbaiah *et al*. (2015) reported that during frozen storage, the sensory qualities decreased because the major cellular components of fish were gradually deteriorated and the leaching of pigments along with drip water resulted a gradual loss of brightness of fish muscle.

Table 4 Sensory evaluation of raw frozen Nile tilapia fillet at 0, 1, 3 and 5 freeze thaw cycles

		Mean ±SD		
Sensory attribute	**Cycle**	**Control**	**STPP^{ns}**	**STPP+NaCl**
Appearance	0^{ns}	6.80±1.02^{a}	6.60±1.26^{a}	7.20±1.04^{a}
	1	6.03±1.56^{ABb}	6.68±1.38^{Aa}	5.40±1.48^{Bc}
	3^{ns}	5.43±1.52^{c}	5.93±1.35^{b}	6.10±1.77^{b}
	5^{ns}	5.00±1.22^{c}	5.28±1.20^{c}	5.50±1.34^{bc}
		Control	**STPP**	**STPP+NaCl**
Texture	0^{ns}	6.88±1.04^{a}	6.45±1.50^{a}	7.08±0.97^{a}
	1	6.00±1.40^{Bb}	6.63±1.10^{Aa}	5.75±1.53^{Bb}
	3	4.85±1.48^{Bc}	5.30±1.91^{ABb}	5.98±1.87^{Ab}
	5^{ns}	4.60±1.30^{c}	4.88±1.68^{b}	5.30±1.56^{b}

Note: control was untreated sample, STPP and STPP+NaCl were treated with phosphate, respectively. mean±SD (n=40) values with different lowercase letters in the column are not significantly different ($p \leq 0.05$) and different capital letters in the row are not significantly different ($p \leq 0.05$) in each properties.

^{ns} are not significantly different in the column or row ($p > 0.05$) in each properties.

Table 5 Sensory evaluation of cooked frozen Nile tilapia fillet at 0, 1, 3 and 5 freeze thaw cycles

		Mean ± SD		
Sensory attribute	**Cycle**	**Control**	**STPP**	**STPP+NaCl**
Appearance	0	5.38±1.98^{Bab}	6.88±1.16^{Aa}	6.63±1.15^{Aa}
	1^{ns}	5.95±1.84^{a}	6.40±1.35^{ab}	6.10±1.28^{ab}
	3	5.28±2.03^{Bab}	6.23±1.39^{ABb}	5.88±1.36^{Bb}
	5	4.60±1.69^{Bb}	5.53±1.09^{Ac}	5.13±1.20^{Bc}
		Control	**STPP**	**STPP+NaCl**
Odor	0	5.90±1.85^{Ba}	7.40±1.24^{Aa}	5.98±1.66^{Ba}
	1^{ns}	5.53±1.60^{b}	5.90±1.92^{b}	5.90±1.69^{a}
	3	5.40±1.55^{Bb}	5.78±1.94^{Ab}	5.48±1.66^{Bb}
	5^{ns}	4.98±1.40^{c}	5.33±1.67^{b}	5.10±1.61^{c}

Table 5 Sensory evaluation of cooked frozen Nile tilapia fillet at 0, 1, 3 and 5 freeze thaw cycles (Continue)

Sensory attribute	Cycle	Mean ± SD		
		Control	**STPP**	**STPP+NaCl**
Flavor	0	6.33 ± 1.46^{Ba}	7.00 ± 1.30^{Aa}	7.10 ± 1.22^{Aa}
	1^{ns}	5.85 ± 1.72^{ab}	6.00 ± 1.66^{b}	5.98 ± 1.44^{b}
	3	5.55 ± 1.48^{Cb}	6.25 ± 1.41^{Bb}	6.68 ± 1.25^{Aa}
	5	5.28 ± 1.22^{Cb}	5.85 ± 1.35^{Ab}	5.68 ± 1.02^{Bb}
		Control	**STPP**	**STPP+NaCl**
Texture	0	6.15 ± 1.56^{Ca}	6.48 ± 1.55^{Ba}	6.93 ± 1.31^{Aa}
	1	5.33 ± 1.53^{Bbc}	5.85 ± 1.69^{Aab}	5.13 ± 1.87^{Cbc}
	3^{ns}	5.58 ± 1.62^{ab}	5.23 ± 1.49^{bc}	5.58 ± 1.50^{b}
	5^{ns}	4.70 ± 1.60^{c}	4.83 ± 1.52^{c}	4.80 ± 1.42^{c}

Note: control was untreated sample, STPP and STPP+NaCl were treated with phosphate, respectively. mean±SD (n=40) values with same lowercase letters in the column are not significantly different ($p\leq0.05$) and same capital letters in the row are not significantly different ($p\leq0.05$) in each properties.

^{ns} are not significantly different in the column or row ($p>0.05$) in each properties.

4. Conclusions

Frozen tilapia fillet treated with STTP and STTP+NaCl solution were less in TVB-N and cooking loss, when the freeze thaw cycle was increased. On the other hand, *L** was gradually increased as increasing in freeze thaw cycle. These results were showed that STTP or STTP+NaCl solution probably can be enhance the physical and chemical properties of frozen tilapia fillet after passed freeze thaw cycle. The sensory evaluation was in line with the physical and chemical properties of frozen tilapia fillet. The raw and cooked samples treated with STTP and STTP+NaCl were accepted at the longer freeze thaw cycles.

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