WALAILAK JOURNAL

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Combination Effects of Phosphate and NaCl on Physiochemical, Microbiological, and Sensory Properties of Frozen Nile Tilapia (*Oreochromis niloticus*) Fillets during Frozen Storage

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Received: 22 October 2017, Revised: 11 July 2018, Accepted: 30 August 2018

Abstract

The objective of this research was to investigate the combination effects of phosphate and sodium chloride (NaCl) on the quality of frozen Nile tilapia fillets (control and treated with sodium tripolyphosphate 1.4 % STPP + 2.7 % NaCl) during storage at -18 °C for up to 8 months. Results showed that moisture content decreased slightly ($P \le 0.05$), while pH gradual decreased, total volatile base nitrogen (TVB-N) increased, and hardness and gumminess decreased with increasing time ($P \le 0.05$). Thiobarbituric acid-reactive substances (TBARS) values were low (0.01 - 0.03 mg malonaldehyde/kg) and phosphate content ranged from 3350 - 3900 mg/kg. There was no significant difference (P > 0.05) in drip loss during storage. The control had higher cooking losses and the L^* value increased with increasing storage time, while a^* , b^* , C^* , and h^* values were not significantly different (P > 0.05). Appearance and texture acceptability scores of treated fish were significantly higher than the control throughout storage ($P \le 0.05$). Total aerobic psychrophilic and mesophilic bacteria were relatively unchanged at about 4 log CFU/g.

Keywords: Phosphate, Nile tilapia, Frozen fish, Tripolyphosphate, Sodium chloride

Introduction

Nile tilapia (*Oreochromis niloticus*) is an economically important freshwater fish in many countries. Nile tilapia had become an important whitefish, which has enabled sector to expand substantially by reaching new consumers both domestically and through exports from the main producing countries. Thailand is one of the major producers of Nile tilapia production in the world [1,2]. In 2015, Thailand exported about 8,000 tons of frozen Nile tilapia with a value of about 18 million US dollars [3].

Freezing is one of the preservation methods used widely for fish and seafood products as an effective and economical way to prolong freshness and flavor. Nevertheless, the low temperatures used in freezing could affect fish muscle protein properties over time, resulting in unacceptable quality changes. The main problems for frozen foods are excessive drip loss and weight loss, which can result in undesirable sensory properties [4-6]. Emire and Gebremarian [7] also showed that significantly quality loss was seen during frozen storage for non-chemically treated tilapia fillets. Total volatile base nitrogen (TVB-N), pH, and total bacterial load increased significantly. However, the fish remained microbiologically acceptable for human consumption. Subbaiah *et al.* [8] reported that tilapia muscle

proteins were gradually degraded during frozen storage, resulting in the loss of functional properties and decrease in sensory acceptability.

To solve those problems, raw fishery products have often been treated with phosphate compounds before freezing to aid in water retention both during thawing and cooking [4,9]. Phosphate compounds are legally permitted additives that are widely used in a variety of fishery products. However, the residual compounds in the final products should not exceed 5,000 mg/kg [10,11]. In general, 1 - 6 % phosphate solutions with 2 - 5 %NaCl are used to retain moisture and protect the taste of fishery products [12-14]. Wangtueai and Vichasilp [14] reported successful optimization of the processing conditions for phosphate and salt in Nile tilapia fillets previously frozen.

However, the effects of phosphates and NaCl on frozen Nile tilapia quality with longer storage periods has not be extensively studied. More comprehensive evaluations of the physical, chemical, and microbiological properties, as well as sensory changes during storage, with longer storage is needed. Therefore, the objectives of this research were to investigate changes in the physiochemical, microbiological, and sensory attributes of frozen Nile tilapia fillets during 8 months of frozen storage untreated or treated with combinations of sodium tripolyphosphate and NaCl.

Materials and methods

Raw materials

Live Nile tilapia (*Oreochromis niloticus*) samples were purchased from a farm at Nakhon Pathom, Thailand. The fish was cut from the gill using a knife, followed by descaling, eviscerating, filleting, and skinning by hand. Individual fillets ranged from 100 - 150 g. The fillets were packed in polyethylene bags (~1 kg/bag), and placed in ice with a fish/ice ratio of 1:3 (w/w) for transportation to the laboratory within 2 h. Food grade sodium tripolyphosphate (STPP) was purchased from Haifa Chemicals Ltd. (Bangkok, Thailand). Refined NaCl (99.99 % according to the manufacturer) was purchased from Thai Refined Salt Co., Ltd. (Bangkok, Thailand).

Frozen fillet processing

The optimum conditions of STPP and NaCl application were used as previously reported by Wangtueai and Vichasilp [14]. Following their optimum procedure, a combination of 1.4 % STPP and 2.7 % NaCl brine solution was prepared using distilled water. Completely randomized design (CRD) was applied for study of the effects of additives and storage periods. Nile tilapia fillets were immersed in brine solutions at 4 °C with a fish to solutions ratio of 1:5 (w/w) for 115 min on the same day as when the fish were slaughtered. The samples were then drained in a plastic basket for 1 min. The soaked (STPP+NaCl) and un-soaked fillets (control) were frozen using a cryogenic nitrogen freezer (Mini Batch Freezer 100, Industrial Gas Co. Ltd., Bangkok, Thailand) at about -60 °C for 20 min until the core temperature using thermocouples reached approximately -30 °C. The samples were then manually glazed using cold distilled water (about 1 ± 1 °C) for 10 s. The frozen fillets were individually loosed-packed in polyethylene Zip-lock bags (Siam Makro Public Co. Ltd., Bangkok, Thailand) and kept at -18°C for up to 8 months with regular sampling.

Drip loss and cooking loss

Drip loss on thawing (at 4 °C for 24 h) was determined in triplicate following the method of Gonçalves and Ribeiro [4]. Drip loss was calculated as follows:

Drip loss (%) = [(weight before thawing - weight after thawing)/weight before thawing] $\times 100$ (1)

The treated and untreated yields after cooking were determined using the method of Rattanasatheirn *et al.* [15] with slight modifications. The weights of fish fillets after thawing and after cooking were obtained. Thawed fillets were cooked over steam at approximately 95 ± 2 °C using a thermocouple for about 15 min until the core temperature of the fish reached 70 °C. Cooking loss was calculated as follows:

Cooking loss (%) = [(weight after thawing - weight after steaming)/weight after thawing] $\times 100$ (2)

Proximate composition, pH, and phosphate content

Moisture, ash, crude protein, and fat contents of Nile tilapia fillets were determined according to standard AOAC methods 934.01, 942.05, 954.01, and 991.36, respectively [16]. The crude protein of the fish fillets was expressed as 6.25×nitrogen content. Phosphate content of frozen fish fillets was determined according to AOAC method 986.24 [17]. To determine pH, a fish fillet was put in a blender (Blender, Tefal, Groupeseb Ltd., Bangkok, Thailand) and then 5 g of the chopped fish were mixed with 50 ml distilled water and the pH measured (Metrohm 744, Metrohm Ltd., Herisau, Switzerland). All analyses were done in triplicate.

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

TBARS were determined in triplicate according to the method of Buege and Aust [18]. Chopped fish muscle (0.5 g) was placed into a test tube and mixed with 5 ml of mixture containing 0.375 g/100 ml thiobarbituric acid (TBA) (Merck, Darmstadt, Germany), 15 g/100 ml of trichloroacetic acid (TCA), and 0.875 ml of concentrated HCl. The mixture was heated in boiling water for 10 min, followed by cooling with running tap water. The mixture was centrifuged at 3000×g for 15 min (Hermle ZK 316, Hermle Labortechnik GmbH, Wehingenand, Germany). The absorbance of the supernatant was measured at 532 nm using a UV/Vis spectrophotometer (Jasco 7800, Tokyo, Japan). TBARS were calculated from the standard curve of malondialdehyde-bis-(diethyl acetal) (Merck) assuming 100 % purity and expressed as mg malondialdehyde/kg muscle.

TVB-N in fish muscle was determined in triplicate using the Conway micro-diffusion method as described by Conway and Byrne [19]. The chopped fish fillets (2 g) were blended with 8 ml of 4 % TCA and the homogenate filtered through Whatman No. 1 filter paper (Whatman International Ltd., Maidstone, UK). The filtrate was used for the analyses. A sealing agent (Vaseline petroleum jelly, Unilever Thai Trading Ltd., Bangkok, Thailand) was applied to the edges of the Conway micro-diffusion units to seal them. Then, 1 ml of filtrate was placed into the outer ring of the Conway micro-diffusion unit. One ml of a 1 % boric acid solution containing an indicator (0.01 g bromocresol green and 0.02 g methyl red in 10 ml ethyl alcohol) was then pipetted into the inner ring. To initiate the reaction, 1 ml of saturated K_2CO_3 was carefully pipetted into the outer ring and gently mixed with the sample extract. The unit was closed and incubated at 37 °C for 60 min. The inner ring solution with a green color was then titrated with 0.02 N HCl until the green color became pink. TVB-N was calculated as mg of Nitrogen/100 g of sample.

Color measurements

Color was measured using a Minolta model CM-3500d colorimeter (Minolta, Osaka, Japan). Six measurements were taken for each fish fillet above the lateral line on the side facing the bone. Fillets were in a plastic petri dish and color measurements were taken through the plastic. Color values were expressed as CIELAB coordinates. The L^* represents the lightness on a 0 - 100 point scale from black to white; a^* is the red (+) and green (-) colors; and b^* is the yellow (+) and blue (-) colors. The color intensity is expressed as a chroma value C^* and hue value h^* . These values were calculated accordingly:

 $C^* = (a^{*2} + b^{*2})^{\frac{1}{2}}$ and $h^* = \tan^{-1}(b^*/a^*)$.

(3)

A white tile supplied by the manufacturer was used to calibrate the instrument.

Textural properties

A texture analyzer (Stable Micro System, TA-HD, Surrey, UK) with a 25 kg load cell was used for texture profile analysis (TPA). Following the Hernández *et al.* [20] method, selected parts of the raw fillets (about the lateral line of the fish fillets) were cut using a sharp knife into blocks approximately $30 \times 30 \times 20$ mm³. The fish blocks were oriented with the muscle fibers horizontally (height = 20 mm) and the blocks were compressed using an aluminum cylindrical probe (35 mm diameter) with 25 %

deformation of the initial height at a speed of 50 mm/min with a pause of 5 s between the first and second compressions. Three independent measurements were made for each treatment. The textural parameters of hardness, gumminess, adhesiveness, cohesiveness, chewiness, and springiness were obtained using the instrument software.

Microbiological analysis

Total viable mesophilic and psychrophilic counts were determined using the modified method of Arashisar *et al.* [21]. Approximately 25 g of fish fillets were homogenized with 225 ml sterile 0.85 % normal saline solution using a Stomacher (Seward BA 7021, London, UK). Additional 10-fold dilutions were prepared with sterile normal saline. The pour-plate method using plate count agar (PCA) media (Merck) was used for total viable aerobic bacterial counts. The inoculated plates were incubated at 37°C for 24 h for total viable mesophilic counts, and at 5 °C for 72 h for psychrophilic counts. All analyses were done in triplicate.

Sensory evaluation

Samples preparation was done using the method of Masniyom *et al.* [9] with slight modifications. Before sensory testing, the samples were tested to assure microbial safety. The frozen fillet samples were thawed and cut into $30 \times 30 \times 20$ mm³ cubes. The samples were wrapped tightly with aluminum foil and steamed until the core temperature was approximately 70 °C for 15 min. 40 untrained panelists (students and faculty mostly from the department) evaluated coded samples using a 3-digit random code for whole raw fillets for appearance and texture, and a different 3-digit code for cooked fillets for appearance, odor, taste, and texture using a 9-point hedonic scale with 1 = extremely dislike; 5 = neither like nor dislike; 9 = extremely like [22].

Statistical analysis

Statistical analysis with one-way ANOVA was done using IBM SPSS statistics 20 software (IBM Corp., Armonk, New York, USA). Duncan's new multiple range test (DMRT) was used to test for the differences between means of each month. Comparisons between the means of treated and untreated sample were analyzed using a t-test. The significance level was $P \le 0.05$.

Results and discussion

Chemical properties

The proximate composition of Nile tilapia fillets was 17.3 ± 0.4 % protein, 2.5 ± 0.2 % fat, 75.7 ± 0.6 % moisture, and 0.9 ± 0.1 % ash, accounting for 96.5 % of the total wet weight. The proximate composition of Nile tilapia fillets showed high protein and low fat, which Subbaiah *et al.* [8] suggested placed tilapia in the lean category, with < 2 % fat. However, the tilapia in this study had a somewhat higher fat content.

The pH and moisture contents are shown in **Table 1**. The initial pH values of the treated samples were about 0.6 pH units higher than the untreated samples because the STPP+NaCl solution is quite alkaline (pH about 8). The pH decreased slightly ($P \le 0.05$) with storage time, which was probably due to increasing concentrations of substances in the unfrozen water that modified the acid-base equilibrium, as described by Rodriguez-Turienzo *et al.* [23] and Soares *et al.* [24]. The moisture content of treated fillets was higher than the untreated during the 8 months of storage ($P \le 0.05$), which is consistent with the water retention properties of polyphosphates. The salt and phosphates might have a greater combination effect on the myofibrillar of flesh fish by change repulsion, resulting in increased water retention in fish muscle [14].

The TVB-N changes are shown in **Figure 1**. TVB-N values in treated samples were lower than untreated samples ($P \le 0.05$), suggesting an antimicrobial activity [9] and trimethylamine-N-oxide demethylase (TMAOase) inhibition [6] of phosphates during frozen storage. TMAOase is an enzyme in fish that leads to negative protein changes. TMAOase breaks down trimethylamine-N-oxide (TMAO) into dimethylamine and formaldehyde. TMAO is found in most marine fish, some invertebrates, and some freshwater fish [25]. The TVB-N of the control increased during the first two months, then slightly

decreased at month three, and then became relatively stable with insignificant differences (P > 0.05). The TVB-N of the treated samples showed a smaller increase during the first two months, followed by a larger drop on month three, and more variability over time, although it appeared to essentially level off. In all cases, the TVB-N of the Nile tilapia fillets remained way below the upper acceptable limit of 25 mg/100 g [26].

Table 1 Changes of pH, moisture, drip loss, and cooking loss of frozen Nile tilapia during 8 months storage

Month	р	Н	Moistu	Moisture (%)		loss (%)	Cooking loss (%)	
WIOHUI	Control	STPP+NaCl	Control	STPP+NaCl	Control	STPP+NaCl	Control	STPP+NaCl
0	6.14 ± 0.08^{Bf}	6.72 ± 0.06^{Ab}	75.7 ± 0.6^{Bbcd}	77.8±0.3 ^{Aab}	8.6±0.9 ^{Aab}	8 ± 6^{Aa}	7 ± 5^{Ab}	5±2 ^{Ad}
1	6.74 ± 0.04^{Aa}	6.68 ± 0.04^{Abc}	78.2 ± 0.9^{Aab}	79 ± 3^{Aab}	8 ± 6^{Aab}	10 ± 4^{Aa}	16 ± 6^{Aa}	8 ± 2^{Bbc}
2	6.67 ± 0.02^{Aab}	6.65 ± 0.04^{Ac}	75 ± 4^{Acd}	77.8 ± 0.3^{Bab}	11±3 ^{Aa}	9±5 ^{Aa}	15±2 ^{Aa}	9±2 ^{Babc}
3	6.65 ± 0.03^{Bb}	$6.94{\pm}0.04^{Aa}$	80±1.2 ^{Aa}	$80.0{\pm}0.8^{Aa}$	6 ± 2^{Ab}	7 ± 1^{Aa}	17±1 ^{Aa}	11 ± 2^{Ba}
4	6.39±0.06 ^{Ac}	6.42±0.06 ^{Ad}	71 ± 2^{Be}	77.0 ± 0.6^{Ab}	10 ± 2^{Aab}	9.7±0.3 ^{Aa}	16±1 ^{Aa}	10±1 ^{Bab}
5	6.34±0.01 ^{Acd}	6.40 ± 0.02^{Bde}	74.8 ± 0.4^{Bcd}	78.2 ± 0.6^{Aab}	ND	ND	ND	ND
6	6.30 ± 0.02^{Ade}	6.36±0.04 ^{Ade}	73.1 ± 0.4^{Bde}	77.7 ± 0.4^{Aab}	8.5 ± 0.5^{Aab}	10 ± 2^{Aa}	13 ± 4^{Aa}	11±2 ^{Aab}
7	6.26±0.03 ^{Ae}	6.33±0.01 ^{Ae}	76.7 ± 0.3^{Bbc}	79±1 ^{Aab}	9 ± 3^{Aab}	11 ± 6^{Aa}	13±2 ^{Aa}	$7\pm 2^{\text{Bcd}}$
8	6.12 ± 0.03^{Af}	6.15 ± 0.03^{Af}	74.0 ± 0.6^{Bcde}	78.1 ± 0.9^{Aab}	9±1 ^{Aab}	11 ± 2^{Aa}	13±1 ^{Aa}	$10\pm3^{\text{Babc}}$

Mean±SD (n = 3): values with the same lowercase letter in a column are not significantly different (P > 0.05) and with the same capital letter in a row are not significantly different (P > 0.05). Samples at 5th month were not determined (ND).

The TBARS in fish fillets (**Figure 2**) were not significantly different (P > 0.05) between treated and untreated samples throughout storage and were well below the acceptable limit of 4 - 7 mg malonaldehyde/kg [9]. Kulawik *et al.* [27] also reported that lipid oxidation was minimal in both tilapia and pangasius frozen fillets. This is also consistent with the results for fresh tilapia stored in ice, which showed that even during prolonged storage, the malonaldehyde content in tilapia did not increase [26]. During the 8 months storage, the phosphate content (3,350 - 3,900 mg/kg data not shown) in all samples was lower than the legal limit (5,000 mg/kg) [10], which is consistent with the prior optimization.



Figure 1 Total volatile base nitrogen (TVB-N) of frozen Nile tilapia during 8 months storage



Figure 2 Thiobarbituric acid-reactive substances (TBARS) of frozen Nile tilapia during 8 months storage

Drip loss and cooking loss

Drip loss and cooking loss are shown in **Table 1**. Drip losses were not significantly different (P > 0.05) between treated and untreated fillets throughout storage, consistent with Turan *et al.* [28] using phosphates with frozen trout fillets. The cooking losses of the untreated samples were higher than those of the treated samples ($P \le 0.05$) after 2 months of storage and remained so except for month 6. Drip loss and cooking loss relate to the weight of fish fillets after thawing and cooking. According to Fellow [29] and Burgaard and Jørgensen [30], many factors affect frozen fish qualities, such as the freezing method, storage temperature, and recrystallization. Those factors affect the size of the ice crystals within products. The bigger ice crystals deform and rupture adjacent cell walls, resulting in increased leaching of watersoluble nutrients and drip loss. In addition, Subbaiha *et al.* [8] and Lee *et al.* [6] reported that ice crystal growth and increased ionic strength during crystallization affected the denaturation of protein, resulting in decreased water retention of fish muscle after thawing and after cooking. However, because phosphate use is limited, the added NaCl also has a major effect on ionic strength. Specifically, the chloride ions and the phosphate ions increase the electrostatic repulsion of muscle proteins, which allows more water to be bound within muscle fibers, reducing fluid loss during thawing and cooking [4].

Color and textural properties

Changes in the color of fish fillets are shown in **Table 2**. The L^* values of the treated samples were generally higher than untreated samples. This might be due to the soaking of treated fish fillets, which may have extracted some surface sarcoplasmic proteins, blood, and pigments [31]. The a^* , b^* , C^* , and h^* values of untreated and/or treated samples were not different (P > 0.05) throughout storage, while C^* of the control was higher than that of the treated samples ($P \le 0.05$) for the last two months. Changes in the color of fish fillets were possibly because of more water on the surface of fish fillets during thawing and/or washing out of hemoglobin and myoglobin [8]. However, the a^* could also reflect the accumulation of brown-colored methemoglobin/metmyoglobin due to hemoglobin oxidation in the fish flesh [32]. Thorarinsdottir *et al.* [12] reported that the color of the fish might depend on the tumbling method used during soaking, which affects the uniformity of the process, as well as causing some physical damage (gaping in fish flesh). Tumbling has to be done carefully, controlling time, speed, and temperature.

Month -		L*	<i>a*</i>		<i>b</i> *		<i>C</i> *		h*	
	Control	STPP+NaCl	Control	STPP+NaCl	Control	STPP+NaCl	Control	STPP+NaCl	Control	STPP+NaCl
0	41 ± 1^{Bd}	45 ± 1^{Abcd}	-3.1±0.3 ^{Bb}	-2.0±1.0 ^{Aa}	3.6±2.1 ^{Aa}	1.9±1.8 ^{Aa}	$4.9{\pm}1.7^{Aa}$	3.4±1.2 ^{Aa}	$140{\pm}10^{Abc}$	150±20 ^{Ab}
1	40 ± 1^{Be}	45 ± 2^{Abcd}	-2.9±0.7 ^{Ab}	-3.4±0.2 ^{Ab}	2.7±2.3 ^{Aa}	1.9±2.1 ^{Aa}	$4.3{\pm}1.5^{Aa}$	4.3±1.2 ^{Aa}	140 ± 20^{Abc}	$160{\pm}30^{Aab}$
2	42 ± 1^{Bcd}	47 ± 1^{Abc}	-3.2 ± 0.4^{Ab}	-3.4±0.4 ^{Ab}	2.3 ± 2.4^{Aa}	$0.4{\pm}1.1^{Aa}$	$4.3{\pm}1.5^{Aa}$	$3.6{\pm}0.4^{Aa}$	150 ± 20^{Aab}	170 ± 20^{Aab}
3	42 ± 1^{Ad}	44 ± 3^{Ad}	-3.1 ± 0.1^{Ab}	-3.2±0.1 ^{Ab}	2.7±0.9 ^{Aa}	$0.8{\pm}1.9^{\text{Ba}}$	4.3±0.6 ^{Aa}	$3.7{\pm}0.8^{Aa}$	$140{\pm}10^{\text{Bbc}}$	$170{\pm}30^{Aab}$
4	43 ± 2^{Bcb}	46 ± 2^{Abcd}	-3.2±0.3 ^{Ab}	-3.3±0.1 ^{Ab}	$2.5{\pm}1.4^{Aa}$	2.2±1.3 ^{Aa}	$4.2{\pm}0.7^{Aa}$	$4.1{\pm}0.7^{Aa}$	150 ± 20^{Abc}	150 ± 20^{Ab}
5	$38{\pm}1^{\rm Bf}$	47 ± 0.3^{Abc}	-1.8 ± 1.2^{Aa}	-3.2±0.3 ^{Bb}	$4.0{\pm}2.7^{Aa}$	1.6±1.9 ^{Aa}	$4.8{\pm}2.0^{\text{Aa}}$	$3.9{\pm}0.8^{Aa}$	120 ± 30^{Ac}	$160{\pm}30^{Aab}$
6	44 ± 1^{Ab}	45 ± 1^{Acd}	-3.5 ± 0.2^{Ab}	-2.8±1.3 ^{Ab}	2.1±0.7 ^{Aa}	$2.4{\pm}2.7^{Aa}$	4.1±0.3 ^{Aa}	$4.4{\pm}1.2^{Aa}$	$150{\pm}10^{Aab}$	$150{\pm}40^{Ab}$
7	44 ± 1^{Bb}	47 ± 1^{Aab}	-3.3±0.2 ^{Ab}	-3.2±0.1 ^{Ab}	3.1±2.2 ^{Aa}	1.1±0.9 ^{Aa}	$4.8{\pm}1.4^{\text{Aa}}$	$3.4{\pm}0.4^{\text{Ba}}$	140 ± 20^{Abc}	$160{\pm}10^{Aab}$
8	$47{\pm}0.2^{\text{Ba}}$	49±1 ^{Aa}	-3.5 ± 0.2^{Ab}	-3.5±0.4 ^{Ab}	$3.6{\pm}0.5^{Aa}$	$2.5{\pm}0.3^{\text{Ba}}$	4.3±0.14 ^{Aa}	$3.7{\pm}0.3^{\text{Ba}}$	$170{\pm}10^{\text{Ba}}$	180 ± 1^{Aa}

Table 2 Color measurements during 8 months storage of frozen Nile tilapia fillets

Mean±SD (n = 6): values with the same lowercase letter in a column are not significantly different (P > 0.05) and with the same capital letter in a row are not significantly different (P > 0.05)

Table 3	Instrumental	texture analyses	during 8	months storage of	frozen Nile tilapia fillets
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Month	Hard	ness (g)	Adhesiv	veness (g.s) Springiness		Cohesiveness		Gumminess (g)		Chewiness (g)		
Month	Control	STPP+NaCl	Control	STPP+NaCl	Control	STPP+NaCl	Control	STPP+NaCl	Control	STPP+NaCl	Control	STPP+NaCl
0	1440±120 ^{Aa}	1660±20 ^{Aa}	-18±9 ^{Aab}	-33±8 ^{Aab}	0.71±0.04 ^{Abcd}	0.69±0.03 ^{Abc}	0.56±0.01 ^{Aa}	0.57±0.01 ^{Aabc}	$800{\pm}78^{Aa}$	940±40 ^{Aa}	570±50 ^{Aa}	650±30 ^{Ab}
1	820±320 ^{Abc}	1010±190 ^{Abc}	-14±10 ^{Aab}	-10±14 ^{Aa}	$0.63{\pm}0.02^{\text{Ad}}$	$0.64{\pm}0.05^{Abc}$	0.59±0.01 ^{Aa}	$0.55{\pm}0.03^{Abc}$	480±182 ^{Abc}	550±80 ^{Abc}	310±120 ^{Abc}	290±140 ^{Aef}
2	1030±120 ^{Ab}	1110±210 ^{Ab}	-12±8 ^{Aa}	-43±26 ^{Aabc}	$0.64{\pm}0.01^{\text{Ad}}$	$0.67{\pm}0.16^{Abc}$	0.55±0.01 ^{Aa}	$0.58{\pm}0.03^{Aab}$	570 ± 57^{Ab}	640 ± 90^{Ab}	$360{\pm}30^{Abc}$	$420{\pm}70^{Acd}$
3	860 ± 90^{Abc}	1050±60 ^{Abc}	-15±10 ^{Aab}	-120±17 ^{Bd}	$0.84{\pm}0.04^{\text{Aa}}$	$0.84{\pm}0.05^{\text{Aa}}$	0.57±0.01 ^{Aa}	0.58±0.01 ^{Aab}	490 ± 54^{Bbc}	600 ± 40^{Abc}	$410{\pm}40^{Ab}$	510 ± 50^{Ac}
4	$900{\pm}80^{Abc}$	830±140 ^{Ac}	-8±3 ^{Aab}	-17±7 ^{Aa}	$0.65{\pm}0.02^{Bcd}$	$0.70{\pm}0.01^{Abc}$	0.58±0.01 ^{Aa}	$0.59{\pm}0.00^{Aa}$	520±43 ^{Abc}	490 ± 80^{Ac}	$340{\pm}30^{Abc}$	$340{\pm}60^{Adef}$
5	$920{\pm}170^{Abc}$	1050±120 ^{Abc}	-22±11 ^{Aab}	-110±39 ^{Bd}	0.55±0.03 ^{Aa}	$0.60{\pm}0.11^{Ac}$	0.55±0.02 ^{Aa}	$0.54{\pm}0.01^{Ac}$	510±100 ^{Abc}	560±60 ^{Abc}	$280{\pm}40^{Abc}$	$340{\pm}50^{Adef}$
6	1480±200 ^{Aa}	1590±80 ^{Aa}	-28±21 ^{Ab}	-89 ± 34^{Abcd}	$0.75{\pm}0.09^{\text{Ab}}$	$0.86{\pm}0.04^{Aa}$	0.55±0.04 ^{Aa}	$0.58{\pm}0.03^{Aab}$	810±60 ^{Aa}	920±70 ^{Aa}	610±120 ^{Aa}	$800{\pm}70^{Aa}$
7	$850{\pm}20^{Abc}$	$530{\pm}70^{Bd}$	-14±3 ^{Aab}	-97 ± 64^{Acd}	$0.69{\pm}0.01^{Abcd}$	$0.77{\pm}0.04^{Bab}$	0.57±0.03 ^{Aa}	0.59±0.03 ^{Aa}	490±38 ^{Abc}	$310{\pm}30^{Bd}$	340±20 ^{Ac}	$240{\pm}10^{\rm Bf}$
8	700±50. ^{Ac}	860±110 ^{Abc}	-32±20 ^{Ab}	-80 ± 11^{Bbcd}	0.73±0.08 ^{Abc}	0.75±0.06 ^{Aab}	0.56±0.04 ^{Aa}	0.58±0.01 ^{Aab}	390±37 ^{Ac}	500±60 ^{Ac}	290±40 ^{Abc}	370±40 ^{Ade}

Mean \pm SD (n = 3): values with the same lowercase letter in a column are not significantly different (P > 0.05) and with the same capital letter in a row are not significantly different (P > 0.05).

The texture profile analysis (TPA) of frozen Nile tilapia fillets is shown in **Table 3**. A great deal of variability was observed, so that it is difficult to assign any overall time effects or differences between samples, although adhesiveness of the STPP+NaCl increased significantly ($P \le 0.05$) towards the end of storage. Therefore, the texture of frozen fish fillet was changed. This possibility suggested that texture degradation may be due to ice crystal formation and other factors leading to protein denaturation [6,8], and soaking fish fillets in phosphates and salt solution may help retain moisture during frozen storage or cooking, resulting in firmer fillet texture [14].

Microbiological analysis

Total aerobic psychrophilic and total aerobic mesophilic of treated and control samples were at about 4 log CFU/g (**Table 4**). During frozen storage, the treated samples generally had lower counts than untreated samples ($P \le 0.05$), which is consistent with phosphates being a weak antimicrobial agent in meat and fishery products [9]. However, the microbial loads in both treated and untreated samples were well within the safety standard for consumption. ICMSF [33] recommends a total plate count (TPC) of fish flesh should be lower than 6 log CFU/g wet weight.

Sensory properties

The appearance, texture, and taste of fish fillets are important attributes for freshness and consumer acceptability [34]. The appearance relates to color and overall visual impact of fish fillets. The acceptability scores for appearance and texture of raw fish fillets were generally not significantly different (P > 0.05) each month between treated and untreated samples, and there was no overall trend with storage time (**Table 5**). Although cooked samples were sometimes different between control and treatments or time, no clear trends were observed. This might be due to the effect of using good freezing and storage conditions. However, all products were still well received by the panelists at the end of the 8 months storage.

Table 4 Microbiological evaluation (CFU/g sample) of frozen Nile tilapia during 8 months storage

Month	Psychrophile	(×10 ⁴ CFU/g sample)	de) Mesophile (×10 ⁴ CFU/g sample)					
wiontin	Control	STPP+NaCl	Control	STPP+NaCl				
0	2.6 ± 1.5^{Abc}	2.0 ± 0.5^{Abc}	5.4 ± 2.4^{Acd}	2.9 ± 0.8^{Ab}				
1	9.6 ± 3.4^{Aa}	4.7 ± 1.7^{Ba}	2.5 ± 1.3^{Bd}	$0.1{\pm}0.0^{Aa}$				
2	ND	ND	ND	ND				
3	2.9 ± 1.1^{Abc}	0.6 ± 0.6^{Bc}	4.4 ± 1.2^{Acd}	1.5 ± 0.7^{Bb}				
4	0.9 ± 0.6^{Aac}	1.0 ± 0.8^{Abc}	2.8 ± 1.4^{Ad}	1.7 ± 0.4^{Ab}				
5	3.9 ± 1.0^{Ab}	1.1 ± 0.5^{Bbc}	6.6 ± 1.2^{Abc}	4.3 ± 0.4^{Bb}				
6	4.6 ± 1.0^{Aa}	$0.4{\pm}0.2^{Bc}$	0.1 ± 0.0^{Aa}	2.1 ± 1.0^{Bb}				
7	4.3 ± 0.7^{Aab}	$2.5\pm0.7^{\text{Babc}}$	8.9 ± 3.5^{Aab}	3.0 ± 1.0^{Bb}				
8	2.9 ± 0.5^{Abc}	1.3 ± 0.6^{Bbc}	6.3 ± 2.3^{Abc}	2.5 ± 0.4^{Bb}				

Mean±SD (n = 3): values with the same lowercase letter in a column are not significantly different (P > 0.05) and with the same capital letter in a row are not significantly different (P > 0.05). Samples at 2nd month were not determined (ND).

 Table 5 Sensory properties of frozen Nile tilapia after thawing and cooking during 8 months storage

		Raw fillets					Cooked fillets							
Month	Арр	earance	Те	xture	Арр	earance	0	dor	Т	aste	Те	xture		
	Control	STPP+NaCl	Control	STPP+NaCl	Control	STPP+NaCl	Control	STPP+NaCl	Control	STPP+NaCl	Control	STPP+NaCl		
0	6.8 ± 1.0^{Abc}	7.2±1.0 ^{Aa}	6.9±1.0 ^{Aab}	7.1±1.0 ^{Aa}	$6.6{\pm}1.2^{Aab}$	7.1±1.2 ^{Aa}	$6.2{\pm}1.6^{Ba}$	6.9±1.3 ^{Aa}	5.4 ± 2.0^{Ab}	7.4±1.2 ^{Ba}	$6.0{\pm}1.7^{\text{Bab}}$	7.0±1.3 ^{Aab}		
1	$6.3{\pm}1.5^{Acd}$	6.9±1.6 ^{Aa}	6.3 ± 1.6^{Ab}	6.7 ± 1.4^{Aa}	$6.1{\pm}1.2^{\text{Bb}}$	6.8 ± 1.3^{Aabc}	$5.2{\pm}1.8^{Abcd}$	5.6 ± 1.8^{Abc}	$5.9{\pm}1.9^{\text{Bab}}$	6.6 ± 1.6^{Aab}	$6.2{\pm}1.7^{Aab}$	6.7 ± 1.5^{Aab}		
2	$6.0{\pm}1.5^{\text{Ad}}$	6.1 ± 1.6^{Ab}	5.5 ± 1.6^{Ac}	6.0±1.5 ^{Ab}	5.9 ± 1.4^{Bb}	6.8 ± 1.4^{Aabc}	5.7 ± 1.8^{Aabc}	5.5 ± 1.6^{Abc}	5.4 ± 1.7^{Ab}	6.0 ± 1.8^{Ab}	5.8 ± 1.7^{Ab}	6.1±1.6 ^{Ab}		
3	6.8 ± 1.4^{Abc}	6.7±1.6 ^{Aa}	6.6 ± 1.4^{Ab}	6.7 ± 2.0^{Aa}	$6.2{\pm}2.0^{Bb}$	7.1±1.6 ^{Ab}	5.3 ± 2.1^{Aabc}	6.2 ± 2.5^{Aab}	5.5 ± 1.9^{Ab}	6.3 ± 2.5^{Ab}	5.4 ± 2.1^{Bb}	6.6±2.5 ^{Aab}		
4	7.1 ± 1.0^{Ab}	7.2±1.2 ^{Aa}	6.3 ± 1.1^{Ab}	6.8 ± 1.4^{Aa}	$4.9{\pm}1.8^{Bc}$	6.4 ± 1.4^{Ac}	$4.4{\pm}1.9^{Bc}$	5.6 ± 1.8^{Abc}	$4.0{\pm}1.8^{Bc}$	6.1 ± 1.4^{Ab}	4.6 ± 1.9^{Bc}	6.2 ± 2.3^{Ab}		
6	$5.3{\pm}1.4^{Be}$	7.3±1.1 ^{Aa}	5.4 ± 1.4^{Bc}	6.9±1.3 ^{Aa}	$6.2{\pm}1.6^{Ab}$	6.4 ± 1.4^{Abc}	5.0 ± 1.9^{Acd}	5.1 ± 2.0^{Ac}	$6.4{\pm}1.9^{Aa}$	6.0 ± 2.1^{Ab}	$6.0{\pm}1.8^{Aab}$	$6.5 \pm .1.7^{Aab}$		
7	7.7±1.1 ^{Aa}	6.9 ± 1.3^{Ba}	$7.3{\pm}1.4^{Aa}$	7.1±1.2 ^{Aa}	$7.1{\pm}1.2^{Aa}$	7.3±1.3 ^{Aa}	5.9±1.9 ^{Aab}	6.1±1.9 ^{Aab}	$6.7{\pm}1.6^{Aa}$	7.4±1.5 ^{Aa}	$6.7{\pm}1.5^{Aa}$	7.2 ± 1.4^{Aa}		
8	$6.4{\pm}1.5^{Bcd}$	7.1 ± 1.4^{Aa}	$6.7{\pm}1.3^{Aab}$	6.9±1.5 ^{Aa}	$6.4{\pm}1.4^{Aab}$	6.7 ± 1.5^{Aabc}	$5.5{\pm}2.4^{Aabc}$	$5.4{\pm}2.2^{Abc}$	$5.9{\pm}2.1^{Aab}$	6.6 ± 2.2^{Aab}	$6.1{\pm}1.7^{Aab}$	$6.6{\pm}1.7^{Aab}$		

Mean \pm SD (n = 40): values with the same lowercase letter in a column are not significantly different (P > 0.05) and with the same capital letter in a row are not significantly different (P > 0.05). Samples at 5th month were not determined.

Conclusions

The treatment of frozen Nile tilapia fillets with STPP and NaCl affected some traits during 8 months of storage. The pH of fish fillets decreased slightly with storage time. The moisture content and TBARS of treated and untreated samples were nearly the same throughout. The TVB-N of the controls was higher than that of the treated samples. The use of STPP+NaCl decreased the cooking loss of Nile tilapia fillets. Drip loss of both fish fillets samples were nearly the same during the 8 months of storage. The L^* value of treated samples was higher than that of the control, while color values of both samples remained about the same during storage. The TPA of frozen Nile tilapia fillets after defrosting showed high variability, although adhesiveness of the treated samples increased towards the latter part of storage. The total aerobic psychrophilic and aerobic mesophilic counts of all samples remained lower the recommended value. In addition, the acceptability scores for appearance and texture of the raw fish fillets were unchanged, but the acceptability scores for color and taste of the cooked treated samples were similar to those of the control. It is suggested that a combination of STPP and NaCl can improve the shelf life of frozen Nile tilapia fillets for at least 8 months. These results can be adapted to other fish products.

Acknowledgements

The authors gratefully acknowledge the support of the National Research Council of Thailand (NRCT) provided to the Office of Agricultural Research and Extension at Maejo University, Thailand, with Dr. Sudapron Tongsiri as the principle investigator. This project provided funds to the authors to do the work reported in this paper.

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