Effect of phosphate on frozen Nile tilapia fillets
Sutee Wangtueai1*, Sudaporn Tongsiri2, Jirawan Maneerote3, Wuttipot Supaviriyakorn2

Abstract
The yield and quality of frozen Nile tilapia fillets treated with various individual phosphates including sodium acid pyrophosphate (SAPP), tetrasodium pyrophosphate (TSPP), sodium tripolyphosphate (STPP), sodium hexametaphosphate (SHMP), and different mixed phosphates at 2.0% concentration in the presence of 2.5% NaCl were studied. The 2% STPP gave the greatest weight gain and cooking yield with the least drip loss and cooking loss as well as slightly enhancing the sensory acceptability score of the fillets. The actual phosphate content in the fish fillets never exceeded the European standard of 5 g/kg sample.

Keywords: Phosphate, Nile tilapia, Nile tilapia fillets, Frozen Nile tilapia fillets

1. Introduction
In Southeast Asia, Nile tilapia are one of the most important fishes because of its white flesh and delicate flavor (Sae-leaw et al., 2009). Nile tilapia are an important fresh water species grown using aquaculture as a source of protein in Thailand. Tilapia are not only widely consumed internally but also exported as frozen whole fish and fish fillets. In 2012, Thailand exported 386 tons of frozen Nile tilapia fillets and 6,900 tons of frozen whole Nile tilapia (The Customs Department, 2013).

Freezing is the best and usually the most economical method of seafood preservation as the fish can retain their fresh qualities longer depending on the details of the processing operations. Because seafood is sold by weight, any excessive loss of water in the final product is both a quality and economic issue. Drip loss lowers the qualities of frozen fish products by giving an undesirable appearance, reduced size, and loss of sensory characteristics such as texture and color (Gonçalves and Ribeiro, 2008).

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Phosphate compounds have been accepted as additives in fishery products to improve functional properties during processing and storage, because phosphates can increase the water retention in fresh products, reduce thaw loss and prevent cook loss of frozen fishery products (Chang and Regenstein, 1997; Masniyom et al., 2005). Phosphates have mostly been used with NaCl in fish and seafood to increase moisture retention (Chang and Regenstein, 1997; Thorarinsdottir et al., 2004) and to improve the taste of products. The synergistic effect between NaCl and phosphate can improve water holding capacity (WHC) and cooking yield (Young et al., 1987). However, the excessive using of phosphate will generally result in the formation of slimy texture, translucency, and a soapy taste (Rattanasatheirn et al., 2008). It might also leave illegally high phosphate residues in the final products.

Thus, the purposes of this work was to determine the effect of a combination of NaCl and individual or mixed phosphates on some key quality parameters of frozen Nile tilapia fillets.

2. Materials and methods

2.1 Raw material

Nile tilapia (Oreochromis niloticus) was purchased from a farm in Nakhonprathom, Thailand. Live fish were transported to the fish pre-processing plant in Samutsakhon, Thailand. They were then descaled, eviscerated, filleted, and deskinned by hand. The size of the individual Nile tilapia fillets were 100-150 g/piece. Fish fillets were packed approximately 1 kg/bag in polyethylene bags and placed in crushed ice with a fish/ice ratio 1:3 (w/w) and transported to the Department of Fishery Products, Kasetsart University, Bangkok, Thailand within a 1-1.5 h travel time.

2.1 Chemicals

The food grade tetrasodium pyrophosphate (TSPP), sodium tripolyphosphate (STPP), sodium hexametaphosphate (SHMP), sodium acid pyrophosphate (SAPP) were purchased from Haifa Chemicals Ltd. (Bangkok, Thailand). The 99.99% refined NaCl were obtained from Thai Refined Salt Co., Ltd. (Bangkok, Thailand).

2.3 Brine preparation

The brine solution was prepared using a combination of 2.5% (w/v) NaCl and 2% (w/v) of the individual or mixed phosphates. The 14 treatments included (1) 2% TSPP, (2) 0.5% SAPP and 1.5% TSPP, (3) 1.0% SAPP and 1.0% TSPP, (4) 1.5% SAPP and 0.5% TSPP, (5) 2.0% SAPP, (6) 2% STPP, (7) 0.5% SAPP and 1.5% STPP, (8) 1.0% SAPP and 1.0% STPP, (9) 1.5% SAPP and
0.5% STPP, (10) 2.0% SHMP, (11) 0.5% SAPP and 1.5% SHMP, (12) 1.0% SAPP and 1.0% SHMP, (13) 1.5% SAPP and 0.5% SHMP, and (14) the control (no phosphate, 2.5% NaCl).

2.4 Frozen fillets processing

Nile tilapia fillets were immersed in 5 volumes of brine (4ºC) for 10 min and drained in a plastic basket for 1 min. The soaked fillets were air blast frozen at -60ºC in a Mini Batch Freezer 100 l (Bangkok Industrial Gas Co. Ltd., Bangkok, Thailand) for 20 min until the core temperature reached -30ºC. They were then glazed using cold water (about 1ºC) for 10 s. The frozen fish fillets were then individually packed in Ziploc plastic bag (Aro, Siam Makro Public Co. Ltd., Bangkok, Thailand) and kept at -18 to -20ºC for 48 h before analysis. The thawing was done at 4ºC 24 h.

2.5 Determination of weight gain, cooking loss, cooking yield, and drip loss

Weight gain, cooking loss, and cooking yield were determined according to the method of Rattanasatheirn et al. (2008) with slightly modification. Weight gain was obtained by weighting fish fillets before and after soaking (and draining) in the brine. Weight gain was calculated as follow:

\[
\text{Weight gain} (\%) = \left(\frac{\text{weight after soaking} - \text{weight before soaking}}{\text{weight before soaking}}\right) \times 100
\]

Cooking loss and cooking yield were measured by weighting fish fillets before and after cooking by steaming at 95±2ºC using a hand-held thermometer for about 15 min until the core temperature reached 70ºC. Cooking loss and cooking yield were determined using the following formulas:

\[
\text{Cooking loss} (\%) = \left(\frac{\text{weight after soaking} - \text{weight after steaming}}{\text{weight after soaking}}\right) \times 100
\]

\[
\text{Cooking yield} (\%) = \left(\frac{\text{weight after steaming}}{\text{weight before soaking}}\right) \times 100
\]

Drip loss was determined by using the modification of Goncalves and Ribeiro (2009). Frozen fish fillets were thawed at 4ºC for 24 h. After thawing, excessive water was removed from the fish surface using filter paper to absorb the excessive water on fillets surface and weighting before and after thawing. Drip loss was calculated as follow:

\[
\text{Drip loss} (\%) = \left(\frac{\text{weight before thawing} - \text{weight after thawing}}{\text{weight before thawing}}\right) \times 100
\]

2.6 Chemical analysis

Determination of moisture content (oven-drying procedure at 105ºC), and phosphate content were performed according to AOAC official methods 934.01 AOAC (2000) and 986.24 AOAC (2005), respectively.
2.7 Texture measurement

Texture profile analysis (TPA) was done using a Texture analyzer (Stable Micro System, TA-HD, Surrey, UK) equipped with a 25 kg load cell. The upper lateral line part of the fish fillets was used for texture analysis has been described previously (Hernández et al., 2009). The selected parts of the raw fillets were cut into cubes of 30×30×20 mm. The fish cubes were oriented with the muscle fibers oriented horizontally and those were compressed using an aluminum cylindrical probe (35 mm diameter) until the deformation reached 25% at a speed of 50 mm/min. The pause between the first and second compressions was 5 s. Five independent measurements were made for each treatment. From the force-time curve of the texture profile, the textural parameters including hardness, gumminess, adhesiveness, cohesiveness, chewiness and springiness were obtained as previously described (Bourne, 1978).

2.8 Sensory evaluations

An acceptance test with 40 non-trained panelists was carried out using 9-points hedonic scales. The whole raw frozen fillets were thawed, drained, and then packed one per Ziploc plastic bag, which the panelists use to evaluate the appearance and texture attributes of raw fillets. Cooked fish fillets were prepared according to the method of Masniyom et al. (2005), fish fillets were cut into 30×30×20 mm cubes, wrapped with aluminum foil and cooked in a steaming pot until the core temperature reached 70ºC (approximately 15 min) using a hand-held thermometer. The cooked fillets were packed in small plastic cups into plastic box with a cover for sensory acceptability evaluation of appearance, odor, taste and texture attributes. The 9-points hedonic scales were 1, dislike extremely; 2, dislike very much; 3, dislike moderately; 4, dislike slightly; 5, neither like nor dislike; 6, like slightly; 7, like moderately; 8, like very much; 9, like extremely (Mailgaad et al., 1999).

2.9 Statistical analysis

Statistical analysis was performed using IBM SPSS statistics 20 software (IBM Corporation, USA). Data were subjected to the one-way ANOVA. Duncan’s new multiple range test (DMRT) was used to test for the differences between mean. The significance level was \( P \leq 0.05 \).

3. Results and discussions

3.1 Effects of phosphate on physiochemical properties

The weight gain of Nile tilapia fillets soaked in the 2.5% NaCl control along with different phosphates are show in Fig. 1A. The range of weight gain was between 0.30 to 2.60% with single
phosphates giving a higher weight gain than the blended phosphates. STPP and TSPP gave the highest weight gain ($P \leq 0.05$). The weight of Nile tilapia fillets after soaking should increase if there is a reaction of phosphate that improves the water hydration of protein in fish muscle. Xiong et al. (2000) reported that the combination of NaCl and phosphates influenced the physical changes in chicken muscle. The pyrophosphate and tripolyphosphate worked about the same in enhancing protein extraction leading to improved water hydration of chicken muscle. Rattanasatheirn et al. (2008) found that deveined shrimp treated with 2.5% NaCl and SAPP had a lower weight gain than the combination of NaCl with STPP or TSPP.

In this work the fillets treated with SAPP and SAPP mixed with other phosphates had a lower weight gain that might be influenced by the lower pH of the brine solutions, which may result in less binding between protein and water. The pH of the brine solution was significantly different for the all treatments ($P \leq 0.05$) as showed in Fig. 1B. Thorarinsdottir et al. (2004) reported that pH, ionic strength, and specific interactions of phosphates with divalent cation and myofibrilar proteins affected the water retention of muscle.

Drip loss during thawing is showed in Fig.1C. All treatments were in the range of 1-6% drip loss. The 2% STPP had the lowest drip loss ($P \leq 0.05$) suggesting that STPP gave the highest water binding. Xiong (1997) explained that during freezing the intra- and extra-cellular fluids are frozen to form ice crystals. The non-uniform structure of the ice crystals can damage cell structure leading to water release during the thawing process.

Soaking Nile tilapia fillets in 2% STPP combined with 2.5% NaCl resulted in the least cooking loss and greatest cooking yield ($P \leq 0.05$, Fig.1D, E) but was not significantly different from the other phosphates, e.g. 2% TSPP, 0.5% SAPP and 1.5% TSPP, 2% SAPP, and 1% SAPP and 1% STPP ($P > 0.05$). However, the highest cooking loss was found in the control ($P \leq 0.05$). Similarly, Rattanasatheirn et al. (2008) shows that SAPP mixed with STPP or TSPP resulted in a slightly lower cooking yield and a higher cooking loss than STPP or TSPP alone in peeled and deveined shrimp.

The moisture content of the frozen Nile tilapia fillets ranged from 80 to 82% (Fig. 2A). Fish fillets treated with STPP had highest moisture content ($P \leq 0.05$). Phosphate content in fish fillets was determined as mg of $P_2O_5$/kg sample (Fig. 2B). Fish fillets treated with SAPP or SAPP mixed with other phosphates had higher phosphate content but all treatments were less than 5 g/kg, a standard maximum value for the EU (Official Journal of the European Communities, 1995).

Frozen Nile tilapia fillets were thawed and texture profile analysis (TPA) was done as seen in Table 1. The average values for hardness, gumminess and chewiness displayed significant variations ($P \leq 0.05$). Fish fillets treated with STPP had the highest hardness values ($P \leq 0.05$). Thus,
fish fillets with STPP were firmer with higher hardness. However, a number of intrinsic biological factors influences the texture of fish muscle that related to muscle fiber density, and the composition of fat and collagen as well as the autolysis and microbiological process after fish death that lead to the muscle having a softer texture and less elasticity (Hernandez et al., 2009; Olafsdottir et al., 2004)

3.2 Effects of phosphate on sensory quality

The sensory acceptability scores of raw and cooked Nile tilapia fillets are showed in Fig. 3 and 4, respectively. The appearance score of raw fillets ranged from like slightly to like moderately (6.0-7.0) with significantly differences ($P \leq 0.05$) between treatments, except for TSPP, SAPP, and STPP which were not significantly different ($P > 0.05$) from each other. The texture score of raw fillets after thawing ranged from like slightly to like moderately. There were no significant different ($P > 0.05$) in texture between treated and untreated samples.

Cooked fillets were evaluated for the attributes of appearance, odor, taste, and texture. Appearance scores for cooked fillets were generally like moderately with fillets treated with SAPP and blended phosphates having lower acceptability ($P \leq 0.05$). The odor scores of all samples were not significantly different ($P > 0.05$) except for the treatment of 1% SAPP and 1% SHMP which had the lowest score of like slightly ($P \leq 0.05$). The taste scores were not significantly different ($P > 0.05$).

Those results showed that overall phosphates slightly enhanced sensory scores as was previously reported by Goncalves and Riberio (2009) with shrimp. Phosphates increased water retention in shrimp making them juicier and more similar to fresh shrimp. Goncalves et al. (2008) reported that phosphates were able to retain moisture and enhanced the ability to hold water in cooked fish fillets, mussel, and shrimp which resulted in higher texture scores.
Figure 1. The pH of brine solution containing phosphate and 2.5% NaCl (B), weight gain (A), drip loss (C), cooking loss (D), cooking yield (E) of frozen Nile tilapia fillets treated in different brine solution containing phosphate and 2.5% NaCl. SAPP; Sodium acid pyrophosphate, TSPP; Tetrasodium pyrophosphate, STPP; Sodium tripolyphosphate, SHMP; Sodium hexametaphosphate. Treatments: (1) 2% TSPP, (2) 0.5% SAPP and 1.5% TSPP, (3) 1.0% SAPP and 1.0% TSPP, (4) 1.5% SAPP and 0.5% TSPP, (5) 2.0% SAPP, (6) 2% STPP, (7) 0.5% SAPP and 1.5% STPP, (8) 1.0% SAPP and 1.0% STPP, (9) 1.5% SAPP and 0.5% STPP, (10) 2.0% SHMP, (11) 0.5% SAPP and 1.5% SHMP, (12) 1.0% SAPP and 1.0% SHMP, (13) 1.5% SAPP and 0.5% SHMP, and (14) control (no phosphate, 2.5% NaCl).
Figure 2. Moisture content (A) and phosphate content (B) of frozen Nile tilapia fillets treated in different brine solution containing phosphate and 2.5% NaCl. SAPP; Sodium acid pyrophosphate, TSPP; Tetrasodium pyrophosphate, STPP; Sodium tripolyphosphate, SHMP: Sodium hexametaphosphate. Treatments: (1) 2% TSPP, (2) 0.5% SAPP and 1.5% TSPP, (3) 1.0% SAPP and 1.0% TSPP, (4) 1.5% SAPP and 0.5% TSPP, (5) 2.0% SAPP, (6) 2% STPP, (7) 0.5% SAPP and 1.5% STPP, (8) 1.0% SAPP and 1.0% STPP, (9) 1.5% SAPP and 0.5% STPP, (10) 2.0% SHMP, (11) 0.5% SAPP and 1.5% SHMP, (12) 1.0% SAPP and 1.0% SHMP, (13) 1.5% SAPP and 0.5% SHMP, and (14) control (no phosphate, 2.5% NaCl).
Table 1. Texture profile analysis (TPA) of frozen Nile tilapia fillets after thawing.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Hardness (g)</th>
<th>Adhesiveness</th>
<th>Springiness</th>
</tr>
</thead>
<tbody>
<tr>
<td>2% TSPP</td>
<td>1430±390</td>
<td>-50±10a</td>
<td>0.92±0.01a</td>
</tr>
<tr>
<td>0.5% SAPP and 1.5% TSPP</td>
<td>850±120</td>
<td>-60±20ab</td>
<td>0.91±0.05ab</td>
</tr>
<tr>
<td>1% SAPP and 1% TSPP</td>
<td>1250±80g</td>
<td>-60±20a</td>
<td>0.90±0.03ab</td>
</tr>
<tr>
<td>1.5% SAPP and 0.5% TSPP</td>
<td>930±150g</td>
<td>-40±0a</td>
<td>0.85±0.06bc</td>
</tr>
<tr>
<td>2% SAPP</td>
<td>1790±240bcd</td>
<td>-80±20ab</td>
<td>0.92±0.04a</td>
</tr>
<tr>
<td>2% STPP</td>
<td>2200±270a</td>
<td>-80±30ab</td>
<td>0.90±0.04abc</td>
</tr>
<tr>
<td>0.5% SAPP and 1.5% STPP</td>
<td>1770±270bdeo</td>
<td>-50±10a</td>
<td>0.93±0.01a</td>
</tr>
<tr>
<td>1% SAPP and 1% STPP</td>
<td>1390±110ef</td>
<td>-90±40ab</td>
<td>0.91±0.02ab</td>
</tr>
<tr>
<td>1.5% SAPP and 0.5% STPP</td>
<td>1890±230bc</td>
<td>-50±0a</td>
<td>0.91±0.03a</td>
</tr>
<tr>
<td>2% SHMP</td>
<td>1990±320ab</td>
<td>-120±60bc</td>
<td>0.91±0.02ab</td>
</tr>
<tr>
<td>0.5% SAPP and 1.5% SHMP</td>
<td>1500±110cdef</td>
<td>-90±20ab</td>
<td>0.94±0.01a</td>
</tr>
<tr>
<td>1% SAPP and 1% SHMP</td>
<td>1420±180bc</td>
<td>-100±60ab</td>
<td>0.92±0.01a</td>
</tr>
<tr>
<td>1.5% SAPP and 0.5% SHMP</td>
<td>1110±90fgh</td>
<td>-80±40ab</td>
<td>0.93±0.01a</td>
</tr>
<tr>
<td>2.5% NaCl</td>
<td>1800±70bcd</td>
<td>-50±0a</td>
<td>0.85±0.04c</td>
</tr>
</tbody>
</table>

Table 1. Texture profile analysis (TPA) of frozen Nile tilapia fillets after thawing. (Cont.)

<table>
<thead>
<tr>
<th>Cohesiveness</th>
<th>Gumminess (g)</th>
<th>Chewiness</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.62±0.02a</td>
<td>880±230cdf</td>
<td>810±220cd</td>
</tr>
<tr>
<td>0.60±0.03a</td>
<td>510±100g</td>
<td>460±100f</td>
</tr>
<tr>
<td>0.59±0.01a</td>
<td>740±40def</td>
<td>670±40de</td>
</tr>
<tr>
<td>0.57±0.01a</td>
<td>530±100fg</td>
<td>450±50f</td>
</tr>
<tr>
<td>0.60±0.02a</td>
<td>1070±140abc</td>
<td>980±150abc</td>
</tr>
<tr>
<td>0.59±0.03a</td>
<td>1290±90a</td>
<td>1160±40a</td>
</tr>
<tr>
<td>0.60±0.02a</td>
<td>1050±150abc</td>
<td>980±130abc</td>
</tr>
<tr>
<td>0.59±0.03a</td>
<td>820±30de</td>
<td>740±30de</td>
</tr>
<tr>
<td>0.59±0.04a</td>
<td>1110±150abc</td>
<td>1020±130abc</td>
</tr>
<tr>
<td>0.57±0.03a</td>
<td>1140±170ab</td>
<td>1030±160ab</td>
</tr>
<tr>
<td>0.60±0.01a</td>
<td>900±60cd</td>
<td>840±60bod</td>
</tr>
<tr>
<td>0.57±0.05a</td>
<td>810±140de</td>
<td>740±140de</td>
</tr>
<tr>
<td>0.59±0.03a</td>
<td>650±80efg</td>
<td>610±80ef</td>
</tr>
<tr>
<td>0.58±0.04a</td>
<td>1050±110bcd</td>
<td>890±140bod</td>
</tr>
</tbody>
</table>

Note: SAPP; Sodium acid pyrophosphate, TSPP; Tetrasodium pyrophosphate, STPP; Sodium tripolyphosphate, SHMP; Sodium hexametaphosphate
: average±SD (n=3); same letters in the column are not significantly different (P>0.05).
Figure 3. Sensory evaluation of raw frozen Nile tilapia fillets treated in different brine solution containing phosphate and 2.5% NaCl. SAPP; Sodium acid pyrophosphate, TSPP; Tetrasodium pyrophosphate, STPP; Sodium tripolyphosphate, SHMP; Sodium hexametaphosphate. Treatments: (1) 2% TSPP, (2) 0.5% SAPP and 1.5% TSPP, (3) 1.0% SAPP and 1.0% TSPP, (4) 1.5% SAPP and 0.5% TSPP, (5) 2.0% SAPP, (6) 2% STPP, (7) 0.5% SAPP and 1.5% STPP, (8) 1.0% SAPP and 1.0% STPP, (9) 1.5% SAPP and 0.5% STPP, (10) 2.0% SHMP, (11) 0.5% SAPP and 1.5% SHMP, (12) 1.0% SAPP and 1.0% SHMP, (13) 1.5% SAPP and 0.5% SHMP, and (14) control (no phosphate, 2.5% NaCl).
Figure 4. Sensory evaluation of cooked frozen Nile tilapia fillets treated in different brine solution containing phosphate and 2.5%NaCl. SAPP; Sodium acid pyrophosphate, TSPP; Tetrasodium pyrophosphate, STPP; Sodium tripolyphosphate, SHMP: Sodium hexametaphosphate. Treatments: (1) 2%TSPP, (2) 0.5%SAPP and 1.5%TSPP, (3) 1.0%SAPP and 1.0%TSPP, (4) 1.5%SAPP and 0.5%TSPP, (5) 2.0%SAPP, (6) 2%STPP, (7) 0.5%SAPP and 1.5%STPP, (8) 1.0%SAPP and 1.0%STPP, (9) 1.5%SAPP and 0.5%STPP, (10) 2.0%SHMP, (11) 0.5%SAPP and 1.5%SHMP, (12) 1.0%SAPP and 1.0%SHMP, (13) 1.5%SAPP and 0.5%SHMP, and (14) control (no phosphate, 2.5%NaCl)

Conclusions

The application of 2% solutions of phosphates (SAPP, TSPP, STPP, and SHMP) combined with 2.5% NaCl affected the quality of frozen Nile tilapia fillets. Overall STPP alone gave the best physiochemical and sensory qualities to frozen Nile tilapia fillets.

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