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Effects of different levels of food additives on weight gain, cook-related yield loss, physicochemical and sensorial quality of Nile tilapia fillets (*Oreochromis niloticus*)

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Abstract

The effect of different food additives on weight gain, and thawing/cooking related yield loss of the Nile tilapia fillet was evaluated. Samples were soaked in Water (control), Sodium chloride (NaCl), Sodium tripolyphosphate (STPP), BRIFISOL 512; BRIFISOL NP-30 (2 and 5% - 15, 30 and 60 minutes), drained (1 mins), weighed (% yield), frozen (-30°C, 24hr), vacuum packed, stored (-18°C, 15 days), thawed (4°C, 24 hr), weighed (% drip loss), grilled (200°C, 3 mins) and weighed (% cooking loss). Analyses of moisture, pH, P_2O_5, chloride were performed. Quantitative descriptive analysis and acceptance test were performed (for the best results). All treatments improve the weight gain and minimize drip loss (thawing and cooking). From the results of weight yield (%) after soaking in different food additives, STPP showed the best yield (compared to control group) corresponding to an average of 13.33%, and lower weight loss on thawing (0.88%). All food additives demonstrate smaller drip losses after cooking for Nile tilapia fillets, which confirms the ability in retaining moisture and should be good substitutes for STPP. The phosphates concentration increased with contact time in all phosphate treatments (0.28 to 0.56% for STPP, and 0.2 to 0.46% for BF512), but both within the international limits, i.e. 0.5 to 1%. All food additives promote acceptability index (> 70%), considered accepted by consumers, and should be a viable alternative to reduce the economic losses during freezing-thawing-cooking procedures.

Introduction

Food grade additives are being used in meat products (before freezing process) to improve the overall quality (i.e. reduce cook losses, improve textural properties especially by increasing water holding capacity (WHC), retard oxidative rancidity, develop color) and also supply protection against microbial growth (Knipe, 2004; Ünal *et al.*, 2006; Kilinc *et al.,* 2009a, 2009b; Kin *et al.,* 2010). The moisture controlling during harvest, processing, distribution, storage, and preparation are common practices in seafood industry (Schubring *et al.,* 2003; Toldrá, 2003; Gonçalves and Ribeiro, 2009).

Polyphosphates have been widely used in aqua food species to improve the quality by increasing water retention in fresh fish, reducing thaw loss in frozen products, modifying texture, yielding better color, and reducing cooking loss (Lampila, 1992; Chang and Regenstein, 1997; Rattanasatheirn *et al.*, 2008; Gonçalves, 2012). Although phosphates have a wide application in the seafood industry and are proving many functional uses, particularly sodium tripolyphosphate (STPP), tetrasodium pyrophosphate (TSPP), sodium hexametaphosphate (SHMP), or their blends, there are limited studies on the effect of others additives, like sodium chloride, sodium bicarbonate, sodium carbonate, and sodium citrate on the quality of seafood (Gonçalves, 2012).

Furthermore, the seafood industry in Brazil is well known for its long-standing excellent reputation worldwide, owing to its outstanding characteristics of quality, freshness, variety, and taste; and to maintain the quality of seafood, few allowed food additives have been used, except phosphates. Thus, the aim of the work described in the following sections was to study quality aspects of Nile tilapia fillets treated with different food additives previously submitted to the freezing process, and subsequently thawing and cooking procedures.

Material and Methods

Raw material and sampling

Nile tilapia *(Oreochromis niloticus)* were purchased from Tilapia Farmer Association

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(AQUAPO, Apodi, RN, Brazil), selected by size (total length = 29 cm, total height = 9 cm and width = 9 cm) and weight (650 g) in average, death by heat shock, filleted and skinned according to the good manufacturing practices. The skinned fillets were properly labeled, packed in polyethylene bags (in order to receive later the food additives solutions), cooled on flake ice (1:1) and transported to the Laboratory of Seafood Technology and Quality Control (LAPESC) using an insulated box. The time from the fish samples (harvest/processing) to treatments at the laboratory was no more than 2 hours. No additives were used during pre-shipping.

Food additives application method

Food grade sodium tripolyphosphate (STPP, Astaris), sodium chloride (NaCl, Synth), BRIFISOL-512 (blend of sodium tripolyphosphate and sodium polyphosphate – BK Giulini Chemie-Adicon) and BRIFISOL-NP30 (blend of sodium chloride, sodium bicarbonate, sodium carbonate, and sodium citrate - BKG/Adicon) were individually dissolved in cold distilled water (4°C) to obtain a final concentration of 2 and 5%.

Experimental design

A completely randomized design (CRD) in a 5x3x4 factorial scheme, with 60 experiments was used. Three replications for each group were accomplished. The experiments considered three variables: solutions (Water, NaCl, STPP, BRIFISOL-512, BRIFISOL-NP30), solutions concentrations (0 - control; 2 and 5g 100mL-1) and contact time (0 - control; 15, 30 and 60 minutes). The skinned fillets were separated into five groups, 60 treatments, totaling 180 samples properly labeled in polyethylene bags, and soaking with each solution according to procedures of Gonçalves and Ribeiro (2008, 2009). Control samples were soaked in cold distilled water (4°C). After soaking, samples were drained for 60 seconds, weighted and submitted to a freezing process.

Freezing method

Treated samples fillets were frozen individually using an Ultra freezer (-30°C, 24 hours). Samples were packed in a vacuum bag and stored at -18°C for a period of 15 days (to assure the complete freezing process) before being thawed and cooked.

Determination of weight gain, drip loss (on thawing and after cooking)

Weight gain was calculated according to Gonçalves and Ribeiro (2008; 2009) as follows: Weight gain (%) = $[(B - A)/A] \times 100$, where A = initial weight (before soaking) and B = weight after soaking and draining (5 mins). Drip losses were determined based on a method described by Regenstein *et al.* (1993) and Gonçalves *et al.* (2008). Samples of treated skinned fillets were thawed in a cold room (4°C) for 24 hr and drip loss after thawing (DLT) was measured. DLT was expressed as DLT = $[(W_0 - W_F)/W_0] \times 100$ (%), where: W_0 is the initial sample weight (in grams) and WF is the final sample weight (in grams). Drip loss after cooking (DLC) was measured as described for DLT. Treated samples were grilled using a stainless steel grill (thermostat was set at 200°C) during 3 mins (each side). To ensure uniform grilling/heating, the internal temperature was controlled using a quartz electronic thermometer, and the process ended when fillets internal temperature raised 72°C. Experimental values are reported as the average of three determinations. The initials weights were transformed to 100 g for better visualization of data on the graphics.

Sensory evaluation

A Quantitative Descriptive Analysis (QDA) was performed with the presence of a staff of 50 non-trained panelists randomly recruited and prescreened by a familiarity with eating grilled fish fillets. Judgments were based on sensory attributes of grilled fillets and used an unstructured line scale of 9cm [appearance, i.e. the first impression when looks the product, from bad (0) to very good (9); odor, i.e. the intensity of fish odor, from absent (0) to intense (9); taste, i.e. the characteristic fish flavor, from absent (0) to intense (9); and texture, i.e. the fish texture quality and succulence, from bad (0) to very good (9)]. An Acceptance Test was also carried out using the hedonic scale [(1) dislike extremely to (9) like extremely]. To determine how much a person like or dislike the samples, the Acceptability Index (AI) was employed [AI (%) = (mean value/higher score) x 100]. According to Dutcosky (2007), IA ≥70% were considered accepted by consumers. Both tests were accomplished in a sensory panel room where each person received samples of hot grilled fillet (at 50°C) for evaluation (Teixeira *et al*., 1987; Stone and Sidel, 2004).

Chemical evaluation

The moisture content was followed by AOAC method (AOAC, 2011). The pH value was recorded according to the potentiometric method, a technique described in the User's National Reference Laboratory Animals (Brazil, 1981), which is based on the measurement of the hydrogen ion concentration in the sample using an aliquot of 50 g homogenized

sample with 10 mL of distilled water. The electrode was inserted directly into the sample and the pH was read. The chloride content was accomplished through the quantification of Cl− following the Möhr´s method of direct titration with $AgNO_3$ using K_2CrO_4 as an indicator (Brazil, 1981). The phosphate content was determined by the Adolfo Lutz Institute methodology (IAL, 2008), with modifications as follows. Phosphorus was estimated colorimetrically as phosphor-vanadomolybdate by the spectrometric method (vanadum phosphomolybdate) which is based on the reaction of orthophosphate in an acidic solution with ammonium molybdate and ammonium vanadate in nitric acid. Approximately 5 g of samples of treated skinned fillets were weighed accurately into a silica dish and 1g CaO added. The sample was heated over a low flame until thoroughly charred and then placed in a furnace at 550°C for 3 hr. After cooling, the sample was transferred to a 250 mL beaker and diluted with approximately 10 mL of distilled water. Then approximately 12 mL of concentrated HCl and approximately 5 mL of HNO_3 were added. The solution was heated, cooled, transferred to a 250-mL volumetric flask, and diluted to volume with distilled water. The solution was then filtered and the first 10 to 20 mL of the filtrate was discarded. The absorbance of filtered solution was determined at 420bnm and samples were compared to a calibration curve where the phosphate content was determined (as mg P_2O_5/g sample). A series of a standard solution (vanado-molybdate reagent) was prepared and the absorption measured at 420 nm. The calibration curve obtained was used for the determination of phosphate content in the samples. All analyses were carried out in triplicate.

Statistical analysis

Treated averages were compared through an Analysis of Variance (ANOVA) and effects were considered significant (by Tukey's test) when p-value ≤ 0.05.

Results

Weight determination and yield calculations

The control group, i.e., Nile tilapia fillet soaked in water (15, 30 and 60 mins), showed no significant difference ($p>0.05$) in gaining weight among soaking times. The highest weight gain occurred at 60 minutes (6.67%); however, significant differences in drip loss were observed after cooking between 15 and 30 minutes (p=0.0077) and between 15 and 60 minutes (p=0.0013), while fillets soaked in water for 15 minutes lose more water on thawing (10.72%) and after cooking (30.32%). After cooking, weight loss was significant at all soaking times and with greater intensity at 15 mins of soaking (Table 1 and Figure 1).

The NaCl group, i.e., Nile tilapia fillet soaked in 2 and 5% NaCl solution (15, 30 and 60 mins) showed in both concentrations, increasing of fillet weight but no significant differences ($p>0.05$) were observed between contact times (Table 1 and Figure 1). Greater weight gain occurred only at 60 minutes of soaking (6.67% for 2% NaCl; 11.67% for 5% NaCl). The greatest drip loss was observed on thawing (12.77% for 2% NaCl) and after cooking (17.52% for 2% NaCl) and the lower drip loss was observed on thawing (2.48% for 5% NaCl – 60 mins) and after cooking (5.81% for 5% NaCl – 60 mins). Significant differences in drip loss were observed in 5% NaCl soaking between 15 and 60 minutes on thawing (p=0.012413) and after cooking (p=0.001555). The best performance results were soaking samples in 2% and 5% NaCl solutions for 60 minutes, and comparing these treatments, no significant difference ($p>0.05$) in weight gain after soaking were observed, but significant difference in drip loss on thawing (p=0.002546) and cooking (p=0.001947) was observed. However, the highest weight gain (11.67%) occurred in a 5% NaCl concentration. The 2% NaCl concentration achieved greater drip loss on thawing (10.94%) and after cooking (8.77%) and 5% NaCl concentration had lower drip loss on thawing (4.62%) and after the cooking (1.90%). After cooking process, a significant difference in drip loss between the soaking time of 15 and 60 minutes (p=0.012905) was observed.

The phosphate group, i.e., Nile tilapia fillet soaked in 2 and 5% STPP solution (15, 30 and 60 mins) showed an increase in fillet weight after soaking and differ significantly ($p\leq0.05$) in both concentrations and contact times (Table 1 and Figure 1). However, on thawing step the fillets soaked for 60 minutes in 2% STPP solution lost less water (6.51%) and differed significantly (p=0.047421) between the times of 15 and 30 minutes (drip loss of 10.35% and 10.26% respectively). The same situation was verified after cooking, showing the effectiveness in 60 mins of soaking in 2% STTP, where a significant difference (p=0.001244) was observed between the drip loss in 60 mins (2.64%) and 15 (13.28%) and 30 (13.46%). Samples soaked in 5% STPP solution, significant differences were observed in weight gain between the times 15 and 60 mins after soaking (p=0.007490), in drip loss on thawing (p=0.001313) and drip loss after cooking (p=0.001324). The weight yield (%) after the soaking was greater in the 60 mins

Table 1. Weight gain (WG %), Drip loss (%) on thawing (DLT) and after cooking (DLC)

Treatments	WG (%)	Drip loss (%)	
		DLT	DLC
Control – 15 min	3.33 ± 2.89^{A}	10.72 ± 0.34^{A}	30.32 ± 4.77^{A}
Control – 30 min	5.00 ± 0.01^{A}	10.53 ± 0.01^{A}	14.09 ± 4.03^{B}
Control – 60 min	6.67 ± 2.89^{A}	10.44 ± 5.40^{A}	13.73 ± 3.40^{B}
2%NaCl – 15 min	3.33 ± 2.89^{A}	12.77 ± 3.39^{A}	17.52 ± 1.86^{A}
2%NaCl – 30 min	5.00 ± 0.02^{A}	12.57 ± 3.55^{A}	14.34 ± 3.84^{B}
2%NaCl – 60 min	6.67 ± 2.89^{A}	12.28 ± 3.04^{A}	9.69 ± 3.58^{B}
5%NaCl – 15 min	6.67 ± 2.89^{A}	6.67 ± 2.89^{A}	12.39 ± 2.21^{A}
5%NaCl – 30 min	8.33 ± 2.89^{A,B}	4.84 ± 0.14^{A}	10.72 ± 0.34^{A}
5%NaCl – 60 min	11.67 ± 2.89^{B}	2.48 ± 2.39^{B}	5.81 ± 0.83^{B}
2%STPP – 15 min	6.67 ± 2.89^{A}	10.35 ± 0.30^{A}	13.28 ± 0.31^{A}
2%STPP – 30 min	8.33 ± 2.89^{B}	10.26 ± 5.40^{A}	13.46 ± 0.31^{A}
2%STPP – 60 min	10.00 ± 0.01^{C}	6.51 ± 3.02^{B}	2.64 ± 0.52^{B}
5%STPP – 15 min	5.00 ± 0.01^{A}	2.61 ± 0.58^{A}	3.03 ± 1.79^{A}
5%STPP – 30 min	8.33 ± 2.89^{B}	2.20 ± 0.53^{B}	2.59 ± 0.60^{B}
5%STPP – 60 min	13.33 ± 2.89^{C}	0.88 ± 0.88^{C}	2.12 ± 0.53^{B}
2%BF512 – 15 min	6.67 ± 2.89^{A}	8.51 ± 3.05^{A}	10.91 ± 2.39^{A}
2%BF512 – 30 min	8.33 ± 2.89^{A}	6.59 ± 2.96^{A,B}	4.12 ± 1.16^{B}
2%BF512 – 60 min	11.67 ± 2.89^{A}	4.04 ± 1.07^{B}	2.88 ± 0.07^{C}
5%BF512 – 15 min	8.33 ± 2.89^{A}	6.68 ± 3.33^{A}	4.17 ± 1.95^{A}
5%BF512 – 30 min	8.33 ± 2.89^{A}	3.82 ± 1.63^{A,B}	3.65 ± 1.17^{A}
5%BF512 – 60 min	10.00 ± 5.00^{A}	3.49 ± 1.31^{B}	2.27 ± 0.71^{B}
2%NP-30 – 15 min	3.30 ± 2.89^{A}	7.04 ± 3.53^{A}	11.95 ± 1.46^{A}
2%NP-30 – 30 min	5.00 ± 0.01^{B}	5.00 ± 0.01^{B}	4.54 ± 1.25^{B}
2%NP-30 – 60 min	10.00 ± 0.02^{C}	4.43 ± 0.57^{B}	3.62 ± 1.20^{C}
5%NP-30 – 15 min	6.67 ± 2.89^{A}	6.67 ± 2.89^{A}	11.12 ± 1.23^{A}
5%NP-30 – 30 min	8.33 ± 2.89^{B}	4.84 ± 0.14^{B}	3.33 ± 1.52^{B}
5%NP-30 – 60 min	11.67 ± 2.89^{C}	4.07 ± 1.20^{B}	1.57 ± 0.53^{C}

Note: mean ± sd (n=3); same letter in the column (for each treatment) = not significantly different ($p \geq 0.05$). Control: water; STPP: sodium tripolyphosphate; NaCl: sodium chloride; BF512: BRIFISOL-512 (sodium tripolyphosphate + sodium polyphosphate), NP-30: BRIFISOL-NP30 (sodium chloride + sodium bicarbonate + sodium carbonate + sodium citrate). WG = refers to initial weight = $[(W_{after\ soaking} - W_{initial})/W_{after\ soaking}] \times 100$ | DLT = refers to weight after soaking = $[(W_{on\ thawing} - W_{after\ soaking})/W_{on\ thawing}] \times 100$ | DLC = refers to thawed weight = $[(W_{after\ cooking} - W_{on\ thawing})/W_{after\ cooking}] \times 100$

of soaking (13.33%). At 15 mins of soaking had the greatest drip loss on thawing (2.61%) and after cooking (3.03%); however, at 60 mins lower drip loss on thawing (0.88%), and after cooking (2.12%) were observed. Nevertheless, treatment with phosphates solution caused an increase in weight of both samples, due to a net increase in moisture content (Table 3) because of water binding properties of proteins.

The Brifisol-512 group, i.e., Nile tilapia fillet soaked in 2 and 5% BF512 solution (15, 30 and 60 minutes) showed an increase in fillet weight (WHC) after soaking in both concentration and soaking time (Table 1 and Figure 1), but without significance ($p>0.05$). The drip loss on thawing showed a higher loss in 2% BF512 (8.51%, 15 mins) but did not differ ($p>0.05$) from 30 mins (6.59%), but differ significantly ($p=0.008732$) from 60 mins (4.04%). After cooking significant difference in drip loss between 15 and 60 mins ($p=0.001146$) and between 15 and 30 mins ($p=0.022596$) were observed. Better results in drip loss were observed using 5% BF512 solution, i.e., lower drip loss in 60 mins of soaking (3.49% - on thawing; 2.27% - after cooking), but no significant differences were observed ($p>0.05$).

The Brifisol-NP30 group, i.e., Nile tilapia fillet soaked in 2 and 5% NP-30 solution (15, 30 and 60 mins) showed similar results - an increase in fillet weight after soaking in both concentration and soaking time (similar to phosphate group – Table 1 and Figure 1) and lower drip losses. At 2% NP-30 solution a significant ($p=0.016130$) increase in weight gain between 15 (3.3%) and 60 mins (10%) of soaking was observed. On thawing, 2% NP-30 solution at 60 mins of soaking had lower and significantly ($p=0.016130$) drip loss compared to 15 mins. Using 5% NP-30 solution, no significant differences ($p>0.05$) were observed in drip loss on thawing between 30 and 60 minutes. After cooking, significant differences were observed among contact soaking time (2% NP-30) and the best drip loss result was at 60 mins (3.62%). For 5% NP-30, similar results in drip loss after cooking among soaking time were observed, but higher significant differences between 15 and 30 mins ($p=0.001496$) and 15 and 60 mins ($p=0.002276$) were observed.

According to Table 1, an overview of yields shows that in all treatments, the best results came from the higher soaking time, but in some cases, no significant difference ($p>0.05$) were verified between 30 and 60 minutes. However, the drip loss on thawing

Table 2. Sensory evaluation of grilled treated Nile tilapia fillets (after soaked for 60 min in different food grade additives solutions)

ATTRIBUTES	TREATMENTS				
	Water	5% NaCl	5% STPP	2% BF512	5% NP30
Appearance	4.39 ± 0.43^{A}	6.50 ± 0.39^{B}	8.20 ± 1.08^{C}	5.80 ± 1.05^{D}	6.80 ± 0.95^{B}
Odor	4.70 ± 0.54^{A}	6.90 ± 0.79^{B}	7.60 ± 0.80^{B}	5.50 ± 0.58^{C}	7.40 ± 0.44^{B}
Taste	5.20 ± 0.59^{A}	6.60 ± 0.43^{B}	8.40 ± 0.62^{C}	5.60 ± 0.45^{A}	7.20 ± 0.74^{B}
Texture	4.90 ± 0.79^{A}	6.50 ± 0.75^{B}	8.50 ± 0.78^{C}	6.60 ± 0.64^{B}	7.80 ± 0.37^{D}
Preference	5.80 ± 0.43^{A}	6.80 ± 0.39^{B}	7.90 ± 0.40^{C}	6.50 ± 0.41^{B}	7.50 ± 0.47^{D}
Acceptance index (AI%)	64.44^{A}	75.56^{B}	87.78^{C}	72.22^{D}	83.33^{E}

Mean ± sd (n=50); same letter in the line = not significantly different (p>0.05). Appearance – from bad (0) to very good (9); Odor – from absent (0) to intense (9); Taste – from absent (0) to intense (9); Texture – from bad (0) to very good (9); Preference – from "dislike extremely (1)" to "like extremely (9)".

and after cooking were the parameters that represent more importance in this study, i.e. lower loss values represent best results. The best results among the five groups were presented in Figure 1 and as expected were at the higher soaking time (60 mins). All groups (Water, 5% NaCl, 5% STPP, 2% BF512, 5% NP-30) showed similar results after soaking, i.e. an increase in weight fillet (6.67%; 11.67%; 13.33%; 11.67%; 11.67%, respectively - see Table 1 and Figure 1). In all steps (soaking, thawing, and cooking) the control group (water) had worst results. The best results of weight yield (%) after soaking in different food additives showed that STPP had the best yield (compared to control group) corresponding to an average of 13.33%, and lower weight loss on thawing (0.88%). The food additive STPP was the one that had the greatest weight gain (13.33 %) after soaking and differed significantly to the control group (p=0.047421) but did not differ significantly from the other additives. All food additives demonstrate smaller drip losses after cooking for Nile tilapia fillets, which confirms the ability in retaining moisture and should be good substitutes for STPP.

Sensory evaluation

Table 2 resumes the sensory evaluation for the best results (i.e. after soaked samples for 60 mins in different food grade additives solutions: water; 5% NaCl; 5% STPP; 2% BF512; and 5% NP30). Figure 2 resume the QDA results ("appearance" better and significant (p<0.05) score for STPP followed by NP30 and NaCl; "odor" was similar for STPP, NaCl, and NP30; "taste" was better for STPP followed by NP30 and NaCl, and "texture" was better for STPP and NP30). The preference (by hedonic scale) was higher and differed significantly among the treatments (Table 2). The best result was for STPP followed by NP30, NaCl, BF512, and control (water). The acceptance index was above 70% for all treatments (except to control group) and was accepted by consumers, and are in accordance with the preference results.

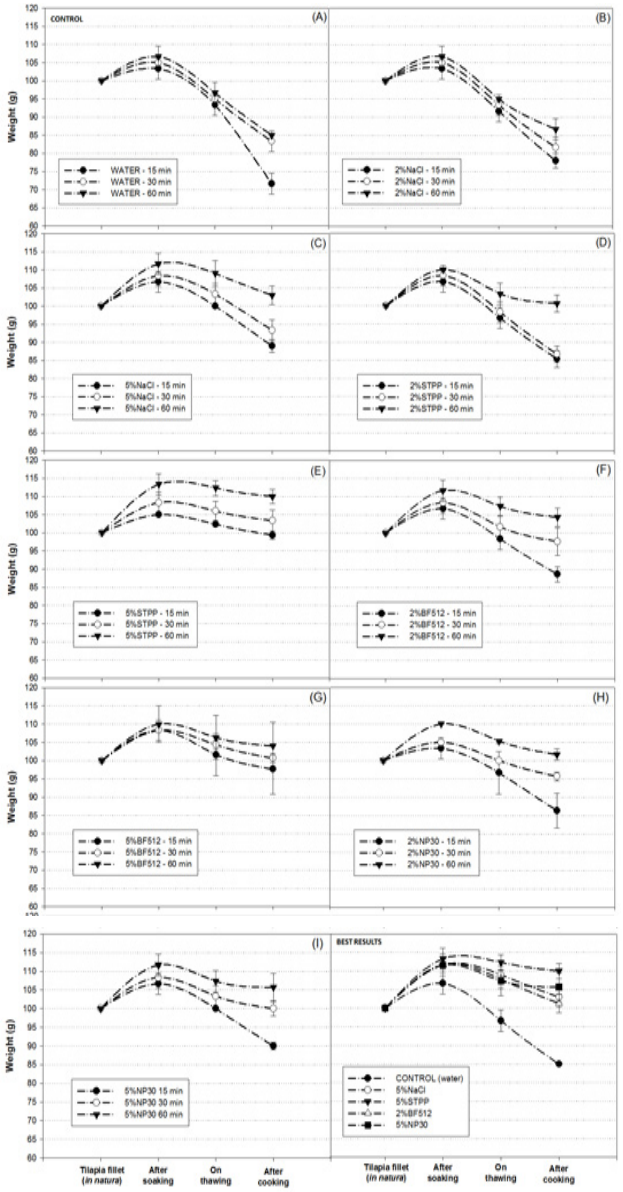

Figure 1. Control (Water), NaCl, Phosphate, Brifisol BF512 and NP-30 groups: Nile tilapia fillet weight variation after soaking in (A) water, (B) 2% sodium chloride, (C) 5% sodium chloride solution, (D) 2% sodium tripolyphosphate, (E) 5% sodium tripolyphosphate solution, (F) 2% BF512, (G) 5% BF512, (H) 2% NP-30, (I) 5% NP-30 (15, 30, 60 minutes), and BEST RESULTS on thawing and after cooking.

Table 3. Nile tilapia fillets moisture (%), pH, phosphate (P_2O_5 %), and chlorine (%) concentrations after soaking in different food grade additives solutions

TREATMENTS	MOISTURE (%) | SOAKING TIME			
	in natura (0 min)	15 min	30 min	60 min
Water (Control)	75.94±0.88^{A}	76.95±0.52^{A}	78.42±0.77^{B}	79.07±0.28^{B,C}
2%NaCl		76.79±0.42^{A}	77.32±0.81^{A}	77.54±0.38^{A}
5%NaCl		76.63±1.17^{A}	77.37±0.65^{A}	78.55±0.44^{B}
2%STPP		78.68±0.40^{B}	78.99±0.08^{B}	80.04±0.42^{C}
5%STPP		80.83±0.99^{B}	80.96±0.25^{B}	81.22±0.45^{B}
2%BRIFISOL-512		77.77±0.99^{B}	79.52±0.75^{C}	79.72±0.42^{C}
5%BRIFISOL-512		77.00±0.25^{B}	77.92±0.08^{C}	79.05±0.47^{C}
2%BRIFISOL-NP30		78.16±0.07^{B}	78.66±0.07^{C}	78.72±0.20^{C}
5%BRIFISOL-NP30		77.61±0.12^{B}	78.46±0.34^{C}	79.06±0.78^{C}
TREATMENTS	**pH | SOAKING TIME**			
	in natura (0 min)	15 min	30 min	60 min
Water (Control)	6.40±0.01^{A}	6.26±0.02^{B}	6.11±0.02^{C}	6.06±0.03^{D}
2%NaCl		6.02±0.04^{B}	6.05±0.03^{B}	6.12±0.04^{C}
5%NaCl		5.98±0.02^{B}	6.01±0.01^{C}	6.02±0.02^{D}
2%STPP		6.39±0.07^{B}	6.41±0.05^{B}	6.45±0.03^{B}
5%STPP		6.51±0.04^{B}	6.54±0.03^{B}	6.63±0.04^{C}
2%BRIFISOL-512		6.43±0.03^{B}	6.47±0.02^{C}	6.52±0.02^{D}
5%BRIFISOL-512		6.44±0.03^{B}	6.52±0.03^{C}	6.57±0.03^{D}
2%BRIFISOL-NP30		6.62±0.02^{B}	6.68±0.04^{C}	6.73±0.05^{C}
5%BRIFISOL-NP30		7.22±0.05^{B}	7.71±0.06^{C}	7.75±0.03^{D}
TREATMENTS	**PHOSPHATE (P_2O_5%) | SOAKING TIME**			
	in natura (0 min)	15 min	30 min	60 min
2%STPP	0,089 ± 0,01^{A}	0,28 ± 0,01^{A}	0,45 ± 0,03^{B}	0,53 ± 0,06^{C}
5%STPP		0,32 ± 0,02^{A}	0,52 ± 0,00^{B}	0,56 ± 0,05^{C}
2%BRIFISOL-512		0,20 ± 0,01^{A}	0,43 ± 0,01^{B}	0,36 ± 0,10^{C}
5%BRIFISOL-512		0,21 ± 0,02^{A}	0,32 ± 0,01^{B}	0,46 ± 0,09^{C}
TREATMENTS	**CHLORINE (%) | SOAKING TIME**			
	in natura (0 min)	15 min	30 min	60 min
2%NaCl	0,03 ± 0,01^{A}	0,46 ± 0,11^{B}	0,68 ± 0,10^{C}	0,71 ± 0,07^{C}
5%NaCl		0,72 ± 0,14^{B}	0,83 ± 0,03^{C}	1,22 ± 0,06^{D}
2%BRIFISOL-NP30		0,38 ± 0,03^{B}	0,44 ± 0,02^{C}	0,49 ± 0,03^{D}
5%BRIFISOL-NP30		0,43 ± 0,01^{B}	0,47 ± 0,04^{C}	0,48 ± 0,01^{C}

Note: Mean ± sd (n=3); same letter in the line are not significantly different (p≥0.05); STPP: sodium tripolyphosphate; BRIFISOL-512: sodium tripolyphosphate + sodium polyphosphate; NaCl: sodium chloride; BRIFISOL-NP30: sodium chloride + sodium bicarbonate + sodium carbonate + sodium citrate.

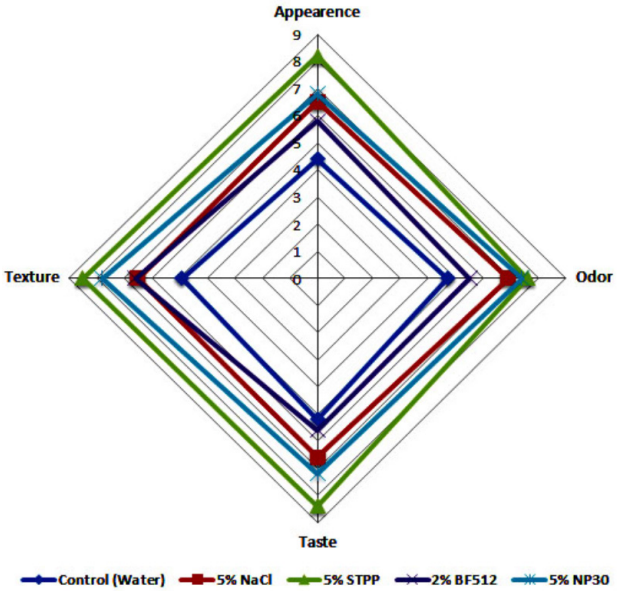

Figure 2. Quantitative descriptive analysis (QDA) for grilled Nile tilapia fillets and used an unstructured line scale of 9 cm to register their evaluations: appearance, i.e. the first impression when looks the product, from bad (0) to very good (9); flavor, i.e. the intensity of fish odor, from absent (0) to intense (9); taste, i.e. the characteristic fish flavor, from absent (0) to intense (9); and texture, i.e. the fish texture quality and succulence, from bad (0) to very good (9).

Chemical evaluation

Table 3 resumes the moisture content, pH value, phosphate (% P_2O_5), and chlorine (%) in thawed samples after soaking in different additives solutions and contact time.

Moisture content was in accordance with the results of drip losses presented in Table 1, i.e. lower drip loss higher moisture content. The pH decrease in control group should be due to a protein denaturation, drip formation, which subsequently causes hydrogen ions release and the increase of solutes concentration by drip loss. The pH decreased with the first 15 mins of soaking in NaCl solution (2% and 5%) and maintain lower up to 60 mins (with lower increase). Changes in pH affected by phosphate pretreatment were observed. The initial pH of the fish sample was 6.40 and increase with soaking time. The phosphates concentration increased with contact time in both treatments, were between 0.28 to 0.56% (for STPP) and 0.2 to 0.46% (for BF512), and within the international limits, i.e. 0.5 to 1% (Gonçalves, 2012). The % chlorine results are comprised between 0.46 and 1.22% (NaCl group) and from 0.38 to 0.49% (BRIFISOL NP30 group). It was observed that there was a progressive and significant increase in chloride content (%) with increasing soaking time in

all treatments. The higher values of chlorides were observed in 5% NaCl (1.22) and 2% BRIFISOL NP30 (0.49) both under soaking 60 minutes.

Discussion

Weight determination and yield calculations

The intention to compare all treatments with the control group (water) was only to have an idea if the soaking samples in water could increase the water retention in the fillets. Gonçalves and Ribeiro (2008; 2009) and Gonçalves *et al.* (2008) also found an increase in water retention after the samples soaked in water, but no detailed information on how water retention occurred in fillets was found. The effects of salt on WHC, and thereby yield, have been described by many authors (Warrier *et al.*, 1975; Offer and Knight, 1988; Fennema, 1990). A final concentration of 0.8 to 1M (4.70 to 5.85%) sodium chloride gives maximum water uptake, although a somewhat lower concentration (2%) is more often used in the manufacture of meat products. Phosphate compounds, particularly pyrophosphate and triphosphate, are also added, usually at concentrations of about 0.3%, to enhance this uptake (Offer and Trinick, 1983; Gonçalves and Ribeiro, 2008, 2009; Gonçalves, 2012).

Asli *et al.* (2013) noted an increase in the amount of chlorides with increasing NaCl concentration (5%, 15% and 25%) with 2.5% Blend (NaCl + sodium bicarbonate) after injection of salmon fillets for 24 hours; where NaCl levels ranged from 0.2 to 2.4%, and was significantly affected by the salt concentration. The addition of $NaHCO_3$ resulted in the brine NaCl concentrations similar to those salted fillets with only NaCl brine.

The present result corroborates with some authors (Thorarinsdottir *et al*., 2001; Shaviklo *et al.*, 2012) due that WHC increases with increasing salt concentration up to about 6%, and when the salt concentration reaches levels above 10%, denaturation of proteins leads to decreased WHC of the muscle. On the other hand, according to Xargayó *et al*. (2004), meat proteins may increase up to twice its size in the presence of salt concentrations. This occurs because salt reduces the protein isoelectric range that increases the separation between the chains, allowing chlorine ions (negative charge) to join with the positively charged protein chains, thus increasing the repulsive force between them. Likewise, the dimensional matrix proteins open, giving rise to a greater number of charges to be exposed to bind with water molecules. Other external factors also influence the protein's ability to bind to water. According to Damoradan *et al.* (2010), these factors are the pH, ionic strength, temperature, salt type and conformation of the protein. The proteins are less hydrated at its isoelectric pH (5.1) in which the increase of protein-protein interactions results in minimal interaction with water. Above and below the isoelectric pH, due to the increase in net charge and repulsive forces, proteins swell and bind more with water. The ability to bind water of most proteins is higher at pH 9-10 than at any other pH. The salts at low concentrations (< 0.2 M or <1.2%) increase the water binding ability of the protein, because of the hydrated salts ions, especially the anions, bind weakly to charged groups in proteins. At this low concentration, the ion binding to proteins does not affect the hydration layer of the charged groups in the protein, and the increase of connection (bonds) with the water should be essentially related to the ions bound. However, at high salt concentrations, most of the existing water is bound to the salt ions, resulting in dehydration of the protein. The ability of proteins to bind water tends to decrease as the temperature rises due to the reduction in hydrogen bonding and to the decrease of the hydration of ionic groups. The ability of a denatured protein to bind water is usually 10% larger than a native protein. If the denaturation results in protein aggregation, then its WHC may decrease due to the displacement of water by the increase of protein-protein interactions.

Thorarinsdóttir *et al.* (2001) proved that the effectiveness of phosphates in the properties of water retention in meat products depended on the type and on a number of phosphates, as well as on the type of product that was processed with their addition. In recent years, the use of additives in food production has been increasing (Gonçalves, 2012). The addition of polyphosphates to meat and seafood products has induced water retention during processing and thereby increased the weight of these products. It should be emphasized that phosphates act on the muscle fiber, depolymerize the myosin filaments and facilitate the dissociation of the actomyosin complex, increasing the protein dissolution and a number of electric charges of the system and increasing the meat WHC (Offer and Trinick, 1983; Xiong, 2005).

Wangtueai *et al*. (2014) studying the yield and quality of frozen Nile tilapia fillets treated with various individual phosphates, showed that 2% STPP (10 mins) 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, and also, the phosphate content in the fish fillets never exceeded the international standard of 5g kg^{-1} sample. Wangtueai and Vichasilp (2015) optimized a critical process and a soaking

step, using phosphates with a combination of NaCl and soaking time, concluded that phosphates and NaCl concentration, and soaking time are significant factors for the physical and sensory properties of frozen Nile tilapia fillets.

It could be observed that at any given time of frozen storage phosphate-treated Nile tilapia fillets also showed lower drip loss and cooking loss percentages and higher moisture retention after cooking, as compared with control samples. In addition drip loss on thawing and after cooking of both samples are found to increase significantly ($p<0.05$) at the time of soaking progressed. These results confirmed the findings of Turan *et al*. (2003), Kolbe and Kramer (2007), Boonsumrej *et al*. (2007) and Gonçalves *et al*. (2008). Concerning drip loss, it is worth mentioning that drip results from the inability of the thawed muscle to reabsorb all of the separated water, which had been previously frozen. Formation of drip brings about the loss of weight, nutrient and flavor components, an unpleasant appearance of seafood, and a tough texture.

The variability of the results on phosphate treatments has been reported in the scientific literature. Dyer (1969) has found that treatment with sodium tripolyphosphate (STPP) had no effect on drip loss although the net weight of the muscle increased, and in some species, such as Dover sole *(Microstomus pacifucus)*, Pacific cod *(Gadus macrocephalus),* halibut *(Hippoglossus stenolepis)* and red snapper *(Sebastodes ruberrimus)*, STPP was effective in reducing thaw-drip in comparison with water-dipped controls but was not effective in others, e.g. Chinook salmon *(Oncorhynchus tschawytscha)*. Sutton (1969) has found that STPP (4; 6; 8%) definitely reduced the weight losses due to processing and that it was more effective than pyrophosphate (2.2; 3.4; 4.5%) in cod fillets. Only a light treatment in the STPP, i.e. 0.5 mins dip in a 4% solution was required to produce improvements in both the water retention and texture properties of the muscle. The addition of sodium chloride did not produce any improvement over phosphate alone.

Kilinc *et al.* (2009) studied the effect of treatment with sodium– monophosphate, diphosphate, and tripolyphosphate solutions for quality improvement of saithe *(Polachius virens)* and seabass *(Dicentrarchus labrax)*. They found that treatment with the solution of the 5% STPP at 10 mins of soaking was the best result to improve the quality and reduce the weight loss after cooking compared to the control group. Moawad *et al.* (2013) also studied the efficacy of Tri-Sodium Phosphate (TSP, Na_3PO_4) on WHC and drip loss after the white shrimp (*Penaeus* spp.) soaked in cold 5% TSP for 10 mins (3-5°C) prior to freezing and frozen storage, and observed advantages in the use of this food grade additive. Similar results were observed by Gonçalves *et al*. (2008) using the treatment of 2% STPP solution in searobin *(Prionotus punctatus)* fillets and 5% STPP solution in pink cusk-eel *(Genypterus brasiliensis)* fillet, which prevented large thawing and cooking-related yield losses. The weight gain using 2% STPP was also observed by Etemadian *et al.* (2012), where kutum fillets *(Rutilus frisii)* were soaked in 2% STPP solution (4°C) for 10 minutes, drained and vacuum packed. After freezing, these authors observed increasing in weight gain and reduction of exudate (drip loss), due to phosphates action by increasing the repulsive forces between the protein molecules and thereby promoting the increase of water retention.

Chantarasuwan *et al*. (2011) studied the effects of weight yield after freezing and cooking using white shrimp *(Litopenaeus vannamei)* immersed in 2% sodium carbonate (Na_2CO_3) and 2% sodium bicarbonate ($NaHCO_3$) and 2.5% NaCl (chloride sodium) at various pH levels. This study verified the effectiveness of this mixture in the improvement of water retention and reduction the weight loss after cooking.

Data showed in Figure 1 (Best results) and Table 1 corroborates to the conclusion drawn by Tenhet *et al.* (1981a, 1981b) and to the study published by Gonçalves *et al.* (2008) for fish and shellfish. However, after cooking the additive BRIFISOL-NP30 exhibited lower weight loss (1.55%) after cooking when compared with phosphate and blend (BF512) groups.

Carneiro *et al.* (2013) observed similar results where shrimp were immersed in 2% and 5% STPP (60 and 120 mins) for performance evaluation of weight after freezing and after cooking. It was observed that soaking in 5% STPP (60 and 120 mins) had less weight loss after cooking and had higher moisture content. However, there were no differences in results between soaking times, concluding that the soaking time of 60 mins is sufficient for the additive acts. Erdogdu *et al.* (2007) found in their study that the STPP is an important functional additive used in meat products, as it reduces losses after cooking, especially by increasing the water holding capacity of the protein. However, an increasing heating temperature or time causes the denaturation of the meat protein, resulting in a reduced ability to retain water.

The results presented in this study confirm considerations of Gonçalves *et al.* (2008), i.e., the effect of phosphate's type after soaking presented the

same trend observed with the control group (water soaking). However, on thawing and after cooking, weight yield remained constant, and the tilapia fillet immersed in 5% BF512 (phosphate blend) solution had lower yield compared with samples treated with 5% STPP. That can be explained by blend composition. The blend incorporates other components (sodium tripolyphosphate, NaCl, and sodium tetra pyrophosphate) should be able to improve the response compared to STPP.

Schnee (2000) affirm that cooking losses for frozen seafood are usually 10 to 30%. The data presented in Table 1 had high drip loss (on thawing/ after cooking) for the control group (water) but when treated with phosphates and other food additives lower losses could be observed. Phosphates blend were more effective to prevent yield losses (retained more moisture) compared to STPP, but in this study STPP alone was more effective. Furthermore, Aitken (2001) comment that fish treated with phosphates before freezing often reduces the amount of thaw drip (i.e, liquid released when frozen fish is thawed).

Commercial experiences and researchers have been demonstrating that phosphates can improve sensory quality and increase consumer's preference (Gonçalves *et al.,* 2008; Gonçalves and Ribeiro, 2008, 2009; Wangtueai *et al.*, 2014; Wangtueai and Vichasilp, 2015). Those studies reveal that consumers prefer seafood appropriately treated with phosphate. Panelists generally felt the phosphated samples meet their expectations and they liked and judged the products to be high quality and valued more than the non-phosphated product. These expectations were in accordance with results obtained by Applewhite *et al.* (1993). However, all processors should have in mind that inadequate use (lacks or excess) could bring in poor appearance, undesirable texture, and consumer's rejection. In the present study, lower food additives concentrations were used and the results on weight gain, reduce drip losses, and sensorial quality was satisfactory.

Wangtueai *et al.* (2014) studying the sensory quality (appearance, odor, taste, and texture) of cooked Nile tilapia fillets treated with various individual phosphates, showed that the appearance scores 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% sodium acid pyrophosphate (SAPP), and 1% sodium hexametaphosphate (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 Gonçalves and Ribeiro (2009) with shrimp.

The acceptance indexes were 87.78%, 83.33%, 75.56% and 72.22% for STPP, NP30, NaCl and BF512 group, respectively. In all groups, acceptance indexes were above 70%, which demonstrated overall acceptance by panelists. The control group (water) the AI < 70% and corroborate to lower sensory values. Some authors have been demonstrated that consumer prefers cooked seafood with high moisture content (Applewhite *et al.*, 1993; Gonçalves *et al.*, 2008; Gonçalves and Ribeiro, 2008, 2009). These studies help to explain why in the present study the panelist preferred treated samples compared to control group (water). The retention of moisture and ability to hold water in the cooked product can provide a consumer benefit in terms of texture (higher sensorial responses). All treated groups showed lower drip loss after thawing and cooking, showing at the same time the water (moisture) retention (Table 3 and Figure 1) and excellent sensorial responses (Table 2 and Figure 2).

Significant sensorial differences between nontreated (control) and treated groups were observed (Table 2 and Figure 2) showing better appearance, taste, and texture. Studies carried out by Lampila (1993) demonstrated the synergic effect of the NaCl and phosphate combination in seafood processing, indicating an improvement in water retention and, consequently, in product sensory attributes. The NaCl group showed good results but was below the STPP and NP30 groups.

Texture attribute for treated samples with STPP and NP30 revealed the highest scores (8.50 and 7.80, respectively). It can be observed that soaking samples in the food additives solutions prior to cooking resulted in their tenderization compared to non-treated samples (controls). Some sensory panelists described the texture for those treated with all food additives as juicy or similar to fresh fish. Chantarasuwan *et al*. (2011) soaking shrimp in brine containing 2.0% sodium carbonate had the lower likeness score for texture compared to the control (non-treated samples) and those soaked in brine containing 2.0% sodium bicarbonate. Gonçalves *et al.* (2008) and Gonçalves and Ribeiro (2008; 2009) found similar results for fish fillets, shrimp, and mussel, and concluded that the retention of moisture and ability to hold water in the cooked product can provide benefits in terms of texture (higher consumer evaluation).

Chemical evaluation

Sodium tripolyphosphate (STPP) is one of the

phosphates, which belong to the family used in the seafood industry that can be used as a humectant, i.e., substances that keep the moisture of the product. As expected, the moisture retention (Table 3) increase over time of soaking and with the increase of additives concentrations. The water holding capacity is normally associated with increased pH in the alkaline region and certain phosphates, when added in low concentrations, raise the pH of the muscle (Knipe, 2004; Thorarinsdottir *et al*., 2001).

Kin *et al*. (2010) found that catfish fillets injected with BRIFISOL-512 at 4°C showed an average pH of 6.4 equal to control. Carneiro *et al.* (2013) found that the pH had to influence the contact time in the shrimp's phosphate solutions, where treatment with 5% STPP for 60 mins led to a pH value significantly higher (pH 7.2). The pH increase with the use of phosphate is an important factor to increase the fish WHC, once the protein moves at a distance from its isoelectric range (pH~5.1), which increases the distance between the polypeptide chains, and then increase the space for water retention (Ordónez-Pereda *et al*., 2005; Carneiro *et al.*, 2013).

Sodium bicarbonate has the ability to partially solubilize the myofibrillar proteins and increase its electrostatic repulsion by increasing the pH. Thus, the concentration of hydrogen ions decreases and shifts the pH of the aqueous phase intramuscularly away from the isoelectric point of myosin (pH~5.2). This leads to transverse expansion of myofibrils, enabling greater water uptake and retention. Due to the expansion, the protein surface area increase, which further promotes hydrogen bonding and electrostatic interactions between the water and the muscle protein (Xiong, 2005).

Protein-rich foods, such as seafood contain phosphorous compounds such as nucleotides, phospholipids, together with naturally occurring orthophosphates [0.11-0.48% (0.026%-1.1% in terms of phosphorous content)], and makes it hard to detect added phosphates by quantitative analysis alone (Lawrie, 1988; Lee *et al.*, 1998; Ünal *et al.,* 2004). The phosphate content of fresh cod *(Gadus morhua* and *Gadus macrocephalus*) is approximately 4.4g P_2O_5 kg^{-1} of muscle (Thorarinsdottir *et al.*, 2001; Schröder, 2010).

There is a risk of producing false negative and positive results when inorganic polyphosphates are to be quantified in fish and meat. Inorganic di- and triphosphates (limit of detection < 0.5mg kg^{-1}) are naturally present neither in fish nor in the product of a degradation reaction during storing/transporting of the animal/tissue or a processing of the sample. Traces of inorganic polyphosphates are to be considered as an indication that the sample has undergone a previous treatment with polyphosphates (Kaufmann *et al.*, 2005). We cannot forget that the Codex Committee on Food Additives and Contaminants had endorsed earlier the level of 10g kg^{-1} total phosphates, to take into account approximately 5g kg^{-1} of phosphates naturally present (Codex, 1990).

Phosphates (P_2O_5 %) were detected in all samples but at a lower percentage than the international legislation (0.5% to 1%). According to FDA (USFDA, 2004), there is neither prohibition of the phosphates used in seafood nor a limit for their use. They can be used as a multifunctional substance without restrictions for specific alimentary products. The appropriate use will be controlled by the Good Manufacturing Practices. On another hand, International legislation (Gonçalves, 2012) liberates the use of phosphates in different seafood species, with multiple uses, but it cannot exceed the concentration from 0.1% to 1% with some restrictions. Gonçalves *et al.* (2008) treated fish fillets pink cusk-eel (Genypterus brasiliensis) and searobin (Prionotus punctatus) with (STPP and Blend) to 5% for 120 mins, had levels of P_2O_5 (%) below the 0.5% limit allowed by international legislation.

Conclusion

All food additives evaluated in this study promotes lower drip loss (on thawing and after cooking), with the maintenance of the initial tilapia fillets weights; promotes acceptability index up to 70% (considered accepted by consumers); and should be a viable alternative (according to the cost effectiveness) to reduce the economic losses during freezing-thawing-cooking procedures. To assess and confirm the viability of the alternative food additives, further studies are needed to determine their effects on microbiological quality and lipid stability of seafood products during refrigerated storage.

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