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Optimization of phosphate and salt application to physical and sensory properties of frozen Nile tilapia fillets

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Abstract

This study was optimized a critical process, a soaking step, using phosphates with a combination of NaCl and soaking time, with response surface methodology (RSM). A Box-Behnken's design with three-factors, three-levels was used to design the experiment. Physiochemical properties (weight gain, cooking loss, cooking yield, drip loss, moisture, phosphate content) and sensory properties of raw fillets (appearance, texture) and cooked fillets (appearance, odor, taste, texture) were investigated. Three factors of phosphate concentration (X_1), NaCl concentration (X_2) and soaking time (X_3) were optimized. It was found that a significant effect with high R^2 of models was observed in weight gain, cooking yield, phosphate content and appearance (raw), taste (cooked) and texture (cooked). Those responses were optimized and a goal condition was obtained at 1.4% of phosphate concentration, 2.7% of NaCl concentration, and 115 min of soaking time. This solution gave a high satisfaction, with a high weight gain of 6.52%, cooking yield of 81.12%, low phosphate content of 3876 mg/kg, and high appearance score of 7.5, taste of 6.2, and texture of 6.6, respectively by using 9-point hedonic scaling. It is concluded that phosphates concentration, NaCl concentration, and soaking time are significant factors for the physical and sensory properties of frozen Nile tilapia fillets.

Introduction

Nile tilapia is one of most important freshwater fishes in Southeast Asia, and has attracted consumers due to its white meat and delicate flavor. At present, there is a high demand for this fish in the market. In Thailand, Nile tilapia is not only consumed internally, but also exported as frozen products, such as whole fish and fish fillets. Generally, a frozen product is the best and most economical production in terms of fish preservation, as it prolongs freshness and flavor. However, a low temperature used in the freezing process could affect the meat properties, resulting in weight loss and drip loss (excessive loss of water) in the final product. Drip loss affects the quality of frozen fishery products by giving an undesirable appearance, a reduced size, and a lower customer acceptance of texture and color (Gonçalves and Ribeiro, 2009).

For decades, phosphates and related compounds have been used as food additives for the frozen food industry to improve meat properties during frozen food processing and storage. Phosphates can increase water retention in fresh products, reduce weight loss from the thawing process, and prevent cooking loss (Chang and Regenstein, 1997; Masniyom *et al.*, 2005). The effect of phosphates on water-retention in meat products depends on the quantity and type of phosphate, as well as the kind of food product. The mechanism of phosphate for increasing water retention is affected by pH and ionic strength, resulting in specific interactions between phosphate anions with divalent cations and myofibrillar proteins (Thorarinsdottir *et al.*, 2004). In general, phosphates are used with NaCl, over a specified soaking time for each individual fishery product, to increase moisture retention and to improve the taste of the product (Chang and Regenstein, 1997; Thorarinsdottir *et al.*, 2004). The concentration about 1-6% phosphates and 2-5% NaCl concentration are generally used and soaking time until the residual contents of phosphates reach approximately 0.5% (Sigurgisladottir *et al.*, 2000; Gonçalves and Ribeiro, 2008). The synergistic effect between NaCl and phosphates results in an increase in the water holding capacity (WHC) and cooking yield (Young *et al.*, 1987). On the other hand, excessive usage of phosphates causes the formation of a slimy texture, a translucent quality, and a soapy taste (Rattanasatheirn *et al.*, 2008). Therefore, the appropriate solution of using phosphates, NaCl, and

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soaking time is a key factor for commercial frozen Nile tilapia products.

Response surface methodology (RSM) is an effective tool to optimize conditions with high or low targets for food processing. It has been widely used to improve the quality and yield in fishery products (Zhou and Regenstein, 2004; Wangtueai and Noomhorm, 2009; You *et al*., 2010; Norziah *et al*., 2014). This study aims to optimize the conditions of phosphate, NaCl, and soaking time for treatment of Nile tilapia fillets, previously subjected to freezing by RSM technique. Important factors in consumer acceptance were chosen for possible responses, including physical and chemical properties, and sensory characteristics of raw and cooked fillets.

Materials and Methods

Raw materials

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

Experimental design

The optimization of brine immersed conditions was done using response surface methodology (RSM) with a three-level and three-factor Box-Behnken design. Three factors of phosphate concentration (X_1), NaCl concentration (X_2) and soaking time (X_3) were selected for the optimization. The selected responses were weight gain (%, Y_1), cooking yield (%, Y_2), phosphate content (mg/kg, Y_3), and scores for sensory evaluation using 9-point hedonic scaling, include appearance of raw fillets (Y_4), taste of cooked fillets (Y_5), and texture of cooked fillets (Y_6). The coded and real values of each level of the three factors are shown in Table 1. The fifteen treatments of the experiments, including twelve incomplete factorial points and three replicates of the central point are showed in Table 2.

Frozen fillet processing

The fifteen brine solutions were prepared with several concentrations of STPP and NaCl, according to Table 2. The fish fillets were immersed in brine solutions (4ºC) with the ratio of fish meat to solutions 1:5 (w/w) with different soaking times as following Table 2. The samples were then drained in a plastic basket for 1 min. The soaked fillets were frozen using an air blast freezing machine (Mini Batch Freezer 100, Industrial Gas Co. Ltd., Bangkok, Thailand) at -60ºC for 20 min until the core temperature reached -30ºC. The samples were then glazed using cold water (about 1ºC) for 10 s. The obtained frozen fish fillets were individually packed in polyethylene zip lock bags (Siam Makro Public Co. Ltd., Bangkok, Thailand) and kept at -18 to -20ºC for 48 h before analysis.

Table 1. Factors and their levels in the 3-factors, 3-levels Box-Behnken design

Factors	levels		
	-1	0	+1
Phosphate concentration (%, w/v): X_1	1.00	2.25	3.5
NaCl concentration (%, w/v): X_2	0.00	1.50	3.00
Soaking time (min) : X_3	10	65	120

Physical properties determination

The weight gain, cooking loss, and cooking yield were determined according to the method of Rattanasatheirn *et al.* (2008) with slight modifications. The frozen fillets were thawed in a refrigerator at 4ºC for 24 h. The weight of each fillet was measured and the weight gain was calculated as per the following:

Weight gain (%) = [(weight after soaking – weight before soaking)/weight before soaking] × 100

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

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

Cooking yield (%) = (weight after steaming/ weight before soaking) × 100

Drip loss was determined by using the method of Gonçalves and Ribeiro (2009). In brief, frozen fish fillets were thawed at 4ºC for 24 h. After thawing, excessive water was removed from the fish surface,

Table 2. The experiment design and response variables for physical and chemical properties, and sensory evaluation of frozen Nile tilapia fillets

Treatments	Factors			physical and chemical responses[A]						sensory evaluation responses[B]					
	X_1	X_2	X_3	Weight gain (%)	Cook loss (%)	Cook yield (%)	Drip loss (%)	Moisture content (%)	Phosphate content (mg/kg)	Appearance (Raw)	Texture (Raw)	Appearance (Cooked)	Odor (Cooked)	Taste (Cooked)	Texture (Cooked)
1	1.00	0.00	65	5.14 ± 0.98^{bc}	24.74 ± 1.60^{abc}	69.78 ± 1.49^{g}	1.04 ± 0.04^{g}	81.07 ± 0.08^{a}	3522 ± 39^{h}	6.40 ± 1.32^{cd}	6.43 ± 1.36^{abcd}	6.65 ± 1.48^{abcd}	6.88 ± 1.22^{ab}	5.53 ± 1.65^{de}	5.95 ± 1.38^{de}
2	3.50	0.00	65	5.04 ± 0.82^{c}	18.52 ± 0.81^{e}	81.48 ± 0.81^{bc}	2.37 ± 0.06^{e}	80.48 ± 0.30^{b}	6292 ± 136^{a}	6.70 ± 1.40^{cd}	6.28 ± 1.65^{bcd}	6.30 ± 1.42^{cd}	6.63 ± 1.27^{ab}	5.50 ± 1.89^{de}	5.98 ± 1.69^{bcd}
3	1.00	3.00	65	6.58 ± 0.42^{abc}	22.63 ± 0.67^{c}	78.67 ± 0.68^{de}	3.59 ± 0.11^{ab}	79.68 ± 0.33^{fg}	3569 ± 36^{h}	7.45 ± 0.93^{ab}	6.48 ± 1.20^{abcd}	7.20 ± 1.02^{a}	6.88 ± 1.09^{ab}	5.95 ± 1.65^{bcde}	6.53 ± 1.30^{abc}
4	3.50	3.00	65	6.63 ± 0.67^{ab}	19.97 ± 0.23^{de}	81.40 ± 0.24^{bcd}	2.93 ± 0.28^{d}	80.10 ± 0.00^{de}	5921 ± 31^{b}	7.80 ± 0.89^{a}	7.10 ± 1.26^{a}	6.23 ± 1.31^{d}	6.53 ± 1.13^{ab}	6.50 ± 1.34^{ab}	6.50 ± 1.55^{abc}
5	1.00	1.50	10	3.56 ± 1.27^{d}	26.74 ± 0.49^{ab}	72.17 ± 0.49^{g}	3.95 ± 0.09^{a}	79.41 ± 0.13^{fg}	3546 ± 1.7^{h}	6.40 ± 1.34^{cd}	6.10 ± 1.24^{bcd}	6.45 ± 1.20^{bcd}	6.65 ± 1.23^{ab}	5.65 ± 1.39^{cde}	6.20 ± 1.24^{abcd}
6	3.50	1.50	10	2.46 ± 0.50^{d}	22.41 ± 0.90^{c}	75.56 ± 0.88^{f}	3.28 ± 0.32^{bcd}	79.24 ± 0.03^{g}	4571 ± 105^{f}	6.78 ± 1.42^{cd}	5.95 ± 1.50^{cd}	6.88 ± 1.42^{abcd}	6.30 ± 1.29^{b}	6.40 ± 1.69^{abc}	6.35 ± 1.69^{abc}
7	1.00	1.50	120	5.95 ± 0.66^{abc}	27.23 ± 1.02^{a}	72.21 ± 2.31^{g}	3.48 ± 0.34^{abc}	79.37 ± 0.50^{fg}	3670 ± 39^{h}	6.90 ± 1.26^{bc}	6.78 ± 1.51^{ab}	7.15 ± 1.12^{ab}	6.63 ± 0.95^{ab}	6.24 ± 1.45^{abcd}	6.20 ± 1.52^{abcd}
8	3.50	1.50	120	6.48 ± 0.39^{abc}	17.78 ± 2.31^{e}	83.45 ± 2.34^{b}	0.72 ± 0.16^{g}	80.14 ± 0.00^{bc}	5989 ± 70^{b}	6.70 ± 1.18^{cd}	6.50 ± 1.22^{abcd}	6.53 ± 1.71^{abcd}	6.70 ± 1.07^{ab}	6.65 ± 1.44^{ab}	6.55 ± 1.43^{abc}
9	2.25	0.00	10	2.41 ± 0.93^{d}	26.37 ± 2.05^{ab}	71.67 ± 2.00^{g}	3.72 ± 0.26^{ab}	79.77 ± 0.09^{ef}	4172 ± 36^{g}	6.48 ± 1.41^{cd}	6.25 ± 1.72^{bcd}	6.43 ± 1.38^{bcd}	6.75 ± 1.21^{ab}	5.65 ± 1.49^{cde}	5.95 ± 1.58^{de}
10	2.25	1.50	10	2.91 ± 0.55^{d}	25.89 ± 0.57^{ab}	71.23 ± 1.80^{g}	3.61 ± 0.40^{ab}	79.57 ± 0.04^{efg}	4220 ± 31^{g}	6.90 ± 1.33^{bc}	6.36 ± 1.75^{abcd}	6.73 ± 1.45^{abcd}	6.75 ± 0.95^{ab}	5.98 ± 1.72^{bcde}	6.50 ± 1.66^{abc}
11	2.25	0.00	120	6.80 ± 1.44^{a}	24.28 ± 1.07^{bc}	77.18 ± 1.09^{ef}	3.08 ± 0.63^{cd}	80.17 ± 0.14^{bc}	4897 ± 0.3^{cd}	7.03 ± 0.92^{cd}	6.70 ± 1.11^{abc}	6.80 ± 1.49^{abcd}	6.55 ± 1.04^{ab}	5.23 ± 1.35^{e}	5.49 ± 2.21^{e}
12	2.25	1.50	120	6.70 ± 0.76^{ab}	14.66 ± 1.40^{d}	86.90 ± 1.43^{a}	1.60 ± 0.15^{f}	80.38 ± 0.13^{bc}	4768 ± 37^{de}	7.60 ± 1.50^{a}	6.68 ± 1.12^{abc}	6.93 ± 1.49^{abcd}	6.90 ± 1.13^{a}	6.45 ± 1.45^{ab}	6.76 ± 1.33^{ab}
13	2.25	1.50	65	6.19 ± 0.21^{abc}	22.16 ± 1.03^{cd}	78.83 ± 1.05^{cde}	2.22 ± 0.25^{e}	80.97 ± 0.03^{a}	4943 ± 138^{c}	6.33 ± 1.54^{cd}	5.90 ± 1.53^{d}	6.68 ± 1.56^{abcd}	6.80 ± 0.79^{ab}	6.40 ± 1.34^{abc}	6.49 ± 1.38^{abc}
14	2.25	1.50	65	6.70 ± 0.92^{ab}	22.53 ± 2.07^{c}	78.74 ± 1.94^{cde}	2.05 ± 0.04^{ef}	80.48 ± 0.02^{b}	4795 ± 136^{cde}	6.15 ± 1.49^{c}	6.55 ± 1.58^{abcd}	6.73 ± 1.32^{abcd}	6.75 ± 1.10^{ab}	6.78 ± 1.29^{a}	6.63 ± 1.29^{abc}
15	2.25	1.50	65	6.90 ± 0.79^{a}	23.19 ± 2.04^{c}	78.38 ± 2.08^{e}	2.97 ± 0.21^{d}	79.80 ± 0.13^{ef}	4669 ± 36^{ef}	6.48 ± 1.41^{cd}	6.08 ± 1.70^{bcd}	7.00 ± 1.32^{abc}	6.88 ± 1.04^{ab}	6.56 ± 1.48^{ab}	6.86 ± 1.33^{a}

Note : X_1: concentration of phosphate (%, w/v), X_2: concentration of salt (%, w//v), X_3: soaking time (min)
: [A]mean±SD (n=3), [B]mean± SD (n=40); same letters in the column are not significantly different (*P*>0.05)

using filter paper to absorb the excessive water on the fillet surface, and the weight of each fillet were measured before and after thawing. Drip loss was calculated as per the following:

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

Chemical analysis

Moisture and phosphate contents of frozen fillets were determined according to the standard method of 934.01 AOAC (2000) and 986.24 AOAC (2005), respectively.

Sensory evaluations

The frozen fillet samples were thawed and cut into 30×30×20 mm cubes. The samples were wrapped with aluminum foil and cooked in a steaming pot until the core temperature reached 70ºC (measured with a hand-held thermometer) for 15 min (Masniyom *et al.*, 2005). The 40 non-trained panelists evaluated raw fillets by attributes of appearance and texture, and cooked fillets by appearance, odor, taste, and texture, using 9-point hedonic scales as per the following; 1, extremely dislike; 2, dislike very much; 3, moderately dislike; 4, slightly dislike; 5, neither like nor dislike; 6, slightly like; 7, moderately like; 8, like very much; 9, extremely like (Mailgaad *et al.*, 1999).

Statistical analysis

The experiment was designed using RSM and optimization was by Minitab (Trial version 16, Minitab Inc., State College, PA, USA). The regression model was conducted, and the model proposed was $Y = b_0 + \Sigma b_i x_i + \Sigma b_{ii} x^2 + \Sigma\Sigma b_{ij} x_i x_j$, where x_i and x_j are the code independent variables, while b_0, b_i, b_{ii}, and b_{ij} are the coefficients for intercept, linear, quadratic, and interaction, respectively. Statistical analysis with one-way ANOVA was performed using IBM SPSS statistics 20 software (IBM Corporation, USA). Duncan's new multiple range test (DMRT) was used to test for the differences between means. The significance level was $P \leq 0.05$.

Results and Discussions

Physical and chemical properties of frozen Nile tilapia fillets

The results of weight gain, drip loss, cooking loss, and cooking yield, moisture, and phosphate content are shown in Table 2. It was found that each treatment was significantly different ($P \leq 0.05$). Before freezing, fillets were treated in brine solution as a combination of phosphates and NaCl. Weight gain was observed, of which treatment no.11 and no.15 had the highest gain. For cooking loss, treatment no.7 had the highest value. Treatment no.12 showed the highest cooking yield, while the highest drip loss was observed in treatment no.5. The highest moisture was in treatment no.1 and no.13. Xiong *et al.* (2000) reported that the combination of NaCl and phosphates influenced the physical changes in chicken muscle. In this study,

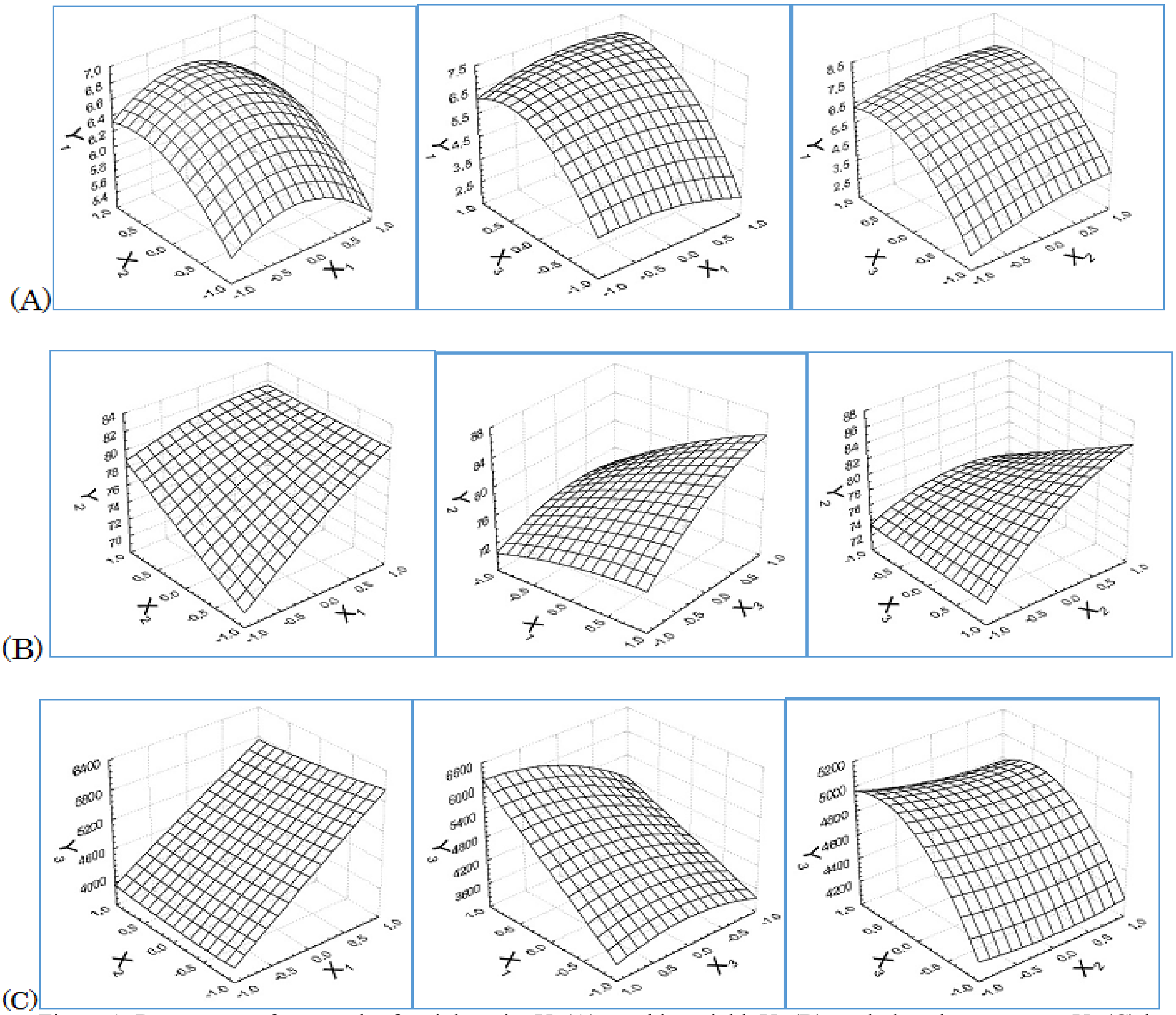

Figure 1. Response surface graph of weight gain; Y_1 (A), cooking yield; Y_2 (B), and phosphate content; Y_3 (C) by effects of phosphate concentration (X_1), salt concentration (X_2), and soaking time (X_3) with code values.

the brine solution contained both salt and phosphates and resulted in an increased fillet weight gain. This confirmed the previous study of Thorarinsdottir *et al.* (2004) which studied the combinations of sodium chloride and phosphate on qualities of frozen cod fish fillets. By the combination of salt and phosphates might have a greater affect on the myofibrillar of fish meat by opening the structure, resulting in increased water retention and yield than if used separately. Moreover, if the addition of phosphates are limited in the residual final product, then addition of salt has a major effect on ionic strength that results in a reduction of fluid loss during cooking, because chloride ion assists in affecting electrostatic repulsion of muscle protein then allows more water to be bound or trapped within muscle fibers or cells (Gonçalves and Ribeiro, 2009).

The remaining phosphate content in the products, treatment no.2, 4, and 8 that used a high concentration of phosphate (3.5%) in brine solution had a higher value than the standard level referenced by the European Parliament and Council (2006) of not over 5000 mg/kg per fish fillet product. The time of immersion and concentration are main effect phosphate content in products, which low concentration take longer time than high concentration to penetrate into the products (Gonçalves and Ribeiro, 2008).

Sensory evaluations

The sensory evaluation of raw and cooked fillets is shown in Table 2. From the results, it was found that the raw sample appearance score was between slightly like (6.0) and like very much (8.0). Treatment no.4 had the highest score in the raw sample texture category. The cooked fillets, treatment no.3, 12, 14 and 15 had the highest appearance, odor, taste, and texture scores, respectively. Gonçalves and Ribeiro (2009) reported that phosphates can increase water retention in shrimp, making them juicier and more similar to fresh shrimp. Gonçalves *et al.* (2008)

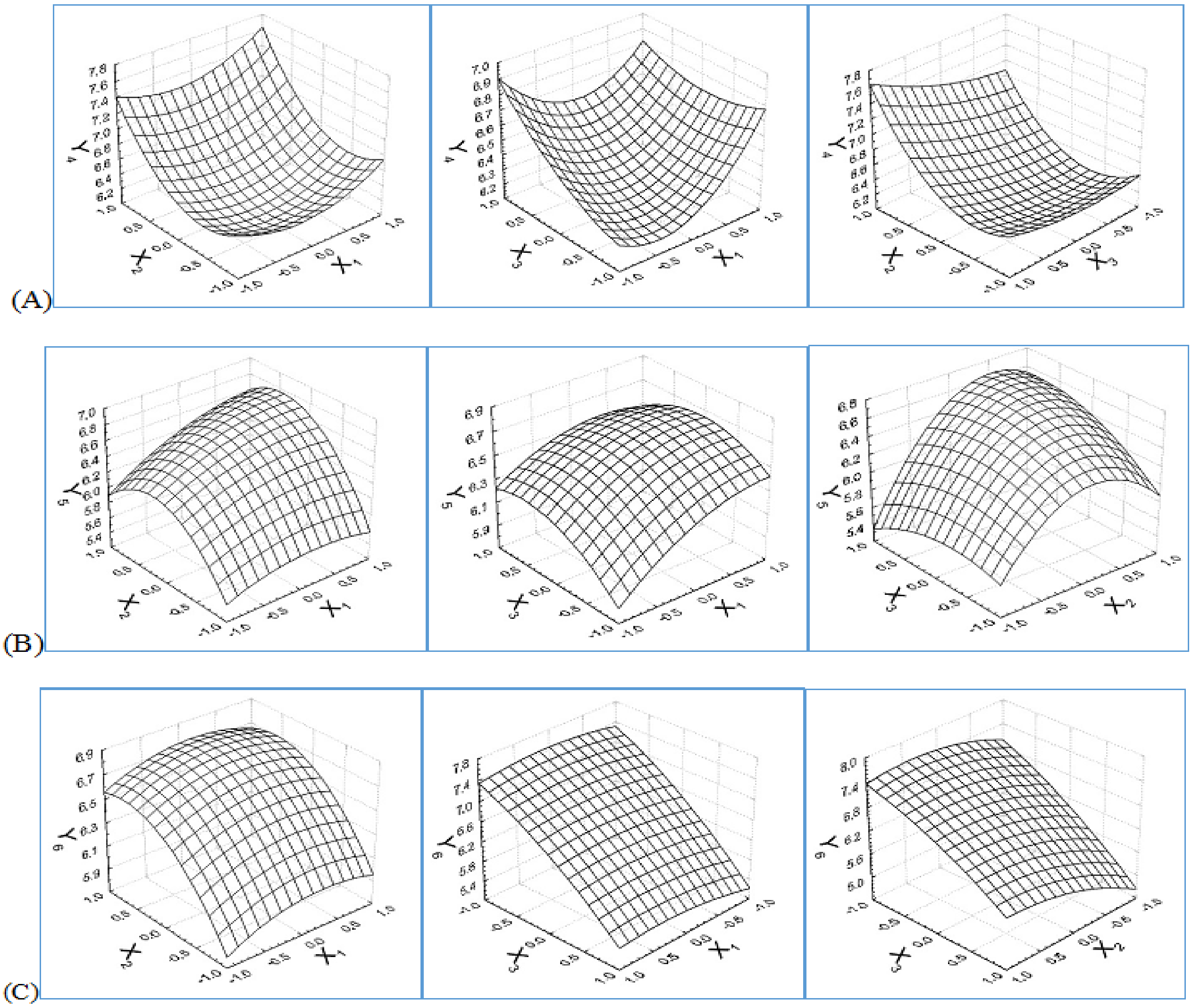

Figure 2. Response surface graph of appearance score of raw fillets; Y_4 (A), taste score of cooked fillets; Y_5 (B) and texture score of cooked fillets; Y_6 (C) by effects of concentration of phosphate (X_1), concentration of salt (X_2) and soaking time (X_3) with code values

mentioned 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. In addition, the impact of freezing on sensory attributes is a deterioration of textural quality of cooked fish fillets, and the rate of freezing affected the color of frozen meat, i.e., the most desirable color was obtained when freezing at -34 to -40ºC, while color tended to be pale at -73 to -87ºC (Gonçalves *et al*., 2012)

As per the results, it could be observed that the obtained response had low and high values. The ranges of response levels covered satisfaction and non-satisfaction with the fillet products. It implies that phosphates, NaCl, and soaking time might be crucial factors, and the level range was appropriate to evaluate the physical and sensory properties of frozen fillets in this study.

Regression models

The designed treatment (Box-Behnken design) and obtained responses were used to calculate a regression model concluding linear model (X_1, X_2, X_3), interaction (X_1X_2, X_1X_3, X_2X_3), and quadratic (X_1^2, X_2^2, X_3^2) equations. It was found that the regression of weight gain (Y_1), cooking yield (Y_2), and phosphate content (Y_3), and the sensory acceptability scores of raw fillet appearance (Y_4), cooked fillet taste (Y_5), and cooked fillet texture (Y_6) showed high values of R^2 of 0.961, 0.937, 0.960, 0.939, 0.938 and 0.901, respectively. The regression model, the higher values of R^2 show a higher percentage of response prediction and how the model is appropriate. Analysis of variance (ANOVA) was used to explain the response of dependent variables of the experiment (Wangtueai and Noomhorm, 2009). All of the models were significant ($P \leq 0.05$) at a 95% probability level, and the lack of fit for all models was not significant ($P > 0.05$), data not show. These can be considered as satisfactory optimum models for this experiment. The full regression models were created as per the following:

Table 3. Actual and predicted results of verification under optimized conditions

Responses	Predicted value	Actual value[a]
Weight gain* (%)	6.52	5.30±0.61
Cooking yield[ns] (%)	81.12	87.41±6.02
Phosphate content* (mg/kg)	3876	3450±50
Appearance score[ns] (raw)	7.5	7.20±1.04
Taste score* (cooked)	6.2	7.40±1.24
Texture score[ns] (cooked)	6.6	7.00±1.30

Note: [a]mean ± SD (weight gain, cooking yield, phosphate content n=3, appearance (raw), taste and texture score (cooked) n=40) [ns]= non-significantly different (P>0.05), *=significantly different (P≤0.05)

$$\text{Weight gain } (\%, Y_1) = 6.597 - 0.078X_1 + 0.429X_2 + 1.824X_3 - 0.421X_1^2 - 0.328X_2^2 - 1.563X_3^2 + 0.038X_1X_2 + 0.408X_1X_3 - 0.150X_2X_3$$

$$\text{Cooking yield } (\%, Y_2) = 78.530 + 3.633X_1 + 2.261X_2 + 3.639X_3 - 0.798X_1^2 + 0.100X_2^2 - 1.885X_3^2 - 2.243X_1X_2 + 1.963X_1X_3 + 2.540X_2X_3$$

$$\text{Phosphate content } (\text{mg/kg}, Y_3) = 4802 + 1059X_1 - 50.66X_2 + 351.9X_3 - 22.94X_1^2 + 47.12X_2^2 - 334.8X_3^2 - 104.5X_1X_2 + 323.5X_1X_3 - 44.58X_2X_3$$

$$\text{Appearance score } (Y_4) = 6.320 + 0.104X_1 + 0.434X_2 + 0.167X_3 + 0.271X_1^2 + 0.496X_2^2 + 0.104X_3^2 + 0.013X_1X_2 - 0.145X_1X_3 + 0.120X_2X_3$$

$$\text{Taste score } (Y_5) = 6.580 + 0.210X_1 + 0.371X_2 + 0.111X_3 - 0.151X_1^2 - 0.559X_2^2 - 0.194X_3^2 + 0.145X_1X_2 - 0.085X_1X_3 + 0.223X_2X_3$$

$$\text{Texture score } (Y_6) = 6.660 + 0.063X_1 + 0.365X_2 - X_3 - 0.135X_1^2 - 0.285X_2^2 - 0.200X_3^2 - 0.015X_1X_2 + 0.050X_1X_3 + 0.180X_2X_3$$

Optimization for soaking process of fish fillets

To optimize the best solution with target response, a function of response optimizer in Minitab software was used. The target value of all responses was set as a maximum value, except phosphate content, which was set as a minimum value. After calculation, the results found that, at the optimum conditions, the code value of phosphate concentration was X_1= -0.72; NaCl concentration, X_2 = 0.82; and soaking time, X_3 = 0.92, with 0.82 of composite desirability. The actual values were as per the following; 1.4% phosphate concentration, 2.7% NaCl, and soaking time for 115 min. It gave a high satisfaction, with a high weight gain of 6.52%, cooking yield of 81.12%, low phosphate content of 3876 mg/kg, and high appearance score of 7.5, taste of 6.2, and texture of 6.6, respectively.

Response surface plots

The response surface plots of physical and sensory evaluation are shown in Figure 1 and Figure 2. In terms of the effect of phosphate concentration (X_1) and NaCl concentration (X_2) on weight gain (Figure 1A), it was found that, when X_1 and X_2 was close to 0 of code value, weight gain was high. It might be that phosphates and NaCl have an interaction between fish muscle and water, so that muscle protein is affected by having a good absorption of water, resulting in a high weight gain (Thorarinsdottir *et al*., 2004). Rattanasatheirn *et al.* (2008) compared the weight gain of shrimp taken by different cutting methods during the preparation of raw materials (peeled and peeled-deveined shrimp). They reported that the peeled-deveined shrimp, treated in phosphate-brine solutions that made the shrimp muscle become exposed to phosphates for absorption into shrimp muscle easily, affected the higher weight gain of the samples. On the other hand, this experiment (high X_1 and X_3) showed a higher phosphate content remained in the fillet, as expressed in Figure 1C. The cooking yield, when X_1 and X_2 was high, the cooking yield increased (Figure 1B). It might be a co-effect of phosphates and NaCl. NaCl could increase the solution of protein, particular myofribrillar protein such as actin and myosin, while phosphates could protect the denaturation of proteins and prevent water loss during the freezing process. Similarly, Thorarinsdottir *et al.* (2004) reported that the co-action of phosphates and NaCl can increase the cooking yield of frozen cod fish. Jittinandana *et al.* (2002) mentioned that the cooking yield was affected by NaCl because NaCl increased the solubility of protein and changed myofibrillar protein.

Sensory evaluation, high X_1, X_2 and X_3 tended to increase the appearance score (Figure 2A). The taste score of fish fillets received a high score when X_2 and X_1 got close to code 0 (Figure 2B), as did the results of texture (Figure 2C). Lampila (1993) explained that the mixed action of phosphates and NaCl results in a high water absorption and moisture in fish fillets. This water absorption is an important factor that directly impacts the human senses in both appearance observation and taste evaluation. Gonçalves *et al.* (2008) reported that phosphates can improve moisture and water holding capacity in fish fillets, shrimp and shellfish, resulting in higher customer satisfaction.

Verification of actual experiment

Verification experiments were conducted under optimal conditions (1.4% phosphate, 2.7% NaCl, and soaking time 115 min) to compare the predicted values and the actual values of the responses (Table 3). The actual values almost coincided with the predicted values. Therefore, the estimated response surface model can be adapted for the optimization of soaking conditions and for the previous freezing for production of frozen Nile tilapia fillets.

Conclusions

This study was optimized the conditions of phosphates, NaCl, and time for the soaking process to produce frozen Nile tilapia fillets. RSM with a three-level and three-factor Box-Behnken design was used to find the optimized conditions, which are at 1.4% phosphate (-0.72 code value), 2.7% NaCl (0.82 code value), and 115 min soaking time (0.92 code value). The highest satisfaction with high predicted value weight gain of 6.52%, cooking yield of 81.12%, low phosphate content of 3876 mg/kg, high appearance score of 7.5, taste score of 6.2, and texture score of 6.6 were obtained. The results concluded that phosphates concentration, NaCl concentration, and soaking time are significant factors for the physical and sensory properties of frozen Nile tilapia fillets.

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