Physiochemical Changes and Optimization of Phosphate-Treated Shrimp (*Litopenaeus vannamei*) Using Response Surface Methodology

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ABSTRACT: The objective of this study was to determine the factors responsible for the changed physiochemical properties of unpeeled shrimp treated in cold phosphate solution ($2\sim4^{\circ}C$) with the intervention of 4 factors: phosphate concentration, dipping time, rotation speed, and volume of brine solution. Response surface analysis was used to characterize the effect of the phosphate treatment on shrimps by running 33 treatments for optimizing the experiment. For each treatment, phosphate amount, moisture content, and weight gain were measured. The results showed that phosphate concentration is the most important factor than other factors for facilitating phosphate penetration in the meat of the shrimp and for getting the best result. The optimum condition of phosphate-treated shrimp in this study was 110 to 120 min dipping time, 500 to 550 mL brine solution for 100 g shrimp sample, and 190 to 210 rpm agitation speed. The studied conditions can be applied in fisheries and other food industries for good phosphate treatments.

Keywords: shrimp, phosphate, response surface methodology

INTRODUCTION

Shrimp is one of the popular seafoods in the world including East Asian countries such as Korea, China, and Japan, because it has several applications in foods. However, seafood quality is easily lost due to microbiological contamination and/or chemical reactions because shrimp has high water content, large quantities of free amino acids, and autolytic enzymes among other factors (1). After the capture of shrimp, a series of complex changes occurs in the seafood, resulting in a decrease of quality (2). Therefore, shrimp should be frozen or kept at a cold temperature to limit or reduce enzymatic and microbial activities to keep its good quality (3). Also, other treatments such as dipping or spraying food additives are being developed. To reduce the undesirable biochemical and physical changes, the kinds of food additives for dipping solutions in seafood are sodium acetate, sodium lactate, sodium citrate (4), and phosphate derivatives (3,5,6). Although phosphate derivatives are commonly used among other additives, only a few researchers have studied the

effects of phosphate derivatives in shrimp.

Phosphate derivatives used for shrimp are sodium tripolyphosphate (STPP), tetrasodium pyrophosphate (TSPP), sodium hexametaphosphate (SHMP), or their blends (3). Moawad et al. (7) found that the good soaking condition of STPP for peeled white marine shrimp was 5% STPP for 10 min by evaluating chemical, physical, sensorial, and microbiological qualities. In the case of a blend treatment, the mixture of STPP and TSPP showed better quality than the STPP treatment in the condition of 5% solution for 120 min (8). In the case of a blend of STPP and SHMP, the peeled and dipped shrimp showed a cryoprotective effect in the treatment of 4% blend for 5 min (9). The most common chemical compound for phosphate treatment in shrimp is STPP and the differences between STPP and other blends were not largely different (8,9). Also, the shrimp samples were mainly de-headed, peeled, and deveined in most studies. Few studies of unpeeled shrimp and phosphate treatment were found and, the quality changes of unpeeled shrimp should be studied by the effect of the dipping conditions

with phosphate treatment.

Response surface methodology is mainly used to investigate the optimum condition for biochemical reactions and physical changes, and to know the effect of the independent variables for the reactions and changes (10). The independent variables for phosphate treatments in shrimp are size and type of shrimp, type of treatment, kinds of phosphate, phosphate concentration, agitation method and speed, dipping time, and solution temperature, among others (11). Therefore, the objective of this study was to assess the effects of dipping whole white marine shrimp (*Penaeus* sp.) in a cold (2~4°C) tripolyphosphate solution with the intervention of 4 factors (phosphate concentration, dipping time, rotation speed, and volume of solution brine) to determine which factor or factors are more responsible to affect the physicochemical properties of shrimp.

MATERIALS AND METHODS

Samples treatment
Five kg middle size (80~95 shrimp/kg) of white marine shrimp (*Penaeus* sp.) were purchased from a seafood processing company (Au Vung Seafood Processing and Exporting Joint Stock Company, Bac Lieu, Viet Nam). The shrimps were frozen without any treatments before starting the experiment.

Experimental design and procedure
Response surface analysis was used to characterize the effect of the phosphate treatment on shrimps. Four factors were treated. The first one was the dipping time in the phosphate solution because the dipping time affects the amount of phosphate absorbed when the shrimp contact the phosphate solution. The second one was the phosphate concentration between 1% to 5% of phosphate (tripolyphosphates and polyphosphates). The third one was the agitation speed, which is the number of rotations per minute for ensuring more contact. Then, the fourth one was the ratio between the shrimp amount and the volume of brine solution.

With these 4 factors, 33 treatments were run to optimize the experiment as shown in Table 1 and 2. All these operations were conducted under low temperature of 0°C to 4°C. For each treatment, phosphate amount (PA), moisture content (MC), and weight gain (WG) were measured.

Determination of WG and MC
The weight gain is the difference between the weight of shrimp before and after treatment. Then, the WG was calculated using the formula below:

$$WG\ (\%) = \frac{W_{before} - W_{after}}{W_{before}} \times 100$$

where W_{before} is the weight of the shrimp before treatment and W_{after} is the weight of the shrimp after treatment.

To determine the MC the sample was dried in an oven under a temperature of 105°C for 10 to 12 h. The difference of the weight before and after is the water evaporated during the drying time, and the MC was calculated with this formula:

$$MC\ (\%) = \frac{W_s - W_d}{W_s} \times 100$$

where W_s is the weight of the sample before drying and W_d is the weight of the sample after drying.

Determination of PA
Quantification of the total phosphate content is usually measured by spectroscopic analysis. The sampling preparation is based on the decomposition of polyphosphates to orthophosphates in the presence of sulphuric or trichloroacetic acid as described by Jastrzębska et al. (12). The orthophosphates react with ammonium molybdate and ammonium vanadate in nitric acid (HNO$_3$), and a yellow rash is formed. The concentration of phosphovanadomolybdate is used to calculate the content of phosphate or phosphorus (13).

In the presence of reducing agents, molybdenum yellow is condensed to a molybdenum blue complex, which

Table 1. Experimental values and coded levels of the independent variables utilized for the full-factorial design in our experiment

Independent variable[1]	Symbol		Levels				
	Uncoded	Coded	−2	−1	0	+1	+2
Dipping time (min)	X_1	x_1	30	60	90	120	150
Phosphate concentration (%)	X_2	x_2	1%	2%	3%	4%	5%
Agitation speed (rpm)	X_3	x_3	100	150	200	250	300
Volume (mL)	X_4	x_4	300	400	500	600	700

[1]Dipping time, the total dipping time of shrimp in phosphate solution; phosphate concentration, the phosphate concentration; agitation speed, the number of rotations in one minute; volume, the volume of brine solution for 100 g shrimp sample.

Table 2. Experimental results of their responses in 33 treatment conditions of four independent variables of phosphate treatment in shrimp by using a full-factorial design

Treatment	Variation levels[1]				Response function[2]		
	X_1	X_2	X_3	X_4	WG (%)	MC (%)	PA (g/kg)
1	120	4	150	600	3.45±0.05[3]	77.78±0.04	3.87±0.07
2	120	2	150	400	2.64±0.06	76.45±0.12	2.38±0.10
3	60	4	150	400	2.52±0.05	77.89±0.13	3.78±0.12
4	120	4	250	400	3.22±0.04	77.79±0.09	3.9±0.13
5	60	2	250	400	2.32±0.05	76.34±0.04	2.32±0.19
6	120	2	250	600	2.42±0.07	76.66±0.08	2.39±0.05
7	60	4	250	600	2.99±0.03	78.02±0.06	4.05±0.06
8	60	2	150	600	2.53±0.07	76.57±0.06	2.51±0.09
9	90	3	200	500	2.88±0.04	77.33±0.05	3.34±0.17
10	90	3	200	500	3.09±0.04	77.23±0.08	3.49±0.11
11	90	3	200	500	3.12±0.06	77.45±0.10	3.4 ±0.10
12	120	4	150	400	3.67±0.10	77.92±0.10	3.85±0.15
13	120	2	250	400	2.44±0.08	76.56±0.02	2.47±0.16
14	60	4	150	600	3.24±0.06	77.62±0.03	3.96±0.09
15	60	2	150	400	2.35±0.06	76.34±0.01	2.23±0.09
16	60	2	250	600	2.38±0.08	76.54±0.10	2.34±0.07
17	120	2	150	600	2.45±0.09	76.66±0.14	2.46±0.05
18	60	4	250	400	3.69±0.06	77.57±0.10	3.86±0.04
19	120	4	250	600	3.93±0.03	78.19±0.09	4.11±0.01
20	90	3	200	500	3.03±0.05	77.36±0.07	3.55±0.04
21	90	3	200	500	2.89±0.03	77.48±0.06	3.49±0.03
22	90	3	200	500	3.12±0.10	77.61±0.07	3.61±0.06
23	30	3	200	500	2.98±0.05	77.43±0.06	3.43±0.05
24	150	3	200	500	3.05±0.04	77.59±0.06	3.63±0.12
25	90	1	200	500	2.02±0.02	76.22±0.05	2.11±0.10
26	90	5	200	500	4.33±0.03	78.24±0.08	4.16±0.06
27	90	3	100	500	3.02±0.07	77.57±0.06	3.49±0.06
28	90	3	300	500	3.20±0.09	77.62±0.06	3.57±0.15
29	90	3	200	300	3.17±0.06	77.34±0.05	3.52±0.07
30	90	3	200	700	3.33±0.05	77.64±0.09	3.64±0.08
31	90	3	200	500	3.13±0.09	77.68±0.06	3.48±0.09
32	90	3	200	500	3.19±0.07	77.59±0.06	3.54±0.04
33	90	3	200	500	3.22±0.06	77.58±0.07	3.52±0.06

[1] X_1, X_2, X_3, and X_4 are uncoded variables as shown in Table 1.
[2] WG, weight gain; MC, moisture content; PA, phosphate amount in the phosphate-treated shrimp.
[3] Values are mean±standard deviation of 4 replicates.

shows an intense light absorption and the maximum absorbance occurs at longer wavelengths (14). The benefit of the myolybdenum blue procedure is its high sensitivity and smaller interferences from coexisting ions. The wet digestion method (nitric acid-perchloric acid method) was used to determine the phosphorous amount using a spectrophotometer at 650 nm. The formula below was used to obtain the phosphate amount:

$$PA\ (g/kg) = 0.05 \times \frac{A}{A_s} \times \frac{1}{S} \times V \times 100$$

where A_s is the standard absorbance, A is the absorbance of the sample, S is the sample amount, and V is the dilution.

Statistical analysis

All experiments were carried out in triplicates. Regression analysis and analysis of variance (ANOVA) were conducted to examine the statistical significance at the 95% significant level using the SAS software program (ver. 9.3, SAS Institute, Cary, NC, USA). The three dimensional graph was drawn using the Maple software program (ver. 8, Maplesoft, Waterloo, ON, Canada).

RESULTS AND DISCUSSION

WG in phosphate-treated shrimp

The first dependent variable of weight gain in phosphate-treated shrimp was experimentally obtained using the response surface methodology, and the results are shown

Table 3. Result of statistical analyses for dependent variables of weight gain, moisture content, and phosphate amount

Response surface for variable	WG (%)	MC (%)	PA (g/kg)
Response mean	3.00	77.33	3.32
Root MSE	0.2813	0.2182	0.2214
R^2	0.8214	0.9192	0.9313
Coefficient of variation	9.3768	0.2822	6.6741

WG, weight gain; MC, moisture content; PA, phosphate amount in the phosphate-treated shrimp.

in the Table 3. The mean of WG was 3.00% with a R^2 value of 0.82. This means that the phosphate treatment increased the weight of the shrimp by 3.00%. The lowest value was 2.02±0.02% under 1% phosphate concentration, 200 rpm rotation speed, 1/5 ratio, and 90 min. The highest value was 4.33±0.03%, under 5% phosphate concentration, 200 rpm rotation speed, 1/5 ratio, and 90 min (Table 2). In the present study, the phosphate treatment increased the shrimp weight by 3%. Other reports show that phosphate treatment increases the weight of the product, because the contact between the meat and the phosphate solution increased the uptake of phosphate (15,16).

Fig. 1 shows the 3 dimensional figure of the effect of 2 different factors on the WG using the following formula and by the drawing from the Maple 8 software program. The predicted formula of WG (%)=0.930972+0.012972×T−0.175417×P+0.007103×R+0.000257×V−0.000062346×T×T+0.003042×P×T−0.016111×P×P−0.000039167×R×T+0.001700×R×P+0.000012944×R×R+0.000000417×V×T+0.000300×V×P−0.000005500×V×R+0.000000264×V×V, which was obtained from a statistical analysis shown in Table 2. Two factors were fixed in their central value, and the effect of the other factors was presented (T=90 min, P=3%, R=200 rpm, and V=500 mL) as shown in Fig. 1.

A positive correlation between the dipping time and
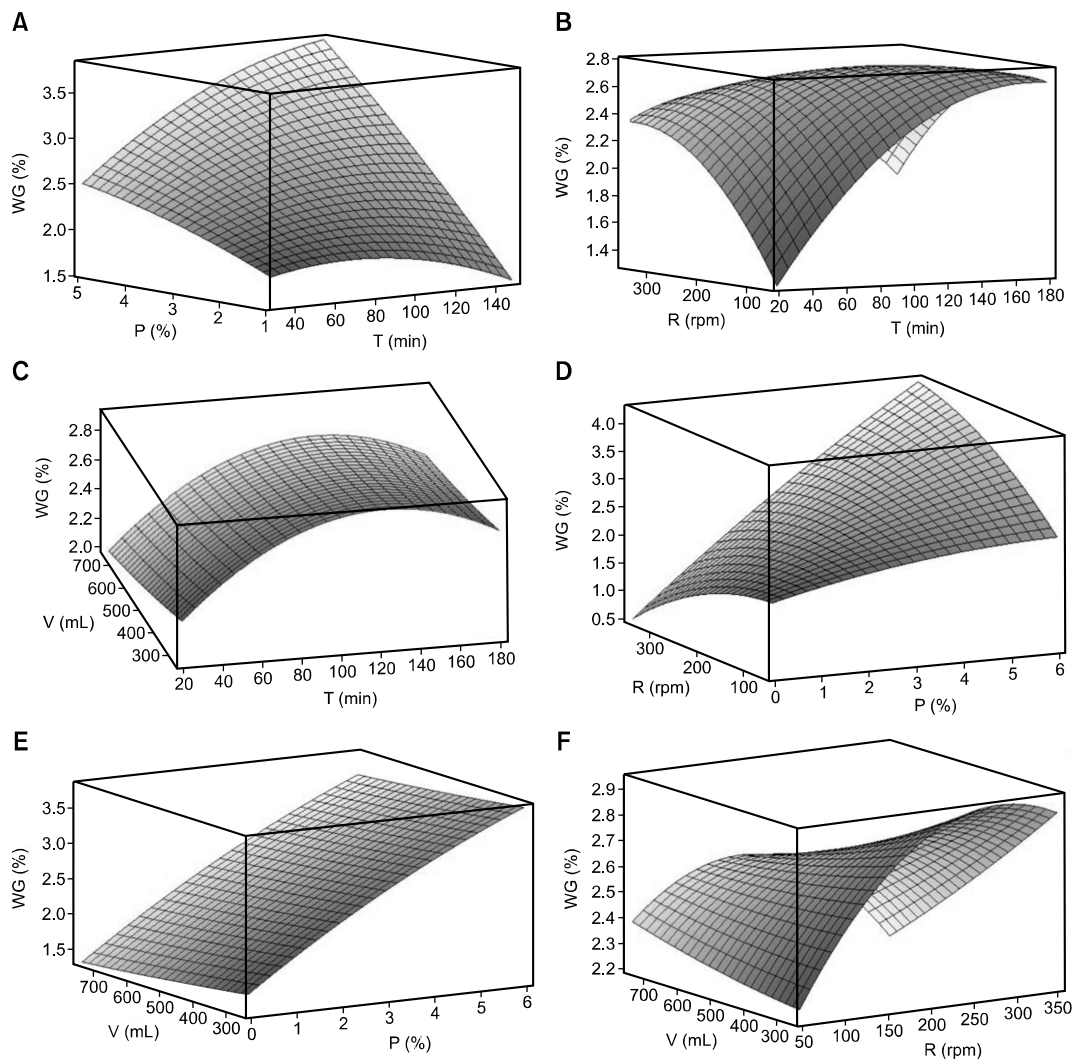
Fig. 1. Three-dimensional plots on effect of weight gain (WG, %) at two other factors in phosphate-treated shrimp by drawing by Maple 8. T, dipping time (min); P, phosphor concentration (%); R, rotation per minute (rpm); V, volume of phosphor solution for 100 g of shrimp (mL).

the phosphate concentration is shown in Fig. 1A. The highest value of the WG was found in the highest value of both, the dipping time and the phosphate concentration. The results of this study agree with Xiong and Kupski (15) in which they found that both, the concentration and the dipping time affected the WG of white shrimp and both factors had an interaction effect.

The same results are shown in Figs. 1D and 1E. The phosphate concentration correlates with the rotation speed and the volume of the brine solution, respectively. The highest value of the WG was found in the highest value of both of them with the strongest in the side of the phosphate concentration. Tenhet et al. (17) found that penetration of the phosphate solution into the shrimp muscle depends on the concentration of phosphate.

The same results were found in Fig. 1B. But for Fig. 1C, the highest value of the WG of the shrimp sample was found in the middle of 110 min and 120 min dipping time and after that, the value declined. Two hours is enough to get the desired result. Fig. 1F shows a complicated relationship between the rotation speed and the volume of the brine solution. The rotation speed gave a good response with a small volume, and it was inversed with a high volume. This means that the small volume needs the rotation for good penetration of phosphate into the shrimp meat, and the big volume does not. In conclusion, the first factor responsible for getting a good result in the WG is the phosphate concentration. The second one is the dipping time but not for a long time (about 2 h, with 3% phosphate). The third position is the rotation speed and the volume of the brine solution.

MC in phosphate-treated shrimp
The second dependent variable of moisture content in phosphate-treated shrimp was experimentally obtained using the response surface methodology, and the results are shown in the Table 3. The moisture percentage mean was 77.33%, with a R^2 value of 0.9192, the lowest value was 76.22 % under 1% phosphate concentration, 200 rpm rotation speed, 1/5 ratio, and 90 min. The highest value was 78.24% under 5% phosphate concentration, 200 rpm rotation speed, 1/5 ratio, and 90 min (Table 2).

The 77.33% MC is within the normal limits for the species, and it is in agreement with that found in the shellfish literature for white shrimp by Sriket et al. (18), which was reported to be 77.2%. Sundararajan (19) found the MC to be 77.36% and Moawad et al. (7) found it to be 77.32%. According to the result of Laura and Garrido (20), the University of Florida has developed tentative standards for shell-on and peeled products that differentiate between phosphate-treated and untreated products. A moisture content of the meat that is higher than 78.5% could be interpreted as a phosphate-treated product. The value is commonly seen in commercial shrimp,

but it may not be applicable to all species of shrimp.

The predictive formula of MC was made as MC (%)＝ 73.909028＋0.006944×T＋1.277083×P－0.006186×R＋0.002885×V－0.000042284×T×T＋0.000083333×P×T －0.108056×P×P＋0.000014167×R×T＋0.000350×R×P－0.000006722×R×R－0.000000833×V×T－0.000187×V×P＋0.000014000×V×R－0.000004306×V×V. By using this formula, the 3 dimensional figure shows the effect of 2 different factors in moisture content using Maple 8. In each subfigure, 2 factors were fixed in their central values, and the effect of the other 2 factors (T＝90 min, P＝3%, R＝200 rpm, and V＝500 mL).

The MC increased when the values of all of the other factors were also increased. The effect of the phosphate concentration was stronger than that of dipping time, rotation speed and volume of the brine solution as shown in Figs. 2A, 2D, and 2E, respectively. Tenhet et al. (17) found that penetration of the phosphate solution into the shrimp muscle depends on the concentration of phosphate in the brine solution. Figs. 2B, 2C, and 2F show that the highest value of moisture was found in the middle range of around 110 to 120 min for the time, 500 to 550 mL volume for 100 g shrimp sample, and 190 to 210 rpm for the rotation speed. In order to maximize the MC, it is not necessary that all of the other factors be at their maximum levels.

The addition of phosphates has been shown to improve water-holding capacity of the product (21). Although much work has been conducted on the effects of polyphosphate treatment on food products including meat and seafood, the actual mechanism of the action of polyphosphates on proteins is not well understood. It is however known that the water-holding capacity of a proteinaceous food involves interactions between protein and water. Increased water-holding capacity is hypothesized to be due, in part, to the increased space between muscle fibres, creating more water-holding capacity (22). Conclusively, it can be said that phosphate concentration is the most important factor than the other factors for getting the best result of MC by facilitating phosphate penetration in the meat of the shrimp.

PA in phosphate treated shrimp
The final dependent variable of the PA in phosphate-treated shrimp was experimentally obtained using the response surface methodology, and the results are shown in the Table 3, which shows the mean value 3.31 g/kg of PA with a R^2 of 0.9313. The lowest value was 2.11±0.10 g/kg under 1% phosphate concentration, 200 rpm rotation speed, 1/5 ratio, and 90 min. The highest value was 4.16±0.06 g/kg under 5% phosphate concentration, 200 rpm rotation speed, 1/5 ratio, and 90 min (Table 2). Little information is available in the literature about the naturally occurring levels of phosphates in crustaceans

A

B

C

D

E

F
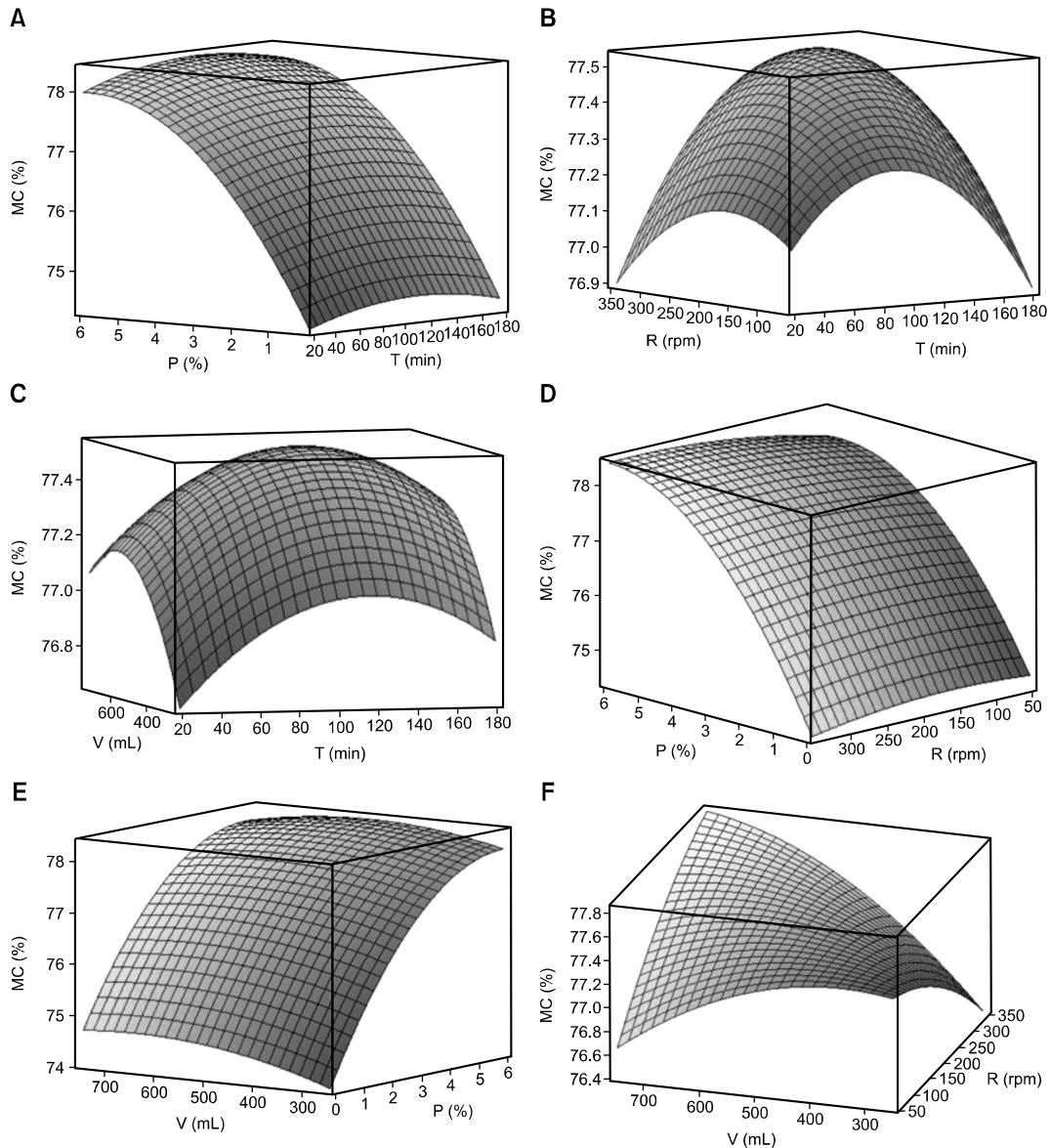
Fig. 2. Three-dimensional plots on effect of moisture content (MC, %) at two other factors in phosphatetreated shrimp by drawing by Maple 8. T, dipping time (min); P, phosphor concentration (%); R, rotation per minute (rpm); V, volume of phosphor solution for 100 g of shrimp (mL).

and molluscs. This is because the levels change rapidly depending on temperature, pH, storage conditions, and/ or enzyme activity. There are also differences between the levels in different species, between individuals of the same species (23), and between the same species but in different geographical locations. Differences can additionally occur depending on how the animals have been caught and handled (22).

By analysing the data on Table 2, this formula was obtained PA $(g/kg) = -1.801736 + 0.012889 \times T + 1.322917 \times P + 0.005211 \times R + 0.003878 \times V - 0.000042670 \times T \times T - 0.000458 \times P \times T - 0.137153 \times P \times P + 0.000009167 \times R \times T + 0.000650 \times R \times P - 0.000015361 \times R \times R - 0.000009167 \times V \times T + 0.000188 \times V \times P - 0.000002750 \times V \times R - 0.000002590 \times V \times V$. As shown in Fig. 3, 3 dimensional figures show the effect of 2 different factors in PA. Two factors

were fixed in their central values, and the effect of the other 2 variables (T=90 min, P=3%, R=200 rpm, and V=500 mL) are shown in the subfigures of Fig. 3.

According to the results mentioned earlier in this study, the mean of PA was found to be 3.31 g/kg. The lowest value was 2.11±0.10 g/kg under 1% phosphate concentration, 200 rpm rotation speed, 1/5 ratio, and 90 min. The highest value was 4.16±0.06 g/kg under 5% phosphate concentration, 200 rpm rotation speed, 1/5 ratio, and 90 min. The effect of changing the phosphate concentration in the brine solution from 1% to 5% was 2.05 g/kg PA in shrimp meat. Crawford (24) found that a 6% polyphosphate solution increased the phosphate content of the shrimp by as much as 1.10 g/kg the control samples. Therefore, the quantity of phosphate added to the shrimp is within the range of the phosphate levels

naturally occurring in these animals. Laura and Garrido (20) concluded that raw shrimp could be considered as phosphate-treated product, if the total PA is higher than 2.6 g/kg of meat.

The phosphate concentration in the brine solution makes a positive correlation between the dipping time, the agitation speed, and the volume of brine solution. As shown in Figs. 3A, 3D, and 3E, respectively, the PA increased when the values of the other factors were increased. The highest value of phosphate amount appeared in the highest value of all of the other factors. Tenhet et al. (17) found that the penetration of phosphate solution into shrimp muscle depends on the concentration of phosphate in the solution, time of application, and the thickness of the muscle. The phosphate content of the solutions regularly increased during dipping due to the diffusion of orthophosphates from within the samples (25). As shown in Figs. 3B, 3C, and 3F, the highest

value of PA appeared in the middle range of the other factors. With the same value as the moisture content before, the optimum condition was 110 to 120 min dipping time, 500 to 550 mL volume of brine solution for 100 g shrimp sample, and 190 to 210 rpm agitation speed. It can be concluded that the phosphate concentration in the brine solution is the first factor to attain the highest value of PA, and the phosphate amount increases when the concentration of phosphate in the brine solution increases.

CONCLUSION

The most important factor that is accountable for better results of WG is the phosphate concentration. The second one is the dipping time (about 2 h with 3% P), and the third is the rotation speed and the volume of the
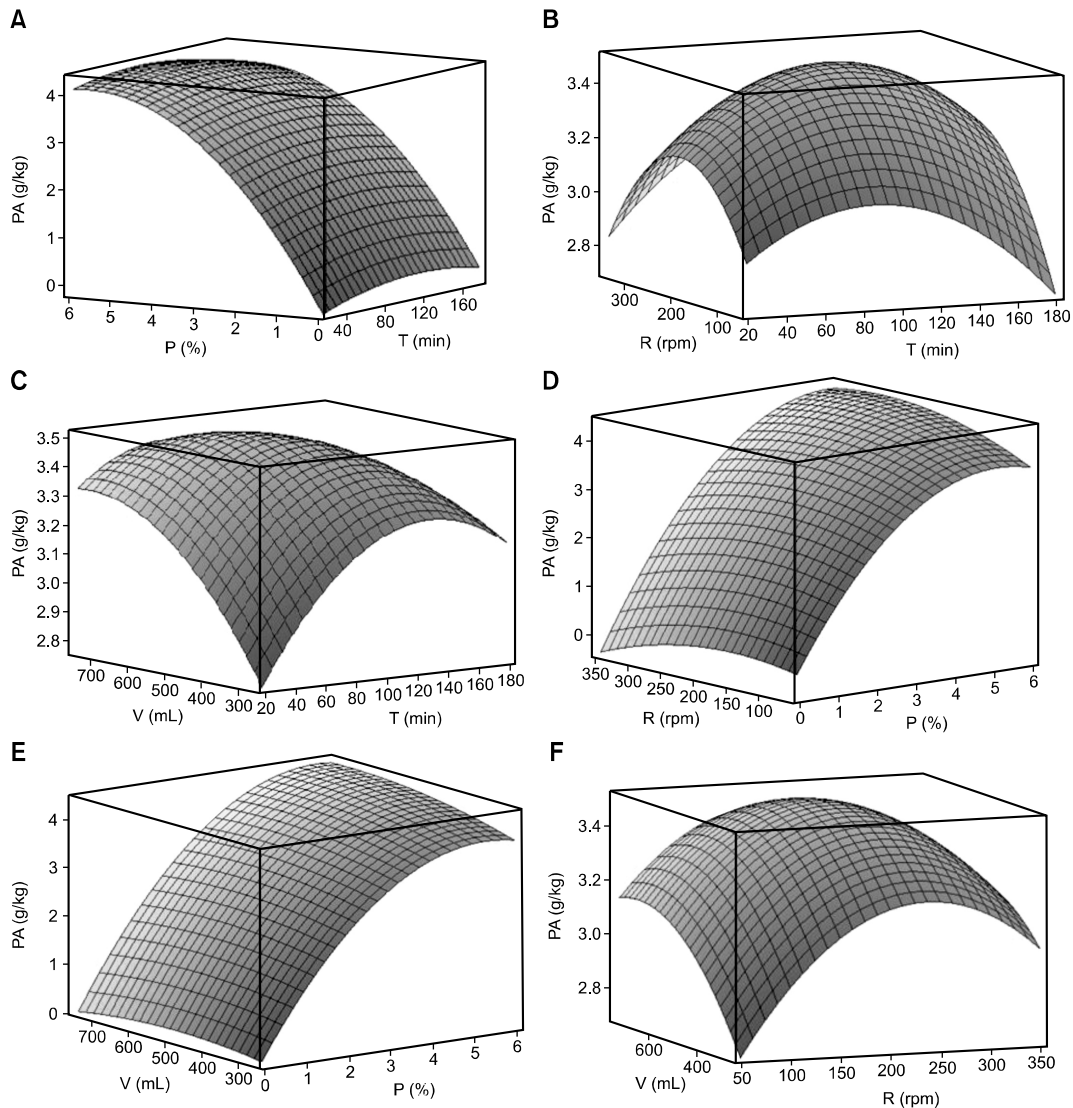
Fig. 3. Three-dimensional plots on effect of phosphate amount (PA, g/kg) at two other factors in phosphatetreated shrimp by drawing by Maple 8. T, dipping time (min); P, phosphor concentration (%); R, rotation per minute (rpm); V, volume of phosphor solution for 100 g of shrimp (mL).

brine solution. As for MC, it can be most definitely said that the phosphate concentration is the most important factor than the other factors for getting the best result by facilitating the phosphate penetration in the meat of the shrimp. In the case of PA, it can be concluded that the phosphate concentration in the brine solution is the first factor responsible for getting the highest value of PA. That is to say the PA in shrimp meat increases when the concentration of phosphate in the brine solution increases. The optimum condition for getting the best result is 110 to 120 min dipping time, 500 to 550 mL brine solution for 100 g shrimp sample, and 190 to 210 rpm agitation speed.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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