Thesis for the Degree of Master of Fisheries Science

Quality Evaluation and Optimization of Phosphate-treated Shrimp *(Litopenaeus vannamei)* Using Response Surface Methodology

by

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KOICA-PKNU International Graduate Program of Fisheries Science

Graduate School of Global Fisheries

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인산염 처리한 새우의 반응표면법에 의한 품질평가와 최적화

Advisor: Professor Yang-Bong LEE

by

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Abstract

The objective of this study was to determine factors which are more responsible for improving the shrimp quality in cold (2-4°C) phosphate solution with the intervention of four factors such as 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 (g/kg), moisture content (%) and weight gain (%) were measured. The results assure that phosphate concentration is more important factor than other factors for facilitating phosphate penetration in the meat of the shrimp and for getting the best result. For phosphate concentration in brine solution, international law reports that its final product should be less than 5‰.

Key words: shrimp, phosphate, response surface methodology

Introduction

Shrimp is very popular in the world, with high nutritional value that explains the high demands on the part of consumers. On the other hand, seafood are highly susceptible to both chemical and microbiological deterioration due to its high water content, neutral pH, large quantities of free amino acids, and naturally presence of autolytic enzymes (Fang et al., 2013). Shortly after the capture, a series of complex alterations occurs on the surface and inside of the edible portion of all seafood, resulting in a decrease of its quality (Tsironi et al., 2009). Therefore, shrimp should be frozen to limit microbial and enzymatic activities which cause deterioration, and consumers should be able to obtain “frozen seafood products” of high quality, best appearance and little weight loss (Gonçalves and Ribeiro, 2008).

Shrimp muscle proteins are very sensitive to denaturation during freezing and frozen storage, and the denaturation of myofibrillar proteins is more pronounced than sacroplasmic and stroma proteins (Sirket et al., 2007 and Diaz-Tenorio et al., 2007). This frozen denaturation can lead to changes in texture, water-holding capacity, and protein solubility (Hui et al., 2006). However, these undesirable biochemical and physical changes can be reduced by dipping seafood into solutions recommended by food regulations. Sodium acetate, sodium lactate and sodium citrate (Sallam, 2007), as well as phosphate derivatives, can be used for improving seafood quality (Goncalves et al., 2008; Kilinc et al., 2009 and Paul et al., 2012).

Phosphates are present normally in all living things and are therefore present in almost all food. They are multi-purpose, generally recognized as safe (GRAS), and legally permitted additives to improve the quality of many foods, particularly that of meat and fish products (Campden BRI Report, 2012). In USA, according to FDA (USDA, 2004), there is neither prohibition of the phosphates use in seafood nor a limit for their use in accordance with good manufacturing practices (GMP), whilst in the EU and Canada, phosphates can be added to frozen molluscs and crustaceans to a level of 5g/kg (ECD, 1995 and CFIA, 2004). However, Codex Alimentarius (92-1981) is a little more flexible and tolerates a higher percentage of phosphates (1%) in the final product. Phosphates should never be used to mask inferior or deteriorated quality products. Also, phosphate solutions should only be used once (Lampila, 1992). Functional properties changed by the treatment of phosphates in seafood and its products are: (a) the retention of the moisture and natural flavor by inhibiting the loss of fluids during the distribution and the commercialization, (b) to stabilize the protein structure of seafood, to form a surface layer of coagulated (solid) protein, to swell muscle fibers and to solubilize muscle proteins, (c) enhancing water-holding capacity and tenderness of seafood by restricting protein denaturation, (d) the inhibition of the process of lipid oxidation (by the quenching of metallic ions), (e) the stabilization of the color, and (f) the cryoprotection of decreasing populations of pathogens, and preventing growth of spoilage microorganisms, which contributes to the extension of its shelf life (Love and Abel, 1966; Applewhite et al., 1993; Turan et al., 2003; Goncalves et al., 2008; Kilinc et al., 2009; Rajkowski and Sommers, 2012). Thus, there could be some advantages to treat fresh shrimps with such food additives before freezing and frozen storage.

Although phosphates have a wide application in the seafood industry and are proving many functional uses by using sodium tripolyphosphate (STPP), tetrasodium pyrophosphate (TSPP), sodium hexametaphosphate (SHMP), or their blends, there are limited studies on the effect of trisodium phosphate (TSP) on the quality of seafood during storage. Therefore, The objective of this study was to assess effects of dipping whole white marine shrimp (*Penaeus spp.*) in cold (2-4°C) triphosphate solution with the intervention of four factors (phosphate concentration, dipping time, rotation speed, and volume of brine solution), to determine which factors or factor are responsible to improve the shrimp quality.

Materials and Methods

Samples and treatments

Five kg middle size (80-95 shrimp/kg), white marine shrimp *(Penaeus spp.)*, were ordered from a seafood processing company (Au Vung Seafood Processing and Exporting Joint Stock Company – Au Vung Seafood - DL 446) from Vietnam. The shrimps were frozen without any treatments within one month before starting the experiment.

Experiment procedure

Response surface analysis (RSA) was used to characterize the effect of the phosphate treatment on shrimps. Four factors were treated. The first one is dipping time in phosphate solution because the dipping time affects the amount of phosphate absorbed when the shrimp contacted the phosphate solution. The second one is phosphate concentration with the interval between 1% to 5% of phosphate (tripolyphosphates and polyphosphates).

The third one is agitation speed which is the number of rotation by minute for ensuring more contact. Then, the fourth one is the ratio between shrimp amount and volume of brine solution.

With these four factors, 33 treatments were running to optimize the experiment as shown in Table 1 and 2. All this operations were conducted under low temperature of 0° to 4 °C. For each treatment, phosphate amount (P g/kg), moisture (M%) and weight gain (G%) were measured.

Determination of weight gain

The weight gain is the difference between the weight of shrimp before and after treatment. Then, weight gain percentage was calculated by using the below formula.

$$\textbf{Weight gain \%} = \{(W_b - W_a) / W_b\} \times 100$$

Where W_b is weight of shrimp before treatment and W_a is weight of shrimp after treatment.

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

Independent variable	Symbol		Levels				
	Uncoded	Coded	-2	-1	0	+1	+2
Time (min)	X_1	x_1	30	60	90	120	150
P concentration (%)	X_2	x_2	1	2	3	4	5
Agitation speed (rpm)	X_3	x_3	100	150	200	250	300
Ratio	X_4	x_4	1/3	1/4	1/5	1/6	1/7

- Time: total time of immersion.
- P concentration: phosphate concentration.
- Agitation speed: number of rotation in one minute (rpm).
- Ratio: 100 g of shrimp /volume of brine solution (100 ml).

Table 2. Experimental design of phosphate treatment of shrimp using response surface methodology

Run no.	Factor				Variation level			
	x_1	x_2	x_3	x_4	X_1	X_2	X_3	X_4
1	1	1	-1	1	120	4	150	600
2	1	-1	-1	-1	120	2	150	400
3	-1	1	-1	-1	60	4	150	400
4	1	1	1	-1	120	4	250	400
5	-1	-1	1	-1	60	2	250	400
6	1	-1	1	1	120	2	250	600
7	-1	1	1	1	60	4	250	600
8	-1	-1	-1	1	60	2	150	600
9	0	0	0	0	90	3	200	500
10	0	0	0	0	90	3	200	500
11	0	0	0	0	90	3	200	500
12	1	1	-1	-1	120	4	150	400
13	1	-1	1	-1	120	2	250	400
14	-1	1	-1	1	60	4	150	600
15	-1	-1	-1	-1	60	2	150	400
16	-1	-1	1	1	60	2	250	600
17	1	-1	-1	1	120	2	150	600
18	-1	1	1	-1	60	4	250	400
19	1	1	1	1	120	4	250	600
20	0	0	0	0	90	3	200	500
21	0	0	0	0	90	3	200	500
22	0	0	0	0	90	3	200	500
23	-2	0	0	0	30	3	200	500
24	2	0	0	0	150	3	200	500
25	0	-2	0	0	90	1	200	500
26	0	2	0	0	90	5	200	500
27	0	0	-2	0	90	3	100	500
28	0	0	2	0	90	3	300	500
29	0	0	0	-2	90	3	200	300
30	0	0	0	2	90	3	200	700
31	0	0	0	0	90	3	200	500
32	0	0	0	0	90	3	200	500
33	0	0	0	0	90	3	200	500

Determination of moisture content

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

$$\textbf{Moisture \%} = ((W_s - W_d) / W_s) \times 100$$

Where Ws is the weight of sample before drying operation; and Wd is the weight of sample after drying operation.

Determination of phosphate amount

Quantification of total phosphate content is usually carried out by spectroscopic analysis. The sampling preparation is based on a decomposition of polyphosphates to orthophosphate in the presence of sulphuric or trichloroacetic acid as described by Jastrzebska and others (2008). The orthophosphates react with ammonium molybdate and ammonium vanadate in nitric acid (HNO_3) and yellow precipitates are formed. The concentration of phosphovanadomolybdate is used to calculate the content of phosphate or phosphorus (Hanson, 1950; Sutton and Ogilvie, 1967).

In the presence of reducing agents, molybdenum yellow is reduced to molybdenum blue complex which shows a strong light and the maximum occurs at longer wavelengths (Jastrzębska, 2009). The advantage of the myolybdenum blue procedure is higher sensitivity and smaller interferences from coexisting ions.

The effects of three different reducing agents of the molybdenum blue method on detection limits and precision and accuracy of the quantification of phosphate have been tested. The reducing agents were ascorbic acid (AA), hydrazine sulphate (HS), hydroquinone and hydrazine sulphate (HHS). The use of HSS proved to be the most accurate procedure for determination of the total phosphorus content in biological materials. The results showed high recoveries, reasonable repeatability and accuracy in comparison with AA and HS (Jastrzębska, 2009). Wet digestion method (Nitric acid – perchloric acid method) was used to determine the phosphate amount, and by using the spectrophotometer at 650 nm, the below formula was used for getting phosphate amount.

$$P\ (mg/100g) = 0.05 \times (A/As) \times (1/S) \times V \times 100$$

Where As is the standard absorbance; A is the absorbance sample; S is the sample amount; and V is the dilution.

Statistical analysis

All experiments were carried out in triplicate. Regression analysis and analysis of variance (ANOVA) were conducted to examine the statistical significance at the 95% significant level by using SAS software program (version 9.3). The three dimensional graph was drawn with Maple 8 software program.

Results

Weight gain in phosphate-treated shrimp

Table 4 shows that the mean of weight gain is 3.00 % with R^2 value of 0.82. It means that the phosphate treatment increases the weight of shrimp with the value of 3 % at mean in different factors. The lowest value is 2.02±0.02 % under 1 % phosphate concentration, 200 rpm rotation speed, 1/5 ratio and 90 min. The highest value is 4.33±0.03 %, under 5 % phosphate concentration, 200 rpm rotation speed, 1/5 ratio and 90 min (Table 3).

Fig. 1 shows the three dimensional figure on the effect of different two factors in weight gain percentage by using the following formula and by drawing with Maple 8 software program.

Table 3. Results of weight gain in phosphate-treated shrimp

Treatment	Variation levels				Response function
	X_1	X_2	X_3	X_4	Weight gain (%)
1	120	4	150	1/6	3.45±0.05
2	120	2	150	1/4	2.64±0.06
3	60	4	150	1/4	2.52±0.05
4	120	4	250	1/4	3.22±0.04
5	60	2	250	1/4	2.32±0.05
6	120	2	250	1/6	2.42±0.07
7	60	4	250	1/6	2.99±0.03
8	60	2	150	1/6	2.53±0.07
9	90	3	200	1/5	2.88±0.04
10	90	3	200	1/5	3.09±0.04
11	90	3	200	1/5	3.12±0.06
12	120	4	150	1/4	3.67±0.10
13	120	2	250	1/4	2.44±0.08
14	60	4	150	1/6	3.24±0.06
15	60	2	150	1/4	2.35±0.06
16	60	2	250	1/6	2.38±0.08
17	120	2	150	1/6	2.45±0.09
18	60	4	250	1/4	3.69±0.06
19	120	4	250	1/6	3.93±0.03
20	90	3	200	1/5	3.03±0.05
21	90	3	200	1/5	2.89±0.03
22	90	3	200	1/5	3.12±0.10
23	30	3	200	1/5	2.98±0.05
24	150	3	200	1/5	3.05±0.04
25	90	1	200	1/5	2.02±0.02
26	90	5	200	1/5	4.33±0.03
27	90	3	100	1/5	3.02±0.07
28	90	3	300	1/5	3.20±0.09
29	90	3	200	1/3	3.17±0.06
30	90	3	200	1/7	3.33±0.05
31	90	3	200	1/5	3.13±0.09
32	90	3	200	1/5	3.19±0.07
33	90	3	200	1/5	3.22±0.06

Table 4. Result of statistical analyses for weight gain

Response surface for variable of weight gain	
Response mean	3.000303
Root MSE	0.281333
R-square	0.8214
Coefficient of variation	9.3768

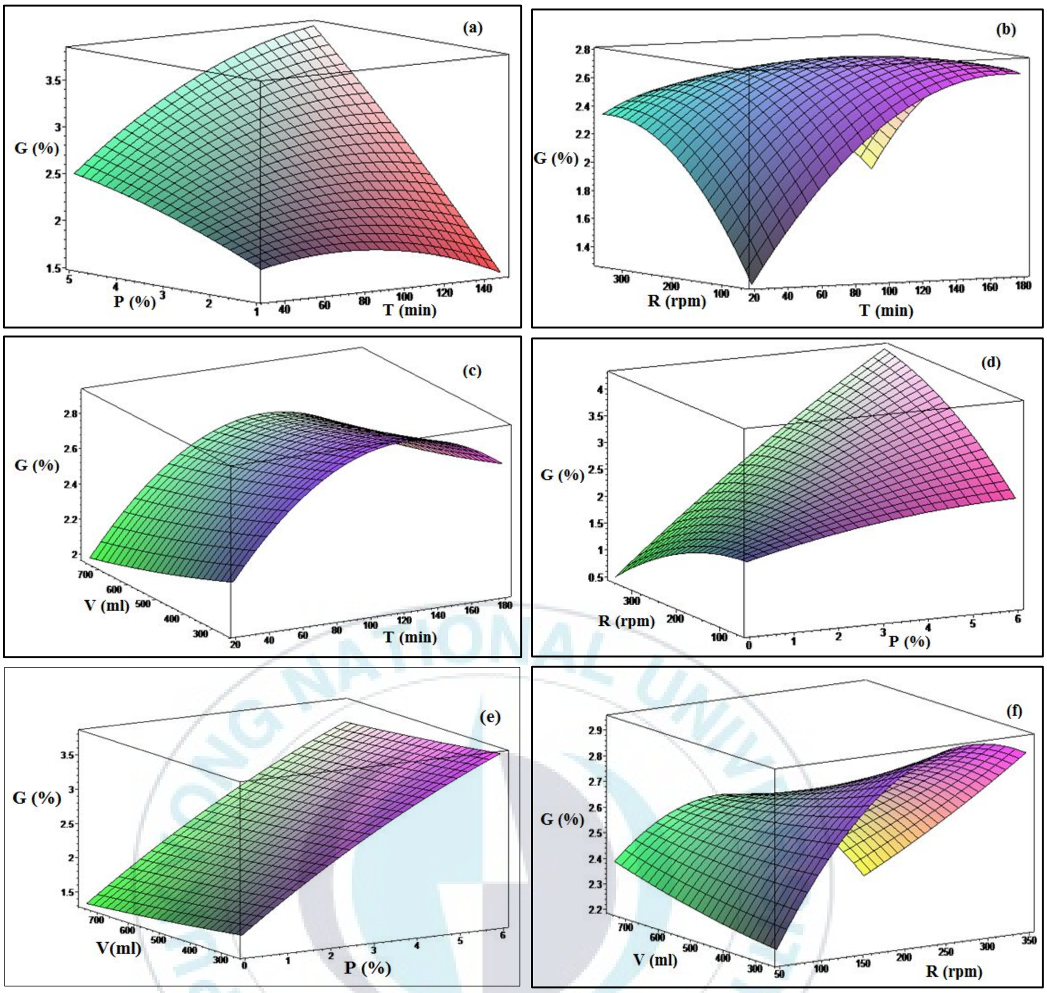

Fig. 1. Three-dimensional plot of effect of two different factors in weight gain percentage drawing by Maple 8. G (%) is weight gain, T (min) is the dipping time, P (%) is phosphate concentration, R (rpm) is rotation per minute, V (ml) is volume of phosphate solution for 100g of shrimp.

The predicted formula of weight gain (G%) = $0.930972 + 0.012972 \times T - 0.175417 \times P + 0.007103 \times R + 0.000257 \times V - 0.000062346 \times T \times T + 0.003042 \times P \times T - 0.016111 \times P \times P - 0.000039167 \times R \times T + 0.001700 \times R \times P + 0.000012944 \times R \times R + 0.000000417 \times V \times T + 0.000300 \times V \times P - 0.000005500 \times V \times R + 0.000000264 \times V \times V$ which was obtained from Table 5. In each subfigures, two factors were fixed in their central value and the effect of the others was presented (fixed values are T=90 min, P= 3 %, R=200 rpm and V=500 ml) as shown in the subfigures of Fig. 1 - (a), (b), (c), (d), (e) and (f).

Fig. 1a appeared a positive relationship between dipping time and phosphate concentration. The highest value of gain weight was appeared in the highest value of both of them. A little stronger effect of phosphate concentration was shown than the dipping time, because the value of weight gain was higher on the side of phosphate concentration than the dipping time, when the effect of the highest value of one of them with the lowest value of another was drawn. The same result is shown in Fig. 1(d) and (e) where the phosphate concentration makes a good relationship with the rotation speed and the volume of brine solution, respectively. The highest value of weight gain appeared in the highest value of both factors with the

Table 5. Coefficient regression model estimated by multiple regression analysis for gain weight

Parameter	DF	Estimate	Standard Error	t value	Pr > |t|	Parameter estimate from coded data
Intercept	1	0.930972	3.005342	0.31	0.7603	3.074444
T	1	0.012972	0.019568	0.66	0.5158	0.195000
C	1	-0.175417	0.587046	-0.30	0.7685	0.983333
R	1	0.007103	0.012379	0.57	0.5732	0.075000
V	1	0.000257	0.006577	0.04	0.9693	0.071667
T*T	1	-0.000062346	0.000056773	-1.10	0.2866	-0.224444
C*T	1	0.003042	0.002344	1.30	0.2109	0.365000
C*C	1	-0.016111	0.051096	-0.32	0.7562	-0.064444
R*T	1	-0.000039167	0.000046889	-0.84	0.4145	-0.235000
R*C	1	0.001700	0.001407	1.21	0.2425	0.340000
R*R	1	-0.000012944	0.000020438	-0.63	0.5345	-0.129444
V*T	1	0.000000417	0.000023444	0.02	0.9860	0.005000
V*C	1	0.000300	0.000703	0.43	0.6748	0.120000
V*R	1	-0.000005500	0.000014067	-0.39	0.7004	-0.110000
V*V	1	0.000000264	0.000005110	0.05	0.9594	0.010556

(T) total time of plunge; (C) concentration of phosphor,(R) rotation in one minute; (V) volume of brine solution

strongest one in the side of phosphate concentration by the same reason as the first one. In Fig. 1b, the same results can be shown with the same degree of effect between the dipping time and the rotation speed. Fig. 1c indicates special effect of the dipping time because the highest value of weight gain in the middle (110 <T<120) is shown and after the value is declined. But Fig. 1f shows a complicate relationship between the rotation speed and the volume of the brine solution, where the rotation speed gives a good response with small volume and inverses with big volume. It means that the small volume needs the rotation for good penetration of phosphate in the shrimp meat and the big volume doesn’t need.

Moisture content in phosphate-treated shrimp

The mean of moisture percentage is 77.33 %, with R-Square in the value of 0.9192 (Table 7). The lowest value is 76.22±0.05 % under 1% phosphate concentration, 200 rpm rotation speed, 1/5 ratio and 90 min. The highest value is 78.24±0.08% under 5% phosphate concentration, 200 rpm rotation speed, 1/5 ratio and 90 min (Table 6).

Table 6. Results of moisture content in phosphate-treated shrimp

Run no.	Variation levels				Response function
	X_1	X_2	X_3	X_4	Moisture content (%)
1	120	4	150	1/6	77.78±0.04
2	120	2	150	1/4	76.45±0.12
3	60	4	150	1/4	77.89±0.13
4	120	4	250	1/4	77.79±0.09
5	60	2	250	1/4	76.34±0.04
6	120	2	250	1/6	76.66±0.08
7	60	4	250	1/6	78.02±0.06
8	60	2	150	1/6	76.57±0.06
9	90	3	200	1/5	77.33±0.05
10	90	3	200	1/5	77.23±0.08
11	90	3	200	1/5	77.45±0.10
12	120	4	150	1/4	77.92±0.10
13	120	2	250	1/4	76.56±0.02
14	60	4	150	1/6	77.62±0.03
15	60	2	150	1/4	76.34±0.01
16	60	2	250	1/6	76.54±0.10
17	120	2	150	1/6	76.66±0.14
18	60	4	250	1/4	77.57±0.10
19	120	4	250	1/6	78.19±0.09
20	90	3	200	1/5	77.36±0.07
21	90	3	200	1/5	77.48±0.06
22	90	3	200	1/5	77.61±0.07
23	30	3	200	1/5	77.43±0.06
24	150	3	200	1/5	77.59±0.06
25	90	1	200	1/5	76.22±0.05
26	90	5	200	1/5	78.24±0.08
27	90	3	100	1/5	77.57±0.06
28	90	3	300	1/5	77.62±0.06
29	90	3	200	1/3	77.34±0.05
30	90	3	200	1/7	77.64±0.09
31	90	3	200	1/5	77.68±0.06
32	90	3	200	1/5	77.59±0.06
33	90	3	200	1/5	77.58±0.07

Table 7. Result of statistical analyses for moisture content

Response surface for variable moisture content	
Response mean	77.33
Root MSE	0.2182
R-square	0.9192
Coefficient of variation	0.2822

The same observation was shown as the weight gain percentage. The highest value of the moisture was given by the highest value of one of four factors. Table 6 shows that weight gain increases when all of the four factors increase.

From Table 8, the formula was made as $M\%=73.909028+0.006944\times T+1.277083\times P-0.006186\times R+0.002885\times V-0.000042284\times T\times T+0.000083333\times P\times T-0.108056\times P\times P+0.000014167\times R\times T+0.000350\times R\times P-0.000006722\times R\times R-0.000000833\times V\times T-0.000187\times V\times P+0.000014000\times V\times R-0.000004306\times V\times V$. By using this formula, the three dimensional figure shows the effect of two different factors in moisture content using Maple 8. In each sub-figure, two factors were fixed in their central value and the effect of the others two (fixed values are T=90 min, P= 3 %, R=200 rpm and V=500 ml) was drawn as the sub-figures shows (a), (b), (c), (d), (e) and (f).

The phosphate concentration in the brine solution makes a positive relationship between the time of plunge, the rotation speed and the volume of brine solution as shown in Fig. 2a, 2d and 2e, respectively. The moisture content increases when the values of our factors increase. The highest value of moisture was appeared in the highest value of one of them. With the

Table 8. Coefficient regression model estimated by multiple regression analysis for moisture

Parameter	DF	Estimate	Standard error	t value	Pr > |t|	Parameter estimate from coded data
Intercept	1	-1.801736	2.364674	-0.76	0.4560	3.491111
T	1	0.012889	0.015397	0.84	0.4135	0.065000
C	1	1.322917	0.461902	2.86	0.0103	1.365000
R	1	0.005211	0.009740	0.54	0.5992	0.046667
V	1	0.003878	0.005175	0.75	0.4633	0.095000
T*T	1	-0.000042670	0.000044670	-0.96	0.3521	-0.153611
C*T	1	-0.000458	0.001845	-0.25	0.8066	-0.055000
C*C	1	-0.137153	0.040203	-3.41	0.0031	-0.548611
R*T	1	0.000009167	0.000036893	0.25	0.8066	0.055000
R*C	1	0.000650	0.001107	0.59	0.5643	0.130000
R*R	1	-0.000015361	0.000016081	-0.96	0.3521	-0.153611
V*T	1	-0.000009167	0.000018447	-0.50	0.6253	-0.110000
V*C	1	0.000188	0.000553	0.34	0.7387	0.075000
V*R	1	-0.000002750	0.000011068	-0.25	0.8066	-0.055000
V*V	1	-0.000002590	0.000004020	-0.64	0.5275	-0.103611

(T) total time of plunge; (C) concentration of phosphor,(R) rotation in one minute; (V) volume of brine solution

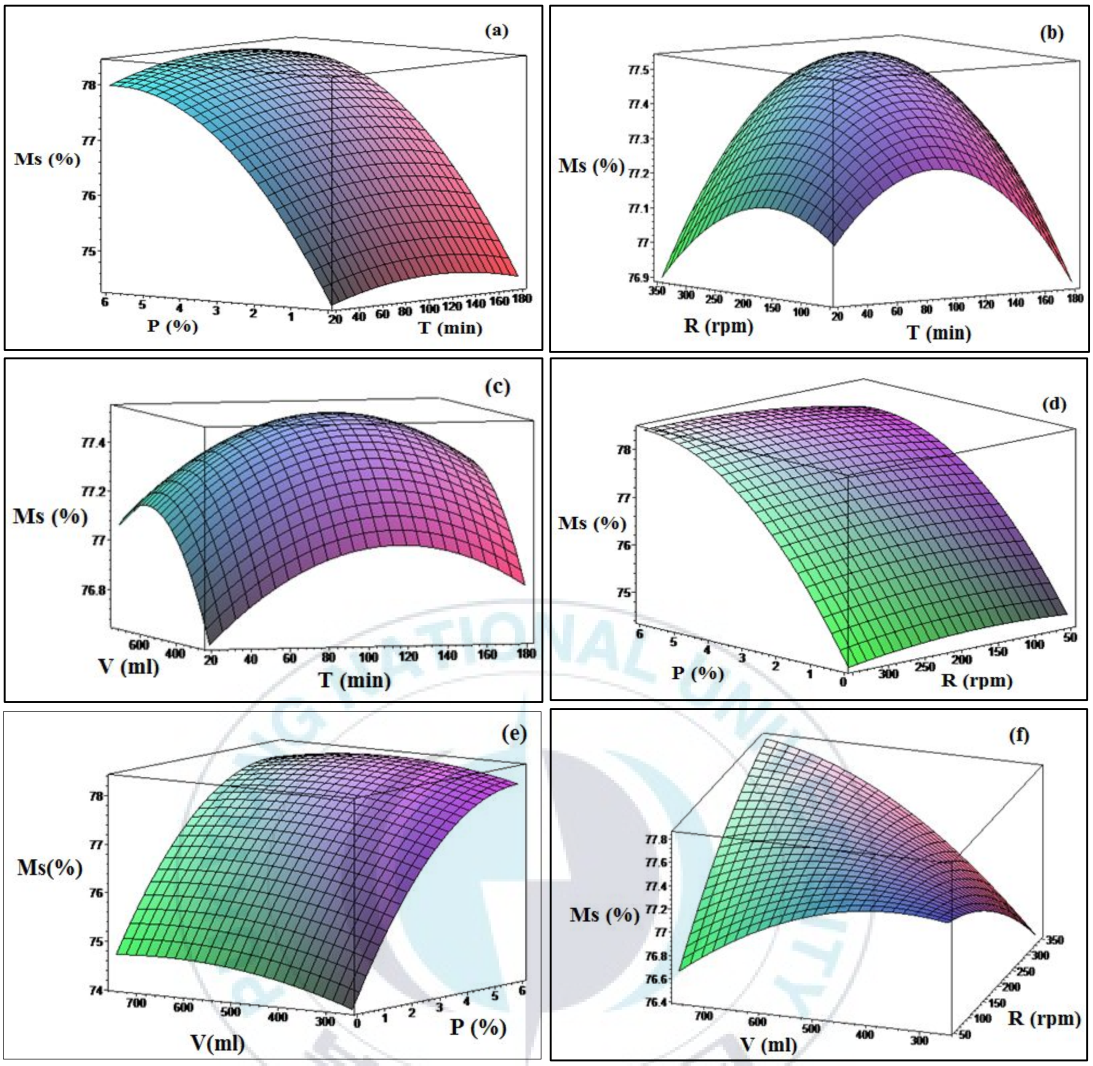

Fig. 2. Three-dimensional plot of effect of two different factors in moisture content drawing by Maple 8. M(%) is moisture content, T(min) is dipping time, P(%) is phosphate concentration, R(rpm) is rotation per minute, and V(ml) is volume of phosphate solution for 100g of shrimp.

strongest effect of phosphate concentration than time, the rotation speed and the volume of brine solution, because the higher value of moisture is in the side of phosphate concentration than the others, when taking the highest value of one of them with the lowest value of other. But in the Fig. 2b, 2c and 2f the highest values of moisture content was in the middle range, 110 to 120 min for the dipping time, 500 to 550 ml for the volume and 190 to 210 rpm for the rotation speed.

Phosphate amount in phosphate-treated shrimp

The data from Table 9 was statistically analyzed and the results are presented in Table 10 and 11, which show the mean value 3.31 (g/kg) of phosphate amount, with R-square in the value of 0.9313. The lowest value is 2.11±0.10 (g/kg) under 1 % phosphate concentration, 200 rpm rotation speed, 1/5 ratio and 90 min. The highest value is 4.16±0.06 (g/kg) under 5 % phosphate concentration, 200 rpm rotation speed, 1/5 ratio and 90 min (Table 9).

Table 9. Results of phosphor amount in phosphate-treated shrimp

Run no.	Variation level				Response function
	X_1	X_2	X_3	X_4	Phosphate amount (g/kg)
1	120	4	150	1/6	3.87±0.07
2	120	2	150	1/4	2.38 ±0.10
3	60	4	150	1/4	3.78±0.12
4	120	4	250	1/4	3.9±0.13
5	60	2	250	1/4	2.32±0.19
6	120	2	250	1/6	2.39±0.05
7	60	4	250	1/6	4.05±0.06
8	60	2	150	1/6	2.51±0.09
9	90	3	200	1/5	3.34±0.17
10	90	3	200	1/5	3.49±0.11
11	90	3	200	1/5	3.4 ±0.10
12	120	4	150	1/4	3.85±0.15
13	120	2	250	1/4	2.47±0.16
14	60	4	150	1/6	3.96±0.09
15	60	2	150	1/4	2.23±0.09
16	60	2	250	1/6	2.34±0.07
17	120	2	150	1/6	2.46±0.05
18	60	4	250	1/4	3.86±0.04
19	120	4	250	1/6	4.11±0.01
20	90	3	200	1/5	3.55±0.04
21	90	3	200	1/5	3.49±0.03
22	90	3	200	1/5	3.61±0.06
23	30	3	200	1/5	3.43±0.05
24	150	3	200	1/5	3.63±0.12
25	90	1	200	1/5	2.11±0.10
26	90	5	200	1/5	4.16±0.06
27	90	3	100	1/5	3.49±0.06
28	90	3	300	1/5	3.57±0.15
29	90	3	200	1/3	3.52±0.07
30	90	3	200	1/7	3.64±0.08
31	90	3	200	1/5	3.48±0.09
32	90	3	200	1/5	3.54±0.04
33	90	3	200	1/5	3.52±0.06

Table 10. Result of statistical analyses for phosphate amount

Response surface result for dependent variable of phosphate amount	
Response mean	3.32
Root MSE	0.221359
R-square	0.9313
Coefficient of variation	6.6741

Table 11. Coefficient regression model estimated by multiple regression analysis for phosphate amount.

Parameter	DF	Estimate	Standard Error	t Value	Pr > |t|	Parameter Estimate from Coded Data
Intercept	1	73.909028	2.331441	31.70	<.0001	77.478889
T	1	0.006944	0.015180	0.46	0.6528	0.120000
C	1	1.277083	0.455411	2.80	0.0117	1.225000
R	1	-0.006186	0.009603	-0.64	0.5276	0.045000
V	1	0.002885	0.005102	0.57	0.5788	0.148333
T*T	1	-0.000042284	0.000044043	-0.96	0.3497	-0.152222
C*T	1	0.000083333	0.001819	0.05	0.9640	0.010000
C*C	1	-0.108056	0.039638	-2.73	0.0139	-0.432222
R*T	1	0.000014167	0.000036375	0.39	0.7015	0.085000
R*C	1	0.000350	0.001091	0.32	0.7521	0.070000
R*R	1	-0.000006722	0.000015855	-0.42	0.6766	-0.067222
V*T	1	-0.000000833	0.000018187	-0.05	0.9640	-0.010000
V*C	1	-0.000187	0.000546	-0.34	0.7351	-0.075000
V*R	1	0.000014000	0.000010912	1.28	0.2158	0.280000
V*V	1	-0.000004306	0.000003964	-1.09	0.2917	-0.172222

(T) total time of plunge; (C) concentration of phosphor,(R) rotation in one minute; (V) volume of brine solution

By using the data of Table 11, this formula was obtained $P\ (g/kg) = -1.801736 + 0.012889 \times T + 1.322917 \times P + 0.005211 \times R + 0.003878 \times V - 0.000042670 \times T \times T - 0.000458 \times P \times T - 0.137153 \times P \times P + 0.000009167 \times R \times T + 0.000650 \times R \times P - 0.000015361 \times R \times R - 0.000009167 \times V \times T + 0.000188 \times V \times P - 0.000002750 \times V \times R - 0.000002590 \times V \times V$). As shown in Fig. 3, three dimensional figures show the effect of two different factors in phosphate amount. In each subfigure, two factors were fixed in their central values and the effect of the other two variables (the fixed values are T=90 min, P= 3 %, R=200 rpm and V=500 ml) are shown in the subfigures of Fig. 3 (a), (b), (c), (d), (e) and (f).

Also, the phosphate concentrations in the brine solution make a positive relationship between dipping time, rotation speed and volume of brine solution as shown in Fig. 3a, 3d and 3e, respectively. The phosphate amount increases when the values of factors increase. The highest value of phosphate amount was appeared in the highest value of both of them. The effect of phosphate concentration was stronger than other factors with the same reason as before. In Fig. 3b, 3c and 3f, the highest values of phosphate amount was appeared in the middle value with the same value as the

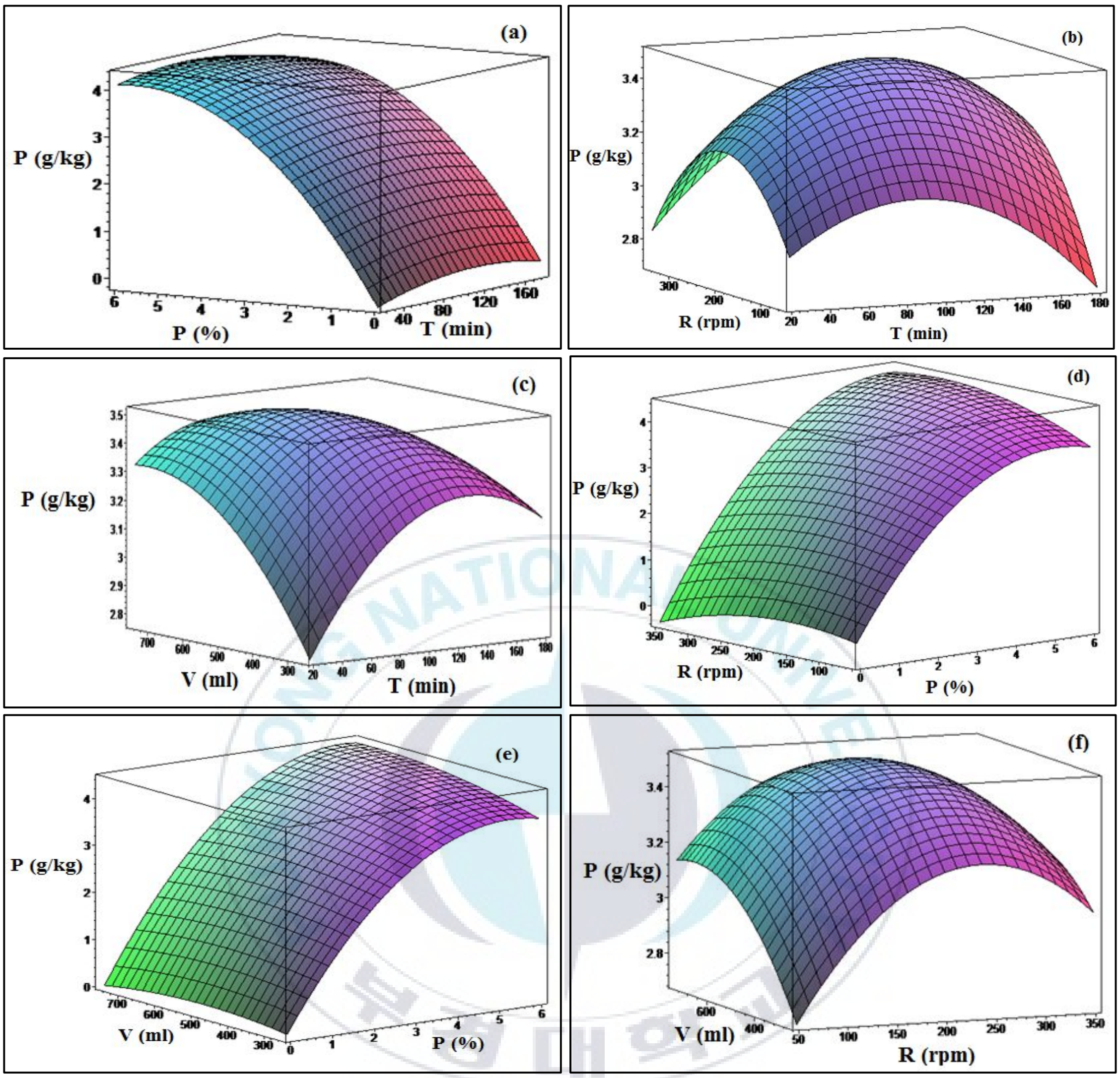

Fig. 3. Three-dimensional plot of effect of two different factors in phosphate amount drawing by Maple 8. P(g/kg) is phosphate amount, T(min) dipping time, P(%) is phosphate concentration, R(rpm) is rotation per minute, and V(ml) is volume of phosphor solution for 100g of shrimp.

moisture content before. The optimum condition was 110 to 120 min dipping time, 500 to 550 ml volume of brine solution and 190 to 210 rpm agitation speed.

Discussion

Weight gain in phosphate-treated shrimp using response surface analysis

In this present study, phosphate treatment increases shrimp weight with the value of 3 % at mean in different factors. Vovcsko, J. (1997) found 5 % weight gain. Many others reports showed that phosphate treatment increase the weight of product, because the contact between the meat and the phosphate solution increases the uptake of phosphhate (Young and Lyon, 1997; Young and Smith, 2004; Xiong and Kupski, 1999).

A positive relationship between the dipping time and the phosphate concentration was appeared in Fig.1a. The highest value of weight gain was appeared in the highest value of both of them. This result was similar to the work reported by Xiong and Kupski (1999). They found that both concentration and dipping time affected weight gain of white shrimp and both factors had interaction effect.

The same results are shown in Fig. 1d and e. The phosphate concentration makes a good relationship with the rotation speed and the volume of brine solution, respectively. The highest value of weight gain was appeared in the highest value of both of them with the strongest in the side of phosphate concentration. Tenhet, V. et al (1981) found that penetration of phosphate solution into shrimp muscle depends on the concentration of phosphate.

The same results were found in Fig. 1b. But for Fig. 1c, a special effect of the dipping time was found because the highest value of weight gain in the middle (110<T<120) was shown and after the value was declined. It means that a good result does not need a long time. Only around two hours can get a maximum of the result.

Fig.1f showed a complicate 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 big volume. It means that the small volume needs the rotation for good penetration of phosphate in the shrimp meat and the big volume does not need or a small speed is needed.

In conclusion, it can say that the first factor responsible for getting a good result of weight gain is phosphate concentration. The second one is dipping time but not a long time (about 2 hours, with 3% P). The third position is both of the rotation speed and the volume of the brine solution.

Moisture content in phosphate-treated shrimp using response surface analysis

The mean of moisture content of phosphate-treated shrimp is 77.33 % presented in Table 7. The value is within the normal limits for the species and is in agreement with that found in the shellfish literature for white shrimp by Sriket et al., (2007), who reported 77.21 %. Sundararajan (2010) found that white shrimp exhibited 77.36%. Moawad et al (2013) found 77.32%. According to the result of Laura (2002), University of Florida has developed tentative standards for shell-on and peeled products that differentiate between phosphate-treated and untreated products. Moisture content of higher than 78.5% of meat could be interpreted as phosphate-treated product. The value is commonly seen in commercial shrimp, but it may not be applicable to all species of shrimp.

The moisture content was increased when the values of all the other factors were increased. The highest value of moisture was appeared in the highest value of one of them. The effect of phosphate concentration was stronger than that of dipping time, rotation speed and volume of brine solution as shown in Fig.2.a, d and e, respectively. With the same reason, Tenhet, et al (1981) found that penetration of phosphate solution into shrimp muscle depends on the concentration of phosphate in the brine solution.

In Fig 2b, c and f, the highest values of moisture was found in the middle range such as 110 to 120 min for the time, 500 to 550 ml for the volume and 190 to 210 rpm for the rotation speed. To maximise the moisture content, all the factors were not needed to make other factors maximize.

The addition of phosphates has been shown to improve water-holding capacity of the product (Rippen et al. 1993). 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 the protein and water. Increased water holding capacity is hypothesized to be due, in part, to increased space between muscle fibres, creating more water-holding capacity (Campden, 2012).

To conclude, it can assure that phosphate concentration is the most important factor than others factors coming for getting the best result of moisture by facilitating the phosphor penetration in the meat of the shrimp.

Phosphate amount in phosphate-treated shrimp using response surface analysis

Little information is available in the literature about the naturally occurring levels of phosphates in crustaceans and molluscs. It is because levels change rapidly depending on temperature, pH, storage conditions and/or enzyme activity. There are also differences between levels in different species, between individuals of the same species (Gibson and Murray, 1973) and between the same species but in different geographical locations. Differences can additionally occur depending on how the animals have been caught and handled (Campden, 2012).

In the result part before, the mean of phosphate amount is 3.31 (g/kg). The lowest value is 2.11±0.10 (g/kg) under 1 % phosphate concentration, 200 rpm rotation speed, 1/5 ratio and 90 min. The highest value is 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 brine solution from 1 % to 5 % was 2.05 (g/kg) phosphate amount in shrimp meat. Crawford (1980) found that a 6% polyphosphate solution increased the phosphate content of the shrimp by as much as 1.10 g/kg over the control samples. Therefore, the quantity of phosphate added to the shrimp is within the range of phosphate levels naturally occurring in these animals. Laura (2002) concluded that raw shrimp could be interpreted as phosphate-treated product, if the total phosphorus amount is higher than 2,6 g/kg of meat.

Also, the phosphate concentration in the brine solution makes a positive relationship between the dipping time, the agitation speed and the volume of brine solution. As shown in Fig. 3 a, d and e, respectively, the phosphate amounts increased when the values of other factors were increased. The highest value of phosphate amount was appeared in the highest value of all the other factors. Tenhet et al (1981) 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. Phosphate content of the solutions regularly increased during dipping due to the diffusion of orthophosphates from within the samples (Ünal, et al 2004).

In Fig 3 b, c and f, the highest value of phosphate amount was appeared in the middle range of 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 and 190 to 210 rpm agitation speed. Therefore, their highest values for phosphate amount were not in the optimum values of factors.

It can be concluded that the phosphate concentration in the brine solution is the first factor for getting the highest value of phosphate amount, and the phosphate amount increases when the concentration of phosphate in the brine solution increases.

Conclusion

In conclusion, it can say that the first factor responsible for getting a good result of weight gain is the phosphate concentration, and the second one is dipping time (about 2 hours, with 3% P). The third position is both of the rotation speed and the volume of the brine solution. For moisture content, it can be sure that phosphate concentration is the most important factor than other factors for getting the best result by facilitating the phosphate penetration in the meat of the shrimp.

In case of phosphate amount, it can be concluded that the phosphate concentration in the brine solution is the first factor for getting the highest value of phosphate amount. The phosphate amount 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 and 190 to 210 rpm agitation speed.

References

Applewhite, L.A., W.S. Otwell and L. Garrido. 1993. Consumer evaluations of phosphate shrimp and scallops.Proceedings of the 18th Annual Tropical and Subtropical Fisheries Technological Conference of the Americas. Virginia USA 101-106.

Campden BRI Report. 2012. SR654: Review of polyphosphates as additives and testing methods for them in scallops and prawns. ISBN no. 978-1-906634-60-5.

CFIA. 2004. Canadian Food Inspection Agency 2004. List of Permitted Additives in Fish and Fish Products. Animal Products Directorate, Fish, Seafood and Production. Accessible at http://active.inspection.gc.ca/eng/anima/fispoi/product/additi/fispoiadd_dbe.asp (Accessed 04/18/2008).

Crawford, D.L. 1980. Meat yield and shell removal functions of shrimp processing. Oregon State University Extension Marine Advisory Program. Land Grant/Sea Grant Cooperative Special Report 597.

Diaz-Tenorio, M., F. Garcia-Carreno, R. Pacheco-Aguilar. 2007. Comparison of freezing and thawing treatments on muscle properties of whiteleg shrimp (*Litopenaeusvannamei*). Journal of Food Biochemistry 31: 563-576.

ECD. 1995. The European Community Directive 95/2/EC "Food Additives Other Than Colors and Sweeteners" (which continues to apply, until 1 June 2013, under the transitional provisions of Regulation (EC) No 1333/2008) states in Annex IV that a maximum level of 5g/kg of additives E338-E452 which includes phosphates).

Fang, X., H. Sun, B. Huang and G. Yuan. 2013. Effect of pomegranate peel extract on the melanosis of Pacific white shrimp (*Litopenaeus vannamei*) during iced storage. Journal of Food Agriculture and Environment 11(1): 105-109.

Gibson, D.M. and Murray, C.K. 1973. Polyphosphates and fish: some chemical studies. International Journal of Food Science and Technology 8 (2): 197-204.

Gonçalves, A.A. and J.L.D. Ribiero. 2008. Do phosphates improve the seafood quality? Reality and Legislation. Pan-American Journal of Aquatic Sciences 3(3): 237-247.

.Hui, Y.H., E.C. Perez, L.M. Cunha, G. Legaretta, H. Liang, Y.M. Lo, D.L. Marshall, W.K. Nip, F. Shahidi, K. Sherkat, R.J. Winger and K.L. Yam. 2006. Handbook of Food Science, Technology and Engineering, Vol. 4, CRC Press.

Henson, L.S. and Kowalewski, K.M., 1992. Use of phosphates in seafood. Info Fish International 5: 52-54.

Jastrzębska, A. 2009. Modifications of spectrophotometric methods for total phosphorus determination in meat samples. Chemical Papers, 63 (1): 47-54.

Jastrzębska, A. and Szlyk, E. 2009. Application of 31P NMR for added polyphosphate determination in pork meat. Chemical Papers. 63 (4): 414-419.

Jastrzębska, A. 2011. Capillary isotachophoresis as rapid method for determination of orthophosphates, pyrophosphates, tripolyphosphates and nitrites in food samples. Journal of Food Composition and Analysis 24: 1049-1056.

Jastrzębska, A., Hol, A. and Szlyk, E. 2008. Simultaneous and rapid determination of added phosphorus (V) compounds in meat samples by capillary isotachophoresis. LWT-Food Science and Technology 41: 2097-2103.

Jastrzębska, A., Hrynczyszyn, P. and Szłyk, E. (2009). Comparison of cITP and 31P NMR methods for determination of polyphosphates in meat product and sea fruits. Czech Journal of Food Science 27: S340.

Kilinc, B. and S. Cakli. 2004. Chemical, microbiological and sensory changes in thawed-frozen fillets of sardine (Sardinapilchardus) during marination. Food Chemistry 88: 275-280.

Lampila, L.E. 1992. Functions and uses of phosphates in the seafood industry. Journal of Aquatic Food Product Technology 1(3): 29-41.

Laura R, Garrido M.S., 2002, phosphate and shrimp processing compounds improve taste other products. Global aquaculture advocate aug02: 78-80.

Love, R.M. and G. Abel. 1966. The effect of phosphate solutions on the denaturation of frozen cod muscle. Journal of Food Technology 1: 323-327.

Moawad, et al. 2013. Effect of food grade trisodium phosphate and water dip treatments on some quality attributes of decapitated white marine shrimp (*Penaeus spp*.) during frozen storage. Journal of Applied Sciences Research 9(6): 3723-3734.

Paul, S., M.S. Reza, A.S.M.S. Mandal, I.M. Ahmed, M.N.A. Khan, M.N. Islam and M. Kamal. 2012. Effect of sodium tripolyphosphate (STPP) and foreign materials on the quantity of giant freshwater prawn *(Macrobrachium rosenbergii)* under ice storage condition. Journal Food and Nutrition Sciences, 3: 34-39.

Rajkowski, K.T. and C. Sommers. 2012. Effect of trisodium phosphate or water dip on the survival of Salmonella and Listeria monocytogenesinoculated catfish before and after freezing. Journal of Aquatic Food Product Technology 21: 39-47.

Rippen, T., Sutton, H., Lacey, P., Lane, R., Fisher, R. and Dupaul, W. 1993. Functional, microbiological and sensory changes in sea scallops (*Placopecten megallanicus*) treated with sodium tripolyphosphate during iced storage. Proceedings of the 18th Annual Tropical and Subtropical Fisheries Technological Conference of the Americas. Virginia USA 51-71.

Sallam, K.I. 2007. Antimicrobial and antioxidant effects of sodium acetate, sodium lactate and sodium citrate in refrigerated sliced salmon. Food Control 18: 566-575.

Sriket, P., S. Benjakul, W. Visessanguan, K. Kijroongrojana. 2007. Comparative studies on chemical composition and thermal properties of black tiger shrimp (*Penaeusmonodon*) and white shrimp (*Penaeusvannamei*) meats. Food Chemistry 103: 1199-1207.

Sundararajan, S., 2010. Evaluation of green tea extract as a glazing material for shrimp frozen by cryogenic and air-blast freezing. M. Sc.Thesis, Faculty of the Louisiana State University and Agricultural and Mechanical College.

Tenhet, V., Finne, G., Nickelson., R. and Toloday, D. 1981. Penetration of sodium tripolyphosphate into fresh and prefrozen peeled and deveined shrimp. Journal of Food Science 46: 344-349.

Tsironi, T., E. Dermesonlouoglou, M. Giannakourou and P. Taoukis. 2009. Shelf life modelling of frozen shrimp at variable temperature conditions. Journal of Food Sciences Technology 42: 664-671.

Turan, H., Y. Kaya and I. Erkoyuncu. 2003. Effects of glazing, packaging and phosphate treatments on drip loss in rainbow trout during frozen storage. Turkish Journal of Fisheries and Aquatic Sciences 3: 105-109.

Vovcsko, J. 1997. When does the use of tripolyphosphates equal economic fraud Seafood Supplier. Available at: http://www.southeasternfish.org/ Members /Documents/stpreg.htm Accessed: January 2012.

Ünal, S.B., Erdo, F., Ekiz, H.B, Özdemir, Y. 2004. Experimental theory, fundamentals and mathematical evaluation of phosphate diffusion in meat. Journal of Food Engineering 65 (2): 263-272.

USDA. 2004. U.S. Code of Federal Regulations 2004. Title 21 -Food and Drugs. Chapter I - Food and Drug Administration, Department of Health and Human Services. Part 182 -Substances Generally Recognized as Safe. Title 21, Volume 3, Revised as of April 1, 2004, 21CFR182, Page 456-469. Accessible at http://www.cfsan.fda.gov/~lrd /FCF182.html (Accessed 04/18/2008).

Xiong YL., Kupski DR. 1999. Monitoring phosphate marinade penetration in tumbled chicken filets using a thin-slicing, dye-tracing method. Poultry Science 78: 1048-1052.

Young, L.L. and C.E. Lyon. 1997. Effect of postchill aging and sodium tripolyphosphate on moisture binding properties, color and Warner-Blatzler shear values of chicken breast meat. Poultry Sci. 76: 1587-1090.

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