# Using microwave-assisted phosphorylation to improve foaming and solubility of egg white by response surface methodology

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**ABSTRACT** The purpose of this study was to establish optimal conditions for microwave-assisted phosphorylation modification of egg white. Response surface methodology was used to model and optimize the degree of phosphorylation, solubility, foaming ability, and foaming stability of egg white powder. The concentration of sodium tripolyphosphate, microwave power, and microwave time were selected as the main processing conditions in the phosphorylation modification of egg white protein. The following 3 conditions for optimal phosphorylation modification of egg white are the concentration of sodium tripolyphosphate of 33.84 g/L, microwave power of 419.38 W, and microwave time 90 s for maximum functional properties (solubility, foaming ability and foaming stability) and the concentration of sodium tripolyphosphate of 32.97 g/L, microwave power of 429.29 W and microwave time of 90 s for maximum foaming properties (foaming ability and foaming stability), respectively. We consequently succeeded in phosphorylation modification with microwave assistance and confirmed the various desirable properties of optimal phosphorylation modification.

**Key words:** albumen, response surface, solubility, foam

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## INTRODUCTION

Egg white is a key ingredient in food industry due to its high nutritional quality (Lechevalier et al., 2017) and excellent functional properties (Xiong et al., 2016; Stefanovi et al., 2018). Owing to its high foaming properties, egg white is widely used in baking goods, such as meringues, cakes, cookies, and chocolate mousses (Li et al., 2018). Egg white powder has become a popular production as commercial, which is substitute for fresh and liquid eggs (Wang et al., 2018; Lechevalier et al., 2017), because the powder could prevent microbiological and oxidative degradation, have lower packaging expenditures, and be more convenient to storage and transport (Sponton et al., 2018).

Egg white powder is usually dehydrated by freeze drying or spray drying. Spray drying is the most economic technique for maintaining quality by rapid dehydration, and spray dried powder has been a more common form. The material was dispersed into fine liquid droplets at first, which can increase the evaporation area of water and accelerate the drying process. And then fine liquid droplets contacted with hot air and most moisture was removed instantly. Finally, the solid in the material was dried into the powder. Compared with the freeze-drying technique, the spray drying process consumed less electricity and shortened drying time at an industrial scale (Hammami and René, 1997).

However, it was reported that many high-protein powder ingredients had poor rehydration properties after storage, including solubility (Carter et al., 2018). The rehydration process of protein powders, which consisted 3 sequential stages (wetting, dispersing and solubilization), was as follows: wetting was the first step where the powders were detached into primary particles; dispersing was the stage where materials were released from particles into the aqueous phase; solubilization was the critical phases where primary particles started to release materials from the particle surface into the liquid (Mimouni et al., 2009; Ji et al., 2016).

Spray-dried egg white powder is a high-protein material and thus has poor rehydration properties. The unique property of solubility could decide other functional properties, such as emulsibility, thermo stability, and gelling property (Sheng et al., 2017; Gouda et al., 2018), and it indicates how well they interact with other components remaining in solution of food (Nayak et al., 2006). Thus, the high-solubility egg white powder would have broader application in food industry.

It was proven in previous reports that proteins modified by phosphorylation had better solubility, foamability, calcium absorption, emulsibility, and gelling property than control (Li et al., 2003, 2004).

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Nayak et al. (2006) studied phosphorylated buffalo milk casein had higher solubility at pH 7.0 and pH 9.0 and co-precipitate near their isoelectric points. Zhang et al. (2006) succeeded in soy protein isolate with sodium tripoly phosphate using a chemical phosphorylation method and found the solubility of modified proteins was greater than that of native protein at pH 3.0 and pH 4.0. In our previous study (Li et al., 2018), egg white protein was phosphorylated with microwave assistance at 500 W for 2 min and its foaming ability and foaming stability increased by 44.2 and 107.2%, respectively. Compared with the conventional dry-heating method, the microwave technique can significantly shorten reaction times and accelerate phosphorylation process (Li et al., 2018).

Response surface methodology (**RSM**) is a statistical procedure frequently used to evaluate more experimental conditions for the optimization of various parameters and their interaction with respect to response variables (Zheng et al., 2014). Response surface methodology has successfully been employed in optimizing ultrasonic-assisted extraction conditions of pueraria isoflavonoid, hydrolysis conditions for bovine plasma protein, and dry heat treatment of egg white related to its functional properties (Talansier et al., 2009; Seo et al., 2015; Wong et al., 2017).

Therefore, the present study was undertaken to establish the optimal conditions for phosphorylation with microwave assistance of egg white protein (the concentration of sodium tripolyphosphate, microwave power, and microwave time), and these conditions were established on the basis of obtaining high solubility (%), foaming ability (%), and foaming stability (%). The results of this study will provide theoretical and practical references in food proteins of drying process and enhanced functional properties.

## MATERIALS AND METHODS

### Materials

Fresh eggs were bought from a local farm. Potassium dihydrogen phosphate, ammonium molybdate, hydroquinone, sodium sulfate, and citric acid monohydrate were provided from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Sodium tripolyphosphate was purchased from Shanghai Aladdin Industrial Co. Ltd. (Shanghai, China).

### Preparation of the Egg White Protein Solution

The egg white protein solution was prepared as previously reported (Li et al., 2018). The 400 mL egg white separated from eggs was stirred for 12 h at 4°C and then added with 800 mL of deionized water. Finally, the mixed solution was stirred for 6 h at 4°C, and its insoluble protein was removed for the preparation of powder.

### Preparation of the Phosphorylated Egg White Protein Powder

Sodium tripolyphosphate was added to the egg white solution to prepare a mixed solution, which was adjusted with citric acid monohydrate (pH 2.0) to pH 8.0. The solutions were placed into a microwave reactor (PLC microwave reactor, Guangzhou Kewei Microwave Energy Co., Ltd.) at certain conditions and then transferred to ice water to avoid further reaction. Finally, the solutions were dialyzed to remove free pyrophosphate for one day against deionized water and dried to the protein powders, in which the outlet temperature and the speed of fluid flow of a spray dryer (SP-1500, Shanghai Sunyi tech. Co., Ltd) were 170°C and 1,200 mL/h. The microwave-assisted phosphorylation modification was performed by varying the concentration of sodium tripolyphosphate (20, 30, and 40 g/L), microwave powder (300, 400, and 500 W), and microwave time (90, 120, and 150 s).

### Response Surface Methodology

The Design Expert Software (Trial Version 8.0.6, Stat-Ease Inc) was used to optimize the microwave-assisted phosphorylation modification of egg white protein. Box-Behnken was used for evaluating the effect of concentration of sodium tripolyphosphate ($X_1$), microwave power ($X_2$), and microwave time ($X_3$) on the modification of egg white. Phosphorus content, solubility, foaming ability, and foaming stability were determined as the response variables. The experimental design had 3 replicates at the central point for a total of 15 runs (Wang and Shahidi, 2018). The runs in a random manner were carried out to decrease the probability of unexpected variations. A quadratic polynomial regression model was applied to analyze the data as follows:

$$Y = b_0 + \sum_{i=1}^{3} b_i X_i + \sum_{i=1}^{3} b_{ii} X_{ii}^2 + \sum_{i<j=1}^{3} b_{ij} X_i X_j \quad (1)$$

where Y is the response variable, $b_0$ is constant, $b_i$, $b_{ii}$, and $b_{ij}$ are the linear, quadratic, and interactive coefficients determined by the model, and $X_i$ and $X_j$ are the independent variables.

### Determination of Phosphorylation Degree of Egg White Powder

The total degree of phosphorylation of egg white powder was measured as previously reported (Chen et al., 1956). The powder ingredients were digested in the ratio of 10:2:1 of nitric acid, sulfuric acid, and perchloric acid, while the digestive fluid became clear and transparent. The amount of phosphorus in the digest was regarded as the total phosphorus of the protein. For the determination of inorganic phosphorus (Pi), 5 mL of 10 mg/mL sample solution was added with the same volume of 15% trichloroacetic acid and then centrifuged at 3,000 g for 20 min. The amount of phosphorus bound to proteins was evaluated as the difference between the total phosphorus and the Pi content.

## *Determination of Solubility of Egg White Powder*

A portion of 2 g of egg white powder was dissolved in the 30 mL water in the centrifuge tube and then the tube was shaken for 3 min by a vortex oscillator (Vortex 2, IKA works Guangzhou Co., Ltd.) and placed into centrifuge at 6,000 *g* for 10 min at 4°C. When the centrifugation finished, the supernatant in the tube was removed and the precipitation would be dried to constant weight at 105°C in the oven. The solubility was calculated according to the following equation:

$$\mathrm{X} = 100 - (\mathrm{m}_2 - \mathrm{m}_1) \times 100/\left((1 - \mathrm{B}) \times \mathrm{m}\right) \tag{2}$$

Where X was the solubility of egg white powder, m was the weight of sample, $\mathrm{m}_1$ was the weight of centrifuge tube, $\mathrm{m}_2$ was the sum weight of centrifuge tube and precipitation, B was the moisture of sample.

## *Determination of Foaming Ability and Foaming Stability of Egg White Powder*

The foaming properties of egg white powder were determined by following the method of Sheng et al. (2019) with slight modification. First, the 15 mL of 10 mg/mL egg white powder solution was poured into the cylinder and then homogenized at a speed of 10,000 r/min at room temperature for 1 min. After 30 s, the volumes of whipped samples were recorded. The foaming ability was calculated according to the following formula:

$$\text{Foaming capacity}\ (\%) = (\mathrm{A} - \mathrm{B}) \times 100/\mathrm{B} \tag{3}$$

where A was the volume after whipping (mL) and B was the volume before whipping (mL).

The foaming stability was calculated as follows,

$$\text{Foaming stability}\ (\%) = (\mathrm{C} - \mathrm{B}) \times 100/\mathrm{B} \tag{4}$$

where C was the volume of whipped samples after standing (mL) at 20°C for 30 min.

## *Statistical Analysis*

The experiment was executed in triplicates. The ANOVA test was employed to evaluate the statistical significance of the regression coefficients. Once the fitted regression equations were determined, the response surface plots were drawn using the Design Expert Software (Trial Version 8.0.6, Stat-Ease Inc).

# RESULTS AND DISCUSSION

The experimental values of response variables for Box-Behnken are presented in Table 1. The model was calculated using the least square technique, and the results were considered statistically significant at $P < 0.05$. The coefficient of variables of the models and $\mathrm{R}^2$ of statistical parameters for $\mathrm{Y}_1$, $\mathrm{Y}_2$, $\mathrm{Y}_3$, and $\mathrm{Y}_4$ are shown in Table 2. The adjust $\mathrm{R}^2$s were 0.9362, 0.8563, 0.8674, and 0.8919, which suggested that the predictive equation fitted well.

**Table 1.** Experimental values of response variables for the Box–Behnken design.

| | Independent variables | | | | | | Dependent variable | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Coded level | | | Uncoded level | | | | | | |
| Run | −1 | 0 | 1 | A | B | C | $\mathrm{Y}_1$ | $\mathrm{Y}_2$ | $\mathrm{Y}_3$ | $\mathrm{Y}_4$ |
| 1 | −1 | 0 | 1 | 20 | 400 | 150 | 1.43 ± 0.11 | 86.19 ± 3.22 | 72.33 ± 2.21 | 42.22 ± 1.39 |
| 2 | 0 | −1 | 1 | 30 | 300 | 150 | 2.52 ± 0.21 | 88.06 ± 2.63 | 107.87 ± 1.36 | 67.50 ± 2.96 |
| 3 | 0 | 0 | 0 | 30 | 400 | 120 | 2.41 ± 0.18 | 90.22 ± 3.52 | 126.00 ± 3.36 | 60.00 ± 1.47 |
| 4 | −1 | 0 | −1 | 20 | 400 | 90 | 1.03 ± 0.02 | 79.62 ± 1.98 | 75.00 ± 1.67 | 46.13 ± 0.98 |
| 5 | 1 | 0 | −1 | 40 | 400 | 90 | 2.53 ± 0.15 | 89.21 ± 1.52 | 129.00 ± 2.36 | 63.00 ± 1.12 |
| 6 | 0 | 0 | 0 | 30 | 400 | 120 | 2.52 ± 0.09 | 90.58 ± 2.23 | 126.67 ± 3.65 | 62.00 ± 1.63 |
| 7 | 0 | 1 | 1 | 30 | 500 | 150 | 3.00 ± 0.12 | 87.07 ± 2.03 | 92.89 ± 2.52 | 37.33 ± 1.87 |
| 8 | 0 | 1 | −1 | 30 | 500 | 90 | 2.43 ± 0.03 | 87.50 ± 1.22 | 132.67 ± 2.63 | 68.67 ± 0.98 |
| 9 | 0 | −1 | −1 | 30 | 300 | 90 | 3.29 ± 0.08 | 89.52 ± 0.63 | 115.83 ± 2.85 | 64.00 ± 1.74 |
| 10 | 1 | −1 | 0 | 40 | 300 | 120 | 2.89 ± 0.01 | 81.83 ± 0.96 | 95.52 ± 2.07 | 64.76 ± 1.35 |
| 11 | −1 | −1 | 0 | 20 | 300 | 120 | 1.40 ± 0.03 | 90.58 ± 0.36 | 68.13 ± 1.28 | 38.67 ± 1.36 |
| 12 | 1 | 1 | 0 | 40 | 50 | 120 | 2.84 ± 0.02 | 86.52 ± 1.85 | 101.86 ± 1.68 | 35.71 ± 1.17 |
| 13 | −1 | 1 | 0 | 20 | 500 | 120 | 1.34 ± 0.05 | 77.68 ± 0.36 | 86.44 ± 1.39 | 42.44 ± 1.87 |
| 14 | 0 | 0 | 0 | 30 | 400 | 120 | 2.46 ± 0.09 | 89.04 ± 0.88 | 133.33 ± 2.24 | 60.00 ± 1.29 |
| 15 | 1 | 0 | 1 | 40 | 400 | 120 | 2.41 ± 0.16 | 80.74 ± 0.36 | 86.67 ± 1.71 | 53.78 ± 1.41 |

Independent variables: $\mathrm{X}_1$ and A, the concentration of sodium tripolyphosphate (g/L); $\mathrm{X}_2$ and B, microwave power (W); $\mathrm{X}_3$ and C, microwave time (min). Dependent variables: $\mathrm{Y}_1$, degree of phosphorylation (mg/g); $\mathrm{Y}_2$, soubility (g/100 g); $\mathrm{Y}_3$, foaming ability (%); $\mathrm{Y}_4$, foaming stability(%).

**Table 2.** Regression coefficients and $R^2$ for 3 dependent variables.

| | Coefficient | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Factors | $Y_1$ | *P*-value | $Y_2$ | *P*-value | $Y_3$ | *P*-value | $Y_4$ | *P*-value |
| Intercept | 4.0661 | 0.0003 | 32.6547 | 0.0020 | −703.8039 | 0.0016 | −290.9216 | 0.0010 |
| $X_1$ | 0.5111 | <0.0001 | 2.7610 | 0.2549 | 25.2261 | 0.0012 | 11.4194 | 0.0019 |
| $X_2$ | –0.0383 | 0.2138 | –0.0882 | 0.0190 | 1.2056 | 0.1793 | 0.8182 | 0.0014 |
| $X_3$ | –0.0420 | 0.8091 | 0.6080 | 0.2984 | 3.4779 | 0.0028 | 0.5106 | 0.0037 |
| $X_1$ $X_2$ | 5.9630E-007 | 0.9926 | 4.3946E-003 | 0.0006 | −2.99455E-003 | 0.3642 | −8.2063E-003 | 0.0021 |
| $X_1$ $X_3$ | –4.3161E-004 | 0.0027 | 8.7194E-005 | 0.6712 | –2.65101E-003 | 0.0455 | –2.9028E-003 | 0.0016 |
| $X_2$ $X_3$ | 1.1160E-004 | 0.0027 | 8.7194E-005 | 0.6712 | –2.65101E-003 | 0.0455 | –2.9028E-003 | 0.0016 |
| $X_1^2$ | –6.5214E-003 | 0.0001 | –0.0494 | 0.0004 | –0.3112 | 0.0002 | –0.1168 | 0.0005 |
| $X_2^2$ | 3.0407E-005 | 0.0049 | –8.5158E-005 | 0.2179 | –9.55657E-004 | 0.0281 | –3.5891E-004 | 0.0586 |
| $X_3^2$ | 4.4595E-005 | 0.5542 | –1.1803E-003 | 0.1391 | −7.55125E-003 | 0.0816 | 2.5527E-003 | 0.1791 |
| $R^2$ | | 0.9362 | | 0.8563 | | 0.8674 | | 0.8919 |

$Y_1$, degree of phosphorylation (mg/g); $Y_2$, soubility (g/100 g); $Y_3$, foaming ability (%); $Y_4$: foaming stability(%).

## Degree of Phosphorylation

Regarding the degree of phosphorylation results, as presented in Table 2 and Figure 1, strong linear effects ($P < 0.01$) of the concentration of sodium tripolyphosphate and quadratic effects ($P < 0.01$) of the concentration of sodium tripolyphosphate and microwave powder contributed to the degree of phosphorylation. In addition, the interaction of microwave power and microwave time had a significant influence ($P < 0.01$) on the degree of phosphorylation. The response surface plots of the degree of phosphorylation were illustrated in Figure 1. The maximum point (3.45 mg/g) was obtained at value of 36.23 g/L (the concentration of sodium tripolyphosphate), 300 W (microwave power), and 90 s (microwave time).

In our study, the degree of phosphorylation of egg white powders in this model, which were phosphorylated with microwave assistance, varied from 0.9005 to 3.4471 mg/g protein. In this model, the degree of phosphorylation varied from 1.0336 to 3.2882 mg/g protein. Egg white had some native phosphorylated proteins and phosphorylated sites. Xiong and Ma (2017) had studied phosphorylated ovalbumin in the presence of sodium tripolyphosphate and had been identified 23, 21, and 18 phosphorylation sites at pH 5.0, 7.0, and 9.0, respectively, including some natural phosphorylation sites Ser68 and Ser 344. Additionally, the amino acids which had the possibility to be phosphorylated were considered to be Ser, Thr, Tyr, Arg, and Lys. This result suggested that the naturally phosphorylated peptide was not readily phosphorylated further.

From the response surface 3D figure (Figure 1a and b), at a certain power (400 W) or a certain reaction time (90 s), as the concentration of sodium tripolyphosphate increases from 20 to 40 g/L, the degree of phosphorylation of egg white protein first increases and then decreases. The improvement at first might be attributed to the collision between the side chain bound to egg white protein and phosphate groups of free sodium tripolyphosphate. However, as the amount of phosphate groups increased further, electrostatic repulsion might occur between phosphorylated proteins and excess phosphate groups, while resulted in the reduction of the degree phosphorylation of egg white powder. In addition, when the microwave time varied from 90 to 150 s, the conditions of microwave power of 500 W level provided the most enhancement of the degree of phosphorylation. The data might suggest that moderate microwave assistance could promote the progress of the phosphorylation reaction.

After the model analysis, we carried out the verification experiment under the optimal conditions, and the results showed the degree phosphorylation was 3.32 mg/g protein. The phosphorus content of native egg white was 0.6 mg/g because egg white contains some naturally phosphorylated proteins, such as ovalbumin, egg white riboflavin-binding protein, etc. The content of phosphate groups had increased more than 5 times.

## Solubility

The solubility of egg white powder under different concentrations of sodium tripolyphosphate, microwave power, and microwave time is shown in Table 2 and Supplementary Figure S2. The regression coefficient indicated the linear effect of the concentration of sodium tripolyphosphate ($P < 0.01$), microwave power ($P < 0.01$), and microwave time ($P < 0.05$), and quadratic effect ($P < 0.01$) of concentration of sodium tripolyphosphate. The effect of the monomial on the solubility of egg white powder was $X_2 > X_1 > X_3$.

In this model, the relationship between solubility and experimental factors was not linear, and the quadratic term and the interaction had a great influence. The interaction between the concentration of sodium tripolyphosphate and microwave power or microwave time ($P < 0.01$) contributed to the solubility of egg white powder. The response surface plots of solubility of egg white powder were illustrated in Supplementary Figure S2. The regression coefficients indicated the optimal conditions of the concentration of sodium tripolyphosphate of 22.28%, microwave power of 300 W, and microwave time of 149.99 s.

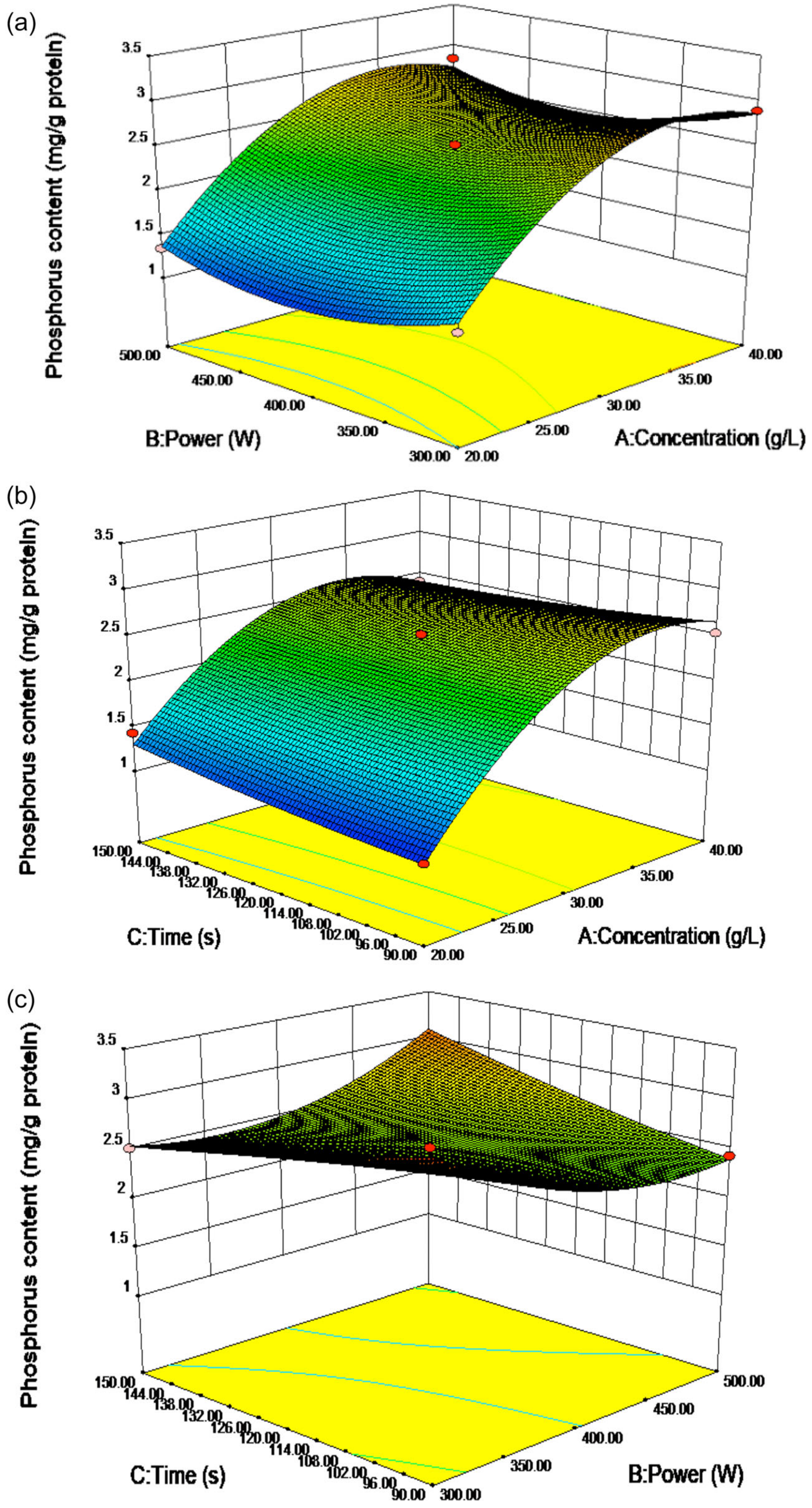

**Figure 1.** Response surface for the effect of independent variables on the degree of phosphorylation of egg white protein powder.

It had been reported in the previous study that phosphorylated proteins had higher solubility under certain conditions (Yin et al., 2014). However, not all phosphorylated proteins under different conditions had better solubility, which might be due to the structural changes of protein, such as the hydrophobicity, secondary structure, and so on. In this study, some samples among 15 runs, such as the solubilities of 79.63 g/100 g powder and 77.68 g/100 g powder for the conditions of 20 g/L ($X_1$), 400 W ($X_2$), 90 s ($X_3$) and 20 g/L ($X_1$), 500 W ($X_2$), and 120 s ($X_3$) were lower than native egg white powder (84.13 g/100 g powder). The results might be attributed that the effect of microwave radiation was greater than that of phosphorylation modification on the solubility of egg white powder. Heating methods could cause a decrease in protein solubility. Yin et al. (2014) found the solubility of egg white protein which was dried heating at 85°C for 3 D decreased 4.9% compared to native egg white protein.

It could be seen in Supplementary Figure S1a. that when the concentration of sodium tripolyphosphate was 20 g/L and the power increased from 300 to 500 W, the solubility of egg white declined continuously. This phenomenon might be ascribed that when the amount of phosphate groups was relatively small, microwave radiation had a dramatic impact on the solubility of the protein. The thermal effect caused by increased microwave power might induce the exposure of the hydrophobic group of the protein, resulting in the reduction of solubility (Sheng et al., 2018). However, when the concentration of sodium tripolyphosphate was 40 g/L and the microwave power varied from 300 to 500 W, the solubility of the egg white powder improved.

After the model analysis, we carried out the verification experiment under the optimal conditions, and the results showed the solubility was 91.08 g/100 g powder, which had improved 7.63% compared with untreated samples (84.13 g/100 g).

## Foaming Ability

The foaming ability of egg white powder under different conditions is shown in Table 2 and Supplementary Figure S2. The regression coefficient indicated the linear effect ($P < 0.01$) and quadratic effect ($P < 0.01$) of the concentration of sodium tripolyphosphate and microwave power. The interaction between microwave time and the concentration of sodium tripolyphosphate or microwave power ($P < 0.05$) contributed to the foaming ability of egg white powder and the solubility of egg white powder. The optimal conditions for phosphorylation modification of egg white suggested by the model to enhance the foaming ability were as follows: the concentration of sodium tripolyphosphate 32.55 g/L, microwave power 415.95 W, and microwave time 90 s. These reaction conditions determined by the RSM model predicted maximum response of the dependent variables.

The microwave power increased from 300 to 500 W, which the concentration of sodium tripolyphosphate was fixed at 20 g/L, and the foaming ability increased continuously. This result was consistent with our previous research (Li et al., 2018). High-power (400, 500, and 600 W) groups had higher surface hydrophobicity and more stretched and flexible structure. It had been reported that the high surface hydrophobicity proteins could adsorb at the interface between air and water strongly and lead to a significant decrease in interface or surface tension, which was used for foaming (Kato et al., 1985).

After model analysis, the optimal reaction conditions for foaming ability were obtained and experiments were carried out under these conditions. The foaming ability of egg white powder reached 139.21%, which had 63.91% improvement compared with native egg white (84.93%).

## Foaming Stability

The foaming stability of egg white powder under different conditions is shown in Table 2 and Figure S3. The regression coefficient indicated the linear effect ($P < 0.01$) of 3 factors and quadratic effect ($P < 0.01$) of the concentration of sodium tripolyphosphate. In this model, the interaction between microwave power and microwave time ($P < 0.01$) or the concentration of sodium tripolyphosphate contributed to the foaming stability of egg white powder. The maximum point (3.45 mg/g) was obtained at value of 35.48 g/L ($X_1$), 300 W ($X_2$), and 150 s ($X_3$).

In our previous study, the foaming stability of phosphorylated egg white protein was not significantly improved under low power treatment (Li et al., 2018). However, as the power was further increased, the foaming property was remarkably improved. When the microwave intensity was too strong, the foaming stability of the phosphorylated egg white was somewhat lowered. This finding was corresponded with our previous study.

Fixed at the concentration of sodium tripolyphosphate 20 g/L, when the power was from 300 to 500 W, the foaming stability first rose and then decreased. However, the foaming ability increased continuously in the same condition. It was analyzed by the data that the group of maximum foaming ability did not have maximum foaming stability. The result might be attributed to excessive microwave power. Duan et al. (2018) found that excessive oxidation could cause an increase in foaming ability of egg white protein, while a decrease in foaming stability.

The continuous increase in foaming stability might be due to the higher electronegativity (Li et al., 2018), which was attributed to the electrostatic repulsion in adjacent films for foam stabilization (Alleoni, 2006). The reduction of foaming stability might be attributed to a decrease in the sterically stabilized and more cohesive interfacial films formed in the presence of aggregates with larger diameters, which subsequently caused a decrease in the coalescence rate and thus resulted in foam decay (Dombrowski et al., 2016).

After the model analysis, we carried out the verification experiment under the optimal conditions, and the results showed that the foaming stability of egg white powder increased from 41.73 to 73.88%. It was not particularly significant to study foaming ability or foaming stability alone, and excellent foaming properties were required in the food industry, which would be discussed later.

**Table 3.** Results of optimization the degree of phosphorylation (mg/g), solubility (g/100 g), foaming ability (%), foaming stability (%), functional properties, and foaming properties of phosphorylated egg white protein manufactured by using the optimum phosphorylation conditions.

| | Optimum phosphorylation | | | Measurements | | | |
|---|---|---|---|---|---|---|---|
| | $X_1$ (g/L) | $X_2$ (W) | $X_3$ (s) | $Y_1$ (mg/g) | $Y_2$ (g/100 g) | $Y_3$ (%) | $Y_4$ (%) |
| **Optimization of 4 responses** | | | | | | | |
| 1 | 36.22 | 300 | 90 | 3.3218 ± 0.0127 | 89.3129 ± 0.0213 | 115.2184 ± 0.0417 | 68.1112 ± 0.0612 |
| 2 | 22.30 | 300 | 150 | 1.7912 ± 0.0182 | 91.0765 ± 0.0317 | 81.9923 ± 0.0165 | 53.2216 ± 0.0543 |
| 3 | 35.58 | 453 | 90 | 2.6014 ± 0.0478 | 89.7658 ± 0.0344 | 139.2113 ± 0.0712 | 67.8921 ± 0.0533 |
| 4 | 35.51 | 300 | 150 | 2.5711 ± 0.0514 | 83.3367 ± 0.0479 | 100.1237 ± 0.0621 | 73.8765 ± 0.0712 |
| **Optimization of functional properties and foaming properties** | | | | | | | |
| 1 | 32.97 | 429 | 90 | 2.3189 ± 0.0173 | 90.0012 ± 0.0439 | 138.1263 ± 0.045 | 69.7691 ± 0.0451 |
| 2 | 33.84 | 419 | 90 | 2.8135 ± 0.0981 | 88.1879 ± 0.0812 | 137.1100 ± 0.0761 | 69.3127 ± 0.0712 |

### Optimization of Phosphorylation Modification with Microwave-Assisted Condition

The optimum phosphorylation modification conditions for egg white were achieved using the desirability function method and determined to obtain maximum degree of phosphorylation, solubility, foaming ability, and foaming stability. The study was designed to obtain the best functional properties (solubility, foaming power, and foaming stability) and corresponded with the condition of the concentration of sodium tripolyphosphate, microwave power, and microwave time. In addition, the model can be a tool for analyzing the foaming properties. After the model analysis, we conducted a verification experiment.

Table 3 shows the practical results of measuring values for the degree of phosphorylation, solubility, foaming ability, foaming stability, functional properties, and foaming properties. The practical results of functional properties and foaming properties were 2.32 mg/g, 90.00 g/100 g, 138.13%, 69.77% and 2.81 mg/g, 88.19 g/100 g, 137.11%, 69.31% for $Y_1$, $Y_2$, $Y_3$, and $Y_4$ under the optimal conditions. The results indicated that the optimal conditions for prediction were suitable for microwave-assisted phosphorylation of egg white protein.

## CONCLUSION

In this work, RSM was established to determine the optimum microwave-assisted phosphorylation modification conditions (the concentration of sodium tripolyphosphate, microwave power, and microwave time). Optimal conditions for maximum functional properties (solubility, foaming ability, and foaming stability) and maximum foaming properties (foaming ability and foaming stability) of phosphorylated egg white protein were obtained. We found 2 solutions: the concentration of sodium tripolyphosphate of 33.84 g/L, microwave power of 419.38 W, and microwave time 90 s for maximum functional properties and the concentration of sodium tripolyphosphate of 32.97 g/L, microwave power of 429.29 W and microwave time of 90 s for maximum foaming properties, respectively. Therefore, we successfully phosphorylated the egg white protein under these conditions and confirmed various desirable properties of egg white protein phosphorylation modification.

## SUPPLEMENTARY DATA

Supplementary data are available at *Poultry Science* online.

**Supplementary Figure S2.** Response surface for the effect of independent variables on the foaming ability of egg white protein powder.

**Supplementary Figure S3.** Response surface for the effect of independent variables on the solubility of egg white protein powder.

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