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OPTIMIZING FOAM PROPERTIES OF EGG WHITE POWDER-BASED FOAM SYSTEM BY RESPONSE SURFACE METHODOLOGY

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Abstract: Foam is a thermodynamically unstable multiphase system consisting of a continuous liquid and discontinuous air phases. Therefore, the interfaces between the air and water phases need to be stabilized by surfactant components. For this purpose, egg white proteins are the most widely used interfacial agent in the food sector; however, the foaming properties of egg white powders produced for technological purposes are negatively affected by thermal treatment. Thus, in this study, the impact of process variables such as saponin extract (0.05-0.15%), guar gum (0.1-0.4%), and mixing time (3-9 min) on foaming capacity (FC) and foam stability (FS) was investigated using response surface methodology via Box-Behnken design to enhance the foaming characteristics of protein powders. The analysis of variance results demonstrated that all process parameters had a highly significant effect on the quadratic FC model. However, it was found that the effect of guar gum on the FS model was dominant ($P < 0.05$). Furthermore, the results showed that increasing saponin content and mixing time, as well as guar gum and mixing time resulted in increased values of FC and FS, respectively. Moreover, Design Expert 13.0 was used as a statistical tool to perform multi-response optimization of both responses via the desirability function approach. The findings indicated that the optimal combination for maximizing responses was at 0.15% saponin content, 0.37% guar gum, and 9 min of mixing time.

Keywords: Optimization, Box-Behnken design, Foaming capacity, Foam stability, Egg white powder

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1. Introduction

Foam can be defined as a multiphase system formed by trapping gas bubbles in a liquid phase. Such systems are thermodynamically unstable, and therefore maintaining the equilibrium between the two phases is of great importance for practical applications. Reducing the surface tension between the liquid and gas interface in a food foam is one of the most important parameters in foam production. For this purpose, saponins and/or proteins with a foaming ability are commonly used (Kezwon and Wojciechowski, 2014). Egg white is the most widely used foaming material in food processing. To meet the industrial demand for egg albumen, egg whites are usually dried using a spray drying method to produce egg white powder (EWP). However, this process generally causes a reduction in the foaming properties of proteins in EWP. Therefore, further research is still needed to improve the foaming properties of EWP (Chang et al., 2020).

Numerous studies have been conducted on the influence of colloidal materials on the properties of protein-based foams. Hydrocolloids exert this effect by increasing the viscosity of the continuous phase and altering the structural, rheological, and amphipathic characteristics of proteins (Li et al., 2023). Therefore, the features of the gums might affect the distribution of the bubbles in liquid matrices in the foam formulations and this might alter the air fraction, as well as the density of the foam system (Ptaszek et al., 2014). Guar gum is considered to be a non-ionic colloidal saccharide obtained from the *Cyamopsis tetragonolobus* (Hamdani et al., 2018). Compared with other commercially available gums, guar gum has a relatively higher molecular weight and a larger hydration ability. Thus, it affects the foaming characteristics of protein foams by substantially increasing the viscosity of the foam solution (Dickinson, 2011).

Saponins are used in formulations where rapid foam formation is desired since they adsorb at air/water interfacial films faster than globular proteins. In the food industry, saponin components are preferred to obtain stable foam in certain confectionery products (e.g., halva, meringue, marshmallow, etc.), beers, and some carbonated beverages. In comparison to saponins, proteins have been shown to contribute considerably to the FS by increasing the elasticity of interfacial films during the adsorption process at foam interfaces. Consequently, the incorporation of saponins and proteins in such systems has been shown to enhance foam properties through synergistic interactions (Wojciechowski et al., 2014). *Gypsophila spp.* possess notable concentrations of saponins within their rhizomes. It was reported that the substitution of egg white with soapwort extract at a ratio of 50% and 75% did not result in a significant change in the physical characteristics of the sponge cake dough (Çelik et al., 2007).

Foaming capacity (FC) and foam stability (FS) are the most important parameters in the assessment of the quality evaluation of food foams. The FC refers to foamability which indicates the amount of air trapped in the foam structure. However, The FS provides information about the period of time the trapped air is retained in the foam structure. The performance of protein foams depends on a combination of internal factors, such as the structure and composition of the protein, and external parameters, such as environmental factors and processing techniques (Indrawati et al., 2008). Thus, it is essential to define the parameters involved in the production of foam and then optimize the process using an appropriate methodology. Response Surface Methodology (RSM) is an empirical modeling approach that integrates statistical and mathematical methods to describe the relationship between response variables and input factors. RSM provides significant advantages, especially in chemical and biological processes where several process variables impact the response. However, it is critical to select an appropriate experimental design to evaluate the effects of process factors on the target variable (Rawat et al., 2024).

Therefore, the objective of this study was to enhance the foaming properties of EWP-based foam using a BBD with RSM. Accordingly, the quality properties of the resulting foams produced by mixing pre-foaming solutions containing various concentrations of saponin extract (0.05-0.15%), guar gum (0.1-0.40%), and different times (3-9 min) were optimized using the RSM. The findings are expected to contribute to the development of improved foaming systems for food industry applications.

2. Materials and Methods

2.1. Material

The EWP and guar gum were supplied by Tito (Izmir, Türkiye). The root of *Gypsophila arrostii var. nebulosa* was procured from a local store in Sakarya, Türkiye. The materials were stored at room temperature. The chemicals used in this research were analytical grade and were of obtained from Sigma-Aldrich (Germany).

2.2. Preparation of Biopolymer Solutions

A total of 20 g of protein powder was gradually transferred to 100 mL of distilled water, which was stirred in a magnetic stirrer (Heidolph MR Hei-Tec, Scwabach, Germany) at 400 rpm. The mixture was left to dissolve for a total of 2 h. The guar gum solution was prepared as 0.5% (w/v) under conditions similar to those described above. Until foam production, the protein powder was stored under refrigerator conditions, while the guar gum solution was mixed at 200 rpm for 12 h at room temperature.

2.3. Saponin Extraction

The saponin extract was prepared from *Gypsophila bicolor* according to the method proposed by Güldane (2023). This method involves subjecting the powdered sample to an extraction process using a soxhlet apparatus for a period of eight hours. Following the extraction process, the saponin extract was stored in an amber glass bottle in the refrigerator. This step was performed after the removal of insoluble components from the solution.

2.4. Foaming Procedure

The production of model foams was carried out in accordance with the specifications outlined in table 2. Throughout the process of foam production, the protein content of the samples was maintained at a constant level of 5% (w/v). Accordingly, 20% protein solution, saponin extract (0.05-0.15%), and guar gum solution (0.1-0.4%) were added into a beaker in calculated amounts, yielding a total of 200 mL solution. This prefoaming mixture was subsequently exposed to a mixing process for 10 min at a speed of 200 rpm using a magnetic stirrer at room temperature. Subsequently, the resulting mixture was whipped using a domestic mixer (Kenwood KM070, England) at 158 rpm at various times (3-9 min). The final foam was analyzed immediately following the whipping process.

2.5. Statistical Analysis

2.5.1. Foaming capacity (FC)

Once the whipping process was completed, the mixing apparatus was gently removed, and the foam sample was poured into the preweighed measuring cup. The surface of the samples was leveled with a plastic spatula and the weights were recorded. The FC values of the samples were then calculated by the following equation 1 (Wang et al., 2015).

$$FC\ (\%) = \frac{(m_s - m_f)}{m_f} \times 100 \qquad (1)$$

where m_s and m_f indicate the weights of the prefoaming solution and the foam, respectively.

2.5.2. Foam stability (FS)

The stability of the model foams determined by the procedure proposed by Güldane (2023). The values for FS were given in min.

2.6. Experimental Procedure

In the current study, the BBD with RSM was used to optimize selected operational parameters, which have an influence on the foaming properties of EWP. RSM is a multivariate statistical approach that can be a useful tool for the interpretation and analysis of experimental data involving more than one variable. It is especially useful in developing regression-based response surface models, which facilitate experimental optimization and predictive analysis (Li et al., 2024).

The RSM experiments were developed using Design Expert software (ver. 13.0, Stat-Ease Co., Minneapolis, MN, USA) based on the BBD model. The process factors with corresponding levels used for the RSM experiments are given in table 1. The experiments consist of three factors with three levels and fifteen response surface tests that include three center points are summarized in table 2. The present research investigated the effects of saponin extract (A, 0.05-0.15%), guar gum (B, 0.10-0.40), and mixing time (C, 3-9 min) on key response variables, FC and FS, using a second-order polynomial equation (equation 2) to establish correlations between the variables.

$$Y = \beta_0 + \sum_{j=1}^{k} \beta_j x_j + \sum_{j=1}^{k} \beta_{jj} x_j^2 + \sum_{i=1}^{k} \sum_{j \neq i}^{k} \beta_{ij} x_i x_j + \varepsilon \qquad (2)$$

where Y is the response variable, β_j, β_{ij} and β_{jj} refer to the coefficients of linear, interaction and quadratic terms, respectively.

Table 1. Process factors and levels

Process variables	Unit	Symbol	Level		
			-1	0	+1
Saponin extract	%	A	0.05	0.1	0.15
Guar gum	%	B	0.1	0.25	0.40
Mixing time	min	C	3	6	9

Statistical analysis was conducted using Design Expert software, which produced three-dimensional surface response plots derived from the polynomial equations based on experimental data. These plots offer a visual representation of the interaction between process variables and response outcomes. The significance of the model was evaluated through analysis of variance (ANOVA), with the statistical relevance of linear, quadratic, and interaction terms determined using Fisher's F-test and associated p-values. The coefficient of determination (R^2) was calculated to evaluate the accuracy and reliability of each model.

Table 2. Box-Behnken design (BBD) matrix and experimental results

No	Process variables			Experimental results	
	Saponin extract %	Guar gum %	Mixing time min	Foaming capacity (FC)	Foam stability (FS)
1	0.05	0.25	3	712.0 ± 12.25	44.99 ± 1.24
2	0.15	0.25	3	468.0 ± 10.22	52.38 ± 1.45
3	0.10	0.25	6	329.0 ± 8.34	59.46 ± 1.89
4	0.15	0.1	6	629.5 ± 15.00	55.26 ± 0.26
5	0.15	0.40	6	632.4 ± 14.23	53.56 ± 1.85
6	0.05	0.25	9	538.2 ± 12.45	46.54 ± 0.80
7	0.10	0.25	6	824.6 ± 14.80	58.61 ± 0.65
8	0.10	0.25	6	542.0 ± 13.00	74.35 ± 1.53
9	0.10	0.40	3	644.4 ± 8.25	53.95 ± 0.41
10	0.05	0.40	6	447.8 ± 10.25	45.31 ± 0.26
11	0.10	0.40	9	399.4 ± 9.54	67.0 ± 1.00
12	0.05	0.1	6	598.7 ± 11.50	51.3 ± 0.54
13	0.10	0.1	3	468.9 ± 13.40	46.11 ± 0.23
14	0.15	0.25	9	648.4 ± 12.84	48.44 ± 0.46
15	0.05	0.1	9	619.4 ± 10.43	69.3 ± 1.12
Minimum	-	-	-	329.0	44.99
Maximum	-	-	-	824.6	74.35

4. Results and Discussion

3.1. Model Fitting

In the study, a BBD was conducted to improve the foaming properties of EWP. A total of fifteen experiments with three center points were carried out in the laboratory to determine optimal levels of the process factors. The mean experimental results obtained from the experiments are presented in table 2. The data was analyzed by using Design Expert 13 software to develop an optimal empirical model that established a relationship between the foaming characteristics, FC and FS, and the selected foaming process variables, saponin extract, guar gum, and mixing time. The fitted quadratic equations for FC (equation 3) and FS (equation 4) are given below.

$$FC = +635.43 + 59.95A - 57.08B + 130.13C + 8.10AB + 56.25AC + 6.55BC - 20.22A^2 - 83.22B^2 - 25.17C^2 \qquad (3)$$

$$FS = +54.26 + 1.28A + 10.60B + 2.62C + 1.36AB + 3.39AC + 2.54BC + 1.08A^2 + 3.74B^2 - 3.24C^2 \qquad (4)$$

The results obtained for the FC and FS from the foaming experiment were analyzed using an analysis of variance to illustrate the validation of the obtained experimental result. The statistical significance and adequacy of the developed model were determined by the statistical tools, F value and P value, which were derived from the sum of squares (SS) and mean squares (MS) statistics. A higher F-value and a lower P-value than 0.05 indicate the statistical significance of the effect of the relevant parameter on the response variable (Dang et al., 2024). The ANOVA results in table 3 summarize the statistical significance of the effects of process parameters (saponin extract, guar gum, and mixing time) and their interactions on FC. The F-value of 137.37 and the P-value ($P < 0.0001$) of the model show that the developed model for the FC is highly significant. However, the coefficient of determination (R^2) values (R^2=0.9960 and R^2_{adj}=0.9887) of this model indicate that the data fit the model. The linear (A, B, C), interaction (A*C) and quadratic (B*B and C*C) terms have a significant impact on FC.

Table 3. ANOVA results for FC

Source	SS	Df	MS	F-value	P-value
Model	230800.00	9	25646.79	137.37	< 0.0001
A-Saponin extract	28752.02	1	28752.02	154.00	< 0.0001
B-Guar gum	26060.45	1	26060.45	139.58	< 0.0001
C-Mixing time	135500.00	1	135500	725.54	< 0.0001
A*B	262.44	1	262.44	1.41	0.2890
A*C	12656.25	1	12656.25	67.79	0.0004
B*C	171.61	1	171.61	0.9192	0.3817
A*A	1509.10	1	1509.10	8.08	0.0361
B*B	25569.28	1	25569.28	136.95	< 0.0001
C*C	2338.56	1	2338.56	12.53	0.0166
Lack-of-fit	808.71	3	25646.79	137.37	< 0.0001
R^2	0.9960		269.57	4.32	0.1937
Adjusted R^2	0.9887				

SS= sum of squares; DF= degree of freedom; MS= mean square.

In the same context, the ANOVA result of the FS model is presented in table 4, which shows that the P-value is desirable, thereby resulting in excellent significance of the regression model. The higher R^2 values (R^2=0.9843 and R^2_{adj}=0.9561) and insignificant lack of fit ($P > 0.1271$) demonstrate that the FS response can be explained by the model terms. The analysis indicates that among all the process parameters, guar gum is the most critical factor influencing the FS response, as supported by a P-value less than 0.0001 and an F-statistics value of 245.65. Furthermore, the linear effect of mixing time, the interaction effect of guar gum and mixing time (B*C), and the quadratic effect of guar gum (B*B) and mixing time (C*C) on FS are found to be significant. On the other hand, all the terms for saponin extract except the interaction effect of A and C have no meaningful impact on the FS.

Table 4. ANOVA table for FS

Source	SS	Df	MS	F- value	P-value
Model	1148.82	9	127.65	34.86	0.0006
A-Saponin extract	13.21	1	13.21	3.61	0.1160
B-Guar gum	899.52	1	899.52	245.65	< 0.0001
C-Mixing time	54.86	1	54.86	14.98	0.0118
A*B	7.43	1	7.43	2.03	0.2137
A*C	46.04	1	46.04	12.57	0.0165
B*C	25.81	1	25.81	7.05	0.0452
A*A	4.32	1	4.32	1.18	0.3270
B*B	51.76	1	51.76	14.14	0.0132
C*C	38.66	1	38.66	10.56	0.0227
Lack-of-fit	16.72	3	5.57	7.03	0.1271
R^2	0.9843				
Adjusted R^2	0.9561				

SS= sum of squares; DF= degree of freedom; MS= mean square

3.2. Effect of Process Factors on Response Variables

The 3D response surface plots in figure 1 to 6 illustrate the relationship between the FC and FS and the process parameters such as saponin extract, guar gum, and mixing time. These plots offer valuable insights into the interactions between saponin extract (A) and guar gum (B), and their impact on the foaming properties.

Figure 1 provides a visual representation of the impact of saponin extract (A) and guar gum (B) on the FC while maintaining a constant mixing time (C) at 6 min, representing the midpoint value. Initially, as both saponin extract and guar gum increase, the FC also increases, suggesting improved foaming properties. However, beyond certain optimal levels of guar gum, further increases lead to an increase in foam density. The graph illustrates that the highest FC value is achieved at concentrations of saponin extract and guar gum of 0.15% and 0.22%, respectively. In a related study, Çelik et al., (2007) reported that the interaction between EWP and soapwort saponins improved the sensory properties of the foam-based product. In a previous study, Chauhan et al., (1999) observed a deterioration in the interfacial properties of quinoa proteins, including foaming and emulsifying, resulting from the removal of saponins from the proteins. The results from dynamic interfacial tension studies demonstrated that the saponin from quillaja bark adsorbs to the air/water interface in a foam system more rapidly than β-casein proteins, thereby promoting foaming by reducing interfacial tension (Wojciechowski et al., 2014).

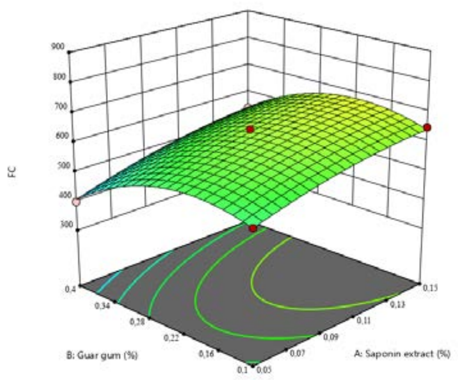

Figure 1. Effect of saponin extract and guar gum on FC.

Figure 2 depicts a relatively linear plane with a slight incline, indicating that both saponin extract and mixing time positively influenced the FC. As saponin extract and mixing time increase, the FC increases consistently, suggesting that higher saponin content and longer mixing improve the foamability of EWP. Foam formation is induced by mechanical agitation of the pre-foaming solution, which causes partial denaturation of the proteins and improvement in interfacial properties (Campbell and Mougeot, 1999). Thus, the foaming potential of a protein solution is measured as the amount of air that can be incorporated into its structure during whipping (Farid et al., 2023).

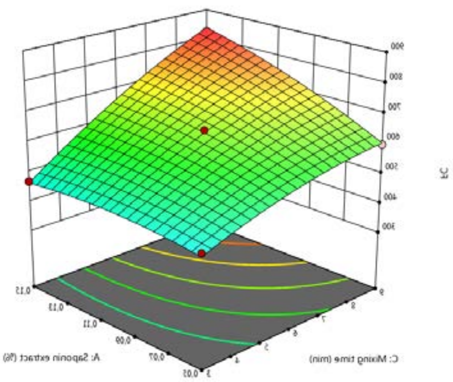

Figure 2. Effect of saponin extract and mixing time on FC.

The interaction effect of guar gum and mixing time on FC, with the saponin extract was maintained at its midpoint of 0.10% is shown in figure 3. It is clear that increasing mixing time in the foaming process increased the foamability of the EWP-base foam model owing to the aeration of the foam system. In contrast, the FC increases slightly as the concentration of hydrocolloid rises from 0.10% to 0.25%. However, a further increase in gum from 0.25% to 0.40% leads to a slight decline in the foaming ability of the foam system. The addition of saccharides into the foaming system causes an increase in viscosity and thus negatively affects the foamability of the pre-foaming solution (Sadahira et al., 2018).

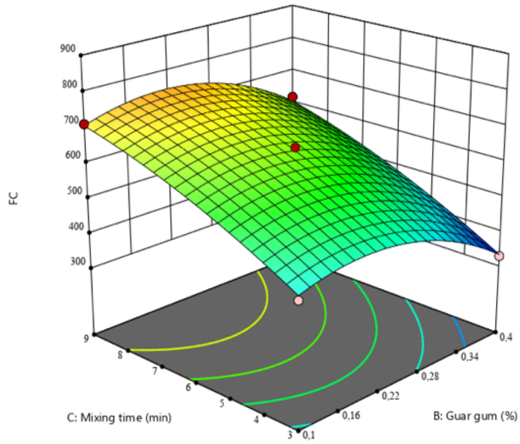

Figure 3. Effect of guar gum and mixing time on FC.

The interactive influence trend of saponin extract and at guar gum on FS in the EWP base foam system is illustrated in figure 4. It can be deduced from the graph that increasing the level of guar gum has a substantial and saponin extract has a positive impact on the FS value. However, the change in the level of hydrocolloid has shown a remarkable effect on the stability of the EWP base foam system than the concentration variation of the surfactant. Similar findings were reported by Tang et al. (2022), who investigated the effects of egg white powder modification with soy peptides on the batter stability of angel food cake. The researchers reported an increasing trend in stability with increasing sucrose concentration up to 36 g/100 ml.

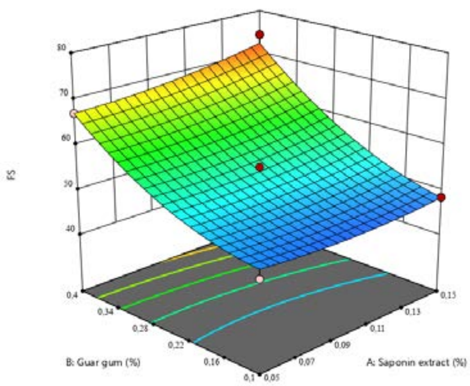

Figure 4. Effect of saponin extract and guar gum on FS.

The hydrocolloid substances play a critical role in FS by binding water molecules within the foam matrix. It has been demonstrated that this action increases the viscosity of the medium, thereby reducing the mobility of water and mitigating water leakage (drainage) from the foam structure. This, in turn, contributes to enhancing the overall structural integrity of the foam (Dickinson 2003). A rheological study conducted by Liszka-Skoczylaset al., (2014) demonstrated that viscosity was a predominant eterminant in whey protein concentrate (WPC)/non-ionic guar gum base foam system. Similarly, Tan et al., (2015) stated a positive correlation between viscosity and FS in WPC foams.

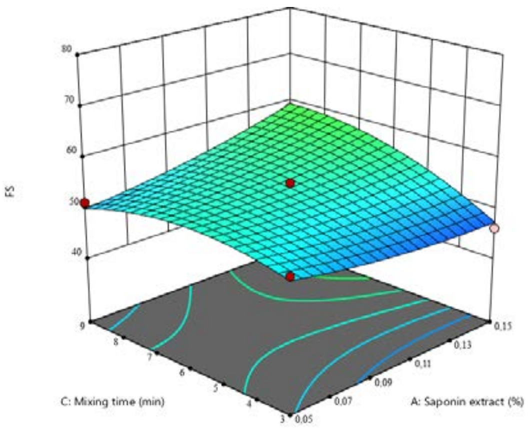

Figure 5. Effect of saponin extract and mixing time on FS.

In figure 6, the impact of guar gum and mixing time on the FS is depicted while maintaining the concentration of saponin extract at 0.15%. It is evident that with an increase in hydrocolloid content, the FS also increases. However, it is noteworthy that beyond a certain whipping time threshold, no substantial enhancement in stability is observed.

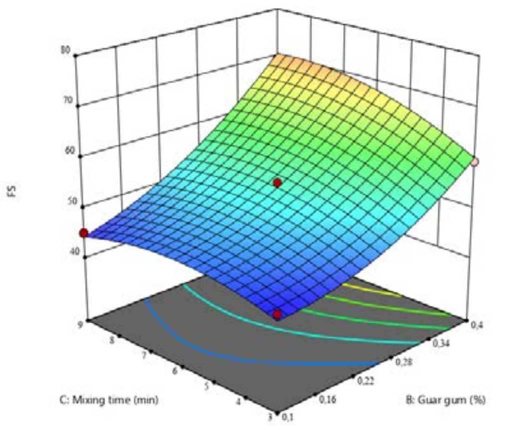

Figure 6. Effect of guar gum and mixing time on FS

Figure 6 also revealed that the maximum FS value can be achieved by selecting levels of guar gum and mixing time of 0.4% and 8 min, respectively. In protein-based foams, the size of bubbles has been shown to have a substantial impact on the stability of the foam. In the EWP/hydroxypropyl methylcellulose foam system, the foams with smaller bubbles have been observed to demonstrate enhanced stability in comparison to those with large and irregular bubble dimensions (Sadahira et al., 2018). Consequently, it has been proposed that prolonging the whipping process leads to the formation of a foam with smaller bubbles that are of similar size in a viscous medium. This, in turn, has been shown to enhance the FS.

3.3. Optimization and Confirmation

In order to optimize the response variables (FC and FS) simultaneously, the desirability function approach was applied. The application of process optimization with desirability functions was introduced by Derringer and Suich (1980). The fundamental principle of the method is to identify "target" operating conditions as the optimal response value. Thus, each response is transformed into an individual desirability function (d_i), which ranges from 0 to 1 (Malghan et al., 2017). However, RSM is often integrated with a desirability function in order to convert the desirability index values of the responses into an overall desirability (D) value (Chiu et al., 2022). The following steps were carried out in the optimization of the foam properties of the EWP-based foam system.

i) The objective of this study was to optimize the FC and FS characteristics. Thus, the d_i value for all responses was computed for each set of experiments using the "larger the better" criterion according to the following equation 5.

$$\begin{cases} 0 & y < L \\ \left(\frac{y-L}{T-L}\right)^{r} & L \le y \le T \\ 1 & y > T \end{cases} \qquad (5)$$

where L and T represent the lower and upper values of the response variables, respectively, while the parameter r refers to the weight value.

ii) The d_i values were then combined, and the overall desirability value (D) was computed using equation 6.

$$D = \left(d_1^{w_1} \times d_2^{w_2}\right) = \left(\prod_{i=1}^{2} d_i^{w_i}\right)^{1/n} \qquad (6)$$

iii) optimal process parameter levels are determined by maximizing the overall desirability values (Kumar et al., 2018).

The desirability function approach integrated with RSM was used to determine the overall desirability value. For this purpose, Design Expert 13.0 software, as a statistical tool, was used to maximize the foaming properties of the EWP-based foam system. The equal weight values for FC and FS were assigned. The index values for FC and FS were calculated by a numerical optimization tool as 0.831 and 1, respectively. Furthermore, the maximum overall desirability (D=0.912) corresponds to the optimal values of the process parameters, i.e., 0.377% guar gum, 9 min mixing time, was identified. Figure 7 shows the optimal parameter settings and predicted response values with red and blue dots, respectively. As illustrated in figure 7, the optimal parameter settings and predicted response values are represented by red and blue dots, respectively. As shown in figure 7, the FC and FS values were predicted as 740.931% and 74.35 min, respectively. To validate the values predicted by RSM, the EWP base foam was produced at the optimal values for the process parameters. Following the performance of three replicate analyses under optimal conditions, the FC and FS values were determined to be 708.32% and 70.86 min, respectively. As the deviations between the observed and predicted values were found to be within 5%, it was concluded that the developed regression model was successful in optimizing the foaming properties of EWP-based foam. Considering the process parameters, the optimization process provides valuable insights into the utilization of response surface methodology as a statistical tool for improving the foaming properties of egg white powder.

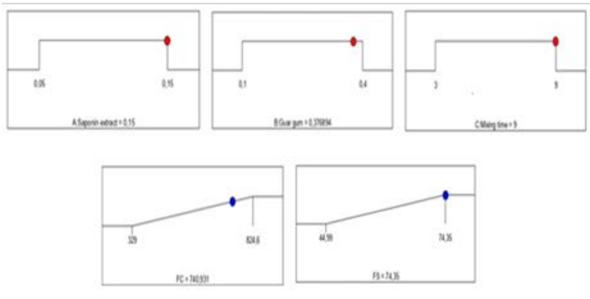

Figure 7. Ramps plot for process factors and responses.

4. Conclusion

The foaming parameters for egg white powder were successfully optimized using response surface methodology with Box-Behnken design. A total of fifteen experiments with three center points were conducted to optimize process factors, including saponin extract, guar gum, and mixing time. Following this, an analysis of variance was performed to develop quadratic models and investigate the impact of each process parameter on the. responses, foaming capacity, and foam stability. The findings indicated a correlation between foaming capacity and all process parameters, while foam stability was found to be associated with guar gum and mixing time. To further explore the synergistic interaction effect between process variables on the selected responses, 3D plots were employed. Multiple response optimization was then executed via a desirability function approach, employing Design Expert software. Confirmation experiments were conducted at optimal process conditions, i.e., 0.15% saponin extract, 0.37% guar gum, and 9 min mixing time. The experimental results obtained in this study were consistent with the predicted results, indicating that there were no statistically significant differences between predicted and observed data. Future research should explore the effect of the interactions with different saponin and/or protein sources on the foam properties of egg white protein powder. Moreover, the impact of optimal foams on final product properties in real food systems must be investigated.

Author Contributions

The percentages of the author' contributions are presented below. The author reviewed and approved the final version of the manuscript.

	M.G.
C	100
D	100
S	100
DCP	100
DAI	100
L	100
W	100
CR	100
SR	100
PM	100
FA	100

C= concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because there was no study on animals or humans.

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