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Functional Characterization of Black Bean Protein with Emphasis on Solubility, Foam Formation and Stability

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ABSTRACT

In the growing food industry landscape, the increasing attention to foam-structured foods as healthy and attractive components necessitates a deeper understanding of the functional properties of plant proteins. Plant proteins, especially black bean (*Phaseolus vulgaris*) proteins, have been considered as healthy and natural alternatives to animal proteins. However, a deep understanding of the environmental parameters affecting the solubility, foaming and stability properties of these proteins plays a key role in improving their performance. In this study, the effects of physicochemical conditions including alkaline pH (9-11), isoelectric pH (4-5) and temperature (4-24 °C) on the solubility, foaming capacity and stability of black bean proteins were investigated. The results showed that increasing alkaline pH and temperature led to a significant improvement in protein structure and the formation of a more stable layer. Higher temperatures caused partial denaturation and the emergence of hydrophobic regions, which create stronger protein networks. Under the optimal physicochemical conditions including alkaline pH (10.08), isoelectric pH (4) and temperature (24°C), the highest solubility (1.15%), highest foaming capacity (253.02%), and highest foam stability at 30 min (111.09%) and 60 min (57.80%) were observed. The software prediction results did not differ significantly from the experimental data ($p>0.05$). These findings not only provide a greater understanding of the mechanisms of foam formation and stability, but also pave the way for the development of plant-based foam products with optimized physical and functional properties.

1- INTRODUCTION

Aerated or foamed foods have long been an important part of the human diet [1]. These types of products have always attracted consumers' attention due to their light texture, attractive appearance and distinctive sensory properties, and their popularity has increased in recent years [2]. The production of these products, which are found in a variety of forms including liquid (such as carbonated drinks, instant coffee), semi-solid (such as ice cream, whipped cream, light desserts) and solid (such as bread, cake, breakfast cereals and foamed chocolates), requires a complex interaction of formulation and processing to achieve and maintain the desired properties [3].

One of the most important challenges in the production of foamed foods is the natural instability of the foam structure, this instability is due to the disinclination between air bubbles and the environment, which leads to the phenomenon of surface tension. The presence of surface-active compounds such as surfactants or proteins plays a key role in reducing the surface tension and stabilizing this structure. The surface properties and foaming ability of proteins have been extensively studied in the last few decades, with the trend towards using proteins instead of low molecular weight surfactants due to their inherent ability to form and maintain foam. In addition, increasing consumer awareness of health and the desire to use natural and sustainable ingredients has increased the use of natural proteins in the food industry [4].

In this context, a growing trend of modern research in the field of food has been directed towards the use and identification of plant proteins in a continuous and targeted manner [5]. Today, attention to plant-based diets is increasing for various reasons, including health considerations, environmental concerns, sustainability principles, and ethical values [6]. Among plant protein sources, black bean (*Phaseolus vulgaris* L.), a member of the Fabaceae family, has received special attention due to its high-quality protein, outstanding nutritional value, and availability [7,8]. Plant proteins can be divided into four main groups based on their solubility and extractability in different solvents [9]: albumins, globulins, prolamins, and glutelins. In the case of bean proteins, globulins constitute the major portion (60–80%), while albumins are the second major protein component (15–25%) and have lower water solubility [10]. In contrast to animal proteins, which generally have low molecular weight and relatively simple globular structures, plant proteins exhibit complex quaternary structures with high molecular weight monomers [11].

Despite the high nutritional value of black beans and their potential functional properties, comprehensive studies on specific properties such as solubility, foaming capacity, and foam stability of proteins extracted from this plant have been reported [12–14]. Many previous studies have focused on nutritional aspects or other functional properties, and less has been done to systematically investigation of the effect of environmental and process parameters (such as alkaline and isoelectric pH, and process temperature) on the mechanism of foam

formation and stability of black bean proteins and solubility. This research gap highlights the need for a comprehensive study to better identification of the factors affecting the functional behavior of black bean proteins. Given the increasing need for sustainable and functional plant protein sources, and the high potential of black beans; the aim of this study was to extract black bean proteins and then investigate their solubility, foaming properties (including foaming capacity and foam stability) under different physicochemical conditions (such as alkaline and isoelectric pH changes, and temperature) to provide an understanding of the factors affecting solubility, foam stability, and optimization of conditions for stable foam production from black bean proteins. The present study can help develop new and innovative applications for black beans in the food industry, especially in the production of aerated products, and provide a better understanding of the interfacial behavior of plant proteins and pave the way for the development of aerated food products based on natural and sustainable ingredients.

2- Materials and Methods

2-1- Raw Materials

All chemicals used were obtained from Merck, Germany, with laboratory grade.

2-2 Sample Preparation

The black beans (*Phaseolus vulgaris* L.) used in this study were obtained from the fields of Khomein (Iran), a native variety. Before starting the experiment, the beans were carefully cleaned of any pests, damage, or external impurities. After ensuring the purity of the product, the beans were ground using an electric grinder (Kip, South Korea) and the resulting powder passed through a sieve with a mesh size of 80 µm to ensure uniform particle size. The resulting powder was packed in polyethylene zipper bags and stored in a freezer (Pars, Iran) at -4 °C until further use [15].

2-3- Protein Extraction

Protein extraction was performed under the influence of alkaline and isoelectric pH and centrifuge temperature. The upper and lower limits of independent variables are presented in Table (1). Black bean powder was mixed with distilled water at a ratio of (1:8 g/ml) and the pH of the mixture was adjusted according to Table (2), using 2 M sodium hydroxide. The resulting mixture was stirred at room temperature for 30 minutes by a magnetic stirrer (PIT300 model, Iran). The suspension was subjected to centrifugation (UNIVERSAL-320R model, Pars Azma Iran) at the temperature specified in Table (2) for each run. The pH of the supernatant was adjusted by adding 2 M hydrochloric acid solution. The mixture was centrifuged for 20 minutes at 9000 rpm to precipitate the protein. The pH of the protein precipitate was adjusted to 7 with sodium hydroxide. The purified protein solution was then freeze dried (Dena Vacuum, Iran) for 24 hours. The dried protein powder was collected and stored in -18 °C until evaluation of functional properties [15].

Table 1- The real and coded values of independent variables for experimental design

Factor	Name	Units	Type	Minimum	Maximum	Coded Low	Coded High	Mean
A	Alkaline pH		Numeric	9.00	11.00	-1 ↔ 9.00	+1 ↔ 11.00	10.00
B	Isoelectric pH		Numeric	4.00	5.00	-1 ↔ 4.00	+1 ↔ 5.00	4.50
C	Temperature	°C	Numeric	4.00	24.00	-1 ↔ 4.00	+1 ↔ 24.00	14.00

2-4- Foaming capacity and foam stability

The foaming property of black bean protein was measured according to the method of Fongtai et al. (2016), with minor modifications [16]. In order to create foam, 10 ml of 1% (w/v) protein solution with a pH of 7 was homogenized in a graduated cylinder in an Ultratorax high-speed homogenizer for 1 min. The total volume of the solution was measured immediately after homogenization (time 0-min). Foam stability was determined by measuring the total volume of the foaming capacity at 30 min and 60 min after foam formation. Foaming capacity and foam stability were calculated in percentage using the following equations:

$$\text{Foam Capacity (\%)} = [(A - B) / B] \times 100 \quad \text{equations 1}$$

$$\text{Foam Stability (\%)} = [(A_{30\ \text{min or}\ 60\ \text{min}} - B) / (A_{0\ \text{min}} - B)] \times 100$$

Where A is the volume after foaming (ml), and B is the volume before foaming (ml).

2-5- Protein solubility

Protein solubility was investigated using the Biuret method. Protein solution preparation was based on the method of Musakhani Ganja et al. (2015) with slight modifications [17]. For this purpose, first 0.5 g of protein was dissolved in 50 ml of deionized water and then stirred for one hour with a magnetic stirrer at room temperature, and then the solution was centrifuged at 5000 g for 20 minutes. The amount of protein in the supernatant was determined by mixing 2 ml of the supernatant with 8 ml of Biuret reagent using a spectrophotometer (UNICO S2100, China). The absorbance was read at a wavelength of 540 nm. Bovine serum albumin solution was used at concentrations of 2 to 10 (mg/ml) in Biuret reagent to provide calibration curve.

2-6- Statistical analysis

In this study, the effect of independent variables including centrifuge temperature (4-24°C), alkaline pH (9-11), and isoelectric pH (4-5) was investigated on solubility, foaming capacity and stability. In this study, Design Expert software version 11, response surface methodology and Box Behnken design were used. Box Behnken design was chosen due to its advantages such as requiring fewer experiments compared to other RSM designs (such as central composite design), not requiring experiments under very severe conditions and its high ability to model nonlinear effects. 18 runs including 6 replicates at the central point were performed according to the design proposal (Table 2). In order to optimize the

conditions predicted by the software for the highest solubility, highest foaming capacity and stability, the experimental conditions were compared to the predicted conditions with the T-student test at a probability level of 5%.

3- Results and Discussion

3-1- Statistical study of optimization of process conditions

The solubility, foam capacity and stability of black bean protein were investigated using the response surface methodology. The results obtained from different treatments are given in Table (2).

The results of the analysis of variance of the effect of independent variables on the solubility, foaming capacity and foam stability of protein at 30 and 60 minutes are shown in Table (3). The p-value for the quadratic model was less than 0.0001, which indicates that the model is very significant and that the independent variables have a strong effect on the dependent variables of the protein. In other words, these variables significantly affect the solubility, foaming capacity and stability of the protein. The high R^2 value indicates that most of the changes in the dependent variables and the relationship between the independent and dependent variables can be well explained by the model. The closeness of the adjusted R^2 and predicted R^2 values indicates that the model is not overfitting and that it can be generalized to new data. In other words, the model not only fits the existing data well, but is also able to accurately predict the solubility and foaming capacity values under new conditions.

Table 2- Values of independent and dependent variables in different runs

	Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3	Response 4
Run	A: Alkaline pH	B: Isoelectric pH	C: Temperature (°C)	Foaming Capacity (%)	Foam Stability 30 min (%)	Foam Stability 60 min (%)	Solubility (mg/ml)
1	9	4.5	4	180	66	30.29	0.117
2	9	5	14	200	60.58	35.76	0.113
3	11	4.5	24	140	115	63	0.105
4	11	4	14	120	97.04	59	0.115
5	10	5	4	280	55	26.57	0.121
6	10	4.5	14	230	55.19	33.33	0.116
7	10	4.5	14	240	55.17	33.33	0.117
8	10	4	4	150	68	33.33	0.126
9	9	4	14	250	50	33.33	0.119
10	10	4	24	260	110	55.54	0.116
11	10	4.5	14	230	53.11	34.47	0.116
12	10	4.5	14	240	55.87	33.33	0.117
13	11	5	14	230	49.56	33.33	0.114
14	9	4.5	24	210	85.16	50.12	0.109
15	11	4.5	4	150	73.56	40	0.115
16	10	4.5	14	240	55.21	37	0.114
17	10	5	24	180	76.93	45.45	0.116
18	10	4.5	14	240	55.18	33.33	0.116

3-2- Evaluation of the foaming capacity response parameter

Foam formation is the process of distributing air bubbles in a continuous liquid or solid phase, resulting in the formation of a flexible surface layer. Foam capacity refers to the ability of a protein to produce foam, which can be evaluated by measuring the increase in foam volume (percentage increase). Various factors affect the foaming process of proteins. These factors include capacity and stability, pH, hydrolysis methods, protein concentration, and extraction techniques [18].

Table 3- Variance analysis of the effect of independent variables on the Solubility, foam formation capacity of black bean and protein foam stability at 30 and 60 minutes after foam formation

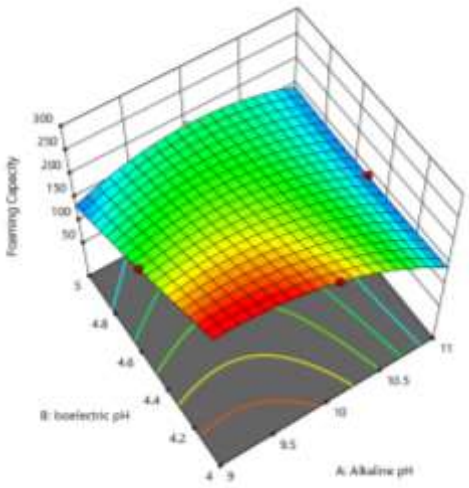
Source	df	Mean Square of Foaming Capacity	Mean Square of Foam Stability 30 min	Mean Square of Foam Stability 60 min	Mean Square of Solubility
Model	9	3970.68***	790.41***	209.53***	0.0000***
A- Alkaline pH	1	5000.00***	673.81***	262.55***	0.0000**
B- Isoelectric pH	1	1512.50***	860.50***	200.90***	0.0000**
C-Temperature	1	112.50*	1938.47***	880.32***	0.0001***
AB	1	6400.00***	842.74***	197.40***	٠,٠٠٠٠**
AC	1	400.00**	124.10***	2.51 ns	0.0000ns
BC	1	11025.00***	100.70***	2.77 ns	0.0000**
A²	1	7728.03***	307.44***	153.27***	0.0001***
B²	1	128.03*	3.91 ns	0.3840 ns	0.0001***
C²	1	2637.12***	2032.37***	146.50***	0.0000ns
Residual	8	19.79	2.07	2.06	0.000
Lack of Fit	3	8.33ns	4.05 ns	1.85 ns	0.0000ns
Pure Error	5	26.67	0.8917	2.18	0.0000
Cor Total	17				
Fit Statistics					
R²		0.9956	0.9977	0.9914	0.9730
Adjusted R²		0.9906	0.9951	0.9816	0.9426
Predicted R²		0.9835	0.9719	0.9452	0.8375
Adeq Precision		47.8750	62.0954	34.9103	26.7796
C.V. %		2.12	2.10	3.63	0.9042

ns: not significant, *: significant at the 5% level ($p < 0.05$), **: significant at the 1% level ($p < 0.01$), ***: significant at the 0.1% level ($p < 0.001$)

Figure (1) shows the three-dimensional graphs of foaming capacity measurements at time zero, or the moment of foam formation. Based on the results of the analysis of variance, the variables alkaline pH, isoelectric pH, the interaction between these two variables, and the interaction between temperature and isoelectric pH showed a significant effect on the foaming capacity of black bean protein. In Figure (1a), the effect of isoelectric pH and alkaline pH on foaming properties has been evaluated. According to the figure, it was observed that with increasing alkaline pH (from 9 to 11), the foaming capacity first increased and then decreased. This decrease could be due to the increase in the net negative charge on protein molecules, which leads to a

stronger electrostatic repulsion between them. This repulsion can prevent the formation of dense and stable layers at the air-water interface and, as a result, reduce the efficiency of foam formation. This behavior is consistent with the findings of previous studies that emphasize the importance of protein surface charge in foam formation [12,19]. According to accepted principles in protein chemistry, decrease in the net surface charge and decrease in the electrostatic repulsion between protein molecules at certain pHs can increase the rate of protein adsorption to the air-water interface. This leads to the formation of more stable protein layers and, consequently, an increase in foaming capacity [20]. In the alkaline pH range, black bean protein reaches an optimal state that maximizes the adsorption capacity and the formation of an efficient layer at the interface. According to the figure 1, it was found that the foaming property decreased with increasing isoelectric pH. At the isoelectric pH, the solubility of the protein is sharply reduced and its tendency to aggregate and precipitate increases. This situation makes it difficult for the protein to adsorb to the air-water interface and form water molecules around it, which is consistent with the results obtained regarding the decrease in foaming capacity at the isoelectric point.

In addition to pH, temperature plays an important role in foaming properties; increasing the temperature to 24°C significantly increased the foaming capacity at time zero (Figure 1c, 1b). This phenomenon can be attributed to the structural changes of the protein under heat, which lead to partial denaturation and exposure of hidden hydrophobic regions of the molecule. These structural changes increase the affinity of protein for adsorption to the air/water interface and enhance its ability to form a stable film on this surface. The increase in surface hydrophobicity enhances the affinity of protein for adsorption to the air/water interface, thereby improving the foaming efficiency. The interpretation of these results regarding the increase in foaming capacity with increasing temperature is consistent with the accepted mechanisms of protein denaturation and exposure of hydrophobic regions, which have been described by other researchers [21].



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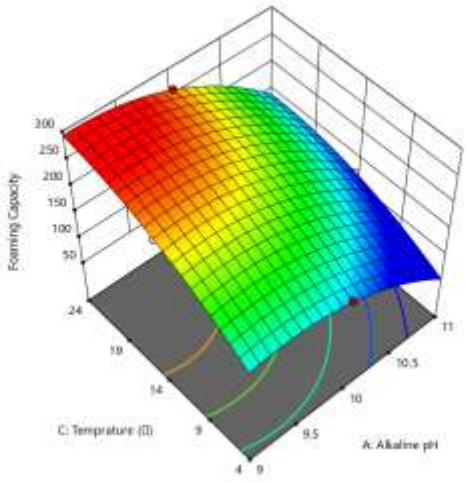

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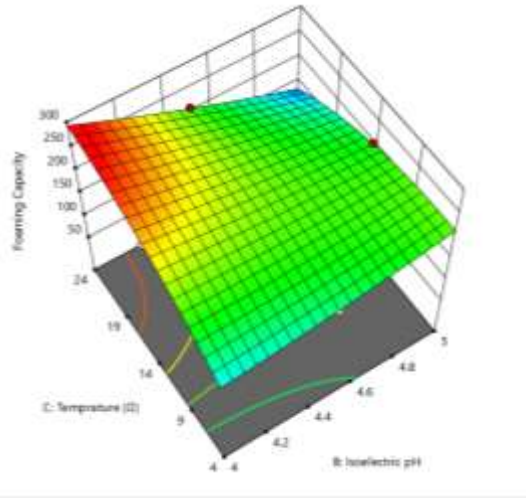


(c)

Fig. 1. The interaction of Alkaline pH and Isoelectric pH (a), temperature and Alkaline pH (b), temperature and Isoelectric pH (c) on the foam formation capacity of black bean protein at the moment of foam formation

The foam formation capacity based on actual values is obtained using equation (1):

$Y = +236.67 – 25.00\ A + 13.75\ B + 3.75\ C + 40.00\ AB – 10.00\ AC – 52.50\ BC - 42.08\ A^2 + 5.42\ B^2 – 24.58\ C^2$ ($R^2 = 0.9956$)

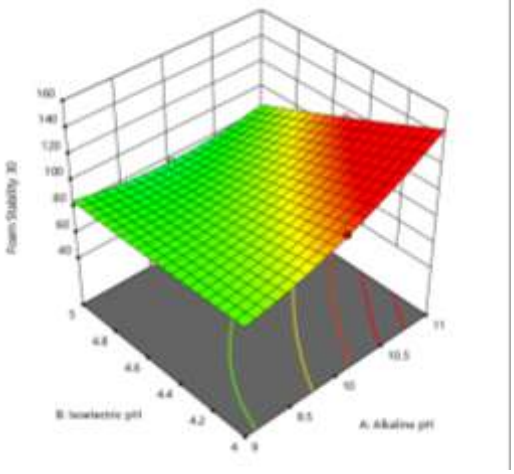
equation 1

In this relation, Y is the foaming capacity (%), A is the alkaline pH symbol, B is the isoelectric pH symbol, and C is the centrifugation temperature (°C).

3-3- Evaluation of the response parameter of foam stability at 30 minutes

The stability of protein foams is determined by the ability of the protein to form an elastic network at the air/water interface [22].
The results of the stability of black bean protein foam 30 minutes after foam formation are presented in Figure (2). Based on the results of the analysis of variance, the variables alkaline pH, isoelectric pH and temperature all had a significant effect on the stability of black bean protein foam at 30 minutes after foam formation. In addition, the interactions between these variables were statistically significant, indicating the complexity and interdependence of these factors in determining the ultimate stability of the foam. According to Figure (2a), increasing the alkaline pH, especially at low levels of isoelectric pH, led to an increase in foam stability, such that the highest foam stability was observed at pH 11 after 30 minutes of foam formation. This is directly related to the strong increase in electrostatic repulsion between protein molecules at the air/water interface. At high pH, a strong negative charge is exerted on the protein side chains, which prevents excessive adsorption and aggregation of proteins. Rather than preventing layer formation, this repulsion helps to stabilize the network and prevents the cell walls of the bubbles from being broken by strong Van der Waals forces of attraction at the interface [21].

The effect of temperature on foam stability was evident. As shown in Figures (2b, 2c), increasing temperature, along with increasing alkaline pH or at low isoelectric pH levels, improved foam stability. These findings are consistent with the results of the studies by Asdebeghi et al., who showed that increasing temperature increased the stability of lentil protein foams at 30 min after foam formation [23]. This agreement suggests that similar mechanisms are involved in the foam stability of different plant proteins at elevated temperatures. The proposed mechanism is that increasing temperature increases the structural flexibility of the protein. This flexibility allows for better and denser arrangement of proteins in the physical interface layer, which in turn leads to improved formation of optimal hydrophobic interactions between protein molecules and reduced water leakage from the foam wall. This balance between partial denaturation (hydrophobicity exposure) and adequate structural retention is the key to increased long-term stability (30 min). However, it should be noted that excessive temperature increases can lead to complete denaturation of proteins and reduced foam stability, so temperature optimization is necessary for each specific protein.



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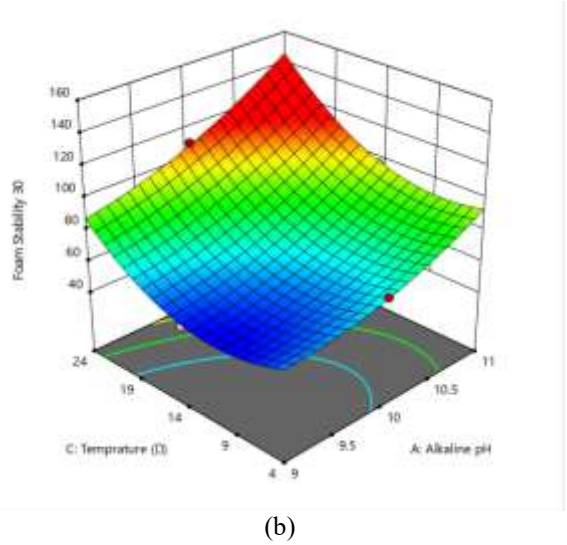

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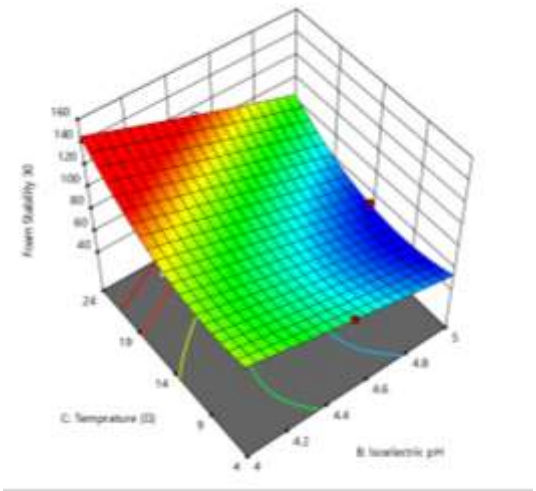


(c)

Fig. 2. The interaction of alkaline pH and isoelectric pH (a), temperature and alkaline pH (b), temperature and isoelectric pH (c) on the stability of black bean protein foam 30 min after foam formation

Equation 2 represents the stability of black bean protein foam 30 minutes after formation based on actual values.

$$Y = +54.96 + 9.18\ A - 10.37\ B + 15.57\ C - 14.51\ AB + 5.57\ AC - 5.02\ BC + 8.39\ A^2 + 0.9463\ B^2 + 21.58\ C^2 \quad (R^2 = 0.9977)$$

equation 2

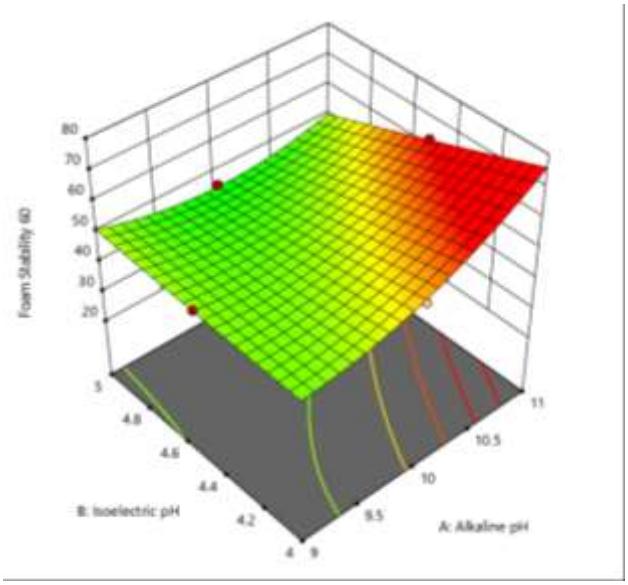
In this relation, Y is the foam stability 30 minutes after formation (%), A is the alkaline pH symbol, B is the isoelectric pH symbol, and C is the centrifugation temperature (°C).

3-4- Evaluation of the response parameter of the foam stability in 60 minutes

In examining the foam stability 60 minutes after formation, the results of the analysis of variance are presented in Table (3). These results show that the selected quadratic model, with a P-value less than 0.0001, is statistically very significant and has a high ability to describe the data. The Adeq Precision value was equal to 34.9103, which indicates the high accuracy of the model in predicting the responses. Considering the coefficient of variation, it can be concluded that the data has a low dispersion and has a good homogeneity. Overall, the results show that the selected model for examining the floor stability 60 minutes after formation has high validity and accuracy and can be used as a suitable tool for predicting and optimizing this feature.

According to the results, the stability of black bean protein foam 60 minutes after formation is affected by the variables of alkaline pH, isoelectric pH and temperature, and these factors significantly affect the foam stability. In addition, the interaction between alkaline pH and isoelectric pH is also significant. As shown in Figure (3a), increasing alkaline pH leads to increased foam stability. This phenomenon can be attributed to the increase in negative charge on protein molecules at higher pH. This negative charge increases the electrostatic repulsion between protein molecules and prevents aggregation and formation of unstable networks. As a result, air bubbles are better protected and the overall stability of the foam is maintained. This finding is consistent with the general principles of protein behavior at different pH values, where far from the isoelectric pH, proteins become more charged and have less tendency to aggregate [24].

As shown in Figure (3b), the combination of increasing alkaline pH and temperature had a positive and increasing effect on foam stability over 60 min. At alkaline pH, this denaturation and exposure of hydrophobic regions can be intensified and lead to the formation of stronger protein networks that improve foam stability over the long term (60 min). According to Figure (3c), improved foam stability was observed at lower isoelectric pH values. This indicates that in this pH range, proteins are in a state that has both a good ability to adsorb to the air/water interface and their structure is such that it can form a stable layer. In summary, a combination of factors such as increased electrostatic repulsion at alkaline pH, partial denaturation and exposure of hydrophobic regions at higher temperatures, as well as exposure of the protein to the optimal isoelectric pH for stable network formation, significantly increase the stability of black bean protein foam after 60 min. These results are consistent with previous studies on various plant proteins and suggest common principles governing the stability of protein foams. In particular, Khan et al. (2011) and Zhang et al. (2011) pointed out that at pH values further from the isoelectric point, proteins are more open and expose more hydrophobic regions for interaction with air, which contributes to increased foam capacity and stability [24-25].



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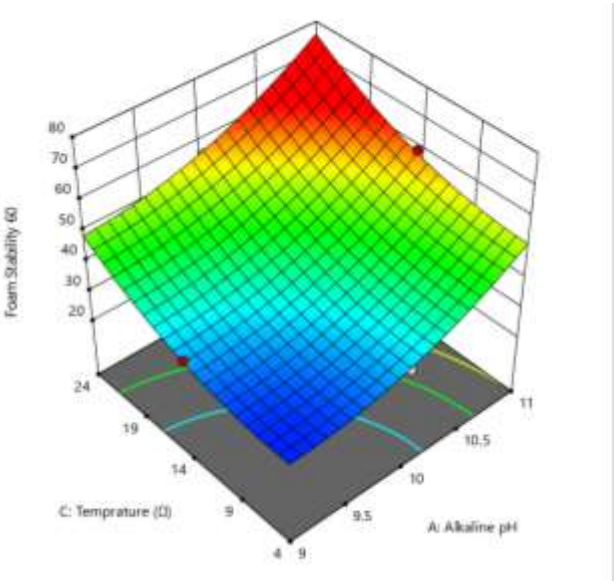

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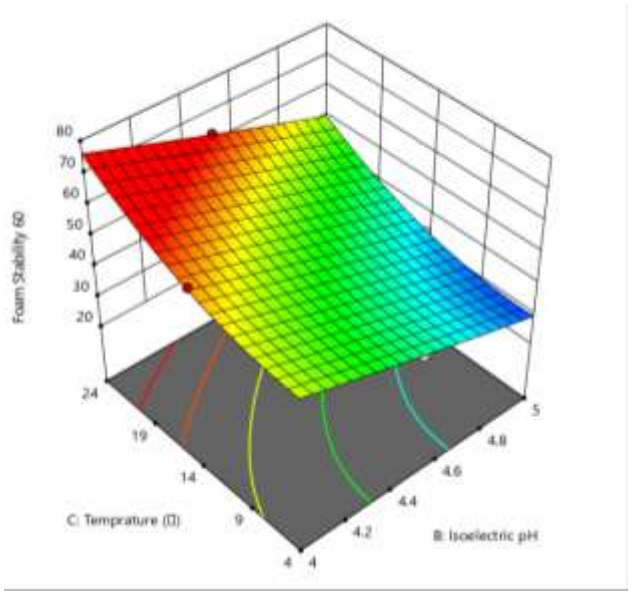


(c)

Fig. 3. The interaction of alkaline pH and isoelectric pH (a), temperature and alkaline pH (b), temperature and isoelectric pH (c) on the stability of black bean protein foam at 60 min after foam formation

The stability of black bean protein foam after 60 minutes was obtained according to equation (3):

$Y = +34.13 + 5.73\ A - 5.01\ B + 10.49\ C - 7.02\ AB + 0.7925\ AC - 0.8325\ BC + 5.93\ A^2 + 0.2967\ B^2 + 5.79\ C^2$ ($R^2 = 0.9914$) equation 3

In this relation, Y is the foam stability after 60 minutes (%), A is the alkaline pH symbol, B is the isoelectric pH symbol, and C is the centrifugation temperature (°C).

3-5- Evaluation of protein solubility

The results of the analysis of variance of the effect of independent variables on the solubility of proteins extracted from black beans are shown in Table (3). The regression model used for protein solubility was expressed using equation (4) where Y and X are the protein concentration (mg/ml) and absorbance at a wavelength of 540 nm, respectively.

equation4

$Y = 0.0498\ X + 0.0935 \quad (R^2 = 0.959)$

As can be seen in Figure (4), protein solubility is completely affected by pH. The data in this graph show three regions for protein solubility: solubility in the acidic region, near the isoelectric pH, and in the alkaline region. The lowest protein solubility was at the isoelectric pH of 4.6, which is similar to the results reported by Abdul et al., 1986; El-Hawari, 1988; Al-Nasri and Al-Tianni, 2007[26-28]. The reason of this phenomenon can be attributed to the zero net charge at the isoelectric pH and the balance between positive and negative electric charges, resulting in a decrease in repulsive forces. In this case, water-protein (hydrophilic) interactions are weakened due to the dominance of internal attraction forces (hydrogen and van der Waals bonds) and proteins begin to aggregate and precipitate due to the decrease in electrostatic repulsive forces; this is an inherent process in the behavior of proteins. The sum of these reasons reduces the solubility of proteins. At alkaline pH and acidic pH, due to the increase in the negative charge from carboxyl groups and the positive charge from amine groups, respectively, the electrostatic repulsive forces between amino acids with the same charges increase, resulting in higher solubility. Figure (4a) shows the interaction effect of alkaline pH and isoelectric pH on the solubility of protein extracted from black beans. According to the figure, it is clear that increasing alkaline pH has significantly increased solubility and its effect is greater than that of isoelectric pH. This is due to the increase in the negative charge of proteins at alkaline pH and the increase in repulsion between them. Figure (4b) shows the interaction effect of isoelectric pH and temperature on the solubility of protein extracted from black beans. According to the figure, the highest solubility was obtained at pH 4. Increasing the centrifugation temperature has reduced solubility. Increasing temperature can lead to protein denaturation and thus decrease solubility. Figure (4c) shows the interaction effect of alkaline pH and temperature on the solubility of protein extracted from black beans. According to the figure, the highest solubility was obtained at pH 10 and low centrifugation temperatures. Increasing alkaline pH and centrifugation temperature, especially at high temperatures, caused a significant decrease in solubility. These results indicate that the optimal conditions for maintaining the solubility of black bean proteins are alkaline pH and low temperature.

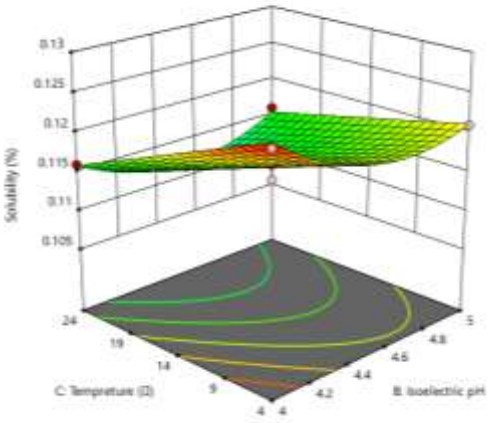
Considering the above, the solubility of black bean protein is obtained based on the coded data according to equation (5):

$Y = +0.1160 - 0.0011\ A - 0.0015\ B - 0.0041\ C + 0.0013\ AB - 0.0005\ AC + 0.0013\ BC - 0.0045\ A^2 + 0.0038\ B^2 \quad (R^2 = 0.9730)$

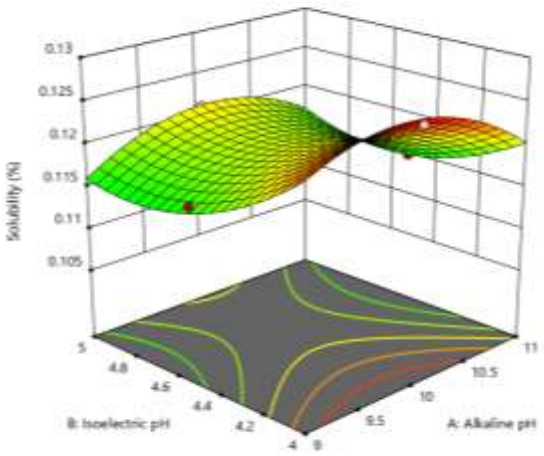
equation 5

In this relation, Y represents the solubility of black bean protein (mg/ml), A represents alkaline pH, B represents isoelectric pH, and C represents centrifugation temperature (°C).

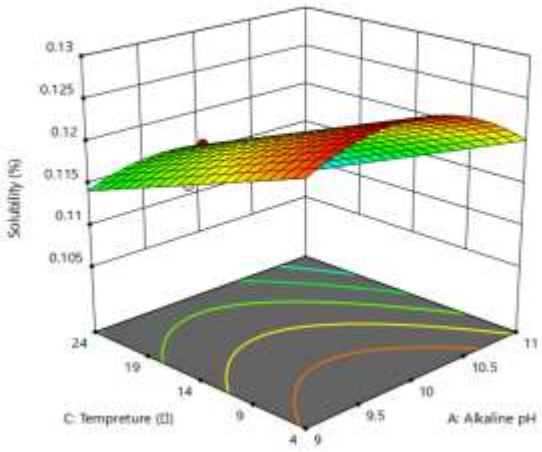
The findings of this study are consistent with previous studies on the solubility of plant proteins from different sources. For example, in a study by Fongtai et al. (2016), it was shown that increasing pH (up to about 10) increased the solubility of rice bran protein. This was explained by a similar reason, namely, increasing the negative charge of the protein and increasing electrostatic repulsion [16].



(a)



(b)



(c)

Fig. 4. The interaction of alkaline pH and isoelectric pH (a), temperature and alkaline pH (b), temperature and isoelectric pH (c) on protein solubility of black bean protein

3-6- Optimization of response parameters

For numerical optimization of foam, foam stability and black bean protein solubility, the values of independent variables were within the defined range and the dependent variables were set at their maximum. According to Table (4) and the result of Student's t-test, no significant difference was observed between the experimental data (in three replicates) and the predicted data ($p>0.05$), which indicates the appropriate accuracy of the model, desirability of 0.886 was obtained.

Table 4. Predicted and experimented optimal conditions

	Alkaline pH	Isoelectric pH	Temperature (°C)	Foam Capacity (%)	Foam Stability 30 min (%)	Foam Stability 60 min (%)	Solubility (mg/ml)
Criteria	Is in range	Is in range	Is in range	Maximize	Maximize	Maximize	**Maximize**
Predicted	10.089	4	24	253.024	111.096	57.803	**0.116**
Experimented	10.089	4	24	251±1^{ns}	110±1 ^{ns}	58.87±0.017^{ns}	**0.115±0.002^{ns}**

ns = not significant

4- Conclusion

Foam stability is a complex phenomenon that is influenced by several factors, including pH, isoelectric point, temperature, and protein structure. This study showed that black bean proteins with favorable foaming capabilities significantly contribute to foam stability under specific physicochemical conditions, especially in the alkaline pH range and at higher temperatures. With an increase in pH to 11, the foam capacity and foam stability of proteins reached their maximum level, which was due to electrostatic changes and partial denaturation of proteins. Temperature, as an effective factor on foaming properties, provided better conditions for foam formation and stabilization with increasing temperature. Under the optimal conditions of alkaline pH (10.08), isoelectric pH (4) and temperature (24 °C), the highest solubility (1.16%), the highest foaming capacity (253.02%) and the highest foam stability at 30 min (111.09%) and 60 min (57.80%) were obtained for black bean protein. The software prediction results did not differ significantly from the experimental data ($p>0.05$). These findings can help increase the use of plant proteins as a natural and healthy alternative in the food industry and produce completely natural and high-quality products.

Data Availability

The data used to support the finding of this study are available from the corresponding author upon request.

Conflict of Interest

The authors have no conflicts of interest to report.

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Author contributions

Saeideh Shafiei Varzaneh contributed to the analysis, funding, resources, methodology, preparation of the main draft, writing– review & editing, and Nafiseh Zamindar contributed to conceptualization, project management, validation, software, writing, and review and editing, Shohreh Vahed and Seyed Nezamoddin Mirsattari contributed to methodology and project consultation;

All authors read and approved the submitted version.

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تحلیل ویژگی‌های عملکردی پروتئین لوبیا سیاه با تأکید بر خواص حلالیت، کف‌سازی و پایداری آن

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چکیده

در چشم‌انداز رو به رشد صنایع غذایی، توجه فزاینده به غذاهایی با ساختار کف به عنوان مولفه‌هایی سالم و جذاب، ضرورت درک عمیق‌تر خواص عملکردی پروتئین‌های گیاهی را ایجاب می‌کند. پروتئین‌های گیاهی، به‌ویژه پروتئین‌های لوبیای سیاه (*Phaseolus vulgaris*)، به عنوان جایگزینی سالم و طبیعی برای پروتئین‌های حیوانی، مورد توجه قرار گرفته‌اند. با این حال، شناخت عمیق‌تر از پارامترهای محیطی مؤثر بر خواص حلالیت، کف‌کنندگی و پایداری این پروتئین‌ها، نقشی کلیدی در بهبود کارایی آنها ایفا می‌کند. در این مطالعه، تاثیر شرایط فیزیکوشیمیایی شامل pH قلیایی(۱۱-۹)، pH ایزوالکتریک (۵-٤) و دما (۲٤-٤ درجه سانتی‌گراد) بر میزان حلالیت، ظرفیت تشکیل و پایداری کف پروتئین‌های لوبیای سیاه مورد بررسی قرار گرفت. نتایج نشان دادند که افزایش pH قلیایی و دما، به بهبود چشمگیر در ساختار پروتئین و تشکیل لایه‌ای پایدارتر منجر شده است. دماهای بالاتر سبب دناتوراسیون جزئی و ظهور مناطق آبگریز شد که شبکه‌های پروتئینی قوی‌تر ایجاد می‌کند. در شرایط بهینه فیزیکوشیمیایی شامل pH قلیایی(۱۰.۰۸)، pH ایزوالکتریک (٤) و دما (۲٤ درجه سانتی‌گراد)، بیشترین میزان حلالیت (۱.۱٦٪)، بیشترین ظرفیت کف کنندگی (۲۵۳.۰۲٪) و بیشترین پایداری کف در ۳۰ دقیقه (۱۱۱.۰۹٪) و در ٦۰ دقیقه (۵۷.۸۰٪) مشاهده شد. نتایج پیش‌بینی نرم افزار با داده‌های تجربی تفاوت معنی‌داری نداشت ($p>0.05$). این یافته‌ها نه تنها درک بیشتری از مکانیسم‌های تشکیل و پایداری کف ارائه می‌دهد، بلکه مسیر را برای توسعه محصولات فومی گیاهی با خواص فیزیکی و عملکردی بهینه هموار می‌سازد.