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Optimizing the functional characteristics of the protein foam stability of Kabuli chickpeas of Anna variety

Haniyeh Rezaee Barzani[1], Nafiseh Zamindar*[2]

1-Master Student, Department of Food Science and Technology, College of Agriculture, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran.

2-Associate Professor, Department of Food Science and Technology, College of Agriculture, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran.

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*Corresponding Author E-n.zamindar@khuisf.ac.ir

ABSTRACT

Chickpea protein is considered a high-quality natural protein and can be used as a nutrient or the main ingredient of foods, useful for human health. In this study, the effect of four independent variables including time (20-60) minutes , temperature (4-35) degrees of Celsius, pH (8.50-10) and solid to solvent ratio of (1:10 – 1:15) was investigated on the optimization of the physicochemical properties of Anna variety chickpea protein, and its functional properties, including foam formation capacity and stability (in 30 and 180 minutes). For this purpose, 30 standard runs were performed using the response surface method, central composite design including 6 repetitions at the central points. The maximum foam formation capacity and stability was obtained with optimal conditions of temperature of 4.055 °C, time of 54.27 min, pH value of 8.517 and solvent to solid ratio of 1:10.220. The highest foam stability was observed after 40 minutes, at pH equal to 8.5. The results of this research showed that the chickpea protein of Ana variety could be used as part of food formulation, which increases the nutritional value and functional characteristics of the product.

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1-Introduction

Protein is one of the essential nutrients that we will face a shortage of it in the future. In recent years, consumer demand for plant proteins has increased significantly due to growing awareness of the negative environmental impacts of animal protein production, the health benefits of plant proteins, and the high cost of animal products [1]. Legumes are an important part of the human diet. They are rich in protein, carbohydrates, dietary fibers, and other bioactive components, and are low in fat, which helps maintain body weight and reduce the risk of cardiovascular diseases, and their consumption is spreading worldwide. Additionally, legumes are rich in vitamins such as folate, thiamine, riboflavin, niacin, and minerals (potassium, calcium, magnesium, phosphorus, and iron) [2]. Chickpeas (*Cicer arietinum* L.) are considered an important source of dietary proteins. Besides their nutritional properties, chickpea seed proteins play a significant role as functional agents in food formulation and processing [3]. One of the essential functional properties of chickpea proteins is their ability to form stable emulsions in various food systems, including cream, sauces, creamy soups, and meat products like sausages [4]. Functional properties related to physical and chemical characteristics may modify the behavior of proteins in food systems during processing, storage, preparation, and consumption. Properties such as protein solubility, water and oil absorption capacity, foaming capacity and stability, emulsifying activity, and gel formation are related to the interaction of proteins with other molecules like carbohydrates, lipids, proteins, salts, volatile compounds, and water [5-8]. Some researchers have studied the functional properties and composition of chickpea protein isolates in relation to their potential use in the food industry [6]. The effect of preparation methods on the physicochemical properties and gel formation of chickpea protein isolates has been investigated by researchers. Chickpea protein isolate was prepared from defatted chickpea flour using two methods: alkaline extraction followed by isoelectric precipitation, and protein extraction in a semi-acidic environment followed by ultrafiltration. It was found that the gel behavior of chickpea protein isolate largely depends on the preparation method [9]. The physical and chemical properties, functional characteristics, and amino acid composition of mung bean protein isolate were determined and analyzed by researchers to explore its potential in the food industry [10]. Commercial sources of chickpea protein in the food industry are limited, while its production is significant both nationally and globally. Therefore, this study aimed to extract and optimize the protein from the Kabuli chickpea variety 'Anna' and evaluate its functional properties, including foaming capacity and stability, in the formation and production of various food systems. The study also examined the impact of factors such as time, temperature, pH, and solid-to-solvent ratio on the main extraction material using response surface methodology to introduce and provide a new source of legume protein.

2-Materials and Methods

2-1. Sample Preparation

The primary material used in this research was the Kabuli chickpea variety 'Anna,' obtained from the Agricultural Research Center of Kermanshah Province. The seeds were free from any damage and pests and were cleaned of any impurities and foreign materials. They were then ground into flour

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(60 mesh) using a household electric grinder and stored at -4°C until use [6].

2-2. Preparation of Acetone Powder

To remove phenolic compounds and lipids, acetone powders were used as sources of protein extract. To prepare the acetone powders, 50 grams of dry ground chickpea sample was homogenized with 200 milliliters of acetone in a blender for 3 minutes. The resulting slurry was filtered using a vacuum pump, Buchner funnel, and ashless filter paper. The residue collected on the filter paper was dried at room temperature and stored at -18°C until used for extraction[11].

2-3. Protein Extraction

To obtain crude protein isolate, the procedure was followed as shown in Figure 1 and Table 2. The produced acetone powder (grams) was mixed with the solvent ratio provided in the RSM table with deionized water (milliliters) and stirred for 30 minutes. At this stage, the initial pH of the mixture was adjusted using 1 M sodium hydroxide (NaOH) according to the RSM table. The mixture was then centrifuged at 9000 rpm for the specified time and temperature according to the RSM table. The supernatant was collected, and the pH was adjusted to 4.5 using 1 M hydrochloric acid. The mixture was centrifuged again at 9000 rpm according to the RSM table. The resulting precipitate was suspended in a small amount of deionized water, and the pH of the precipitate was adjusted to 7 using 1 M sodium hydroxide. It was then poured into a petri dish and freeze-dried at -50°C for 24 hours. Finally, it was stored at -18°C until used for evaluating their functional properties [12].

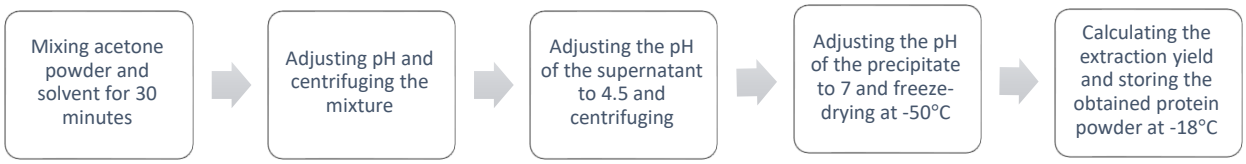

Fig 1- The procedure of chickpea protein isolate extraction

2-3. Foaming Capacity and Foam Stability

۲۵۰mg of protein was mixed with 25 mL of deionized water, and its pH was adjusted to seven using 1 M sodium hydroxide (NaOH). The protein solutions were prepared at room temperature, and then homogenized using an Ultraturrax homogenizer at 11,000 rpm for 1 minute to create foam [12]. Foaming capacity was determined by measuring the volume of foam formed immediately (time zero) in milliliters. Foam stability was determined by measuring the volume of foam in milliliters at 30 and 180 minutes.

2-4. Statistical Analysis

In this study, the response surface methodology (RSM), central composite design (CCD), and Design Expert software were used to investigate the effects of variables such as time (20-60 minutes),

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temperature (4-35°C), pH (8.5-10), and solid (chickpea acetone powder) to solvent ratio (1:10-1:15 g/mL) on the responses of foaming capacity and foam stability at 30 and 180 minutes. The number of runs, as suggested by the design, was 30 runs with 6 repetitions at the central point, according to Table 2. According to the software's prediction, after determining the optimal conditions, the product was produced under the predicted conditions, and the optimized physicochemical properties were compared using the T-Student test at a significance level of 0.05.

3- Results and Discussion

3-1. Statistical Analysis of Process Optimization Conditions Using Response Surface Methodology

Table (1) shows the actual and coded values of the independent variables used for the experimental design of Anna variety chickpea extraction.

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

Factor	Name	Minimum	Maximum	Coded Low	Coded High	Mean	Std. Dev.
A	Time	20.00	60.00	-1 ↔ 20.00	+1 ↔ 60.00	40.00	15.76
B	Temperature	4.00	35.00	-1 ↔ 4.00	+1 ↔ 35.00	19.70	12.21
C	pH	8.50	10.00	-1 ↔ 8.50	+1 ↔ 10.00	9.25	0.5909
D	Water/meal ratio	10.00	15.00	-1 ↔ 10.00	+1 ↔ 15.00	12.50	1.97

Table (2) shows the values of the independent variables used for designing the experiment and the experimental results of the response variables.

Table 2. Experimental design of variables and the test results of responses

		Factor 1	Factor 2	Factor 3	Factor 4	Response 1	Response 2	Response 3
Std	**Run**	**A:Time(min)**	**B:Temperature(°C)**	**C:pH**	**D:Water/meal ratio(ml/g)**	**Foaming capacity(ml)**	**Foam stability 1 (ml/30min)**	**Foam stability 2 (ml/180min)**
10	1	60	4	8.5	15	90	60	55
16	2	60	35	10	15	75	50	40
3	3	20	35	8.5	10	80	55	45
12	4	60	35	8.5	15	85	55	50
15	5	20	35	10	15	40	40	35
28	6	40	20	9.25	12.5	60	45	35
26	7	40	20	9.25	12.5	70	48	40
2	8	60	4	8.5	10	90	60	55
17	9	20	20	9.25	12.5	42	40	35
20	10	40	35	9.25	12.5	80	55	45
14	11	60	4	10	15	85	55	50
5	12	20	4	10	10	38	38	35
19	13	40	4	9.25	12.5	70	48	38
13	14	20	4	10	15	38	38	35
8	15	60	35	10	10	75	50	40
9	16	20	4	8.5	15	80	55	40
29	17	40	20	9.25	12.5	60	45	35
7	18	20	35	10	10	38	38	35
21	19	40	20	8.5	12.5	85	55	50

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22	20	40	20	10	12.5	50	40	35
6	21	60	4	10	10	85	55	50
4	22	60	35	8.5	10	85	55	50
1	23	20	4	8.5	10	80	55	50
24	24	40	20	9.25	15	60	45	35
25	25	40	20	9.25	12.5	60	45	35
23	26	40	20	9.25	10	60	45	35
30	27	40	20	9.25	12.5	60	45	35
11	28	20	35	8.5	15	80	55	45
18	29	60	20	9.25	12.5	60	45	35
27	30	40	20	9.25	12.5	60	45	35

3-2. Evaluation of Foaming Capacity Response Parameter

Foaming capacity is determined based on the ability of protein to reduce surface tension, molecular flexibility, and physical and chemical properties (hydrophilicity, charge and its distribution, and hydrodynamic properties). Increasing the sample concentration intensifies protein-protein interactions, which increases viscosity and facilitates the formation of a multilayer protein film attached to the surface. Additionally, an increase in sample concentration can lead to the production of thicker films [13].

The results of the analysis of variance for evaluating the foaming capacity response parameter for the full quadratic model with interactions show that the F-Value of the quadratic model is 35.09, indicating that the model is significant ($p < 0.0001$) (Table 3). The F-value for Lack of Fit in this model is 0.9617. In other words, the model fits the experimental data. The R-Squared value is 0.9704, very close to 1 indicating a high correlation between the model results and the experimental results. Adeq Precision in this model is 18.5777, indicating an adequate signal. The Pre R-Squared value is 0.8988, and the Adj R-Squared value is 0.9427.

Table3. Variance analysis of the effect of independent variables on the the foam formation capacity of chickpea protein at zero moment

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	7979/35	14	569/95	35/09	< 0.0001	significant
A-Time	2547/27	1	2547/27	156/84	< 0.0001	
B-Temperature	18/00	1	18/00	1/11	0/3091	
C-pH	2963/60	1	2963/60	182/47	< 0.0001	
D-Water/meal ra	0/2204	1	0/2204	0/0136	0/9088	
AB	64/74	1	64/74	3/99	0/0644	
AC	1156/00	1	1156/00	71/18	< 0.0001	
AD	0/2500	1	0/2500	0/0154	0/9029	
BC	4/31	1	4/31	0/2651	0/6141	
BD	0/2482	1	0/2482	0/0153	0/9033	
CD	0/2500	1	0/2500	0/0154	0/9029	
A^2	250/08	1	250/08	15/40	0/0014	
B^2	518/18	1	518/18	31/90	< 0.0001	
C^2	115/45	1	115/45	7/11	0/0176	
D^2	1/76	1	1/76	0/1085	0/7465	
Residual	243/62	15	16/24			

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Lack of Fit		160/29	10	16/03	0/9617	0/5542	not significant
Pure Error		83/33	5	16/67			
Cor Total		8222/97	29				
Std. Dev.	4/03	**R^2**	0/9704				
Mean	67/37	**Adjusted R^2**	0/9427				
C.V. %	5/98	**Predicted R^2**	0/8988				
		Adeq Precision	18/5777				

3-2-1. Three-Dimensional Plots of Response Surface for Foaming Capacity

Figure (2) shows the results of measuring the foaming capacity of chickpea protein at time zero, or the moment of foam formation. According to the analysis of variance, the variables pH and time had a significant effect on the foaming capacity of chickpea protein ($p < 0.0001$), while temperature and the solid-to-solvent ratio were not significant ($p > 0.0001$). The interaction effect of time × pH was significant ($p < 0.0001$).

In Figure (2a), the effect of time and pH on the foaming capacity of chickpea protein is evaluated. It is clear from the figure that with an increase in pH, the foaming capacity of chickpea protein decreased. Additionally, with an increase in time from 20 minutes to 60 minutes, the foaming capacity of the protein slightly increased. Figure (2b) shows the effect of temperature and pH changes on the foaming capacity of chickpea protein. The results indicate that with an increase in temperature, the foaming capacity of chickpea protein did not change. However, an increase in pH led to a decrease in the foaming capacity of chickpea protein ($p < 0.0001$). In a similar study, evaluating the functional properties of hydrolyzed Faba bean protein using a combined hydrolysis method, it was stated that the foaming capacity of Faba bean protein isolate increased with an increase in pH from 4 to 8. The highest foaming capacity of Faba bean protein was observed at pH of 8, and at pH of 10 the foaming capacity decreased in all samples [14]. Figure (2c) shows the effect of temperature and time changes on the foaming capacity of chickpea protein. The results indicate that with an increase in temperature, the foaming capacity of chickpea protein did not change. However, an increase in time slightly increased the foaming capacity of chickpea protein (the highest foaming capacity was at 40 minutes). The results indicate that with an increase in the solid-to-solvent ratio, the foaming capacity of chickpea protein did not change. Based on the mentioned points, the foaming capacity was obtained according to equation (1):

$$Y=1366.732-2.633A-2.323B-251.313C+0.567AC-0.025A^2+0.058B^2+11.43C^2$$

Where, Y represents the foaming capacity (milliliters), A represents the centrifugation time (minutes), B represents the centrifugation temperature (degrees of Celsius), and C represents the pH.

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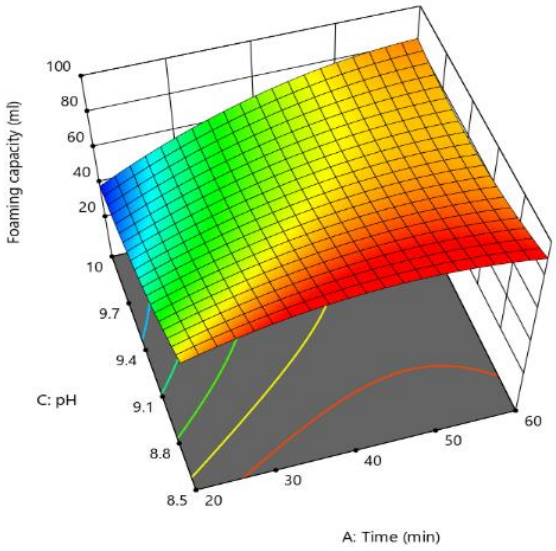

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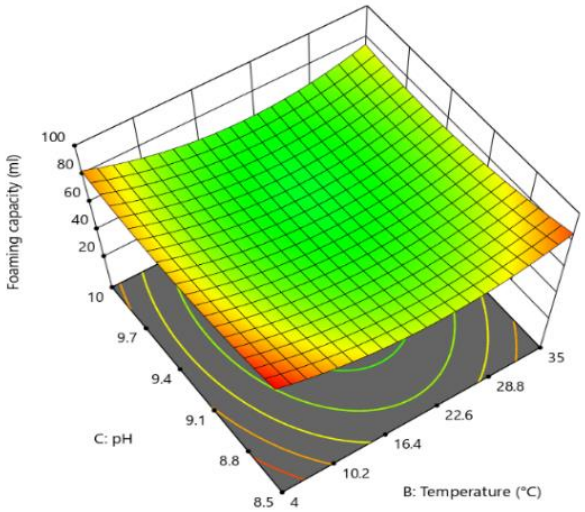

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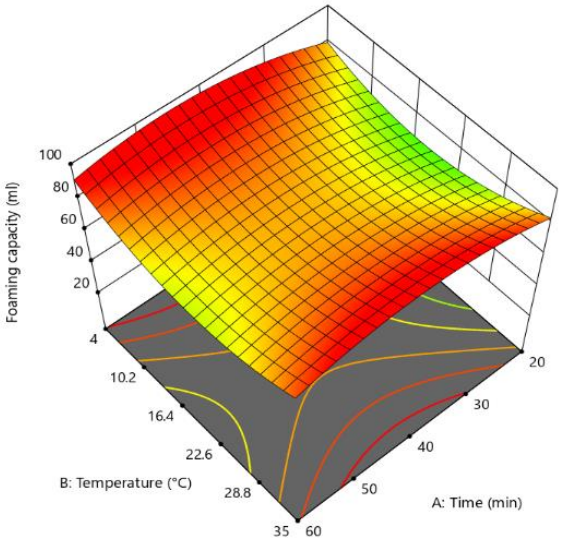

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Fig. 2. The interaction of time and pH (a), temperature and pH (b), temperature and time (b) on the foam formation capacity of chickpea protein at zero moment

3-3. Evaluation of Foam Stability Response Parameter at 30 Minutes

Foam stability is the time it takes the produced foam to reduce to 50% of its initial volume due to factors such as gravity and mechanical stresses [15].

The results of the analysis of variance for evaluating the foam stability for the full quadratic model with interactions show that the F-Value of the quadratic model is 22.99, indicating that the model is significant ($p < 0.0001$) (Table 4). The F-value for Lack of Fit in this model is 3.47. In other words, the model fits the experimental data. The R-Squared value is 0.9555, indicating a high correlation between the model results and the experimental results. Adeq Precision in this model is 16.0575, indicating an adequate signal. The Pre R-Squared value is 0.8217, and the Adj R-Squared value is 0.9139.

Table4. Variance analysis of the effect of independent variables on the stability of chickpea protein foam 30 minutes after foam formation

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1277/15	14	91/22	22/99	< 0.0001	significant
A-Time	280/75	1	280/75	70/75	< 0.0001	
B-Temperature	6/72	1	6/72	1/69	0/2127	
C-pH	566/80	1	566/80	142/84	< 0.0001	
D-Water/meal ra	0/2204	1	0/2204	0/0556	0/8169	
AB	30/50	1	30/50	7/69	0/0142	
AC	132/25	1	132/25	33/33	< 0.0001	
AD	0/2500	1	0/2500	0/0630	0/8052	
BC	0/2204	1	0/2204	0/0556	0/8169	
BD	0/2482	1	0/2482	0/0625	0/8059	
CD	0/2500	1	0/2500	0/0630	0/8052	
A^2	19/66	1	19/66	4/95	0/0418	
B^2	100/41	1	100/41	25/31	0/0001	
C^2	13/07	1	13/07	3/29	0/0896	
D^2	0/1677	1	0/1677	0/0423	0/8399	

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Residual		59/52	15	3/97			
Lack of Fit		52/02	10	5/20	3/47	0/0912	not significant
Pure Error		7/50	5	1/50			
Cor Total		1336/67	29				
Std. Dev.	1/99	**R^2**	0/9555				
Mean	48/67	**Adjusted R^2**	0/9139				
C.V. %	4/09	**Predicted R^2**	0/8217				
		Adeq Precision	16/0575				

3-3-1. Three-Dimensional Plots of Foam Stability at 30 Minutes

The foam stability of chickpea protein was evaluated by measuring the volume of foam produced 30 minutes after foam formation, with the results presented in Figure (3). According to the analysis of variance, the variables pH and time had a significant effect on the foam stability of chickpea protein at 30 minutes ($p < 0.0001$), while the parameters temperature and solid-to-solvent ratio were not significant. The interaction effects of time × pH and time × temperature were significant ($p < 0.05$).

As shown in Figure (3a), with an increase in pH, the foam volume and stability decreased ($p < 0.0001$). The highest foam stability 30 minutes after foam formation was observed at pH of 8.5. Additionally, foam stability increased over time. Figure (3b) shows the effect of temperature and time on the foam stability of chickpea protein 30 minutes after foam formation. The results indicate that foam stability increased with time. As shown in Figure (3c), with an increase in pH, the foam volume and stability decreased, but at very low or very high temperatures, an increase in foam stability was observed. Similar to the results of the present study, Samai et al. reported that the highest foam stability was observed at pH of 8 for the solution, and with an increase in pH to 10, foam stability decreased. These researchers stated that Faba bean protein isolate at pH of 4 had no foam after 60 minutes [14]. Based on the mentioned points, the foam stability of chickpea protein after 30 minutes is obtained according to equation (2):

$$y=178.608-1.105A-1.009B-15.149C-0.004AB+0.192AC-0.005A^2+0.029B^2$$

In this equation, Y represents the foam stability (milliliters) of chickpea protein after 30 minutes, A represents the centrifugation time (minutes), B represents the centrifugation temperature (degrees of Celsius), and C represents the pH.

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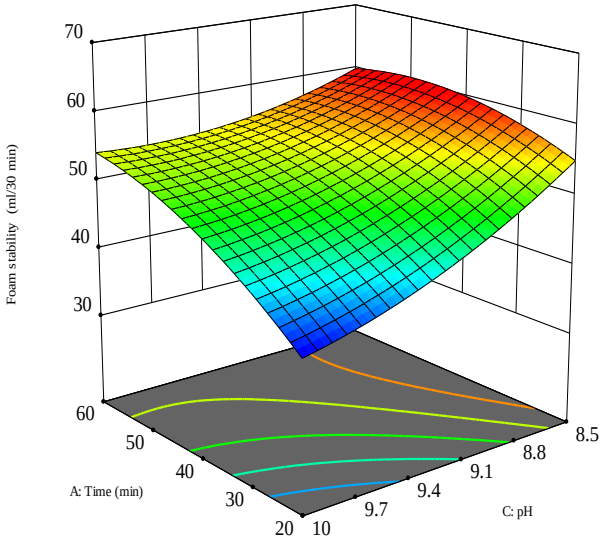

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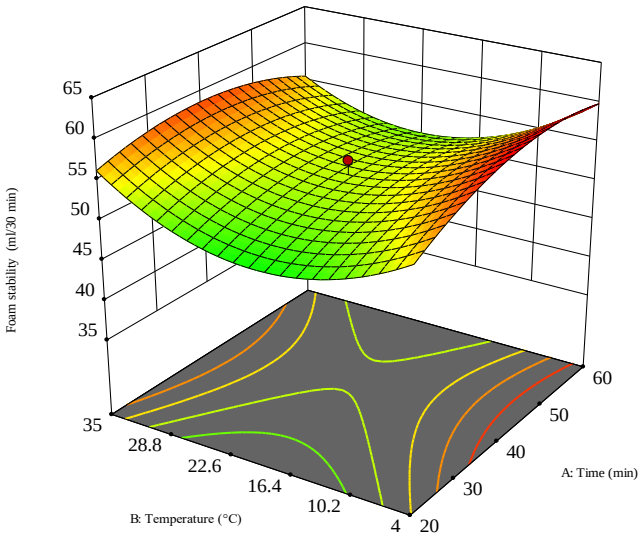

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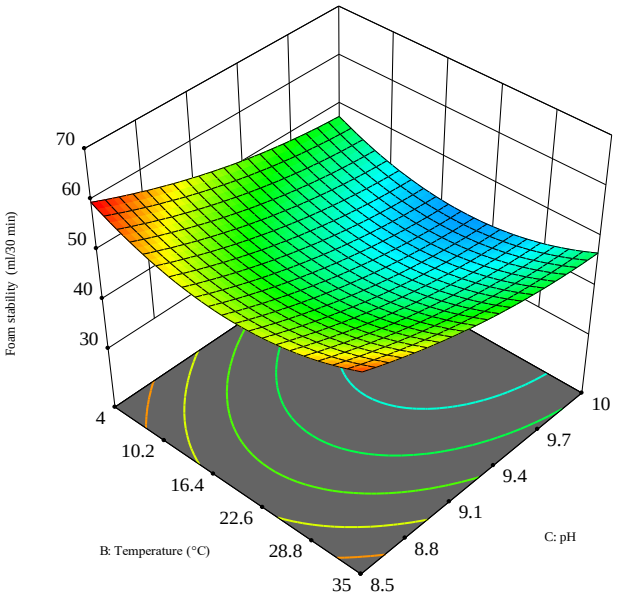

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Fig. 3. The interaction of time and pH (a), time and temperature (b), pH and temperature (c) on the stability of chickpea protein foam 30 minutes after foam formation

3-4. Evaluation of Foam Stability Response Parameter at 180 Minutes

The results of the analysis of variance for evaluating the foam stability response parameter (at 180 minutes) for the quadratic model with interactions show that the F-Value of the quadratic model is 8.86, indicating that the model is significant ($p < 0.0001$) (Table 5). The F-value for Lack of Fit in this model is 3.23. In other words, the model fits the experimental data. The R-Squared value is 0.8922, indicating a high correlation between the model results and the experimental results. Adeq Precision in this model is 11.0888, indicating an adequate signal. The Pre R-Squared value is 0.4880, and the Adj R-Squared value is 0.7915.

Table5. Variance analysis of the effect of independent variables on the stability of chickpea protein foam 180 minutes after foam formation

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1287/11	14	91/94	8/86	< 0.0001	significant
A-Time	273/17	1	273/17	26/34	0/0001	
B-Temperature	29/39	1	29/39	2/83	0/1130	
C-pH	401/00	1	401/00	38/66	< 0.0001	
D-Water/meal ra	5/60	1	5/60	0/5399	0/4738	
AB	57/19	1	57/19	5/51	0/0330	
AC	6/25	1	6/25	0/6026	0/4497	
AD	6/25	1	6/25	0/6026	0/4497	
BC	6/48	1	6/48	0/6243	0/4418	
BD	6/29	1	6/29	0/6068	0/4481	
CD	6/25	1	6/25	0/6026	0/4497	
A^2	5/30	1	5/30	0/5107	0/4858	
B^2	65/52	1	65/52	6/32	0/0239	
C^2	95/47	1	95/47	9/20	0/0084	
D^2	5/30	1	5/30	0/5107	0/4858	
Residual	155/59	15	10/37			
Lack of Fit	134/75	10	13/48	3/23	0/1036	not significant

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Pure Error		20/83	5 4/17
Cor Total		1442/70	29
Std. Dev.	3/22	**R²**	0/8922
Mean	41/10	**Adjusted R²**	0/7915
C.V. %	7/84	**Predicted R²**	0/4880
		Adeq Precision	11/0888

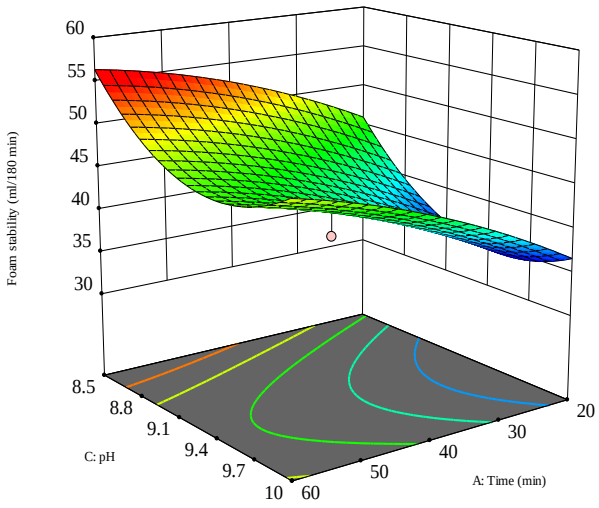
3-4-1. Three-Dimensional Plots of Foam Stability at 180 Minutes

The results related to foam stability at 180 minutes are presented in Figure (4). According to the analysis of variance, the variables pH and time had a significant effect on the foam stability of chickpea protein at 180 minutes ($p < 0.05$); however, the parameters temperature and solid-to-solvent ratio did not have a significant effect ($p > 0.05$). The interaction effect of time × temperature was significant ($p < 0.05$).

As shown in Figure (4a), with an increase in pH, the foam volume and stability decreased ($p < 0.0001$). The highest foam stability 180 minutes after foam formation was observed at pH of 8.5. Additionally, foam stability increased over time. Figure (4b) shows the effect of temperature and time on the foam stability of chickpea protein 180 minutes after foam formation. The results indicate that foam stability increased with time. As shown in Figure (4c), with an increase in pH, the foam volume and stability decreased, but at low temperatures, an increase in foam stability was observed. Based on the mentioned points, the foam stability of chickpea protein after 180 minutes is obtained according to equation (3):

$$y=830.188+0.314A-0.461B-166.497C-0.006AB+0.016B^2+8.660C^2$$

In this equation, Y represents the foam stability (milliliters) of chickpea protein after 180 minutes, A represents the centrifugation time (minutes), B represents the centrifugation temperature (degrees of Celsius), C represents the pH, and D represents the solid-to-solvent ratio (grams per milliliter).



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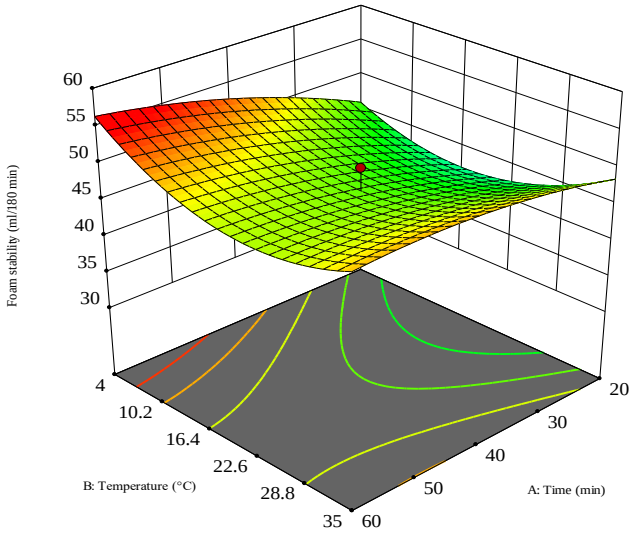
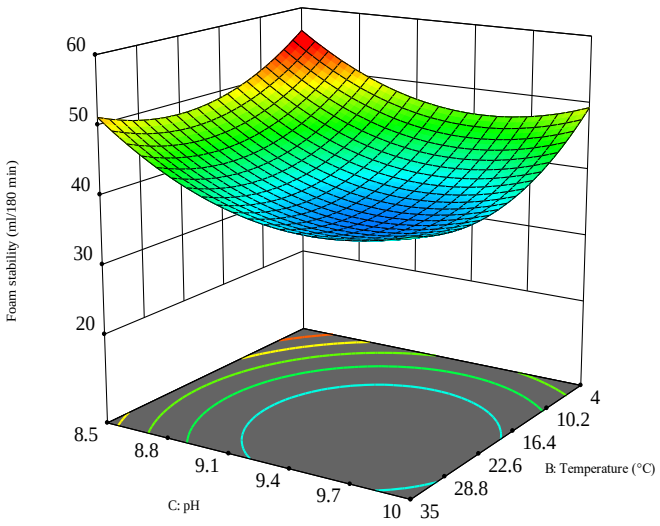
a



b



c

Fig. 4. The interaction of time and pH (a), time and temperature (b), the pH and temperature (c) on the stability of chickpea protein foam 180 minutes after foam formation

3-5. Optimization of Response Parameters

For optimizing the foam and foam stability of Kabuli chickpea protein, the independent variables were defined within the described range, and the dependent variables were maximized.

Table (6) shows the software's suggested conditions for optimizing the extraction process conditions and the results of the test conducted in three repetitions. According to table (6) and the results of the T-student test, there was no significant difference between the conducted test and the suggested conditions of software, indicating the accuracy of the model.

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Table 6. Recommended conditions of the software for optimizing the process and the tests performed

	Time	Temperature	pH	Water/ meal ratio	Foaming capacity	Foam stability (30 min)	Foam stability(180 min)
Predicted conditions	54.27	4.055	8.517	10.22	93.22	60.31	55.22
Experiments performed	54.27	4.055	8.517	10.22	94±5.1^{ns}	60±1.4^{ns}	55±1.2^{ns}

ns: not significant

4-Conclusion

In this study, the optimization of the extraction process conditions for evaluating the functional properties of foam and its stability in the Kabuli chickpea protein variety 'Anna' was carried out using the RSM method. The parameters pH, time, extraction temperature, and solid-to-solvent ratio were selected as independent variables. The results showed that the quadratic statistical model was highly accurate for predicting response parameters, and the optimization and prediction results of the model were in good agreement with the experimental results. The most important findings of this research indicate that the extraction pH of chickpea protein significantly affected the foaming capacity and foam stability at 30 and 180 minutes, and an increase in extraction pH led to a decrease in foaming capacity and foam stability at 30 and 180 minutes. The evaluation of the independent variable centrifugation time on foam and its stability also showed that with an increase in centrifugation time, the foaming capacity and foam stability at 30 and 180 minutes of chickpea protein increased. The independent variables temperature and solid-to-solvent ratio had not significant effects on all responses. The comparison of the optimal conditions suggested by the software with the test results showed no significant difference. Other components present in the sample, such as carbohydrates and fats, also affect the quality of the protein in determining functional properties. The results of this research can provide an effective outlook to use this material as an abundant and accessible source of protein and as a substitute for animal proteins in products such as coffee derivatives to produce suitable foam and improve food properties.

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مقاله علمی_پژوهشی

بهینه‌سازی ویژگی‌های عملکردی پایداری کف پروتئین نخود کابلی رقم آنا

هانیه رضایی برزانی[۱]، نفیسه زمین‌دار*[۲]

۱- دانش آموخته کارشناسی ارشد مهندسی علوم و صنایع غذایی، دانشکده کشاورزی، دانشگاه آزاد اسلامی واحد اصفهان (خوراسگان)، اصفهان، ایران

۲- نویسنده مسئول: دانشیار، گروه علوم و صنایع غذایی، دانشکده کشاورزی، دانشگاه آزاد اسلامی واحد اصفهان (خوراسگان)، اصفهان، ایران

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* مسئول مکاتبات:

n.zamindar@khuisf.ac.ir

چکیده

پروتئین نخود یک پروتئین طبیعی با کیفیت بالا در نظر گرفته می‌شود، که بعنوان یک ماده مغذی یا ماده اصلی غذاهای سودمند برای سلامتی استفاده می‌شود. در پژوهش حاضر تاثیر چهار متغیر مستقل زمان (٦۰-۲۰) دقیقه، دما (۳۵-٤) درجه سلسیوس، pH (۱۰- ۸/۵) و نسبت جامد به حلال(آب دیونیزه) (۱:۱۵-۱:۱۰) بر بهینه‌سازی خصوصیات فیزیکوشیمیایی پروتئین نخودکابلی رقم آنا، و خصوصیات عملکردی آن، شامل ظرفیت تشکیل و پایداری کف (۳۰ و ۱۸۰ دقیقه) در طی ۳۰ اجرای استاندارد با استفاده از روش سطح پاسخ، طرح مرکب مرکزی و ٦ تکرار در نقطه مرکزی، مورد ارزیابی قرار گرفت. حداکثر ظرفیت تشکیل کف و پایداری آن با شرایط بهینه‌ی دمای سانتریفوژ ٤/۰۵۵ درجه سلسیوس، زمان ۵٤/۲۷ دقیقه، مقدار pH ۸/۵۱۷ و نسبت جامد به حلال ۱۰/۲۲۰ :۱ بدست آمد. بیشترین پایداری کف پس از گذشت ٤۰ دقیقه در pH برابر با ۸/۵ مشاهده شد. نتایج این پژوهش نشان داد که پروتئین نخود کابلی رقم آنا قابلیت استفاده به عنوان جزئی از فرمولاسیون غذایی را داشته که ارزش غذایی و ویژگی‌های عملکردی محصول را افزایش می‌دهد.

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