آماده انتشار

Optimizing the functional characteristics of water and oil absorption capacity and the gel forming capacity of Kimia lentil protein isolate

Mojgan Assadbeigi[1], Nafiseh Zamindar[1*], Kimia Sahba[1]

Department of Food Science and Technology, Institute of Agriculture, Water, Food, and Nutraceuticals, Isf. C., Islamic Azad University, Isfahan, Iran

Abstract

As it is essential to adopt a global approach to sustainability, particularly concerning the use of plant proteins. Given the challenges posed by climate change and food security, shifting our focus towards sustainable plant-based protein sources has become more crucial than ever. Generally, animal protein is considered less environmentally sustainable. Therefore, a gradual transition from animal-based to plant-based protein sources may be desirable to maintain environmental stability, uphold ethical standards, ensure food affordability, enhance food safety, meet higher consumer demand, and combat protein-energy malnutrition. For these reasons, plant-based proteins contribute to better lives for people and improve the nutritional quality of our diets. This article comprehensively explores the nutritional quality of lentil proteins, cost-effective extraction and processing technologies, impacts on nutrition. These alternatives require fewer resources than animal protein and help reduce greenhouse gas emissions. By incorporating a variety of plant proteins into our diets, we can enhance nutritional security while supporting agricultural practices that are both environmentally sustainable and socially responsible. I am truly honored to submit my manuscript titled " Optimizing the Functional Characteristics of Water and Oil absorption Capacity and the Gel Forming Capacity of Kimia lentil Protein Isolate" which is exerted from thesis of my student. I encourage the journal to continue highlighting innovative research and case studies that capture these critical perspectives, as they significantly contribute to our understanding of sustainable food systems worldwide.Various plant proteins serve as essential components in food formulations, with lentils exhibiting a notably high protein content. This study evaluated the effects of three independent variables temperature (ranging from 4 to 30 °C), time, and pH (between 8.5 and 10) on the extraction rate of Kimia lentil protein and the functional characteristics of the extracted protein, including water and oil absorption capacity, as well as gel formation. The assessment was carried out through 20 standard runs utilizing response surface methodology, incorporating and six replications at the central points. The maximum protein yield was achieved under optimal conditions of 30 °C, a processing time of 20 minutes, and a pH of 8.6. The highest water absorption capacity of 0.45 g was recorded at 4 °C, while the peak oil absorption capacity of 0.92 g was observed at the corresponding pH level. These findings indicate that lentil protein isolate from the Kimia cultivar can be effectively integrated into food formulations, enhancing both the nutritional value and functional attributes of the product. Moreover, lentil protein isolate is positioned as a high-

quality natural protein, serving as a beneficial nutrient or primary ingredient in foods that promote human health.

Keywords: Functionalities, Extraction, Optimization, Lentil, Plant protein

1. Introduction

Legumes are a significant component of human diets and confer numerous health benefits. They are rich in protein, carbohydrates, fiber, and other bioactive compounds while being low in fat, which aids in weight management and reduces the risk of cardiovascular diseases. Global consumption of legumes is on the rise. Due to their high protein content and cost-effectiveness, legumes are viewed as a viable substitute for meat. Lentils, one of the most important legumes worldwide, are an abundant source of protein, fiber, vitamins, minerals, and other nutrients. They facilitate the production of short-chain fatty acids, which can mitigate obesity and associated health issues. Researchers determined and analyzed the pHysical and chemical characteristics, functional properties, and amino acid composition of isolated mung bean protein (MPI) to explore its potential in the food industry. The results indicated that the extracted protein exhibits good solubility, water retention capacity, oil absorption capacity, emulsifying properties, and foaming capacity. MPI demonstrated water retention and oil absorption capacities comparable to commercially available soy protein isolate [1]. Furthermore, a study indicated that Chinese coriander seed protein isolate (CPI) was extracted using aqueous extraction and isoelectric precipitation methods, revealing its pHysical and chemical properties. The results showed that CPI contains all essential amino acids except methionine and demonstrates high emulsion capacity, foam stability, water retention capacity, oil absorption capacity, and excellent gel formation capacity [2]. In the present study, lentil protein was extracted using alkaline extraction, and response surface methodology (RSM) was employed to optimize the extraction factors (initial pH, extraction time, and temperature) to achieve maximum yield of lentil protein isolate (LPI). The functional properties, particularly water absorption capacity (WAC), oil absorption capacity (OAC), and gel formation of the extracted protein, were analyzed, introducing Kimia lentil protein isolate as a novel source of legume protein.

2. Materials and methods

The raw material utilized in this research comprises green lentil seeds of the Kimia cultivar, procured from the Agricultural Research Center of Kermanshah province. The seeds were devoid of damage and pests and have been thoroughly cleaned of impurities and foreign substances. They were subsequently ground into flour using an electric mill and stored at 4 °C until required [3].

2.1. Preparation of Acetone Powders

To eliminate pHenolic compounds and lipids, acetone powders were utilized as sources of protein extract. The preparation involved homogenizing 50 grams of ground dry lentil samples in a blender with 200 ml of acetone for 3 minutes. The resulting slurry was filtered using a vacuum pump, Buchner funnel, and filter paper. The residue collected on the filter

paper underwent two additional rounds of homogenization with 200 ml of acetone, followed by filtration. The final powder was stored at -18°C for future extraction [4].

2.2. Protein Extraction

To obtain a crude protein isolate, 20 grams of the acetone powder was suspended in 250 ml of deionized water by stirring with a glass rod for 100 strokes. The mixture was then placed on a stirrer for 30 minutes. During this process, the initial pH of the mixture was adjusted to the desired level using one mole per liter of sodium hydroxide (NaOH), as specified in the table no 2. The mixture was subsequently centrifuged at 9000 rpm in accordance with the RSM table. The supernatant was collected and adjusted to pH 4.5 with 1 mole per liter of acetic acid. Following this, the mixture was centrifuged again at 9000 rpm, as indicated in the table no 2. The resulting precipitate was suspended in a small volume of deionized water, and the pH was adjusted to 7 using 1 mole per liter of sodium hydroxide before being transferred to a Petri dish. It was then placed in a freeze dryer at -50°C for 24 hours and subsequently stored at -18°C until characterization of its functional properties no 2.

The yield value of the method was calculated based on Eq.1

$$\text{Protein Yield}(\%) = \frac{\text{Weight}(g)\ \text{of extracted protein powder} \times \text{Protein content}(\%)}{\text{Weight}\ (g) \text{of defatted lentil meal} \times \text{Protein content}(\%)} \times 100 \qquad \text{Eq.1}$$

2.3. Gelling Capacity

The gel formation capacity of the extracted protein was assessed by determining the lowest gel concentration. A series of protein solutions in distilled water, with concentrations ranging from 14 to 19 g/100 g, was prepared. All solutions were made at room temperature and then transferred to test tubes, which were heated in a water bath at 90°C for 1 hour. The tubes were cooled to room temperature and left for 2 hours to facilitate gel formation. Gel formation was evaluated by observing the flow characteristics of the contents when the tubes were inverted. The lowest concentration of the gel that formed a firm structure, remaining intact when inverted, corresponded to the lowest protein concentration [5,6].

2.4. Water and Oil Absorption Capacity

To evaluate the water and oil absorption capacity, 50 mg of extracted protein was combined with 1.5 ml of distilled water or commercial sunflower oil in a centrifuge tube. The mixture was stirred at room temperature for 20 seconds using a glass rod. After mixing, the tubes were sealed and incubated at 30°C for 30 minutes. The tubes were subsequently centrifuged at 25°C for 30 minutes at 9000 rpm, after which the separated water or oil on the surface was carefully removed. The amount of absorbed water and oil was determined by weighing the tubes [7] (Eq. 2)

٢.Eq

$$\text{FAC or WHC} = (W_2 - W_1) / W_1$$

W_1: (Mass of dry sample (gr
$_2$W: like precipitate-Mass of gel (gr)

3. Statistical Analysis

To model and optimize the conditions for protein extraction from Kimia cultivar green lentil seeds, the effects of independent variables such as pH, centrifugation time and temperature were assessed using the response surface methodology and Design Expert software (Table 1). A central composite design with six repetitions at the central point was employed across 20 trials, examining the pHysical, chemical, and functional properties of the green lentil protein isolate. The protein extraction efficiency of the Kimia cultivar lentil was compared between model predictions and laboratory results using a t-test at a significance level of 0.05.

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

Independent variables	Coded values		
	-1	0	1
Extraction time (min)	20	40	60
Extraction Temperature (ºC)	4	17	30
Alkaline pH	8.5	9.25	10

4. Results and Discussion

4.1. Variables Examined in the Present Study

This study evaluated the effects of three independent variables—temperature, time, and pH on the optimization of lentil protein extraction across 20 experimental runs. It also assessed the pHysical and chemical characteristics of the extracted lentil protein, including water absorption capacity, oil absorption capacity, and gel formation capacity, utilizing the response surface methodology (RSM). Table 2 summarizes the treatments, independent and dependent variables, and response levels.

Table 2

Independent and dependent variables in the process of lentil protein extraction

Run	Alkaline pH	Time	Temperature	Yield (gr)	Water absorption capacity(	Oil absorption capacity	Gel capacity(gr)

					gr/ml)	(gr/ml)	
1	9.25	40	17	2.19	0.27	0.59	17
2	10	40	17	2.26	0.19	0.35	16
3	9.25	20	17	2.2	0.38	0.64	17
4	8.5	60	30	2 .08	0.28	0.92	15
5	10	20	4	1.75	0.43	0.78	16
6	10	60	30	2.44	0.22	0.6	16.3
7	9.25	40	17	2.27	0.28	0.55	17
8	9.25	40	4	2.15	0.43	0.83	17
9	8.5	40	17	2.2	0.17	0.43	17
10	8.5	20	30	2.33	0.21	0.8	17
11	10	20	30	2.6	0.3	0.65	17
12	9.25	60	17	2.3	0.35	0.8	16
13	8.5	60	4	2.35	0.44	0.9	18
14	10	60	4	2.04	0.32	0.85	15.3
15	9.25	40	17	2.14	0.26	0.65	17
16	9.25	40	17	2.15	0.31	0.64	17
17	8.5	20	4	2.12	0.37	0.64	19.3
18	9.25	40	17	2.28	0.3	0.61	17
19	9.25	40	30	2.34	0.271	0.9	16
20	9.25	40	17	2.14	0.3	0.68	17

4.2. Optimization of the Lentil Protein Extraction Conditions

The study investigated the effects of temperature (C), time (B), and pH (A) on the efficiency of lentil protein extraction. Analysis of variance indicated that neither pH nor time had a significant impact on the amount of extracted lentil protein ($p > 0.05$). In contrast, temperature significantly influenced protein extraction ($p < 0.05$). According to the results, with the increase in temperature from 4 to 30°C, the amount of lentil protein extraction increased. So that the maximum amount of lentil protein extraction was at 30°C , but the increase in time and pH on lentil protein extraction was not significant ($p > 0.05$). According to the results, with the increase in temperature from 4 to ٣٠°C, the amount of lentil protein extraction increased, such that the maximum amount of lentil protein extraction was at 30 °C . However, the increase in time and pH on lentil protein extraction was not significant ($p > 0.05$). The results indicated that as temperature increased from 4 to30°C, the amount of lentil protein extracted also increased, reaching a maximum at 30°C. However, increases in time and pH did not significantly affect lentil protein extraction ($p > 0.05$). The results showed that increasing the temperature from 4 to 30°C led to a rise in lentil protein extraction, with the maximum extraction occurring at 30°C. However, variations in time and pH did not significantly affect lentil protein extraction ($p > 0.05$). Specifically, as temperature increased from 4 to 30°C, the amount of lentil protein extracted also increased, peaking at 30°C. However, variations in time and pH did not significantly affect lentil protein extraction ($p > 0.05$). The results demonstrated that as temperature increased from 4 to 30 °C, the amount of lentil protein extracted also increased, peaking at 30°C. However, variations in time and pH did not significantly affect lentil protein extraction ($p > 0.05$). Overall, the findings suggest that higher temperatures enhance lentil protein extraction, with the maximum yield observed at 30°C, while changes in time and pH do not significantly impact extraction efficiency ($p > 0.05$).The three dimensional contour plots presented in Figure 1 illustrate the interaction

effects of temperature and pH on lentil protein extraction, while Figure 2 depicts the influence of temperature and time on the extraction process. Figure 1 indicates that the steep slope of the three-dimensional grapH signifies a substantial impact of temperature on the quantity of lentil protein extracted. Notably, at a temperature of 30°C, extraction efficiency increases as pH rises above 9.7. Conversely, Figure 2 reveals that, at a constant pH of 10, a decrease in temperature adversely affects protein extraction, whereas an increase in temperature enhances it. The highest yield of lentil protein extraction is observed at 30°C. Additionally, at a constant pH of 10, the amount of extraction diminishes over time as temperature increases. Ee-San Tan et al. (2014) conducted a study on optimizing protein extraction from pinto beans using the response surface method, achieving a maximum yield of 54.8 mg/g under optimal conditions of 25°C, 1-hour extraction time, and a buffer-to-sample ratio of 20 ml/g.

Eq.3

$$Y= 2.22 + (0.001\times A) + (0.021\times B) + (0.138\ C\times) + (0.1638 \times A \times C) - (0.1162 \times B \times C)$$

1. In this equation, Y is the Extraction of lentil protein (gr), A stands for pH, B for Centrifiugation time and C is the Centrifiugation temperature.

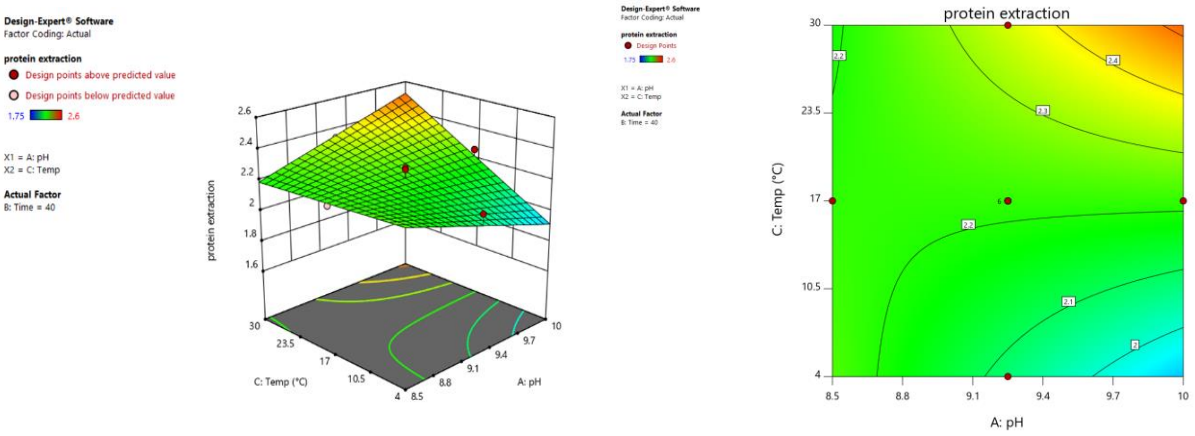

Fig1. The interaction of temperature and pH on the amount of lentil protein extraction

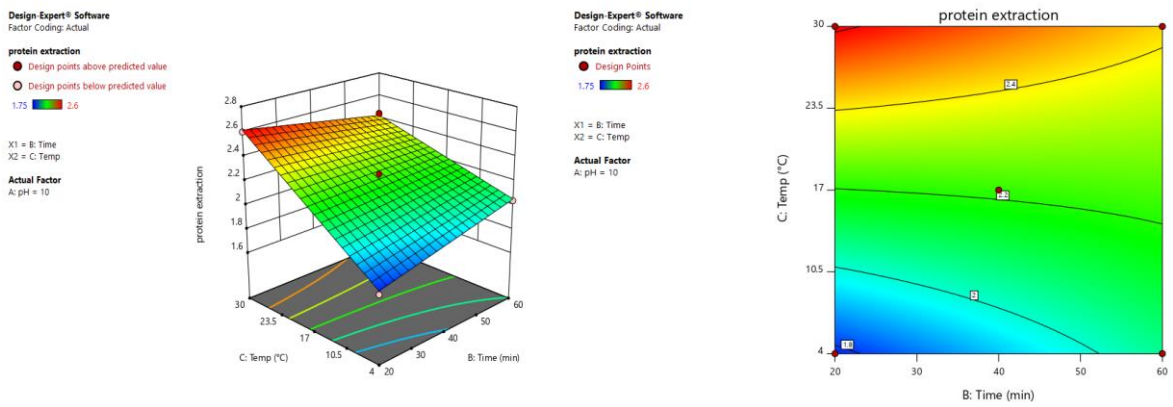

Fig2. The interaction of time and temperature on the amount of lentil protein extraction

4.3. Water Absorption Capacity (WAC)

Water absorption capacity (WAC) quantifies a material's ability to absorb and retain water molecules under conditions of limited water availability. This capacity varies among different foods and is influenced by factors such as amino acid composition, the spatial arrangement of proteins, the levels of hydropHilicity and hydropHobicity of the proteins, and the presence of hydropHilic carbohydrates. Results indicate that increasing temperature significantly affects the water absorption capacity of lentil protein ($p < 0.01$), with a decrease in absorption capacity as temperature rises. The maximum water absorption capacity of lentil protein (0.45 g) was observed at 4°C($p < 0.01$), with a notable decrease in absorption capacity as temperature rises. The maximum water absorption capacity of lentil protein, recorded at 4°C, is 0.45 g ($p < 0.01$), with absorption capacity decreasing as temperature rises. The maximum water absorption capacity of lentil protein, recorded at 4°C, is 0.45 g. Furthermore, the effect of pH on the water absorption capacity of lentil protein shows that increasing pH does not significantly influence this capacity ($p < 0.01$) and with increasing temperature, the water absorption capacity of lentil protein decreased. So that the maximum water absorption capacity of lentil protein (0.45 g) was observed at 4 . The evaluation of the effect of pH on the water absorption capacity of lentil protein also showed that the increase in pH did not have a significant effect on the water absorption capacity of lentil protein ($p > 0.01$), and with increasing temperature, the water absorption capacity of lentil protein decreased. As temperature increases, the water absorption capacity of lentil protein continues to decrease. The maximum capacity of 0.45 g was consistently observed at 4°C. Additionally, the impact of time on water absorption capacity was found to be insignificant ($p > 0.01$). Figure 3 illustrates the changes in water absorption capacity of lentil protein concerning time and pH. Research has reported the water absorption capacity of cable chickpea protein isolate as 2.1 grams of protein, while estimates for the protein water storage capacity of seeds range around 1.4 grams of protein. In contrast, the water storage capacity of lentil protein has been determined to be between 0.43 and 0.48 grams of water per gram of protein. These findings align with the current study, which estimates the water storage capacity of lentil protein to range from 0.17 to 0.44 grams of water per gram of protein.

Eq.4

$$Y = 0.2868 - (0.0709 \times C2) - (0.0413 \times A \times B) + (0.0112 \times A \times C) - (0.107 \times A\,2) + (0.078 \times B\,2) + (0.0635 \times C\,2)$$

4.4. In this equation, Y is the Water holding capacity(gr/gr), A stands for PH, B for Centrifiugation time and C is the Centrifiugation temperature.

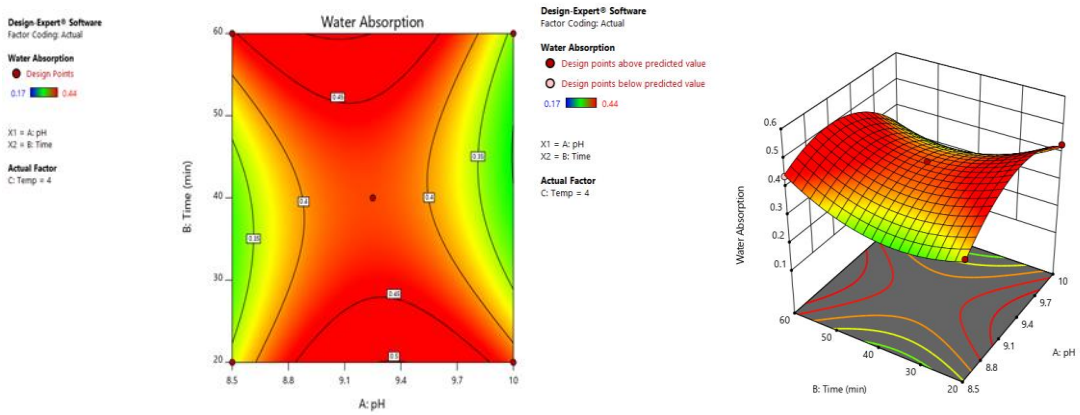

Fig3. The interaction of time and PH on the water absorption capacity of lentil protein

4.4. Oil Absorption Capacity (OAC)

The oil absorption capacity (OAC) is a pHysical pHenomenon defined as the pHysical confinement of oil, attributed to the nonpolar chains and steric shape of proteins [12]. The three-dimensional contours and diagrams presented in Figures 4, 5, and 6 illustrate the effectiveness of lentil protein oil absorption capacity in relation to temperature, PH, and time. Figure 4 investigates the combined effects of temperature and PH on the oil absorption capacity of lentil protein. The grapH shows that, at a fixed time of 40 minutes, increasing the temperature from 4°C to 17°C results in a decrease in oil absorption capacity. However, as the temperature rises further to 30°C, the absorption capacity increases again. Additionally, as the PH rises from 8.5 to 9.25, the oil absorption capacity also increases, but a further increase to PH of 10 leads to a decrease in capacity. Figure 5 presents the results related to the interaction between time and PH on the absorption capacity of lentil protein oil. At a constant temperature of 30°C, the oil absorption capacity increases with PH up to 9.7, but decreases again as the PH continues to rise, making the environment more alkaline. Moreover, as the time increases from 20 to 60 minutes, the absorption capacity of lentil protein oil increases slightly, reaching its highest point at 30 minutes. Figure 6 reports the mutual effects of temperature and time on the absorption capacity of lentil protein oil absorption. At a constant PH of 9.25, increasing the temperature from 4°C to 30°C and the time from 20 to 60 minutes significantly enhances the absorption capacity. [13] Reasearchers reported that the absorption capacity of quinoa protein oil using the freeze-drying method, similar to the method employed in this research, was significantly higher than that achieved through vacuum and spray drying methods, measuring 3.19 grams at PH 7 [13].

Eq.5

$$Y= 0.6153\ (0.046\times A)\ +(0.056\times B)\ -(0.045\times A\times B)-(0.07\times A\times C)+(0.0325\times B\times C)+(0.2182\times A\ 2\ )+(0.118\times B\ 2\ )+(0.2568\times C\ 2\ )$$

4.5. In this equation, Y is the Fat absoption capacity(gr/gr), A stands for PH, B for Centrifiugation time and C is the Centrifiugation temperature.

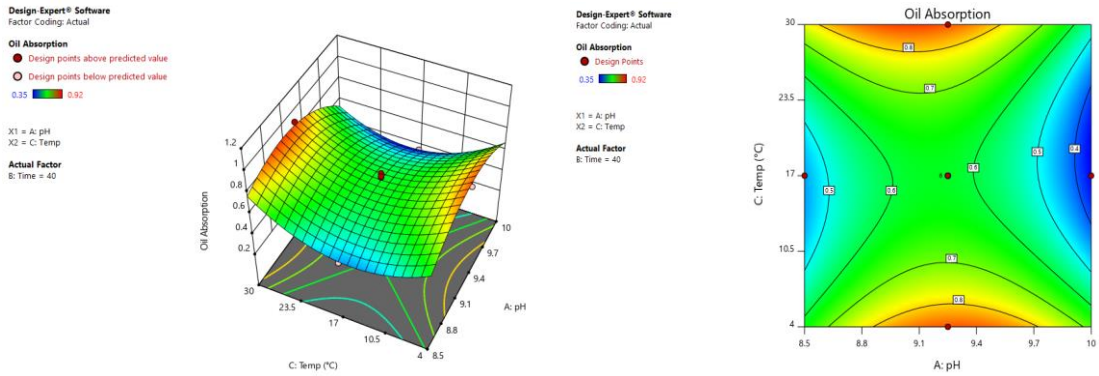

Fig4. The interaction of temperature and PH on the oil absorption capacity of lentil protein

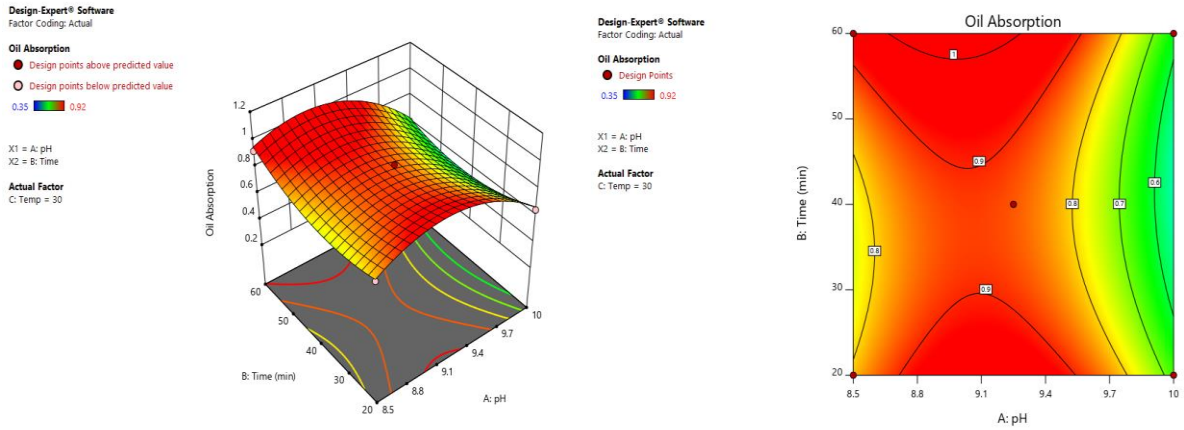

Fig5. The interaction of time and PH on the oil absorption capacity of lentil protein

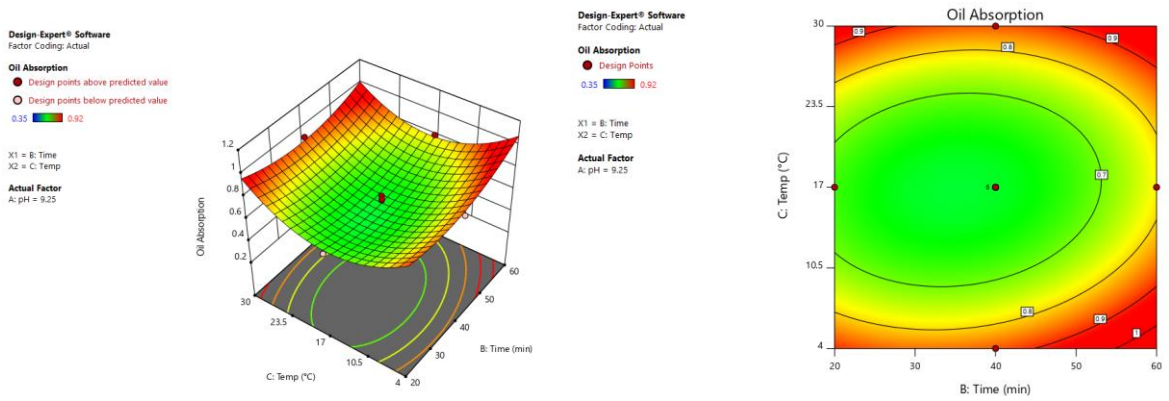

Fig6. The interaction of temperature and PH on the oil absorption capacity of lentil protein

4.5. Gel Capacity

The capacity of proteins to form gel is essential for achieving optimal sensory and textural qualities in food products. Denaturation or the unfolding of the native protein structure is a critical prerequisite for effective gelation. This process can be influenced by various factors, including pHysical variables such as heat and pressure, as well as chemical factors like acidity, ionic strength, and enzymatic action. Figures 7 and 8 provide compelling evidence regarding the relationship between independent variables and protein gel formation capacity. In Figure 7, as PH rises from 8.5 to 10, we observe a significant decline in the capacity for protein gel formation. Furthermore, as time progresses, the gel formation capacity of lentil protein continues to decrease. The subtle slope of the curve suggests that changes in PH and time exert a comparable influence on the protein's ability to form gels, underscoring the importance of both variables. Figure 8 further empHasizes this relationship, at a controlled time of 20 minutes, the capacity for lentil protein gel f'rmation diminishes with increasing temperature and pH. Notably, the maximum gel-forming capacity is achieved at a PH of 8.5 and a temperature of 4°C. The prominent slope of this curve reveals the profound impact of PH on gel formation, highlighting its critical role in the process. Reaserchers have estimated that the gel formation capacity of seed protein is around 8%, suggesting a robust potential for gel development [14]. Additionally, research by Ee-San Tan et al. (2014) found that pinto beans failed to form a gel at concentrations between 6% and 14%. However, at a concentration of 16%, a weak gel was achieved. Reaserchers demonstrates that the protein gel formation capacity is notably within the range of 16%, showcasing its promising potential for high-quality gel formation.

Eq.6

$$Y = 16.74 - (0.57\times A) - (0.57\times B)\ (0.43\times C) + (0.2375 \times A \times B) + (0.9125 \times A \times C) - (0.0875 \times B \times C)$$

1.1. In this equation, Y is the Gel capacity(gr), A stands for pH, B for Centrifiugation time and C is the Centrifiugation temperature.

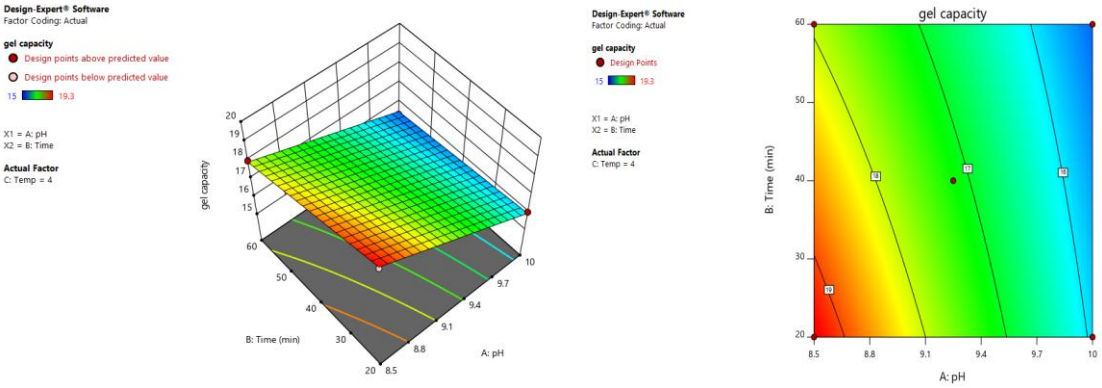

Fig7. The interaction of time and pH on the gel capacity of lentil protein

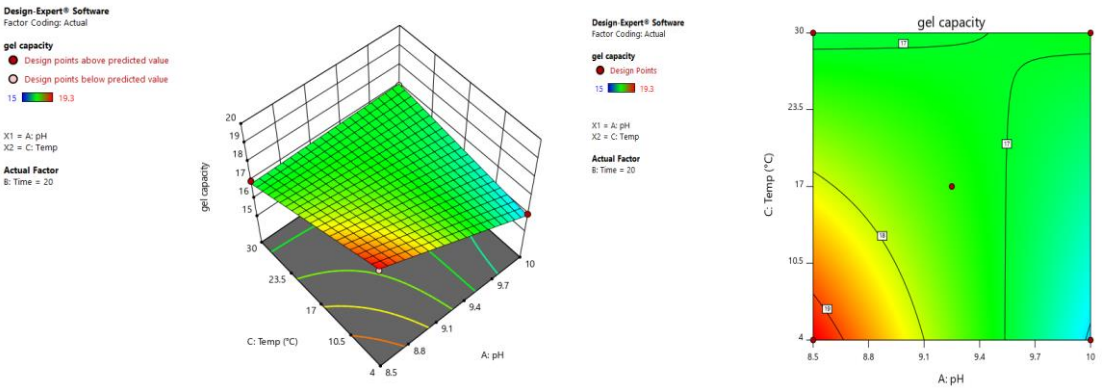

Fig8. The interaction of temperature and pH on the gel capacity of lentil protein

4.6. Optimization of Response Parameters

This study aimed to optimize the extraction process and evaluate the functional properties of lentil protein from the Kimia cultivar using Response Surface Methodology (RSM). After analyzing the variance of the data, we fine-tuned the independent variables based on various pHysicochemical tests to achieve a desired protein with the highest efficiency, water absorption capacity, oil absorption capacity, and gel formation. The optimization and extraction of lentil protein were carried out according to the criteria set by our software. The predicted optimal conditions, summarized in Table 3, included a temperature of 30°C, a time of 20 minutes, and a pH of 8.6. We compared the predicted data with the laboratory results using the T-student test and found no statistically significant differences ($p < 0.05$). This indicates that the model's accuracy was appropriate.

Table 3

Comparison of the predicted values of the software and experimental values in the pHysicochemical properties of lentil protein by T. student test

Parameters	Software prediction	Average of experiments	p-value
Protein extraction	2.312	2.333± 0.47	0.607^{ns}
Gel capacity	16.884	16.886± 0.47	0.562^{ns}
Oil absorption capacity	0.866	0.833± 0.47	0.721^{ns}
Warer absorption capacity	0.238	0.23± 0.21	0.563^{ns}

ns:not significant($p \geq 0.05$)

5. Conclusion

The results demonstrated that the quadratic statistical model is highly effective in predicting the response parameters. Furthermore, the optimization and prediction results from the model aligned well with the experimental data. Statistical analysis showed no significant differences between the predicted and experimental data for protein extraction, water holding capacity, oil absorption capacity, and gel capacity ($p \geq 0.05$). These findings suggest that lentil protein can serve as an abundant and accessible source of nutrition for products in the food industry. Overall, this research indicates that lentil protein possesses favorable functional characteristics, making it a useful component for enhancing the properties of food and demonstrating great potential for new food formulations.

Research involving human participants and/or animals
Not applicable.

Informed consent
Not applicable.

CRediT authorship contribution statement
Mozgan Asadbeigi: Writing – original draft, Software, Reosources, Methodology, Investigation, Data curation. Nafiseh Zamindar: Supervision, Project administration, Conceptualization. Kimia Sahba: Writing – review & editing, Software, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.Data availability

Data availability

All data of this current research are represented in the form of grapHs and the numeric data will be made available upon request.

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