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Optimization of Water and Oil Absorption Capacity of Bilesavar Lentil Protein by Surface Method

Dehghani, M. [1], **Zamindar, N.** [2*]

1. Graduated Master, 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-Mail:
n.zamindar@khuisf.ac.ir

ABSTRACT

The increase in the needs of the population for protein causes research on natural and plant products that can be a good substitute for animal proteins. Lentil is a rich source of protein containing 20/6 to 31/4% protein. As a result, trying to investigate the physicochemical properties of its protein and replacing animal proteins is considered important. In this research, the optimization of water and oil absorption capacity of Bilesawar green lentils was investigated using the response surface method and Design Expert 11 software. For this purpose, central composite design with three independent variables and 6 central points was used. 20 treatments resulting from the combination of three independent variables pH (8/5-10) and centrifugation time (20-60 minutes) and centrifugation temperature (4-30°C) were evaluated. The results showed the decreasing effect of pH and time on the water absorption capacity, indicating that with the increase of pH and time, the water absorption capacity decreased. Temperature and pH had decreasing effect and time had an increasing effect on oil absorption capacity. Applying the optimized conditions, the oil and water absorption capacity for the optimum point were obtained 1/235 and 1/373, respectively. For validation, optimized predicted and experimented data were compared using T student test ($p<0/05$).

1. Introduction

The food system takes inputs from the world in the form of natural resources: land, sunlight, water, fossil energy, and chemicals. These inputs work together to produce food for human societies. The food system also produces undesirable results in the form of solid, liquid and gaseous wastes. The existing human pressure on limited land resources and the simultaneous dynamics of climate change raise serious concerns about the resilience of agricultural food and feed chains [1]. Human and natural disasters and poverty prevent access to food for those who need it the most [2]. Over the past decades, the food processing industry has become a dynamic and complex mix of material flows that are combined into durable and standardized food products and widely distributed to consumers [3]. Technological innovation is often used to solve enduring challenges, and firms reorient by adjusting inputs and processes to deliver new products [4]. The increase in the needs of the population for protein causes research on natural and vegetable products that can be a good substitute for animal proteins [5]. Assessments show that food-related activities cause a major part of global environmental pressure due to continued growth in world population and per capita income [6-8]. The emission of greenhouse gases or the amount of environmental destruction related to the usual meat diets is several times compared to plant diets [9, 10]. Most research on the environmental impacts of dietary patterns has focused on reducing greenhouse gas emissions. Compared to the UK benchmark diet, the reduction in greenhouse gas emissions was estimated to be 22% for vegetarians and 26% for vegans [11]. Protein is one of the main nutrients that we will be lacking in the future. Dietary proteins come from either animal sources (such as meat, eggs, and dairy) or plant sources (such as legumes, grains, and microalgae). In recent years, consumer demand for plant proteins has become more important than animal proteins due to increasing knowledge about the negative environmental effects caused by animal protein production and the relationship between plant proteins and health and the high cost of animal products [12]. Seeds are a special source of edible proteins due to their relative abundance, stable supply and low cost [13]. Proteins as concentrates or isolates are used as a functional ingredient primarily to increase nutritional quality and provide desirable sensory properties such as structure, texture, flavor and color for formulation. Food products, concentrates and extracted proteins that are used in the food industry today are mostly obtained from soy, whey and wheat. Recently, there has been an increased interest in separating grain products into their components (protein, starch and fiber) [14, 13] and using these components as components of food systems [15, 16]. The challenge of traditional extraction methods is their inability to disrupt structural molecules. Therefore, about half of the proteins in a plant system are extracted [17]. Several studies have been done on legume proteins [18-20, 3]. Researchers have proven that the production of animal protein requires 11 times more fossil energy than plant protein for human consumption. However, the ratio of energy consumption to protein production varies greatly. Energy consumption for chicken protein is 4 times and for beef protein 40 times more than grain protein [23-21]. The land required for the preparation of animal protein is 6 to 17 times more than soy protein [24]. Lentils with a scientific name*Culinary lenses* It is a plant from the legume family that is rich in fiber and low in fat. Having 20.6 to 31.4 percent of protein after soybeans and hemp, lentils have the third highest level of plant protein and are an important part of the diet in many regions of the world, especially in the Indian subcontinent, which has a large population of vegetarians. [25 and 26]. Most of these proteins are located in the cotyledon and contain a small percentage of sulfur-containing amino acids. Lentil proteins consist of about 16% albumin, 70% globulin, 11% glutelin, and 3% prolamin [27]. Lentils are an excellent source of several nutritional factors such as dietary fiber, vitamins and minerals [28], [29]. It is also a rich source of important micronutrients such as iron and zinc[30] The nutritional properties of lentils are associated with cholesterol reduction, lipid-lowering effects in humans, and reduced incidence of colon cancer and type 2 diabetes[13]. Lentil protein hydrolysates have antihypertensive effect because they can act as inhibitors of angiotensin I converting

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enzyme[31]. Plant proteins can be separated from seeds and converted into functional elements using suitable commercial extraction and purification methods [21-23]. The present study was conducted in order to optimize the water absorption capacity and the protein oil absorption capacity of Bilehsawar variety of green lentils under the influence of independent variables including pH, centrifugation time and centrifugation temperature.

2- Materials and methods

2-1- sample preparation

Green lentil samples of Bilehsawar variety were obtained from Sablan Corn Research Company located in Ardabil province and used for the experiment. Green lentils were stored in the refrigerator at -4°C. The lentil seeds were cleaned from foreign materials, including stones, and then ground by an electric mortar and turned into powder.

2-2- Design of experiments

2-2-1- Preparation of acetone powders

To prepare acetone powders (AP), according to the method of Arkan and Yemincioglu (2007), first, 50 grams of dry ground lentil sample was mixed with 200 ml of cold acetone for 3 minutes and then filtered. Then it was filtered as in the previous step with two repetitions. Finally, the obtained powders were stored in the freezer at -18°C to be used during extraction. Also, in order to extract protein from the samples under the influence of 3 independent variables of centrifugation time, centrifugation temperature and pH, according to Arkan and Yemincioglu's method based on Table 1, the extracted proteins were transferred to a freezer with a temperature of -18°C to be used in the next experiments. be used

2-2-2- Measurement of water and oil absorption capacity

In order to check water absorption capacity and oil absorption capacity according to the method of Arkan and Yemincioglu [32], 50 mg of extracted protein was mixed with 1.5 ml of distilled water or commercial sunflower oil at room temperature for 20 seconds with a glass rod. After mixing, they were kept inside the incubator at 30°C for 30 minutes. Then they were centrifuged for 30 minutes at 25°C with 9000 revolutions and the separated water or free oil in their supernatants was carefully removed and collected. The amount of absorbed water or oil was determined by measuring the residual sediment inside the falcons. The gram of water or gram of oil absorbed per gram of protein powder was measured.

2-3- Statistical analysis of data

The response surface method is very important as an effective and powerful tool for optimizing chemical and food processes in situations where several independent variables affect the desired response. In this research, response surface method, central compound design, using Design Expert version 11 software, was used to optimize water absorption capacity and oil absorption capacity, and the response equations were obtained in terms of three independent variables, pH, centrifugation time, and centrifuge temperature. According to the prediction of the software, after determining the optimal conditions, the product was produced with the predicted conditions, and the water absorption capacity and oil absorption capacity of green lentils of Bilehsawar variety were compared with the prediction of the model by T-Student test at the 5% probability level.

3- Conclusion and discussion

This research was carried out with the aim of optimizing the water absorption capacity and oil absorption capacity of Bilehsawar variety green lentils using the response surface method (RSM). Water absorption capacity and oil absorption capacity are terms that usually refer to the amount of water and oil that can be absorbed per gram of sample, respectively.

3-1- Water absorption capacity

Water absorption capacity values are useful indicators of protein's ability to prevent liquid leakage from a product during food storage or processing [33, 34]. According to the report of Joshi et al., there are various factors that may explain the difference in water absorption capacity, such as protein extraction method, protein shape and size, surface topography and polarity [35]. It also seems that the presence of carbohydrates and other components may prevent water absorption, although no mechanism has been proposed for this phenomenon [36]. Water absorption should be considered the most important physical characteristic of proteins. This phenomenon not only strongly affects the physical structure and properties of food containing protein (such as drying), but it is also very important from the point of view of food spoilage due to its effect on the level of water activity [37]. The water absorption capacity of different foods depends on the composition of amino acids, the spatial arrangement of protein, the level of hydrophilicity and hydrophobicity of protein, as well as the presence of hydrophilic carbohydrates [40-38]. In addition to the mentioned factors, the presence of raw fiber is also considered as an effective water absorption factor.

Based on the results of analysis of variance, the effect of pH variable ($P < 0.01$) and time ($P < 0.01$) on water absorption capacity of green lentils of Bilehsawar cultivar was estimated to be significant. As shown in Figure 1, the water absorption capacity decreases with increasing pH and time. Based on the results of this test, a suitable regression model for predicting the water absorption capacity of a linear model with an explanation coefficient of 0.88 has been evaluated, which shows that 88% of the changes in the water absorption capacity are explained by changes in pH and time variables. Also, based on this regression model, the coefficient of pH and time were both negative, which indicates the decreasing effect of pH and time on the water absorption capacity, which means that as the pH and time increase, the water absorption capacity decreases.

Table 1 Experimental design of independent and response variables

Water absorption capacity (gram)	Oil absorption capacity (gram)	Temperature (Centigrade Degree)	Time (minutes)	**pH**	Treatment number
1.35	1.38	4.00	20.00	8.50	1
1.21	1.15	4.00	20.00	10.00	2
1	1.42	4.00	60.00	8.50	3
0.92	1.16	4.00	60.00	10.00	4
1.34	0.57	30.00	20.00	8.50	5
1.28	1.4	30.00	20.00	10.00	6
1.11	0.95	30.00	60.00	8.50	7
1	1.57	30.00	60.00	10.00	8
1.23	1.19	17.00	40.00	8.50	9
1.04	1.31	17.00	40.00	10.00	10
1.24	1.01	17.00	20.00	9.25	11
0.94	1.28	17.00	60.00	9.25	12
1.13	1.27	4.00	40.00	9.25	13
1.13	1.18	30.00	40.00	9.25	14
1.17	1.26	17.00	40.00	9.25	15
1.1	1.3	17.00	40.00	9.25	16
1.16	1.28	17.00	40.00	9.25	17
1.11	1.22	17.00	40.00	9.25	18
1.21	1.22	17.00	40.00	9.25	19
1.08	1.15	17.00	40.00	9.25	20

equation (1)

$$Y = (+2.14) - (0.08\ A) - (7.25E\text{-}03B)$$

In this equation, A represents pH, B is centrifuge temperature, and Y is water absorption capacity of green lentils of Bilehsawar variety.

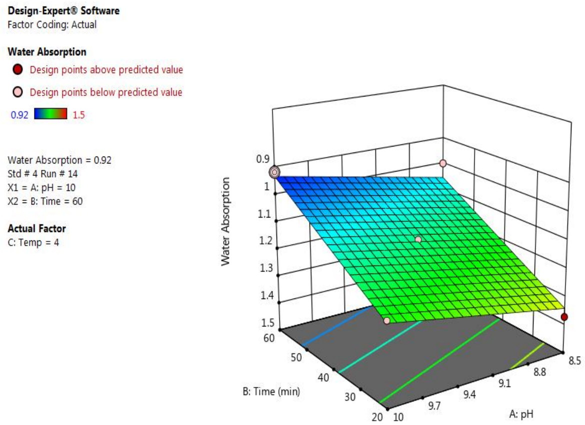

Fig 1 Interaction of pH and centrifuge time on water absorption capacity of lentil protein

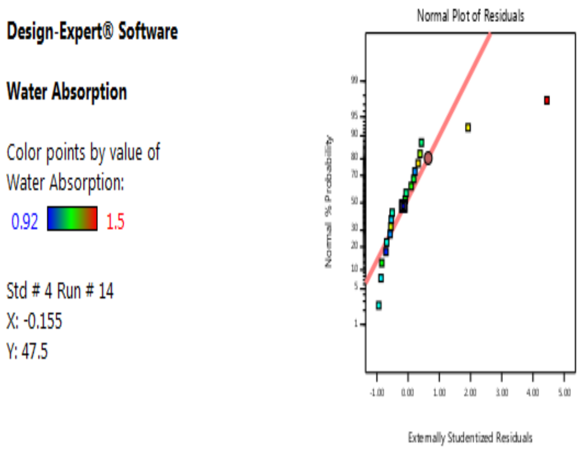

Fig 2 The normalized residuals plot for water absorption capacity of lentil protein

Figure (2) shows the normal diagram of the residuals, as it can be seen that the points are on the right line and follow the normal distribution.

2-3- oil absorption capacity

Kaur and Singh have described the amount of oil absorption as the physical confinement of the oil and attribute it to the non-polar protein chains that may bond with the hydrocarbon side chains of the oil, and also to the spatial shape of the protein, and as a result, the difference between They suggest these factors in different foods such as legumes causing differences in the numbers obtained in oil absorption [41]. Also, Abu and colleagues have come to the conclusion that the more non-polar amino acids in the side chain of proteins, the greater the oil absorption capacity. According to the theory of Adebwal et al., increasing the amount of fat in the sample, water absorption decreases because the presence of fat covers the sites that can be connected with water [42].

Equation (2)

$$Y= (+3.87) - (0.28\ A) + (2.63E\text{-}04\ B) - (0.25\ C) + (0.02\ AM) + (2.40E\text{-}04\ BC)$$

In this equation, A represents pH, B represents centrifugation temperature, C represents centrifugation time, and Y represents oil absorption capacity of green lentil protein of Bilehsawar variety. According to equation (2), which shows the relationship between the independent variables of the research and the oil absorption capacity of green lentils of Bilehsawar variety, it can be seen that the increase in pH and temperature has led to a decrease in the oil absorption capacity. Increasing time has an increasing effect on oil absorption capacity. The interaction of pH and temperature as well as the interaction of time and temperature has led to an increase in oil absorption capacity.

According to Figure (3), the interaction of pH and temperature as well as the interaction of time and pH has led to an increase in oil absorption capacity. This means that with increasing pH and temperature, the oil absorption capacity in lentils increases and with decreasing time, the oil absorption capacity in lentils decreases. Also, the interaction effect of pH and temperature, as well as the interaction effect of time and temperature on the oil absorption capacity have also been increased.

Figure (4) shows the normal graph of the residuals. As can be seen, the points are on the right line and follow the normal distribution.

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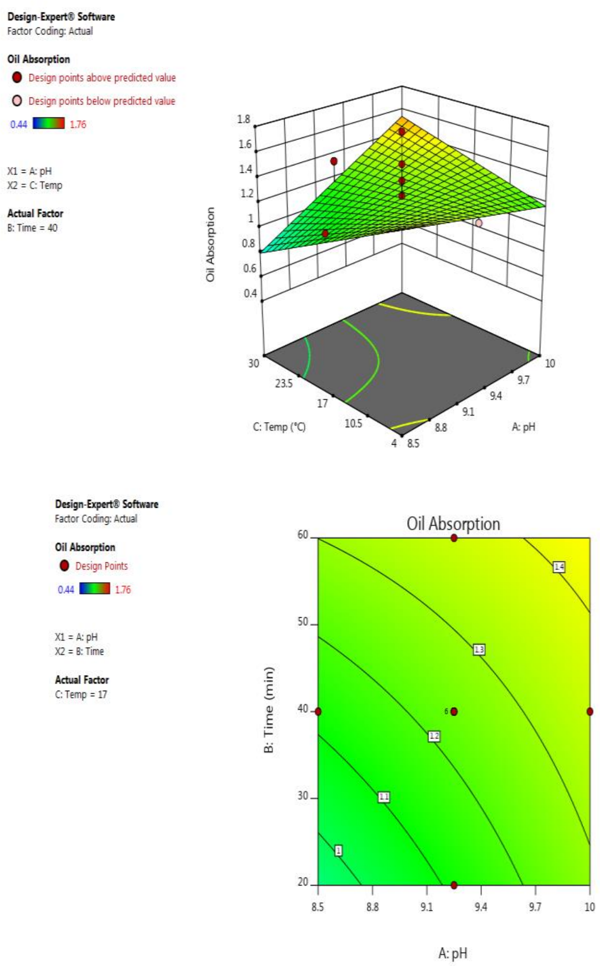

Fig 3 Interaction of pH and centrifuge time and centrifuge temperature on lentil oil absorption capacity

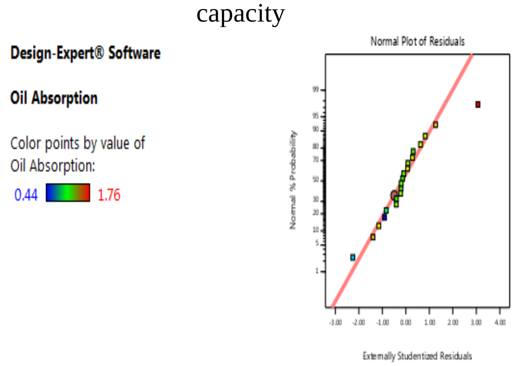

Fig 4 The normalized residuals plot for oil absorption capacity of lentil protein

Changes in protein structure may increase or decrease oil absorption capacity, depending on the effect [43]. Soliman et al explained an improved oil absorption by displaying non-polar groups as a result of a change in protein composition [36]. The mechanism of oil absorption includes the physical connections of oil with protein components and the combined tendency of non-polar protein chains to connect with fat. In the present study, the results of the analysis and analysis of variance of the data obtained from the tests conducted regarding the oil absorption capacity of green lentils of the Bilehsavar variety showed that the effect of pH, centrifugation time and temperature of the centrifuge was significant ($p<0.01$) and the change in this Three variables will change the amount of oil absorption capacity in green lentils of Bilehsawar variety. Also, based on the results of variance analysis, the mutual effects of pH and temperature and the mutual effects of temperature and time on oil absorption capacity were significant. In the following, the second quadratic regression model with an explanatory coefficient of 0.91 is presented to predict the oil absorption capacity, which shows that 91% of the changes in the oil absorption capacity of green lentils of Bilehsawar variety are explained by the change in the stated variables. According to this regression model, it has been determined that pH and temperature have a decreasing effect and time has an increasing effect on oil absorption capacity. This means that with increasing pH and temperature, the oil absorption capacity in lentils increases and with decreasing time, the oil absorption capacity in lentils decreases. Also, based on the presented regression model, the interaction effect of pH and temperature, as well as the interaction effect of time and temperature on the oil absorption capacity were also increasing. Many researchers have attributed the amount of oil absorption to the physical confinement of the oil and attributed it to the non-polar protein chains that may bond with the hydrocarbon side chains of the oil and also to the steric shape of the protein, and as a result, the difference between these factors in food Different types such as legumes cause differences in the obtained numbers in oil absorption [41]. Also, researchers have come to the conclusion that the more non-polar amino acids in the side chain of proteins, the greater the oil absorption capacity. Oil absorption is a physical phenomenon, so that the compounds and polymers in the sample cause the oil droplets to be trapped inside. be.

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3-3- Optimizing response parameters

To carry out the present research based on RSM, five stages of designing experiments, analyzing data, screening and removing non-significant factors, and renewing the mathematical model were continued until reaching an acceptable model, optimization and finding the optimal area and point. In this research, 2 answers suggested by the software were tested (Table 2). For the optimization process of water absorption capacity and oil absorption capacity with three factors of pH, time and temperature, the extracts that had the highest water absorption capacity and oil absorption capacity were considered. The obtained result was evaluated with a level of desirability of 1. By applying the conditions, the optimization results for the first optimum with pH 9.57, time 35.39 minutes and temperature 11.61 degrees Celsius were obtained for the oil absorption capacity equal to 1.235 and for the water absorption capacity equal to 1.135. Also, the optimization results for the second optimum with pH 10, time 20 minutes and temperature 30°C were obtained for the oil absorption capacity of 1.373 and for the water absorption capacity of 1.135. According to the results of Student's T test, no significant difference was observed between the predicted optimal conditions and the laboratory data, and the results of the experiments were very similar to the results of the optimal conditions predicted by the response surface method. The oil absorption capacity and water absorption capacity of green lentils of Bilehsavar variety also indicated the significance and appropriateness of the regression model obtained for these two variables.

Table 2 Actual values of the independent variables used for optimized points

Number	pH	Time	Temperature	Oil Absorption	Water Absorption	Desirability
Point 1	9.57	35.39	11.61	1.235	1.135	**1.000**
Point 2	10.00	20.00	30.00	1.373	0.250	**1.000**

4- General conclusion

In the current research, based on the results of analysis of variance, the effect of pH and time variables on the water absorption capacity of green lentils was estimated to be significant, and the suitable regression model for predicting the water absorption capacity was a linear model with an explanatory coefficient of 0.88, in which the pH coefficient and time in each There were two negatives. The regression model for oil absorption capacity in green lentils of Bilehsavar variety was second order, which explained 91% of the changes in oil absorption capacity by the stated variables. pH and temperature had a decreasing effect and time had an increasing effect on oil absorption capacity. The interaction effect of pH and temperature as well as the interaction effect of time and temperature on oil absorption capacity has also been increased. According to the results, one of the optimal points predicted for the maximum water and oil absorption capacity of green lentil protein of Bilehsavar variety was with a pH of 10 at a temperature of 30 degrees Celsius and a centrifugation time of 60 minutes, and there was a significant difference with the laboratory data. did not have.

5- Resources

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بهینه‌سازی ظرفیت جذب آب و روغن پروتئین عدس سبز رقم بیله‌سوار به روش سطح پاسخ

ملیحه دهقانی [۱] ، نفیسه زمین‌دار[۲]*

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

۲- 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

افزایش نیازهای جمعیت به پروتئین، باعث انجام تحقیقات بر روی محصولات طبیعی و گیاهی می‌شود که می‌تواند جایگزین مناسبی برای پروتئین‌های حیوانی باشد. عدس با داشتن بین ۲۰/۶ تا ۳۱/٤ درصد پروتئین منبع سرشار از پروتئین است. در نتیجه تلاش جهت بررسی خواص فیزیکوشیمیایی پروتئین آن و جایگزینی پروتئین‌های حیوانی امری مهم تلقی می‌شود. در این پژوهش بهینه سازی ظرفیت جذب آب و روغن عدس سبز رقم بیله‌سوار با استفاده از روش سطح پاسخ، با ۲۰ تیمار حاصل از ترکیب سه متغییر مستقل (۸/۵-۱۰)pH، زمان سانتریفیوژ(۶۰-۲۰ دقیقه) و دمای سانتریفیوژ (۳۰-٤ درجه سانتی‌گراد) مورد بررسی قرار گرفت. طرح مرکب مرکزی با سه متغیر مستقل و ٦ نقطه‌ی مرکزی با استفاده از نرم افزار Design Expert۱۱ در روش سطح پاسخ استفاده شد. نتایج به‌دست آمده از آزمایش‌های انجام گرفته در خصوص ظرفیت جذب آب در عدس سبز رقم بیله‌سوار، نشان داد که با افزایش میزان pH و زمان، ظرفیت جذب آب کاهش می‌یابد. همچنین با افزایش pH و دما، ظرفیت جذب روغن در عدس افزایش و با کاهش زمان، ظرفیت جذب روغن در عدس کاهش یافت. پاسخ بهینه پیشنهاد شده توسط نرم‌افزار مورد آزمایش قرار گرفت. با اعمال شرایط بهینه، نتایج ظرفیت جذب روغن و ظرفیت جذب آب برای نقطه بهینه به ترتیب۱/۲۳۵و ۱/۱۳۵ حاصل شد. باتوجه به اعتبارسنجی حاصل از آزمون T student، پاسخ آزمایشات عملی مشابه با شرایط بهینه‌ی پیش‌بینی شده با روش سطح پاسخ بود ($p<0/05$).