



Scientific Research

Optimization of emulsifying capacity and stability of wheat bran protein

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ARTICLE INFO	ABSTRACT
Article History: Received: 2024/8/2 Accepted: 2025/1/21	<p>Recently, the demand of consumers to use vegetable proteins as a substitute for animal proteins has been increased. Cereal bran contains protein, fiber sources, minerals and antioxidants. In this research, optimization of emulsifying capacity and emulsion stability of wheat bran protein was performed using response surface method (RSM), central composite design (CCD) and Design Expert software. The design included the independent variables of centrifugation time (20-60 minutes), centrifugation temperature (4-20°C), alkaline pH (8-12) and 10 replications at the central point. Centrifugation time had a significant effect on emulsion stability ($P < 0.05$); but it had no significant effect on the capacity of emulsion formation ($p > 0.05$). Centrifugation temperature changes had a significant effect on the amount of emulsion formation capacity and stability ($P < 0.05$). Changes in alkaline pH had a significant effect on the amount of emulsion formation capacity and stability ($P < 0.05$). Evaluation of the optimal conditions suggested by the software for the formation capacity and stability of the emulsion were not significantly different from the test results ($p > 0.05$). The results of this research showed that wheat bran protein as a vegetable protein source has a high potential to be used as an emulsifier in the food industry.</p>
Keywords: Emulsifying capacity, Emulsion stability, Wheat bran protein	
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1-Introduction

One of the most important and fundamental challenges of most countries is the increase in waste in the production process of agricultural products, and one of the serious policies of governments in the food security is to reduce waste [1]. Unfortunately, in Iran, despite having appropriate nutritional value, agricultural by-product is wasted or is limited to livestock consumption due to the lack of equipment required by the processing and complementary industries near the production centers [2]. Recently, plant-based proteins have attracted increasing attention as a dietary supplement due to their low cost and perceived health benefits compared to animal-based proteins [3]. Animal proteins are expensive, in limited supply, and are directly linked to freshwater depletion, climate change, and biodiversity loss [4]. Extracting proteins of a low-value by product of wheat milling, such as bran, can increase the value of bran and avoid problems with consumer acceptance of bran-containing foods. Replacing animal proteins with plant proteins of food processing by-products ensures sustainability in food production [3].

The use of a protein as a food ingredient has certain conditions. The functional characteristics of the protein depend on its structural properties, and these structural properties are affected by the environmental conditions of protein preparation [5]. The functional properties of a protein material depend on its suitability for the intended product. Many formulated foods are produced as foams or emulsions, and therefore require proteins with suitable surface properties and solubility, while some other foods require an

insoluble protein with a high capacity to absorb and retain water to achieve a desired texture [6,7]. Emulsions are defined as dispersions of two or more immiscible liquids in which one liquid is dispersed in the other as small droplets. Emulsions are formed in the presence of an emulsifier, which is composed of hydrophobic and hydrophilic components and reduces the surface tension at the oil-water or water-oil interface. Common proteins used in the food industry for their emulsifying properties include: whey protein isolate, ovalbumin, and bovine serum albumin. Also, many legumes, cereals, and oilseeds have been studied by scientists to investigate their potential use as emulsifiers in the food industry [6,8-15]. Few studies are available on the emulsifying properties of wheat bran protein. This study aimed to develop and optimize a method for extracting wheat bran protein in order to investigate the emulsifying properties and stability of wheat bran protein emulsions.

2-Materials and Methods

2-1-Sample Preparation

In this study, wheat bran was obtained from Khoshe Zarrin Borujen Flour Company. The bran was packed in impermeable plastic bags and stored at refrigerator temperature until the experiments were performed. To prevent oxidation of wheat bran, defatting was performed using hexane [13,16].

2-2-Protein Extraction

To extract wheat bran proteins under alkaline conditions, the method described in Figure 1 (according to the experimental design) was used [17,18].

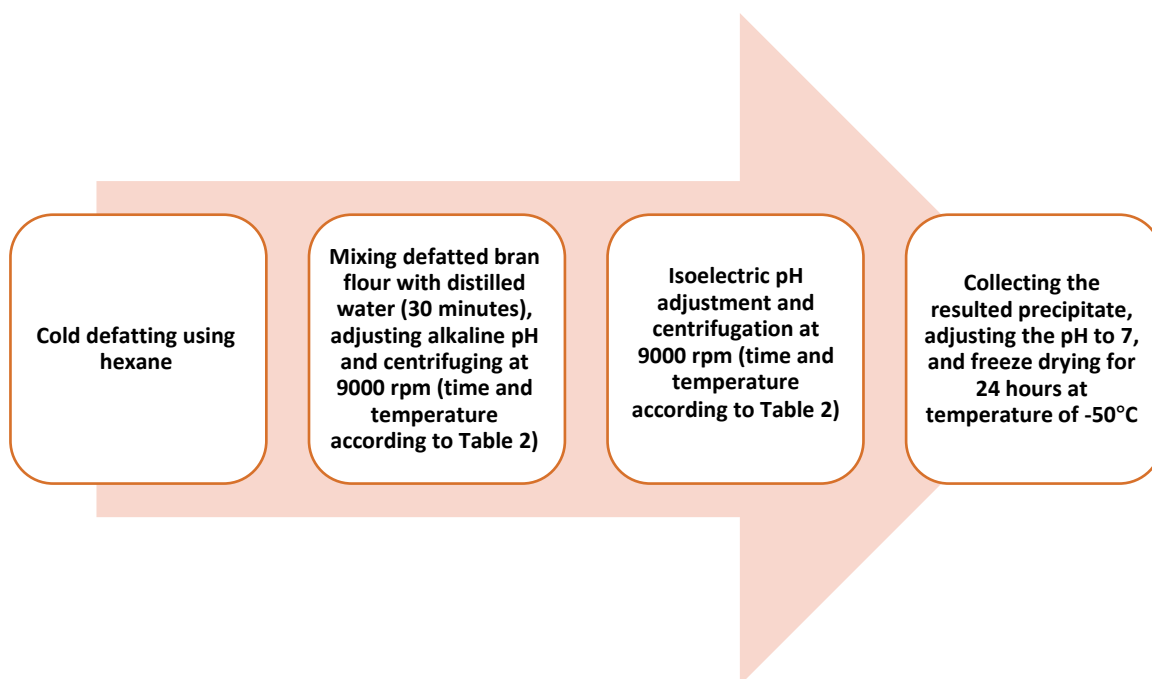


Fig 1- Flowchart of extraction of wheat bran protein

۲-۳- Measurement of emulsifying capacity

and emulsion stability

To measure the emulsion capacity and stability, 0.09 g of extracted protein was mixed with 9 g of water to obtain a 1% clear solution. Then, 1 ml of oil was added to form an oil-in-water emulsion. The pH of this 1% isolate solution was adjusted to 7 using sodium hydroxide and then homogenized using an Ultratorex homogenizer at 11,000 rpm at room temperature for 1 minute and its volume (initial total volume) was recorded. The emulsions were centrifuged immediately after homogenization at 4,500 rpm for 5 minutes and the emulsifying capacity was obtained by dividing the emulsion volume by the total volume [16].

To calculate the stability, the prepared emulsion was placed in a water bath at 80°C for 30 minutes and then centrifuged at 4500 rpm for 5 minutes at room temperature. The stability of the emulsion was calculated by dividing the

final volume of the emulsion by the volume of the initial emulsion [16].

2.4-Statistical analysis

In this study, the effect of centrifugation time (20-60 minutes), centrifugation temperature (4-20°C), and pH (8-12) on the emulsion formation capacity and its stability of wheat bran protein was investigated (Table 1). Response surface methodology, central composite design, and Expert Design software version 11 were used, and the number of runs was 24 runs with 10 replicates at the central point, as suggested in Table 2. The product was produced and tested according to the conditions predicted by the software (best conditions). In this study, the physicochemical properties of the emulsion formation capacity and stability of wheat bran protein at the optimal experimental conditions were compared with that of optimal predicted conditions by the T-Student test at a probability level of 5%. All measurements were repeated three times for each of the protein samples.

Table 1- Real and coded values of independent variables used for experimental design

Factor	Name	Minimum	Maximum	Coded Low	Coded High	Mean
A	Time	20.00	60.00	-1 ↔ 20.00	+1 ↔ 60.00	40.00
B	Temperature	4.00	20.00	-1 ↔ 4.00	+1 ↔ 20.00	12.00
C	pH	8.00	12.00	-1 ↔ 8.00	+1 ↔ 12.00	10.00

3- Results and Discussion

The optimization of the physicochemical properties of wheat bran was investigated using the response surface methodology, the

results obtained from the independent, and dependent variables were presented in Table 2.

Table 2. Values of independent and dependent variables in different treatments

Run	A: Time min	B: Temperature °C	C: pH	Emulsion Capacity %	Emulsion Stability %
1	40	12	10	0.87	0.66
2	40	12	10	0.86	0.66
3	20	4	8	0.81	0.51
4	60	20	12	0.93	0.92
5	40	12	12	0.92	0.83
6	40	12	10	0.89	0.65
7	40	12	8	0.82	0.55
8	40	20	10	0.91	0.71
9	60	4	8	0.82	0.53
10	20	20	12	0.93	0.91
11	40	12	10	0.85	0.6
12	40	12	10	0.87	0.66
13	40	12	10	0.88	0.65
14	40	12	10	0.87	0.64
15	20	4	12	0.91	0.72
16	20	12	10	0.86	0.61
17	60	12	10	0.91	0.69
18	40	12	10	0.9	0.65
19	40	4	10	0.85	0.6
20	40	12	10	0.88	0.58
21	60	20	8	0.85	0.6
22	60	4	12	0.91	0.8
23	20	20	8	0.84	0.56
24	40	12	10	0.88	0.57

3.1- Wheat bran protein emulsion forming capacity

The emulsifying property of protein products has been attributed to the amount of total protein and the amount of non-protein compounds. In addition, the number of hydrophilic and hydrophobic groups, the protein dispersion index, and the degree of protein solubility have been introduced as other factors affecting the emulsifying property. Protein, as one of the main components affecting the emulsifying property, causes the formation of a thin film around oil droplets in an aqueous medium and prevents them from binding, creaming, coagulation, and sedimentation. The amount of oil in the sample and the presence of other components such as carbohydrates, fats, and salts affect the emulsifying activity due to their interaction with protein or with each other [19]. Table 3 and Figure 2 show the interaction of time, temperature, and pH on the emulsifying

capacity of wheat bran protein. Based on the statistical analysis and the results of the analysis of variance, the full quadratic model was significant for the dependent variable of emulsifying capacity ($p < 0.0001$). According to Figure 2(a), it is observed that at a constant temperature of 20°C, the emulsifying capacity of wheat bran protein increased with increasing pH. In addition, the emulsifying capacity of wheat bran protein increased slightly with increasing time, so that at pH of 11 to 12 and time of 60 minutes, wheat bran protein had the highest emulsifying capacity. According to Figure 2(a,b), it is clear that the effect of temperature and time on the emulsifying capacity of wheat bran protein is small and according to Table 3, the effect of the time variable is not significant ($p > 0.05$). Increasing pH increased the emulsifying capacity, with wheat bran protein having the highest emulsifying capacity at pH 12. The researchers stated that the increase in emulsifying capacity at higher pHs is due to increased solubility [19].

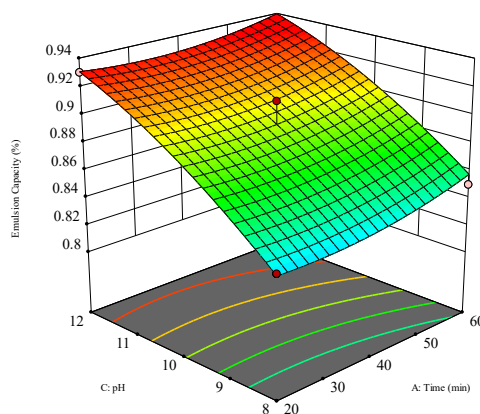
In line with the present study, researchers evaluated the emulsifying capacity and emulsion stability of grain protein isolate at different pHs and reported that with increasing pH from 2 to 4, the emulsifying capacity decreased rapidly and the lowest emulsifying capacity was observed at pH 4 near the isoelectric point. However, with increasing pH from 5 to 10, the emulsifying capacity increased sequentially and reached its highest value at pH 10 [20]. In another study, the emulsifying capacity of protein from three different bean genotypes was investigated and it was found that the emulsifying capacity is dependent on the type of protein and pH. The protein obtained from all three different bean genotypes showed the minimum emulsifying capacity at pH of five. The highest emulsifying

capacity was obtained at pHs of 5 and 7 for the protein from the Speckled Sugar bean, which could be due to the higher solubility of this protein at these pHs [21]. In another study, researchers stated that the maximum protein yield for Kimia lentil was obtained under optimal conditions of 30°C, 20 minutes, and pH of 8.5, and the emulsifying capacity was highest at pH of 10 [22]. The emulsifying capacity of wheat bran protein based on actual data was obtained according to equation 1; in this equation, Y is the emulsifying capacity, B is the centrifugation temperature (degrees of Celsius), and C is the pH symbol.

$$\text{equation 1: } Y = 0.621 + 0.002B + 0.023C \quad (R^2=0.8537)$$

Table 3. Variance analysis of the effect of independent variables on the emulsion capacity of wheat bran protein

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0245	9	0.0027	11.79	< 0.0001	significant
A-Time	0.0005	1	0.0005	2.12	0.1677	
B-Temperature	0.0026	1	0.0026	11.06	0.0050	
C-pH	0.0212	1	0.0212	91.45	< 0.0001	
AB	0.0000	1	0.0000	0.0000	1.0000	
AC	0.0000	1	0.0000	0.2161	0.6492	
BC	0.0000	1	0.0000	0.2161	0.6492	
A²	0.0001	1	0.0001	0.5168	0.4840	
B²	6.652E-06	1	6.652E-06	0.0287	0.8678	
C²	0.0002	1	0.0002	0.8633	0.3686	
Residual	0.0032	14	0.0002			
Lack of Fit	0.0014	5	0.0003	1.35	0.3266	not significant
Pure Error	0.0019	9	0.0002			
Cor Total	0.0278	23				
Std. Dev.		0.0152	R²			0.8834
Mean		0.8758	Adjusted R²			0.8085
C.V. %		1.74	Predicted R²			0.5491
			Adeq Precision			14.0546



a

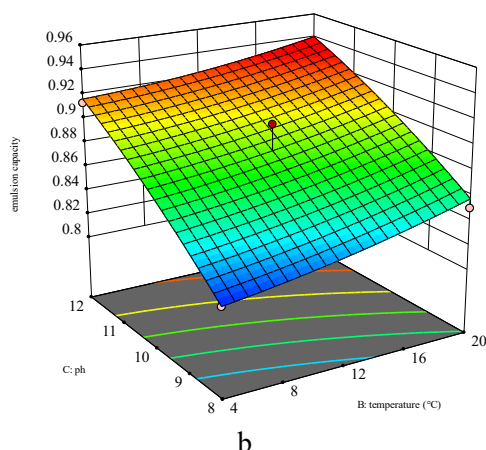


Fig 2. Three-dimensional diagram of the interaction effect of time and pH (a), temperature and pH (b), on the emulsion capacity of wheat bran protein

3.2- Stability of wheat bran protein emulsion

Differences in the spatial shape of proteins and ionic strength can explain the differences in the stability of emulsions in different samples. Increased bidirectional adhesion between fat and protein due to protein deformation can lead to increased resistance and strength of the oil-water interface, thereby increasing the stability of the emulsion. In addition, the decrease in emulsion stability during storage may be due to increased contact between molecules, resulting in flocculation and coalescence of oil droplets [19]. Based on the statistical analysis and the results of the analysis of variance, the quadratic model was significant for the dependent variable of emulsion stability ($p < 0.0001$). Examining the effect of each of the independent variables on the stability of wheat bran protein emulsion showed that, assuming other factors as constant, changes in time, temperature, and pH had a significant effect on the stability of the emulsion ($p < 0.05$). The effect of simultaneous changes in temperature \times pH variables on the stability of wheat bran protein emulsion was significant ($p < 0.05$). The results of the emulsion stability measurements are given in Table 4 and Figure 3. As shown in Figure 3, the effect of time and pH variables on the dependent variable of emulsion stability was evaluated. The results indicated that with increasing pH and increasing time up to 60 minutes, the stability of wheat bran protein emulsion increased. In addition, the effect of

temperature and pH on the stability of wheat bran protein emulsion shows that at a fixed time of 60 minutes, with an increase in temperature from 4 to 20 °C and an increase in pH from 8 to 12, the stability of the emulsion increased ($p < 0.05$). In line with the present study, researchers stated that, at a fixed time of 40 minutes, with an increase in temperature from 4 to 30 °C and an increase in pH from 8.5 to 10, the stability of the emulsion increased [22]. The researchers also stated that the stability of the grain protein emulsion gradually increased with increasing pH to 10. These researchers stated that the lowest emulsion stability was observed at pH 4 near the isoelectric point, which could be due to poor solubility, improper hydration, and weak electrostatic repulsion between protein molecules. However, with increasing pH to 10, the stability of the emulsion increased [20]. The researchers' results showed that the emulsion stability increased slightly over time, but temperature changes did not have a significant effect on the stability of the lentil protein emulsion [22]. The emulsion stability of wheat bran protein based on real data was obtained according to equation 2; in this equation, Y is the stability of the wheat bran protein emulsion, A is the centrifugation time (minutes), B is the centrifugation temperature (degrees of Celsius), and C is the pH.

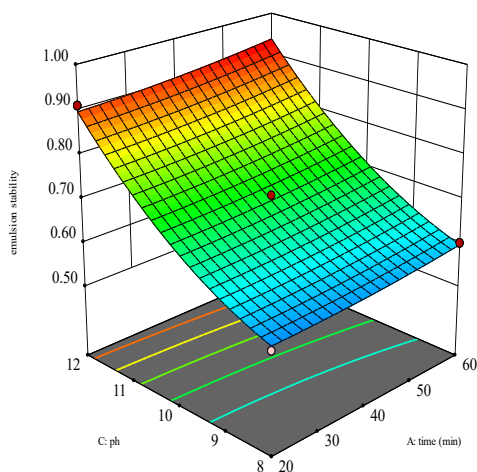
$$\text{equation 2: } Y = 1.35 + 0.0011A - 0.008B - 0.222C + 0.001BC + 0.013C^2 \quad (R^2=0.9478)$$

Table 4. Variance analysis of the effect of independent variables on the wheat bran protein emulsion stability

Source	Sum of Squares	df	Mean Square	F-value	p-value
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Model	0.2622	9	0.0291	30.42	< 0.0001	significant
A-Time	0.0053	1	0.0053	5.52	0.0339	
B-Temperature	0.0292	1	0.0292	30.45	< 0.0001	
C-pH	0.2045	1	0.2045	213.56	< 0.0001	
AB	0.0003	1	0.0003	0.3264	0.5769	
AC	0.0001	1	0.0001	0.1175	0.7369	
BC	0.0045	1	0.0045	4.71	0.0476	
A²	0.0001	1	0.0001	0.0766	0.7860	
B²	0.0003	1	0.0003	0.2990	0.5931	
C²	0.0057	1	0.0057	5.94	0.0287	
Residual	0.0134	14	0.0010			
Lack of Fit	0.0024	5	0.0005	0.4016	0.8362	not significant
Pure Error	0.0110	9	0.0012			
Cor Total	0.2756	23				
Std. Dev.		0.0309			R²	0.9514
Mean		0.6608			Adjusted R²	0.9201
C.V. %		4.68			Predicted R²	0.8365

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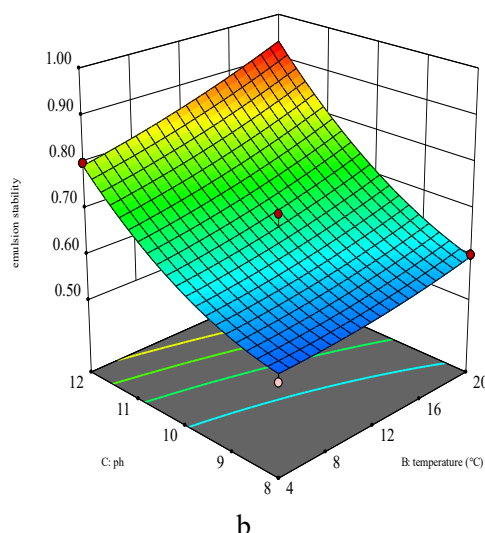


Fig 3. Three-dimensional diagram of the interaction effect of time and pH (a), temperature, and pH (b), on the emulsion stability of wheat bran protein

3-3-Optimization of response parameters

For numerical optimization, the values of independent variables were within the defined range and the dependent variables were at their maximum. According to Table 5, the optimum point was achieved at

centrifugation time of 47.869 minutes, the centrifugation temperature of 19.963 degrees of Celsius, and the pH value of 10. In accordance with the proposed treatment, the tests were performed in three replicates and compared with the t-student test, and no significant difference was shown ($p > 0.05$).

Table 5. Predicted conditions of the software for optimizing the process and the tests performed

	Time	Temperature	pH	Emulsion Capacity	Emulsion Stability	Desirability
predicted conditions	47/869	19/963	12/000	0/931	0/920	1/000
tests performed	47/869	19/963	12/000	0.93 ± 0.05^{ns}	0.92 ± 0.05^{ns}	

4-Conclusion

In the present study, the emulsification ability of wheat bran was optimized using the response surface methodology (RSM). The independent variables included centrifugation time (20-60 minutes), centrifugation temperature (4-20°C), and pH (8-12). The quadratic statistical model was used with high accuracy to predict the dependent variables ($p < 0.05$). Also, the optimization results showed a good agreement with the experiment ($p > 0.05$). The most important results of this research were that changes in centrifugation time had a significant effect on the stability of the emulsion ($p < 0.05$). Changes in temperature and pH had a significant effect

on the emulsion formation and stability ($p < 0.05$). Results declared that pH changes produced the most effective changes in the dependent variables. The evaluation of the optimal conditions suggested by the software was obtained at the centrifugation time of 47.869 minutes, centrifugation temperature of 19.963 degrees of Celsius, and pH of 10. This study showed that wheat bran protein has a high potential for application as an emulsifier in food formulations.

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بهینه سازی ظرفیت امولسیون کنندگی و پایداری امولسیون پروتئین سبوس گندم

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اطلاعات مقاله	چکیده
تاریخ های مقاله : تاریخ دریافت: ۱۴۰۳/۵/۱۲ تاریخ پذیرش: ۱۴۰۳/۱۱/۲ کلمات کلیدی: ظرفیت امولسیون کنندگی، پایداری امولسیون، پروتئین سبوس گندم	چکیده اخیراً، تقاضای مصرف کنندگان برای استفاده از پروتئین های گیاهی به عنوان جایگزین پروتئین های حیوانی زیاد شده است. سبوس غلات حاوی پروتئین، منابع فیبری، مواد معدنی و آنتی اکسیدان ها است. در پژوهش حاضر بهینه سازی ظرفیت امولسیون کنندگی و پایداری امولسیون پروتئین سبوس گندم با استفاده از روش سطح پاسخ (RSM)، طرح مرکب مرکزی (CCD) و نرم افزار Design Expert انجام شد. طرح شامل متغیرهای مستقل زمان سانتریفوژ (۶۰-۲۰ دقیقه)، دمای سانتریفوژ (۲۰-۴ درجه سانتی گراد)، pH قلیایی (۸-۱۲) و ۱۰ تکرار در نقطه مرکزی بود. تغییرات زمان سانتریفوژ تاثیر معنی داری بر میزان پایداری امولسیون داشت ($p < 0.05$)؛ اما بر روی ظرفیت تشکیل امولسیون تاثیر معنی داری نداشت ($p > 0.05$). تغییرات دمای سانتریفوژ تاثیر معنی داری بر میزان ظرفیت تشکیل و پایداری امولسیون داشت ($p < 0.05$). تغییرات pH قلیایی تاثیر معنی داری بر میزان ظرفیت تشکیل و پایداری امولسیون داشت ($p < 0.05$). ارزیابی شرایط بهینه پیشنهادی نرم افزار برای ظرفیت تشکیل و پایداری امولسیون با نتایج آزمون تفاوت معنی دار نداشتند ($p > 0.05$). نتایج این پژوهش نشان داد پروتئین سبوس گندم به عنوان یک منبع پروتئین گیاهی، پتانسیل بالایی برای استفاده به عنوان امولسیفایر در صنایع غذایی دارد.

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