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# Optimization of Cheese Analogue Formulation with Rice Milk, Chia Seed and Hazelnut Oil Applying Response Surface Methodology

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#### Abstract

The physical, chemical and textural properties of cheese analogue depend on their source as well as the formulation conditions. Recently, cheese analogue has attracted the attention of consumers due to its nutritional, textural, sensorial and health properties. The aim of this study was to investigate the interaction effects of independent parameters such as rice milk (70 to 75%), chia seed (15 to 18%) and hazelnut oil (4 to 6%) to evaluate the optimal cheese analogue formulation using the response surface methodology. According to the obtained results, it was observed that with the increase concentration of independent variables, the overall acceptance of the samples initially increased and then showed a downward trend. Also, the textural characteristics of cheese analogue samples such as hardness, cohesiveness, springiness and gumminess showed rice milk and chia seed improved the textural properties, but the concentration of hazelnut oil had the negative effect on these parameters. The concentration of chia seed and hazelnut oil decreased  $L^*$ , while the concentration of rice milk increased brightness index. The  $a^*$  index showed a reduction trend in high concentrations of rice milk. The results observed that the optimum formulations of cheese analogue determined rice milk 71.87%, chia seed 17.62% and hazelnut oil 4.86%. Also, the estimated responses containing  $L^*$ ,  $a^*$ , hardness, cohesiveness, springiness, gumminess, and total acceptability parameters were 68.08, 3.56, 4.79, 2.47, 1.42, 2.96 and 2.67, respectively. Generally, According to the results found in the present study, it can be concluded that the response surface methodology was able to predict the optimal of cheese analogue formulation with high desirability (0.85).

# Introduction

Milk is one of the most commonly consumed food item relished by human since ancient times due to its nutritional value and its versatility in satiating Received: 2022.05.18 Revised: 2022.08.04 Accepted: 2022.09.13 Online publishing: 2022.12.30

#### Keywords

Cheese analogue Hardness Hazelnut oil High desirability Rice milk



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appetite. This ingredient can be obtained from either animal source such as cows, goat, sheep, and buffalos (López-Calleja *et al.*, 2005). Animal sources is expensive, as a result of this, its use for cheese is now limited. Therefore, the continuous search for alternative protein source in order to find cheap resources in developing countries continues (Cheng et al., 2019). On other hands, nowadays milk consumption has raised concerns among the health conscious and risk prone population as clinical studies have demonstrated that some constituents of milk are associated with deleterious health effects such as cow milk allergy, lactose intolerance, and coronary heart diseases (Goulding et al., 2004; Kay et al., 2021; Pereira, 2014; Rangel et al., 2016). Substitution of milk fat with vegetable fat is an alternative to obtain dairy products with a balanced saturated/unsaturated fat (Ejeahalaka & On, 2019; Rojas-Nery et al., 2015). Hazelnut (Corylus heterophylla) is rich in oil and is one of the 4 major dried fruits in the world (Turan et al., 2013). Hazelnut oil is highgrade edible oil that is rich in nutrients, having a wide range of primarily consisting oleic acid (73.6 to 82.6%), linoleic acid (9.8 to 16.6%), palmitic acid (4.1 to 6.8%) and stearic acid (1.6 to 3.7%). The content of unsaturated fatty acids is as high as 90% (Sun et al., 2022). Plant based milk may be a good choice for people looking for dairy free alternatives (Mäkinen et al., 2016). One of the dairy products that can be substituted with the other ingredients is cheese, whose product is called cheese analogue (Aini et al., 2019). The advantage of cheese analogue was the production cost which was relatively lower compared to cheese made from cow's milk. Rice milk is lactose free making them prefect alternative suffering for patients from lactose intolerance (Padma et al., 2019). It can be processed into extract that has milk-like taste, which can then be used to substitute for dairy products, for example, into cheese formulation (Lagrange et al., 2015). Legumes and nuts have the characteristics that make them convenient to combine order to produce dairy-free nutritious and palatable plant based milk alternative. In addition to this ingredients have nutritional and health benefits, because it contains no

cholesterol, lactose and only small amounts of saturated fatty acids (Bridges, 2018). Chia seed come from an annual herbaceous plant, Salvia hispanica L., which consist of 34% fiber, 30% oil, and 16% protein. Chia seeds are also a good source of polyunsaturated fatty acids, dietary fiber, essential amino acids, and micronutrients photochemical, vitamins such as and minerals. Therefore, chia seeds are "functional considered as the food" (Dinçoğlu & Yeşildemir, 2019). Response surface methodology (RSM) as one of the important statistically most efficient methods can be of great help in the diagnosis and analysis of experimental data. This method achieves the best response level by discovering the optimal response level of each of the design variables. In designing experiments, the goal is to identify and analyze the variables affecting the outputs with the least number of experiments. The objective of the study was optimization analogue of cheese formulation including rice milk, chia seed and hazelnut oil by RSM and to investigate physicochemical, textural and its organoleptic properties.

#### Materials and methods Material

All chemicals used in this study were purchased from Sigma (St. Louis, Mo.,USA). Chia seed was purchased from local markets in Sabzevar City, Razavi Khorasan province, Iran.

# Preparation of hazelnut oil

Initially, broken or damaged hazelnuts and other impurities such as stem, skin etc. was removed. Then, seeds were extracted with cold press (BD Company, Iran), (12 to 15 kg/h capacity) without heat treatment. The impurities can inappropriate effect on the color and flavor of hazelnut oil which was attained after pressing. The hazelnut oil was purified from solid impurities bv sedimentation for 4 days followed by filtration. Purified hazelnut oil was kept in closed colored bottle in a refrigerator set at

5 °C (Al Juhaimi *et al.*, 2018).

# Preparation of rice milk

The extraction of rice milk was done from method described by Al Tamimi (2016) with minor modification. Hashemi rice samples were purchased from the local markets in Rasht, Gilan province, Iran. Initially, 100 g of rice samples in water at room temperature overnight. Then, the soaking water was then discarded and the rice were put in an electric blender with 1000 mL of water and blended thoroughly for 5 min until the milk was completely smooth and no chunks of rice remained. The mixture was filtered with a fine strainer lined using a thin fabric muslin in order to remove the shells and impurity particles. The filtrated rice milk was stored in the refrigerator at 5 °C for 24 h (Al Tamimi, 2016).

#### **Production of cheese samples**

In order to produce cheese analogue, the modified method used with Awad et al. (2014). The samples were carefully weighed a balance-type scale (SKX1202 model OHAUS company, Switzerland) according to the statistical design. First, rice milk, chia seed and hazelnut kept overnight at 4 °C. These ingredients were heated to 20 °C. Then, the mixture was homogenized completely using homogenizer (Bosch model, Germany) at 30 MPa for 20 min at an inlet temperature of 30±1 °C. The mixture was then subjected to a temperature of 40 °C for 10 min to remove moisture. In order to pasteurize the mixture, it was done at 80 °C for 1 min. Finally, the temperature of cheese analogue formulated samples was gradually reduced to 2 °C with the help of water and ice, and then mixtures were stored in sterilized Pyrex bottles in the dark. Cheese samples were transferred to the refrigerator and stored at 5 °C until before the tests (Awad et al., 2014).

# Sensory evaluation

In order to study total acceptability, the 5 -point hedonic scoring method was used.

In this method, scoring each of the desired features is from 1 (very bad) to 5 (very good). After coding, the samples were given to a group of trained evaluators (Including 12 male and female evaluators at ages 15 to 40) to rate the overall desirability of each sample (Chung & Han, 2013).

## Texture profile analyzer

Texture changes from the addition of rice milk values, chia seed and hazelnut oil to cheese analogue samples were evaluated by the analysis of the texture profile analyzer CT3 texture analyzer (Brookfield Engineering Lab, Inc., Middleboro, MA). The Brookfield CT3 analyzer was set to a Pre-Speed of 1 mm/s, Test-Speed of 1 mm/s, and Post -Speed of 10 mm/s. The distance was set to 50% strain, the trigger box at 0.49 N, and the acquisition rate to 50 pps. A 75 mm diameter compression plate performed the compression test and was calibrated by the Brookfield CT3 texture analyzer before testing began. The compression test was run once per cheese for a completion of 17 total trials. Prior to testing, the height (mm) and diameter (mm) were measured and recorded for all cheese samples at room temperature (RADOČAJ *et al.*, 2011).

# Color

The color of the cheese analogue samples with rice milk values, chia seed and hazelnut oil were done with Hunter Lab. (Color Flex, USA). Initially, the colorimeter was calibrated using a black and white screen and the cheeses were then moved to the device and evaluated. Color parameters containing  $L^*$ (green) were (brightness),  $a^*$ and determined (Chakraborty et al., 2021).

# Statistical analysis

In order to optimize cheese analogue samples including rice milk (70 to 75%), chia seed (15 to 18%), and hazelnut (4 to 6%) were carry out using response surface methodology (RSM).





Fig. 1. Contour plots of independent variable on total acceptability of cheese analogue including rice milk, chia seed and hazelnut oil

**Table 1.** The experimental range and levels of the variables

Variable	Sumbol	Low	0	High
(%)	Symbol	(-1)	0	(+1)
Rice milk	$X_1$	70.00	72.50	75.00
Chia seed	$X_2$	15.00	16.50	18.00
Hazelnut oil	$X_3$	4.00	5.00	6.00

**Table 2.** Experimental design of independent variables in the central composite rotatable design (CCRD) for the production of cheese analogue

Treatment	A: Rice	B: Chia	C: Hazelnut
	milk	seed	oil
1	70	16.5	4
2	70	15	5
3	70	18	5
4	70	16.5	6
5	72.5	15	4
6	72.5	18	4
7	72.5	16.5	5
8	72.5	16.5	5
9	72.5	16.5	5
10	72.5	16.5	5
11	72.5	16.5	5
12	72.5	15	6
13	72.5	18	6
14	75	16.5	4
15	75	15	5
16	75	18	5
17	75	16.5	6

Analysis of variance (ANOVA) table and contour plots were used with Design Expert Version 10 software. Rice milk (X<sub>1</sub>), chia seed (X<sub>2</sub>) and hazelnut oil (X<sub>3</sub>) were determined to optimize the cheese analogue samples (Table 1). Hardness, cohesiveness, springiness, gumminess, total acceptability,  $L^*$  and  $a^*$  indices were considered as the response (Y) of the experiments. The experimental range was performed with three input variables and 17 treatments with 5 replications at central point and 4 replicates (Table 2).

#### Results and discussion Sensory evaluation

By increasing the rice milk concentration from 70 to 75% and increasing chia seed from 15 to 18%, while the other variable was constant at the central point, the total acceptability initially increased and decreased at higher levels (Fig. 1). The quadratic effects of the three independent variables showed a significant effect on the total acceptability of changes (P<0.01), (Table 3).

Source	Total Hardness	Uardnoog	<i>P</i> -value				
	acceptability	nardness	Cohesiveness	Springiness	Gumminess	L*	a*
Model	0.0011	0.0001	0.0006	0.0001	0.0003	0.0001	0.0001
A-Rice milk	0.0003	0.0004	0.0001	0.0010	0.0003	0.0035	0.0001
B-Chia seed	0.9207	0.0001	0.0001	0.0001	0.0001	0.0054	0.0007
C-Hazelnut oil	0.0984	0.0257	0.0022	0.0130	0.0008	0.0177	0.0644
AB	0.6704	0.0026	0.0768	0.1197	0.1883	0.0537	0.4582
AC	0.8548	0.2917	0.9765	0.5399	0.0060	0.2153	0.7063
BC	0.2378	0.0246	0.6908	0.6437	0.1558	0.4406	0.9697
$A^2$	0.0028	0.2716	0.0638	0.4059	0.3919	0.7285	0.5198
$\mathbf{B}^2$	0.0025	0.0503	0.9406	0.7084	0.4627	0.1500	0.6481
$C^2$	0.0004	0.1284	0.7386	0.6769	0.1557	0.0844	0.2126
Lack of Fit	0.09654	0.4157	0.0534	0.0568	0.2567	0.3172	0.0935

**Table 3** ANOVA results obtained by central composite rotatable design for total acceptability, hardness, cohesiveness, springiness, gumminess,  $L^*$  and  $a^*$  indices cheese analogue including rice milk, chia seed and hazelnut oil

The interaction of rice milk concentration and hazelnut oil content on total acceptability is shown in Fig. (1). According to the contour diagrams, the panelists scored cheese samples at low concentrations of rice milk and hazelnut oil more highly. In other words, rice milk in the range of 70 to 72% and hazelnut oil in the range of 4 to 5% improved the overall acceptance of the samples and in higher concentrations observed an inverse effect on this parameter. The interaction between chia seed and hazelnut oil concentrations on total acceptability of cheese analogue is presented in Fig. (1). In the samples with the lowest amount of chia seed (15%), the total acceptance decreased and with the increase of this compound, it reached its highest level up to 16.2% and then found a downward trend. Safaa (2017) stated that increasing the concentration of chia seed from 4 to 8% total acceptance in the samples increased and at higher levels reduction trend which is consistent with the results of the present study. Also, Arise et al. (2020) stated that increasing almond milk content in almond-soy cheese formulation from 30 to 100% acceptability reduction trend showed (Arise et al., 2020; Safaa, 2017).

#### **Texture profile analyser**

Texture profile analyser (TPA) value of cheese samples shown in Figs. (2) to (5).

With increasing rice milk concentration from 71 to 75 % and increasing chia seed from 15 to 18%, while the other variable was constant at the central point, the hardness increased (Fig. 2), which was due to the presence of starch-protein compounds and the establishment of a strength configuration in the cheese structure (Bravo-Núñez et al., 2019). The effect of independent variables on hardness was significant (P < 0.01). In the recent study, the highest hardness was predicted at about 2.64 (N). On the other hand, with increasing the amount of hazelnut oil in the range of 4 to 6%, the Cheese hardness factor decreased. analogue formulated samples with highest rice milk and chia seed concentrations were higher in gumminess values than hazelnut oil. This could be related to increasing of water binding capacity of protein-carbohydrate-fiber linkage existing in two ingredients which led to rising water holding capacity vegetable cheese. Regarding the effect of vegetable oils on the textural properties of different cheese samples, different results have been published, which indicate that the type of vegetable oil, method of preparation, type of product, temperature and concentration used are important parameters on the textural indicators of the product (Giha et al., 2021).



X1: B: Chia Bean (%) X2: C: Hazelnut oil (%)

Fig. 2. Contour plots of independent variable on hardness of cheese analogue including rice milk, chia seed and hazelnut oil

In a recent study, with increasing the concentration of hazelnut oil (4 to 6%), springiness and gumminess hardness. parameters showed reduction with a dosedependent. The oil initially formed from structure of triglyceride, attach with primary and secondary bonds. Heat treatment and rate of agitation in cheese making processing as important parameters cause these bonds to break. The firmness property of these products depends on the rupture of bond. In this study, an increase degradation of hazelnut oil during cheese analogue processing such as initial heat treatment, rapid homogenization, flash pasteurization, and cooling operations can result in reduction of protein-carbohydrate reaction. These changes can lead to establishing loose cheese analogue structure that is susceptible compression to and disintegration. Therefore, the reduction of texture properties such as the hardness of springiness and gumminess, with the increase in the concentration of hazelnut oil

in the formulation cheese analogue can be justified. Since the chia seed and hazelnut increased. oil the hardness behaved differently in a way that with increasing the ratio of chia seed, the hardness increased and hazelnut oil decreased this parameter. This was directly related to the increase in dry matter of the samples. Rice milk concentration had a direct effect on the hardness of cheese samples. Combination of high amylose and amylopectin starches in cheese mixture increased the hardness, which was referred to hydrogen bonding that leaked out from the starch particles during the heating process of cheese. The results of the present study are consistent with the results of (Hussein & Shalaby, 2018). Zisu & Shah (2005) mentioned that the fat reduction and the presence of starch are important parameters in increasing the hardness.Cohesiveness is related to the strength of the internal bonds in cheese structure, the lower cohesiveness the smoother cheese texture.





X1: B: Chia Bean (%) X2: C: Hazelnut oil (%)

Fig. 3. Contour plots of independent variable on cohesiveness of cheese analogue including rice milk, chia seed and hazelnut oil

The effect of independent variables on cohesiveness was a significant effect. The results showed that with increasing the concentration of rice milk and chia seed, the cohesiveness increased (Fig. 3), which was related to the formation of cross-links in protein-starch structure cheese analogue. Also, hazelnut oil concentration had negative effect on this parameter. Springiness is basically referred to the protein content in cheese formulation. Fat presented as globules contained the protein matrix network in cheese curd, acting as a plasticizer to inhibit the formation of crosslinks between the matrix chains (Roy et al., 2021). Lower protein and higher fat contents allowed cheeses to melt better and thus reduce the springiness. The contour independents plots of variable on gumminess observed in Fig. (4). Cheese samples with a higher ratio rice milk (27.50 to 75.00%) and chia seed (16.50 to 18.00%) had a higher gumminess than hazelnut oil (5.00 to 6.00%), (Fig. 5). This was due to much dense total solids content with more rice milk and chia seed in mixture formulation. which can cause more maintenance of serum entrapment in the aqueous section and an increase degree of cross-linkage of the carbohydrate-fiberprotein in cheese conformation. High gumminess is desirable characterization on the texture of cheese. In such a way that cheeses with high gumminess, due to the high protein content and reduced fat content the formulation. maintained their in structure against the share rate and the texture of the product improved. A number of researchers reported a direct relationship between reducing fat and increasing gum, which is consistent with the results of recent research, so that with the decrease in the amount of hazelnut oil, gumminess showed an upward trend (Cunha et al., 2010; Cunha et al., 2013; Hsieh et al., 2022; Zheng *et al.*, 2016).



X1: C: Hazelnut oil (%) X2: B: Chia Bean (%)

Fig. 4. Contour plots of independent variable on springiness and gumminess of cheese analogue including rice milk, chia seed and hazelnut oil



Fig. 5. Contour plots of independent variable on gumminess of cheese analogue including rice milk, chia seed and hazelnut oil



Fig 6 Contour plots of independent variable on L\* and a\* of cheese analogue including rice milk, chia seed and hazelnut oil

### Color

The cheese color had a key role on marketability, directly influencing acceptability, commercial value, and choosing type of milk. The results showed that with increasing rice milk the brightness index increased (Fig. 6). The interaction of the independent variables on the amount of redness and yellowness is shown (Fig. 6). According to the results, it was found that rice milk increased whiteness and hazelnut oil and chia seed reduced  $L^*$  of cheese samples. White rice was due to high amounts of amylose and amylopectin, as well as the absence of color compounds (Kraithong *et al.*, 2018). On the other hand,

the presence of cartinoid and Xanthophyll compounds in hazelnut oil structure increased  $a^*$  index. Also, Chia seed included chlorogenic, quercetin, caffeic acid and phenolic compounds that could increase yellowness of cheese samples. Anthocyanin is a dark-colored pigment present in black chia seeds (de Falco et al., 2017). The differences observed in cheese samples color demonstrate the linkage between the protein-carbohydrate sources with pigments in cheese structure.

**Optimization of cheese analogue formulation** Optimization results for determination of cheese analogue formulation with rice milk, chia seed and hazelnut oil observed in Fig. (7). Also the coefficient of determination  $(\mathbb{R}^2)$  is stated as the ratio of the changes explains using the model to the total changes, which is determined by the degree of fit. In the present project,  $R^2$ the total acceptability, hardness, for cohesiveness, springiness, gumminess,  $L^*$ and  $a^*$  parameters were 0.94, 0.99, 0.98, 0.95. 0.97 and 0.99 respectively, demonstrating that the quadratic model had significant effect on independent variable.



Fig. 7. Optimization results for determination of cheese analogue formulation with rice milk, chia seed and hazelnut oil

#### Conclusions

The results showed that rice milk, chia seed and hazelnut oil concentration had a significant effect on the total acceptability of cheese analogue. At low levels of rice milk, chia seed and hazelnut oil, this parameter showed an upward trend. The  $L^*$  observed a decreasing trend with increasing hazelnut oil and chia seed, while  $a^*$  index showed an increasing trend. Hardness, cohesiveness, springiness and gumminess parameters by increasing the ratio of rice milk and chia seed in cheese analogue formulation showed a significant effect with an upward trend. The optimum operating conditions for cheese formulation were investigated using numerical optimization technique with Design Expert Version 10 software. Nutritionists emphasis on the negative effects of consuming cheese is due to complications such as anemia and coronary heart diseases. Plant-based cheeses are a suitable and unique solution in order to achieve an economical and functional food. The use of rice milk, chia seed and hazelnut oil in the formulation of cheese analogue and its formulation optimization using the response surface method showed that the obtained product had suitable physicochemical, textural and organoleptic properties and can be utilized as a good substitute for cheeses made of cow milk.

#### **Author contributions**

Negar Golchin: Writing and drafting of the manuscript, Presenting the research idea and study design, Data analysis, Revising and editing the manuscript, Approval of the final version; Sara Jafarian: Revising and editing the manuscript, Approval of the final version, Presenting the research idea and study design; Seyyed Hossein Hosseini Ghaboos: Data analysis, Writing the draft of the manuscript, Revising and editing the manuscript, Supervising the study; Leila Roozbeh Nasiraie: Data analysis and interpretation, Presenting the research idea and study design, Approval of the final version.

## **Conflicts of interest**

There is no conflict of interest based on the writers.

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# بهینهسازی فرمولاسیون پنیر آنالوگ با شیر برنج، دانهٔ چیا و روغن فندق با استفاده از روش سطح پاسخ

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#### چکیدہ

خواص فیزیکی، شیمیایی و بافتی پنیر به منبع آن و همچنین شرایط فرمولاسیون بستگی دارد. بهتازگی پنیر انالوگ بهواسطهٔ داشتن ویژگیهای تغذیهای، بافتی، حسی و سلامتیبخشی موردتوجه مصرفکنندگان قرار گرفته است. این مطالعه با هدف بررسی اثر متقابل پارامترهای مستقل مانند شیر برنج (۲۰ تا ۲۵ درصد)، دانهٔ چیا (۱۵ تا ۱۸ درصد) و روغن فندق (۴ تا ۶ درصد) برای ارزیابی فرمولاسیون بهینهٔ آنالوگ پنیر با استفاده از روش سطح پاسخ بود. باتوجهبه نتایج بهدستآمده مشاهده گردید که با افزایش غلظت متغیرهای مستقل؛ پذیرش کلی نمونهها در ابتدا افزایش یافت و سپس روند نزولی را نشان داد. همچنین ویژگیهای بافتی نمونههای پنیر آنالوگ مانند سختی، چسبندگی، فنریت و صمغیت، نشان داد که شیر برنج و دانهٔ چیا باعث بهبود خواص بافتی شده، اما غلظت روغن فندق بر این پارامترها تأثیر منفی داشت. غلظت دانهٔ چیا و روغن فندق باعث کاهش شاخص روشنایی و غلظت شیر برنج باعث افزایش شاخص روشنایی پارامترها تأثیر منفی داشت. غلظت دانهٔ چیا و روغن فندق باعث کاهش شاخص روشنایی و غلظت شیر برنج باعث افزایش شاخص روشنایی پارامترها تأثیر منفی داشت. غلظت دانهٔ چیا و روغن فندق باعث کاهش شاخص روشنایی و غلظت شیر برنج باعث افزایش شاخص روشنایی پارامترها تأثیر منفی داشت. غلظت دانهٔ چیا و روغن فندق باعث کاهش شاخص روشنایی و غلظت شیر برنج باعث افزایش شاخص روشنایی پارامترها تاثیر منفی داشت. غلظت دانهٔ چیا و روغن فندق باعث کاهش شاخص روشنایی و غلظت شیر برنج باعث افزایش شاخص روشنایی پارامترها تاثیر منفی داشت. غلظت دانهٔ چیا و روغن فندق باعث کاهش شاخص روشنایی و غلظت شیر برنج باعث افزایش شاخص روشنایی پارمایر ما داذ که فرولاسیون بهینه آنالوگ پنیر، ۲۱/۲۷، ۲/۱۶، ۲/۱۶، ۲/۱۶، ۲/۱۶، ۲/۱۶، ۲/۱۶، ۲/۱۶، ۲/۱۶، ۲/۱۶، ۱۵/۱۶، ۲/۱۶ و دری به سخی انور مورنایی، زردی، مطویت بودستآمده در پژوهش حاضر میتوان نتیجه گرفت که روش سطح پاسخ قادر به پیشبینی فرمولاسیون بهینهٔ پنیر آنالوگ با

**واژههای کلیدی**: پنیر آنالوگ، روغن فندق، سختی، شیر برنج، مطلوبیت بالا