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## Scientific Research

## Optimizing gluten-free biscuit formulation using date syrup and date kernel powder

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## ARTICLE INFO

## ABSTRACT

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It appears that celiac patients have more trust in products like biscuits and crackers as sources of carbohydrates. Therefore, this study focused on evaluating and optimizing gluten-free biscuits using date syrup and date seed powder. Sugar levels at 20%, 30%, 40%, and 60% were replaced by date syrup and rice flour at levels of 0, while rice flour levels at 0%, 10%, 13%, and 20% were substituted with date seed powder. Gluten-free biscuits were assessed based on the dough's textural properties, as well as the biscuits' physicochemical properties, including spread ratio, moisture, water activity, color, antioxidant activity, total fiber, total sugar, fat, and protein, using standard methods. Sensory evaluation was conducted with a 5-point hedonic test, and mouthfeel was assessed with Sensomaker software to determine the optimal replacement levels for date syrup and date seed powder. Finally, the proposed optimized formulation was produced, and textural and physicochemical properties, including firmness, total fiber, total sugar, fat, protein, ash, pH, moisture, water activity, color, and antioxidant activity, were measured and compared with a control sample. Replacing rice flour with date seed powder and sugar with date syrup increased the dough's firmness and elasticity. Regarding the textural properties of gluten-free biscuits, firmness significantly increased with higher levels of date seed powder and date syrup. For sensory characteristics, replacing rice flour with date seed powder and sugar with date syrup had a significant effect on taste, crispness, and color.

## 1- Introduction

Celiac disease is a chronic inflammation of the small intestine that leads to various symptoms such as malnutrition, intestinal disorders, skin problems, bone issues, neurological and muscular complications [1]. Celiac disease is a global health concern, affecting approximately 1% of the world's population [2]. This disease results in the malabsorption of several essential nutrients, including iron, folic acid, calcium, and fat-soluble vitamins. Currently, the only effective treatment and management strategy for celiac disease and gluten-related disorders is a strict gluten-free diet [3]. The gluten network in bread and other soft products plays an essential role when processed with biological leavening agents such as yeast [4], whereas it does not have a significant function in biscuits. In these products, the gluten network must develop gradually to achieve a dough that is more or less sticky. It appears that celiac patients have greater confidence in products such as biscuits and crackers as a source of carbohydrates [2].

One way to enrich gluten-free products is by using date-based ingredients. Date syrup is one of the most important date-derived products and is used in the formulation of various food items, including ice cream, beverages, confectionery, bakery products, sesame paste and date syrup mixtures, jams, and butter. It is rich in carbohydrates [5]. Glucose and fructose are the main sugars present in date syrup, with a total sugar content of 88% [6]. In addition to its nutritional components, date syrup is also rich in antioxidants. Researchers have investigated the incorporation of date syrup in sponge cake formulations. The results indicated that replacing sucrose with date syrup had no significant effect on the firmness of the samples [7]. In another study, sucrose was replaced with date syrup at different levels, and the results showed a significant increase in phenolic content and antioxidant activity ( $p < 0.05$ ); however, the color of the samples containing date syrup darkened significantly [7]. Date syrup has also been used as a sucrose substitute in various other products, such as different types of cookies, biscuits, layered cakes, and sponge cakes [8].

Date seed powder is another date-derived product that has gained significant attention in recent years. Date seeds contain high amounts of potassium, sodium, magnesium, zinc, copper, nickel, cobalt, chromium, lead, and cadmium, with potassium, phosphorus, magnesium, calcium, and sodium being present in higher concentrations. Additionally, date seeds are an excellent source of phenolic compounds, gallic acid, and antioxidants. Due to their high polyphenol content and nutritional value, date seeds can be used as a functional ingredient in the food, pharmaceutical, and cosmetic industries

[9]. In one study, the chemical, physical, and sensory properties of biscuits formulated with date seed powder were evaluated. Sensory analysis results indicated that biscuits containing 30% date seed powder had the highest acceptance. However, the specific volume and density of the biscuits decreased as the level of date seed powder increased, while hardness increased [8]. Another study examined the functional properties of flatbread made with date seed powder. The results showed no significant difference in moisture content compared to the control sample, and different levels of date seed powder had no effect on protein content [10]. Due to the absence of gluten in products designed for celiac patients, the sensory characteristics of these products are generally lower compared to similar gluten-containing products. Therefore, sensory evaluation plays a crucial role in assessing their quality. Sensomaker is a software used for analyzing sensory study data and is considered user-friendly due to its various functional domains. The required data can be collected using different methods, such as categorized scales, linear scales, Temporal Dominance of Sensations (TDS), and Time-Intensity (TI) analysis. Data analysis can be performed using various techniques, and the software provides informative curves and charts. Sensomaker is particularly beneficial for products where consumer preference is a priority, making it a valuable tool for product development and improvement in relation to sensory attributes [11]. Given the limited research on the use of date-based products in gluten-free food production, the present study aims to expand the variety of gluten-free products by investigating the effects of incorporating date syrup and date seed powder into the formulation of gluten-free biscuits on their physicochemical and sensory properties.

## 2. Materials and methods

### 2.1. Raw materials for gluten-free biscuit preparation

Rice flour, cornstarch, solid oil, powdered sugar, eggs, rose water, and cardamom powder were purchased from local stores. Date syrup and date seeds were obtained from Noshin-e-Khoy factory.

### 2.2. Preparation of date seed powder

In the first step, date seed powder was prepared. The date seeds were first washed, dried, and then placed on a baking tray. They were roasted in an oven at 160°C for 15 minutes, with stirring every 7 minutes to ensure uniform roasting. After cooling, the roasted seeds were ground using a grinder and passed twice through a 60-mesh sieve. The final powder was stored in zip-lock polyethylene bags in a dry and cool place until use.

### 2.3. Formulation of biscuit dough samples

In this study, gluten-free biscuits were prepared by replacing sugar with date syrup and substituting rice flour with date seed powder. The formulation

was designed using Design Expert software based on the Response Surface Methodology (RSM), following the conditions outlined in Table 1.

Table 1: Formulation of different biscuit samples

Sample code Ingredient s	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Date seed powder	10 %	0%	20%	20%	0%	0%	13 %	0%	10%	0%	20 %	20 %	10%	0%
Date syrup	30 %	40 %	0%	0%	0%	0%	60 %	60%	0%	60%	60 %	20 %	0%	20%
Rice flour	90 %	60 %	80%	80%	100 %	100 %	87 %	100 %	90%	100 %	80 %	80 %	90%	100 %
Sugar	70 %	60 %	100 %	100 %	100 %	100 %	40 %	40%	100 %	40%	40 %	80 %	100 %	80%

## 2.4. Sample preparation

First, egg yolk, powdered sugar, and cardamom were mixed by a hand mixer for 2 minutes using the creaming method. Then, semi-solid oil was added and mixed for 20 seconds. Next, rice flour and cornstarch were incorporated into the mixture to form a dough. Based on the formulation, the amounts of date seed powder, date syrup, powdered sugar, and rice flour were adjusted accordingly. The prepared dough was placed in a zip-lock polyethylene bag and rested in the refrigerator for 30 minutes. After resting, the dough was rolled out to a thickness of 8 mm using a rolling pin. It was then cut into 40 mm diameter shapes and baked in an oven at 150°C for 15 minutes. After cooling, the biscuits were stored in zip-lock bags and then placed in PET disposable containers at room temperature until testing

### 2.4.1. Dough texture evaluation

After preparing the dough, a specific amount was uniformly compressed into glass plates with a diameter of 10 cm and a height of 1.5 cm, then allowed to rest for 15 minutes. The dough texture was evaluated using a Texturometer TA-XT plus (Stable Micro-Systems, UK). The compression test was conducted using a 35 mm stainless steel probe with a probe speed of 0.5 mm/s, performing a two-cycle (compression and return) test with 75% penetration [12].

## 2.5. Physical tests of biscuits

### 2.5.1. Water activity measurement

The water activity (aw) of the gluten-free biscuit samples was measured using a Novasina water activity meter [12].

### 2.5.2. Measurement of dimensions and spread ratio

For this test, the diameter and thickness of five biscuits from each treatment were measured in three replicates. The spread ratio was calculated by dividing the diameter by the thickness using Equation 1 [12].

$$1] \text{ Spread ratio} = (\text{diameter} / \text{thickness}) \times 100$$

### 2.5.3. Color measurement

The surface color of the biscuits was measured using a CHROMA METER CR-400 (Konica Minolta, Japan) [13]. Additionally, the total color difference ( $\Delta E$ ) was calculated using the following formula:

$$2] \Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$$

Where:

$L^*$ ,  $a^*$ , and  $b^*$  represent the color parameters of the sample.

## 2.6. Chemical tests of biscuits

### 2.6.1. Moisture measurement

Moisture content was measured using Oven method [4].

### 2.6.2. Ash content measurement

Ash content was measured using the muffle furnace method at 550°C [12].

### 2.6.3. pH measurement

To measure pH, 10 grams of the grinded sample were thoroughly mixed with 100 mL of freshly boiled distilled water. After 20 minutes, the pH of the water phase was measured using a pH meter [12].

### 2.6.4. Acidity measurement

Acidity was measured using the titration method with a standard sodium hydroxide (NaOH) solution, and the results were expressed as the percentage of free fatty acids (FFA) based on the weight percentage of oleic acid [12].

$$3] \text{ Acidity (gr Oleic Acid / 100 gr)} = (A \times N \times 0.009) \times 2$$

Where A is the volume of NaOH used (mL) and N is the normality of NaOH

#### 2.6.5. Fat content measurement

Fat content was determined using the Soxhlet extraction method [14]. 5 grams of the sample were carefully weighed on filter paper, placed inside a Soxhlet cartridge, and covered with cotton. The fat extraction flask was pre-heated, cooled, and weighed precisely, then filled with hexane and connected to the Soxhlet apparatus and the extraction process was carried out for 4 hours.

#### 2.6.6. Protein measurement

The Kjeldahl method was used to determine the protein content [12].

#### 2.6.7. Total sugar measurement

Total sugar content was determined using the Fehling's method [12].

#### 2.6.8. Total fiber measurement

The fiber content of the gluten-free biscuits was measured according to Iranian National Standard No. 3105 for food and agricultural products [12].

#### 2.6.9 antioxidant activity measurement

The antioxidant activity was determined using the DPPH radical scavenging assay [15].

### 2.7. Sensory evaluation of gluten-free biscuits

#### 2.7.1. Hedonic test

The sensory properties of the gluten-free biscuits were evaluated using a 5-point hedonic scale. A panel of 15 participants (8 women and 7 men, aged 25 to 65 years) assessed the biscuits after 24 hours of storage under identical conditions. The texture, appearance, color, taste, and overall acceptability were evaluated.

#### 2.7.2 Mouth adhesion test

After determining the required test duration, a software-based analysis was used to measure the adhesion time of the biscuit samples to the palate and teeth [16].

## 3. Results and discussion

### 3.1. Biscuit dough texture characteristics

The effect of date seed powder and date syrup on the firmness of gluten-free dough was statistically significant. Figure 1 illustrates the changes in dough firmness as the level of date seed powder increases.

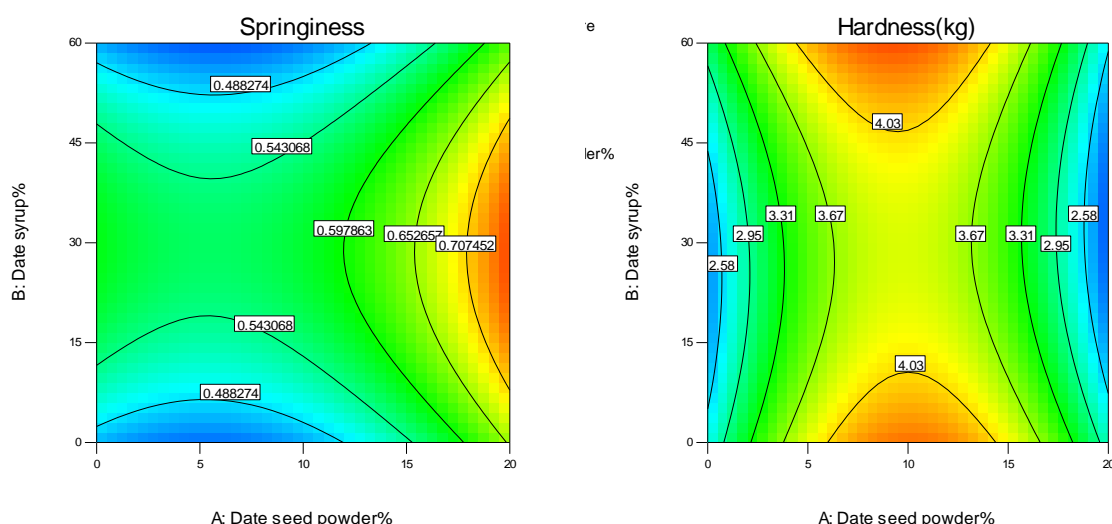


Figure 1: Graph of changes in firmness and Springiness of gluten-free biscuit dough

According to the graph, increasing the amount of date seed powder led to a higher dough firmness. However, the independent effect of date syrup and the interaction between date seed powder and date syrup did not follow a consistent trend. As shown in Figure 1, increasing date syrup levels led to an increase in dough elasticity. However, there was no clear trend regarding the independent effect of date seed powder or its interaction with date syrup on elasticity.

#### 3.2. Changes in biscuit firmness over time after baking

The results indicate that date seed powder and date syrup had a significant effect on the firmness of gluten-free biscuits one day after baking. As shown in Figure 2, increasing the level of date seed powder resulted in an increase in biscuit firmness. Similarly, increasing the amount of date syrup also led to a firmer texture, which aligns with findings from previous research. A study on gluten-free cookies formulated with Shahaneh date flour and rice flour reported a similar trend, where the combination of date flour and rice flour resulted in higher firmness compared to the control dough.

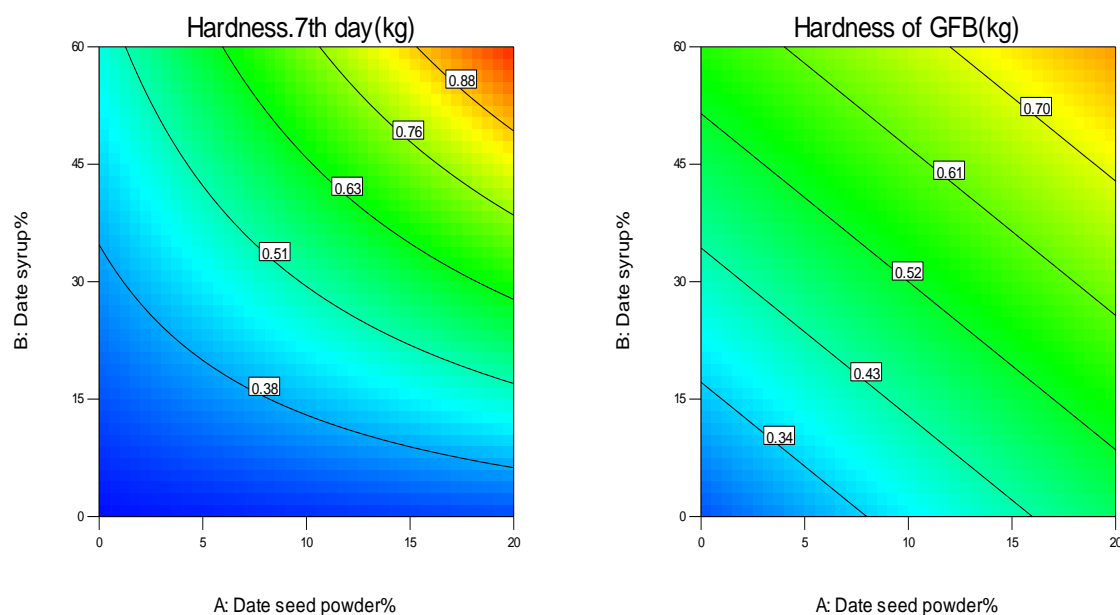


Figure 2: Contour plot graph of changes in firmness of gluten-free biscuit

The study concluded that incorporating date seed powder increased cookie firmness [13]. Another study evaluating the physicochemical, physical, and sensory properties of biscuits containing date seed powder found that specific volume and density decreased as the level of date seed powder increased, while firmness increased [8]. The firmness of gluten-free biscuits containing date seed powder and date syrup was measured one week after baking. As shown in Figure 2, the results indicate that increasing the level of date seed powder led to a higher biscuit firmness. Similarly, with higher levels of date syrup, the firmness of biscuits also increased. Furthermore, regarding the interaction effects between the two factors, it was observed that as both

ingredients increased, the biscuit firmness continued to rise.

### 3.3. Spread ratio

The spread ratio (or diameter) is a key indicator of the flour properties used in biscuit production and plays an important role in biscuit acceptance and desirability [17]. Figure 3 illustrates the effect of date seed powder and date syrup on the spread ratio of gluten-free biscuits. Increasing date seed powder levels resulted in a decrease in the spread ratio. Increasing date syrup levels, on the other hand, led to a higher spread ratio. A study investigating the replacement of sugar with date syrup in cookies found that biscuits containing date syrup exhibited a reduction in thickness, diameter, and spread ratio [4].

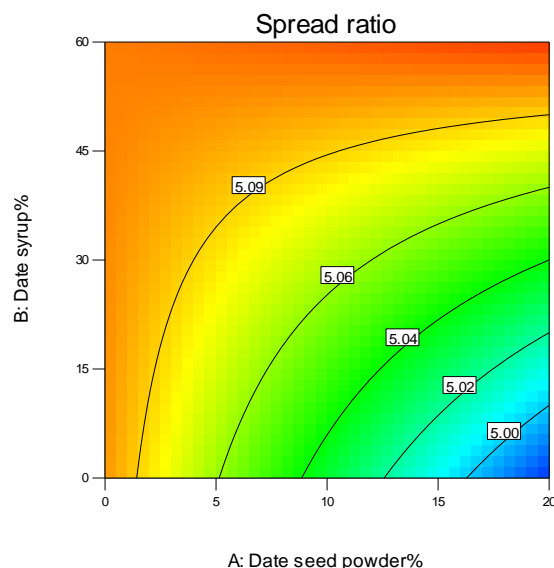


Figure 3: Contour plot diagram of thickness variations in gluten-free biscuits

A study on the nutritional properties of gluten-free biscuits containing date seed powder and chickpea flour revealed that increasing the amount of date seed powder led to an increase in thickness and a decrease in the spread ratio [18]. One possible explanation for the reduction in spread ratio in biscuits containing date seed powder is that gluten-free flours with high protein content tend to absorb more water during dough preparation. This reduces the availability of free water to dissolve sugar during baking, leading to higher dough viscosity and ultimately a lower spread ratio [19].

#### 4.3. Water activity of biscuits over time

The results shown in Figure 4 illustrate the effects of date seed powder and date syrup on the water

activity of gluten-free biscuits one day after baking. The data indicate that increasing the levels of date seed powder led to a decrease in water activity, while increasing the levels of date syrup resulted in higher water activity. Similarly, as shown in Figure 4, the water activity of biscuits one week after baking changed based on the independent effects of each variable. Higher levels of date seed powder further reduced water activity however, higher levels of date syrup increased water activity. A combination of higher date syrup and lower date seed powder resulted in higher water activity. Conversely, higher date seed powder and lower date syrup led to lower water activity.

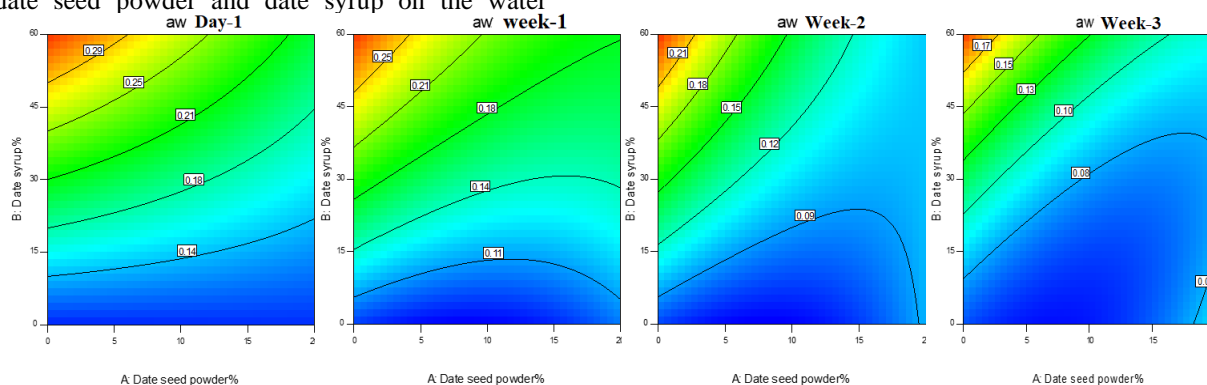


Figure 4: Contour plot of aw changes after baking over time

Figure 4 demonstrates the water activity trends of gluten-free biscuits two weeks post-baking. Water activity decreased with increasing date seed powder content, acting as an independent variable. Similarly, elevated date syrup levels also led to reduced water activity. Particularly, both variables significantly influenced water activity in biscuits

three weeks post-baking. Higher date seed powder concentrations continued to reduce water activity, while increased date syrup levels resulted in elevated water activity. The combination of higher date syrup and lower date seed powder led to increased water activity, while higher date seed

powder with lower date syrup decreased water activity.

### 3.5. Color measurement

#### 3.5.1. Lightness index ( $L^*$ )

Figure 5 illustrates the effects of date seed powder and date syrup on the lightness ( $L^*$ ) of gluten-free biscuits. Based on the graph, increasing each variable independently resulted in a decrease in biscuit lightness, and the combined effect of both variables also led to a further reduction in lightness. This decrease in brightness is attributed to the high concentration of reducing sugars in date syrup, which are more reactive than sucrose and actively participate in the Maillard reaction [20]. Other researchers, in a study on biscuits enriched with date

seed flour and wheat bran flour, also found that increasing the amount of date seed powder led to a decrease in lightness [20].

#### 3.5.2. Redness index ( $a^*$ )

According to Figure 5, the redness ( $a^*$ ) of the biscuits was affected as follows: increasing each variable independently resulted in a higher red intensity, and the combined effect of both variables further intensified the red coloration. The increase in redness may be attributed to the natural pigments present in date seed powder and date syrup, as well as the participation of reducing sugars in the Maillard reaction.

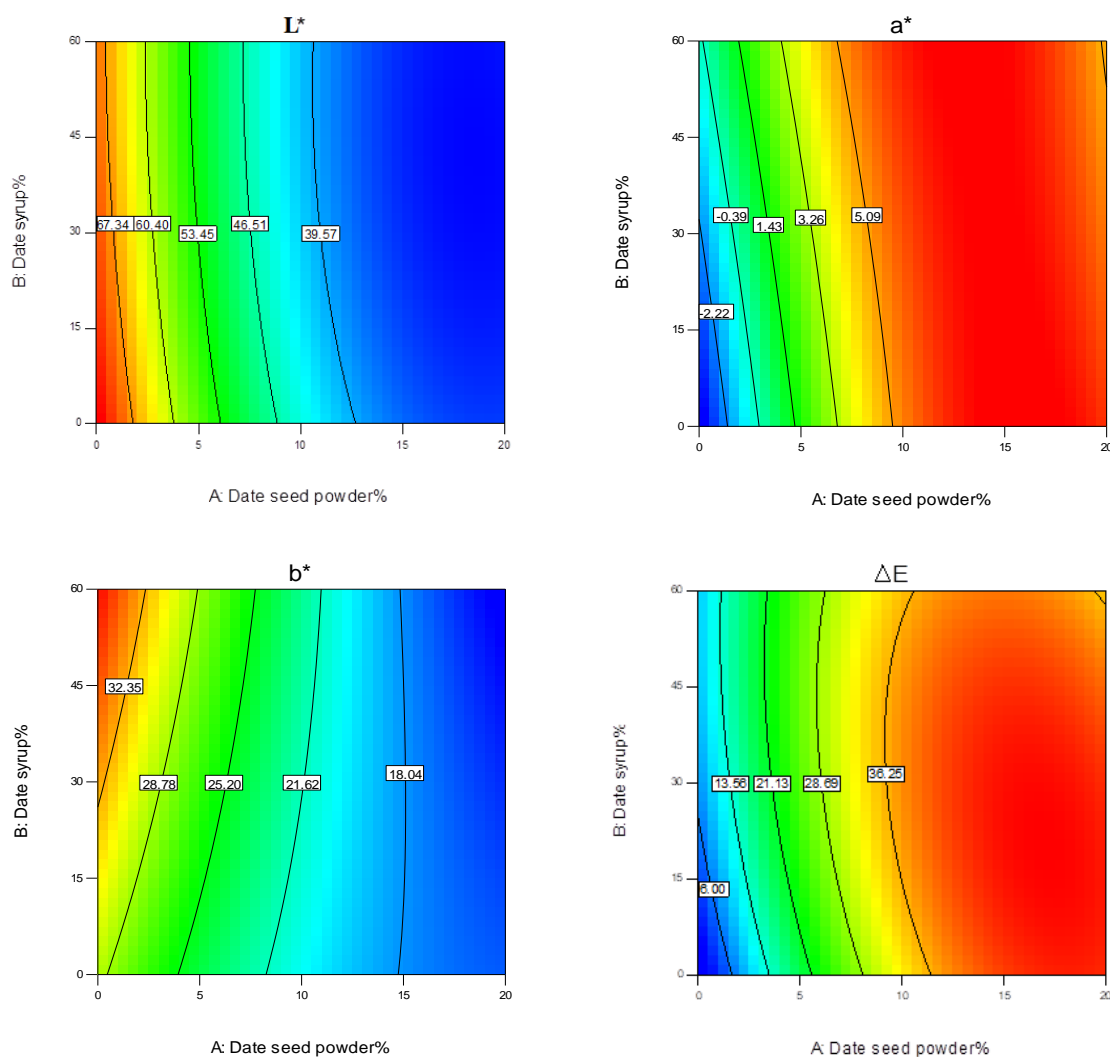


Figure 5: Contour plot of color parameter changes in gluten-free biscuit

#### 3.5.3. Yellowness index ( $b^*$ )

As shown in Figure 5, data obtained from the colorimeter indicate that increasing each variable independently resulted in a reduction in yellowness. The interaction of date seed powder and date syrup also led to a decrease in biscuit yellowness.

#### 3.5.4. Overall color change ( $\Delta E$ )

According to Figure 5, increasing each of the variables (date seed powder and date syrup) independently led to an increase in the  $\Delta E$  value of the biscuits. Regarding the interaction effects of the two variables, it was also observed that increasing the levels of both variables resulted in a higher  $\Delta E$  value in the biscuits.



### 3.6. Chemical properties of gluten-free biscuits

#### 3.6.1. Moisture content measurement

The final moisture content of baked products is one of the most important quality parameters, as it affects the shelf life of the product [21]. Due to its high hygroscopic and hydrophilic properties, date syrup prevents excessive water loss during baking. The sugars in date syrup, including glucose and fructose, dissolve more quickly at lower temperatures compared to sucrose and absorb more water [22]. As shown in Figure 6, the presence of date seed powder and date syrup in the formulation of gluten-free biscuits influenced the moisture content of the samples. With increasing levels of

date syrup, the moisture content increased, whereas higher levels of date seed powder led to a decrease in moisture content. In a study on the effect of replacing date syrup and date powder in cookie formulations, researchers concluded that both ingredients increased the moisture content of samples containing these factors [18]. For samples containing sucrose, when the dough enters the oven, water evaporates easily in the initial stage of baking, creating an open structure in the dough, which facilitates water evaporation from the biscuit. However, for samples containing date syrup, rapid water evaporation is prevented when the dough enters the oven.

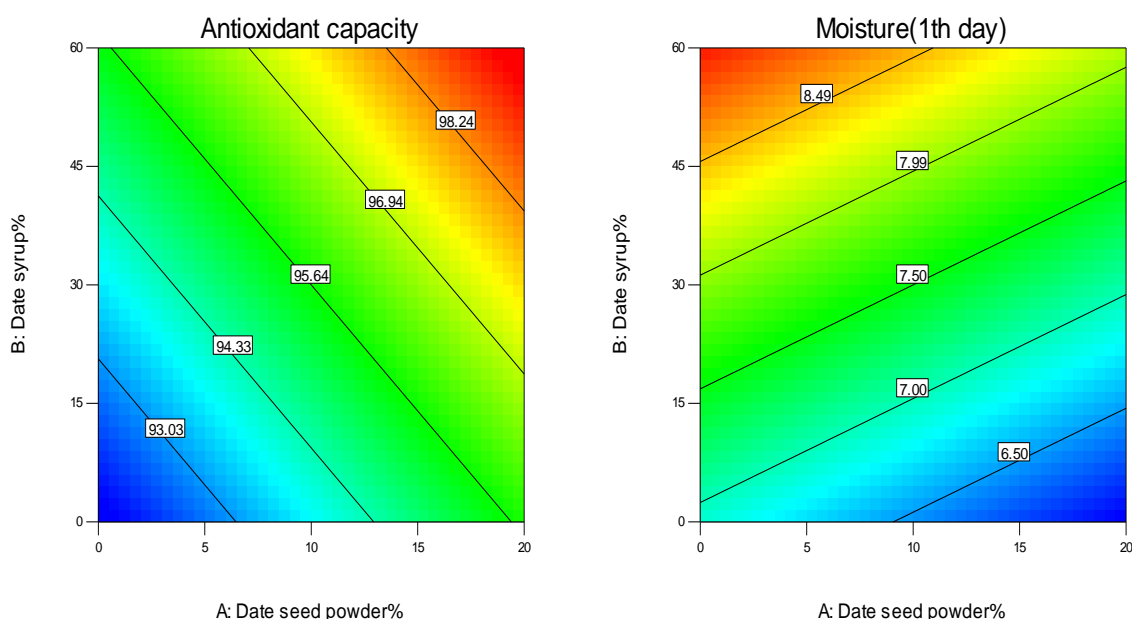


Figure 6: Contour plot of moisture content and antioxidant level changes in gluten-free biscuits

#### 6-3-2 Antioxidant content

Date seeds are recognized as one of the richest plant sources of polyphenols, even surpassing seeds such as flaxseed and linseed [23]. According to the results shown in Figure 6, increasing the levels of both date syrup and date seed powder led to an increase in antioxidant properties. However, the interaction effects between date seed powder and date syrup were not statistically significant. These findings are consistent with the results of other researchers [7].

### 3.7. Sensory properties

#### 3.7.1. Crispiness

Based on the trend observed in the graph in Figure 7, increasing the amount of date seed powder led to higher acceptance of biscuit crispiness. Regarding

the interaction effects between date seed powder and date syrup, it was found that increasing the levels of date seed powder while reducing the levels of date syrup resulted in greater acceptance of crispiness.

#### 3.7.2. Flavor

The results related to the flavor of gluten-free biscuits are presented in Figure 7, showing a significant effect of date seed powder on the flavor of gluten-free biscuits ( $P < 0.05$ ). According to the findings, increasing the levels of date seed powder improved flavor acceptability. In contrast, the effect of date syrup, as another independent variable, on flavor acceptability was not statistically significant ( $P > 0.05$ ).



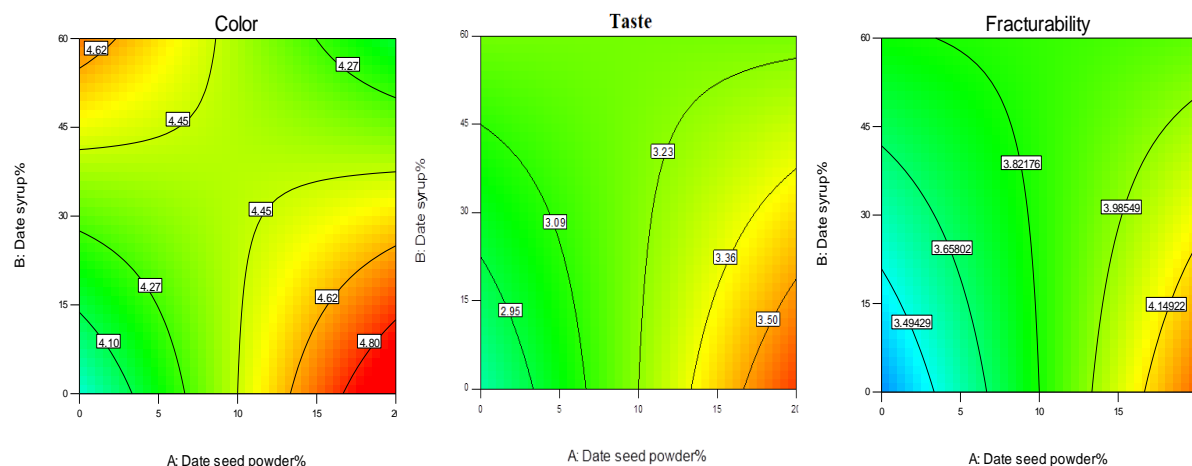


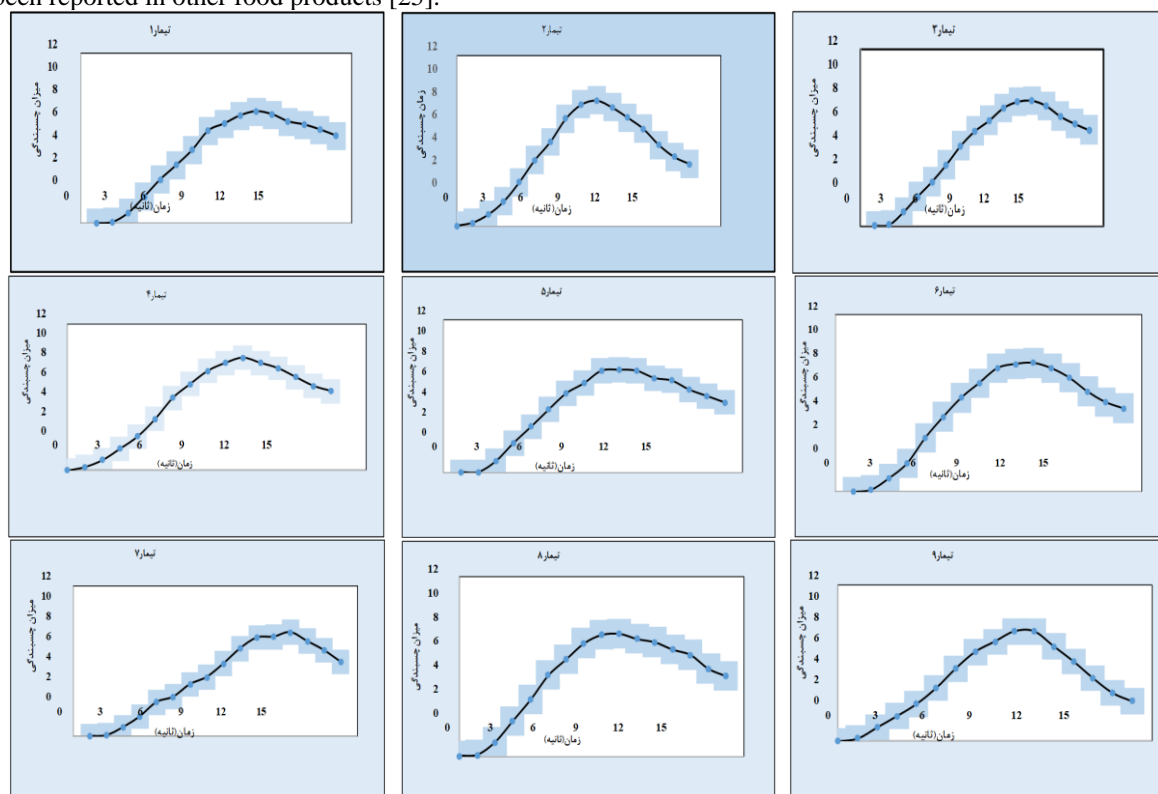
Figure 7: Graph of crispness, flavor, and color acceptance levels in gluten-free biscuits

### 3.7.3. Color

As shown in Figure 7, increasing the amount of date seed powder enhanced the acceptance of biscuit color. Other researchers have also reported that adding sea buckthorn seed powder increased the whiteness of bread and improved its color acceptability [24]. Regarding the interaction effects between date seed powder and date syrup, it was observed that increasing the levels of date seed powder while reducing the levels of date syrup resulted in higher color acceptance. A reduction in brightness due to the addition of date syrup has also been reported in other food products [25].

### 3.7.4. Evaluation of Oral Stickiness Using the SensoMaker Software

Figure 8 presents the graphs related to the degree of oral stickiness of the samples at different time points. According to the results, the level of stickiness varied among different formulations. In treatments No. 1, 3, 4, 6, 7, 12, and 14, the adhesiveness increased until the 12th second, after which the curve followed a decreasing trend. In treatments No. 2, 5, 8, 9, 10, 11, and 13, the adhesiveness increased until the 9th second and then showed a declining trend.



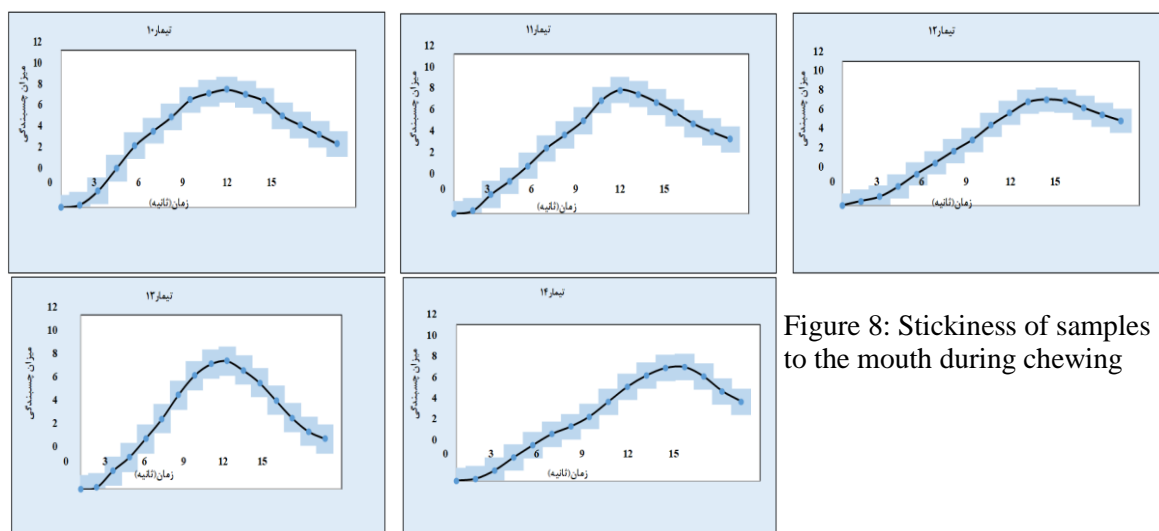


Figure 8: Stickiness of samples to the mouth during chewing

### 3.7.4. Appearance and Overall Acceptance

According to the results, the effects of date seed powder and date syrup on the acceptance of other evaluated parameters, including appearance and overall acceptance, were not statistically significant ( $P > 0.05$ ). The impact of adding powders on the overall acceptance of a food product depends on the type of powder used. While sea buckthorn powder had a negative effect on the overall acceptance of gluten-free bread samples [24], the addition of carrot pomace powder improved the sensory characteristics of gluten-free bread [26].

### 3.8. Optimization

To determine the optimal sample, after conducting tests, analyzing responses, and fitting statistical models, the optimal amounts of date seed powder and date syrup were identified. At this stage, the Design Expert software and the Response Surface Methodology (RSM) were used to obtain the optimal formulation by replacing rice flour with date seed powder and sugar with date syrup. The ideal response values and their importance levels were determined by the software. Based on the results, the gluten-free biscuit with 20% date seed powder and 60% date syrup was identified as the best substitute for rice flour and sugar (Figure 9).



Figure 9: Control sample (right) and optimized sample (left)

### 3.9. Evaluation of Physical, Chemical, and Sensory Properties of the Optimized Biscuit Compared to the Control Sample

The results of the physical, chemical, and sensory properties of the gluten-free biscuits made from the optimized formulation compared to the control sample are presented in Table 2.

Table 2: Results related to the physicochemical and sensory properties of gluten-free biscuits

Evaluated Parameter	Optimized Sample	Control Sample
Moisture	7.8	6.9
Water Activity	0.21	0.10
Spread Ratio	5.08	5.11
L*	32.74	74.08
a*	5.55	-4.03
b*	14.97	29.57
Antioxidant Property	99.25	91.85
Fat	21	19
Protein	6.74	6.22
Ash	1	0.72
Total Sugar content	2	12.55
Total Fiber content	31	26
pH	5.42	6.45

## 4. Conclusion

In this study, date seed powder and date syrup were used as substitutes for rice flour and sugar to enhance the quality of gluten-free biscuits. Based on the results of physicochemical and sensory properties, the optimal formulation included 20% date seed powder, which, due to its high antioxidant activity, high fiber content, desirable physical properties, and consumer acceptance in terms of

taste, color, and appearance, can be considered a functional ingredient for gluten-free food products, particularly for celiac patients, in the food industry.

## 5. Acknowledgment

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## بهینه سازی فرمولاسیون بیسکوئیت فاقد گلوتن با استفاده از شیر خرم و پودر هسته خرما

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به نظر میرسد که بیماران سلیاکی اعتماد بیشتری به محصولاتی مانند بیسکوئیت ها و کراکرها به عنوان منبع کربوهیدرات دارند. از این رو این مطالعه به بررسی و بهینه سازی بیسکوئیت فاقد گلوتن با استفاده از شیر خرم و پودر هسته خرما پرداخته است. شکر مصرفی در سطوح ۰، ۱۰، ۱۳ و ۲۰ درصد به وسیله پودر هسته خرما جایگزین گردیدند. بیسکوئیت های فاقد گلوتن از لحاظ خواص بافتی خمیر و بیسکوئیت و خواص فیزیکی شیمیایی بیسکوئیت شامل ضریب پخش، رطوبت، فعالیت آبی، رنگ، فعالیت آنتی اکسیدانی، فیبرکل، قندکل، چربی و پروتئین بر اساس روش های استاندارد مورد ارزیابی قرار گرفتند. برای تعیین بهترین سطوح جایگزینی شیر خرم و پودر هسته خرما، ارزیابی حسی با استفاده از آزمون هدونیک ۵ نقطه ای و ارزیابی احساس دهانی با استفاده از نرم افزار سنسومیکر انجام گرفت. در نهایت ترکیب بهینه پیشنهادی تولید و ویژگی های بافتی و فیزیکی شیمیایی شامل سفتی، فیبرکل، قندکل، چربی، پروتئین، خاکستر، pH، رطوبت، فعالیت آبی، رنگ، فعالیت آنتی اکسیدانی اندازه گیری و با نمونه شاهد مقایسه شد. جایگزینی آرد برنج با پودر هسته خرما و همچنین جایگزینی شکر با شیر خرم، سفتی و ارتجاعیت خمیر را افزایش داد. در ارتباط با ویژگی های بافتی بیسکوئیت های فاقد گلوتن، سفتی با افزایش سطوح پودر هسته خرما و شیر خرم به طور معناداری افزایش پیدا کرد. در مورد ویژگی های حسی نیز باید اظهار داشت که جایگزینی آرد برنج با پودر هسته خرما و همچنین شکر با شیر خرم بر روی طعم، تردی و رنگ تاثیر معناداری داشت.