



### Effects of Xanthan gum and *Melissa officinalis* seed gum on physicochemical properties, acrylamide formation, and acceptability of baguette bread

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## ABSTRACT

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Acrylamide, as a toxic and carcinogenic compound, is found in many cooked or fried foods. In this work, the effect of *Melissa Officinalis* seed gum (MOSG) in the concentration of 1% (w/w) was investigated on acrylamide formation in bread and compared with baguette bread containing xanthan 1% (w/w) and control sample (without gum). The addition of MOSG increased the moisture level of the bread samples from 22.1% to 23.6% ( $p < 0.05$ ). The acrylamide content of bread and those containing MOSG and xanthan gum were 1180, 836.75, and 1167.6 ng/g, respectively. The addition of MOSG reduced the hardness of the samples from 3380 g to 730 g and the bread containing xanthan gum showed the lowest hardness (410 g). The addition of MOSG to the bread's formulation increased  $L^*$  (brightness) and  $b^*$  (blue/yellow) values and reduced  $a^*$  (red/green) value of the samples. The sensory evaluation results demonstrated that the addition of MOSG improved porosity, softness, flavor and taste, aroma, color, chewiness, and elasticity of the samples. In conclusion, MOSG was found to be a potential candidate with excellent efficiency to be used for the reduction of acrylamide from bread.

## 1- Introduction

Acrylamide (is a toxic and carcinogenic compound found in many cooked or fried foods. Acrylamide is a white, crystalline, solid, colorless, odorless substance classified as a carcinogen. Carbohydrate-rich foods contain more content of acrylamide than protein-rich products. The production of acrylamide occurs mainly by Millard browning reactions, and various factors, including the initial percentage of the precursors, flour quality, processing conditions, and nutrient composition, affect its formation [1, 2]. The formation of acrylamide predisposes to the presence of reducing sugars and asparagine [3]. Asparagine is an important amino acid that interacts with reducing sugars and forms acrylamide [4]. The maximum content of acrylamide formation is produced in an asparagine/glucose ratio of 1:1 [5].

Baking bread at a high temperature and the presence of carbohydrates in this product are the main reasons for the production of acrylamide [6, 7]. Bread is considered a staple foodstuff in many countries and provides energy, protein, minerals, and B vitamins. In Iran, about 60-65% of protein and calories, as well as about 2-3 g of salt, are provided by

bread [8]. Therefore, considering the importance of bread, which is one of the cheapest and most essential foods, the improvement of its quality is necessary. The acrylamide content in bread, cookies/cakes, and breakfast cereals are, on average, 446, 350, and 96  $\mu\text{g} / \text{kg}$ , respectively [9].

The acrylamide content reduction in the foods is essential. Several scientists have been focused on reducing acrylamide formation during food processing while maintaining the quality of products. Apart from modifying the product formulations, heating and processing circumstances such as baking time and temperature, lowering pH, and addition of various ingredients have been tested to decrease the amount of acrylamide formed in fried and dried foods. The reducing effect of some inhibitors on acrylamide formation has been examined in several food systems [10-12].

Recent works have shown that some vitamins have a significant inhibitory effect on acrylamide formation [13-15]. It has been reported that there is a positive relationship between the amount of acrylamide and dry matter in flatbread samples [16]. Liu, Liu, Man and Liu [17] coated the bread with starch and

reduced acrylamide content. These authors also reported that the dough's coating by a combination of glycine and corn starch could reduce acrylamide content. It also has been reported that natural compounds such as bamboo and olive oil had good potential to inhibit acrylamide formation [18, 19].

*Melissa Officinalis* is one of the oldest and most common medicinal plants in the family Mint (Lamiaceae). The Lemon scent is a characteristic of this plant, and for this reason, it is also called Lemon balm. In traditional medicine, this plant is helpful for the treatment of high blood pressure, chronic colds, earaches, and headaches [20]. The phenolic compounds of *Melissa Officinalis* include rosmarinic acid, tannins, and flavonoids such as apigenin-7-oxide-glucoside, luteolin-7-glycoside oxide, rhamnocitrin, and isoquercitrin [21].

To date, various papers have been published on reducing the acrylamide content in bakery products. However, in this work, for the first time, the effect of MOSG to decrease acrylamide content in bread was examined.

## 2. Material and Methods

### 2.1. Materials

*Melissa Officinalis* seeds were provided from the local market in Shiraz, Iran. The seeds were manually cleaned to separate all foreign matter and then kept in plastic bags. Asparagine, glucose, and acetonitrile were provided by Sigma Aldrich Company (Milwaukee, WI, USA). Xanthan gum was obtained from Cargill Company (Shanghai, PR China). All the chemicals with analytical grade were obtained from Merck Company (Germany). Wheat flour and other bread ingredients were obtained from a local market in Shiraz, Iran.

### 2.2. Methods

#### 2.2.1. Gum extraction

The extraction of MOSG was conducted as a study by Mohebbi, Fathi and Khalilian-Movahhed [22]. *Melissa Officinalis* seed gum was extracted using distilled water at a water/seed ratio of 38:1 W/V, a temperature of 49 °C, and a pH of 7 for 30 min. Finally, the swollen seeds were separated from the slurry. The extracted gum was dispersed in deionized water, followed by boiling with ethanol to inactivate enzymes, remove lipophilic substances, and isolate low molecular weight proteins. The precipitated material was

dispersed in distilled water, followed by centrifugation at 5000 g for 10 min. The solutes were then precipitated by ethanol and then subjected to freeze-drying. The extraction yield of the gum was found to be 17.4%.

### 2.2.2. Bread production

About 0.5 g (wt/kg) of asparagine was added to the bread dough, and MOSG (1% (w/w)) and xanthan gum (1% (w/w)) were added to the dough. A sample was prepared without gum as a control.

### 2.2.3. Moisture content

Moisture content (WC) of bread samples was measured after drying at 110 °C for 24 hours.

The following equation computed moisture retention (WR) in bread:

$$\text{WR} = \left[ \frac{\text{WC of treated sample}}{\text{WC of the control sample} - 1} \right] \times 100 \quad \text{Eq. 1}$$

The positive and negative values of WR indicate the positive and negative effects of the gums on moisture retention, respectively.

### 2.2.4. Extraction of acrylamide

First, the whole bread was completely crushed and powdered and then transferred to 50 mL tubes. In the next step, 40 mL of distilled water was added to the tubes under mechanical agitation. Then, the samples were

ultrasonicated, filtrated, and transferred to 50 mL Falcon, followed by adding 10 mL acetonitrile and 2 mL hexane. Afterward, 4 g of magnesium sulfate without water and 0.5 g of salt were added to the samples under mechanical stirring and then centrifuged in 3000 g for 5 min. The hexane layer was discarded, and 5 mL of acetonitrile extract was poured into the flask and dried in a rotary vacuum. The resulting samples were dissolved again in 2 mL of distilled water and injected into the SPE column, previously conditioned with 2 mL of methanol and 2 mL of water [23].

### 2.2.5. HPLC-DAD analysis

The amount of acrylamide was determined by HPLC (Shimadzu LC-20AT system, LC-20AT). The fluid phase was a combination of water (A) and acetonitrile (B) in the following ratios: 0-10 minutes: 100% A and 0% B, 20: 75% A and 25% B, 30-35: 10% A and 90% B. The solutions were degassed using sonication for 10 min, followed by filtration by 0.2-mm cellulose acetate filters. The mobile phase was pumped at the rate of 0.8 mL/min at a column temperature of 25 °C. Detector responses were recorded at 205 nm [24].

### 2.2.6. Texture Analysis

The textural characteristics of the bread samples were analyzed using a texture analyzer (Brookfield, USA) using a probe at 500 N and a speed of 20 mm/min.

### 2.2.7 Color Analysis

The color of the bread was analyzed using Hunter lab (FMS Jansen GmbH & Co. KG, USA), and the values of  $L^*$  (brightness),  $a^*$  (red/green), and  $b^*$  (blue/yellow) were determined.

### 2.2.8. Sensory evaluation

The sensory attributes of the bread were evaluated 2 hours after baking by 12 semi-trained panelists. The sensory parameters, including color, elasticity, porosity, softness, taste, aroma, and chewiness, were assessed based on a 9-point hedonic scale (1 for the worst and 5 for the best).

### 2.2.7. Statistical analysis

The results were analyzed by a completely randomized design in a factorial format using SPSS software (Version 21). A comparison of the mean was done using the Duncan test at the significance level of 0.05. All the tests were conducted in three replications.

## 3. Results and Discussion

### 3.1. Moisture content of bread

Fig. 1 presents the moisture level of the bread samples. The addition of MOSG significantly increased the moisture level of the bread samples ( $p < 0.05$ ). However, the capability of this gum to retain moisture is lower than xanthan gum. This is due to 1) the high ability of hydrocolloids to retain moisture, 2) their tendency to bind water, and 3) the hydrophilicity of these hydrocolloids. Similar findings have been reported regarding the effect of brea gum [2], cress seed gum [25], and a combination of cress seed and xanthan gum [26] on the moisture level of bread.

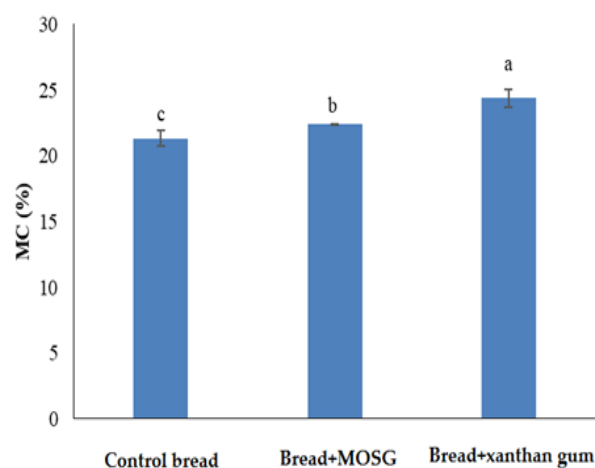


Fig. 1. The moisture content of control bread and the samples containing MOSG and xanthan gum

### 3.2. Acrylamide content of bread

The amount of acrylamide in the bread samples is presented in Fig. 2. The acrylamide

content of the control bread and the samples containing MOSG and xanthan gum were found to be 1180, 836.75, and 1167.6 ng/g, respectively. The addition of MOSG significantly reduced the acrylamide content of the bread samples ( $p < 0.05$ ). This gum had a better ability to reduce acrylamide content than the xanthan sample.

Among the various food products, cereal products such as bread contain the highest acrylamide content. Therefore, the influence of MOSG and xanthan gum on the acrylamide formation of bread was examined.

Zeng, Cheng, Du, Kong, Lo, Chu, Chen, and Wang [23] investigated the impact of different hydrocolloids on the acrylamide formation in some chemical models. They indicated that adding these hydrocolloids decreased the amount of acrylamide ( $p < 0.05$ ). This effect has been associated with the ability of hydrocolloids to increase the viscosity and their ability to bond with other compounds. Their findings showed that alginic acid and pectin were the most potent inhibitors of acrylamide formation in fried potato strips. Sadat Mousavian, Niazmand and Sharayei [27] also stated that hydrocolloids have different abilities to reduce acrylamide amounts in food

models based on their constituent structure. In baked white bread, it was observed that the outer crust had a significantly lower water content, but a higher acrylamide content was observed than the inner crust [28].

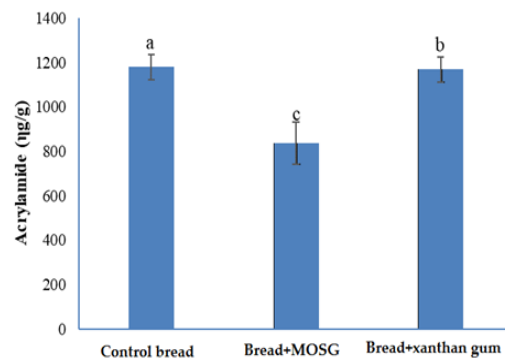


Fig. 2. Acrylamide content of control bread and the samples containing MOSG and xanthan gum

### 3.3. Physical properties of bread

#### 3.3.1. Texture

Figure 3 shows the hardness of different bread samples. The addition of MOSG significantly reduced the hardness of the bread samples ( $p < 0.05$ ). The bread containing xanthan gum had lower hardness than the sample containing MOSG.

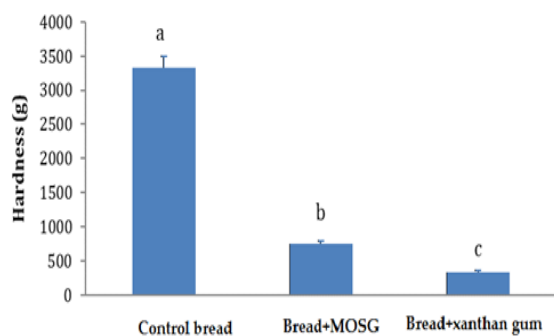


Fig. 3. Hardness of control bread and the samples containing MOSG and xanthan gum

Moisture retention and decrease of gluten network are two critical factors in reducing hardness. With increasing water absorption, moisture content increases, and as a result, hardness decreases. Xanthan's ability to create strong gel-like structures and the power of seed gums to increase the viscosity-induced softness of samples. Our results are in line with those obtained by Sheikholeslami, Mahfouzi, Karimi, Hejrani, Ghiafehdavoodi, and Ghodsi [25] and Sahraiyani, Naghipour, Karimi and Davoodi [29], who indicated a decrease in the hardness of products using gums.

### 3.3.2. Color attributes

Figure 4 shows the color properties ( $L^*$ ,  $a^*$  and  $b^*$ ) of different bread samples. The addition of

MOSG increased  $L^*$  (brightness) and  $b^*$  (red/green) and reduced  $a^*$  (blue/yellow) values of the samples ( $p < 0.05$ ). The increasing  $L^*$  value is due to increased moisture retention in the presence of MOSG. With adding MOSG to the bread formulation, the value  $b^*$  parameter increased, which is due to the increased yellowness of the crust and core of the bread. The color alteration of bread samples is attributed to Millard browning reactions. In other words, during Millard reactions, the color-forming compounds are created that affect the taste and texture properties of bread samples. Improving the color of bread crumbs can be related to the interaction of sugar and protein with amylose [25]. The color difference of the control sample with the bread samples containing MOSG and xanthan gum was evaluated by determination of  $\Delta E$ . The  $\Delta E$  values for the control bread and the sample containing MOSG were found to be 11.8, while this value for the bread containing xanthan gum was 8.86.

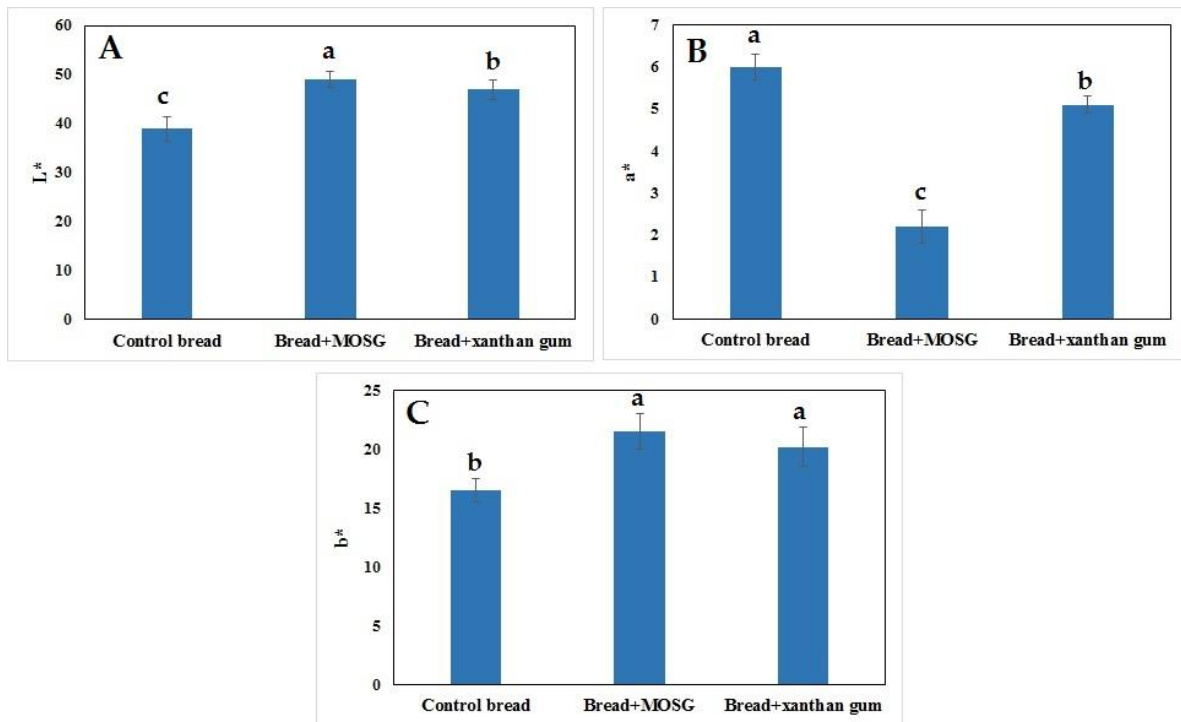


Fig. 4. The values of  $L^*$  (A),  $a^*$  (B), and  $b^*$  (C) of control bread and the samples containing MOSG and xanthan gum

### 3.4. Sensory analysis of bread

The sensory evaluation of the samples revealed the highest and lowest porosity were related to the bread containing the xanthan sample and control sample, respectively (Fig. 5). Xanthan's ability to create strong gel-like structures could be the reason for increasing porosity. The highest softness was obtained in the samples containing MOSG and the lowest was found in the sample incorporated with xanthan gum. The ability of seed gums to increase the viscosity and pure hydrocolloids such as xanthan can create strong textures and

structures-induced softness of samples. Regarding the aroma and taste, the control sample was the best. Regarding the color and chewiness, the bread containing xanthan gum indicated the highest score, which may be associated with the ability of xanthan gum to form a strong texture. The MOSG sample had the same texture and elasticity as the xanthan sample. The production of flavor compounds resulted from the caramelization and Maillard reactions in the control sample. Sheikholeslami, Mahfouzi, Karimi, Hejrani, Ghiafehdavoodi and Ghodsi [25] and Sahraiyan, Naghipour, Karimi and Davoodi



[29] also revealed that the addition of cress seed gum and *Lepidium sativum* enhanced the

sensory properties of bread, respectively.

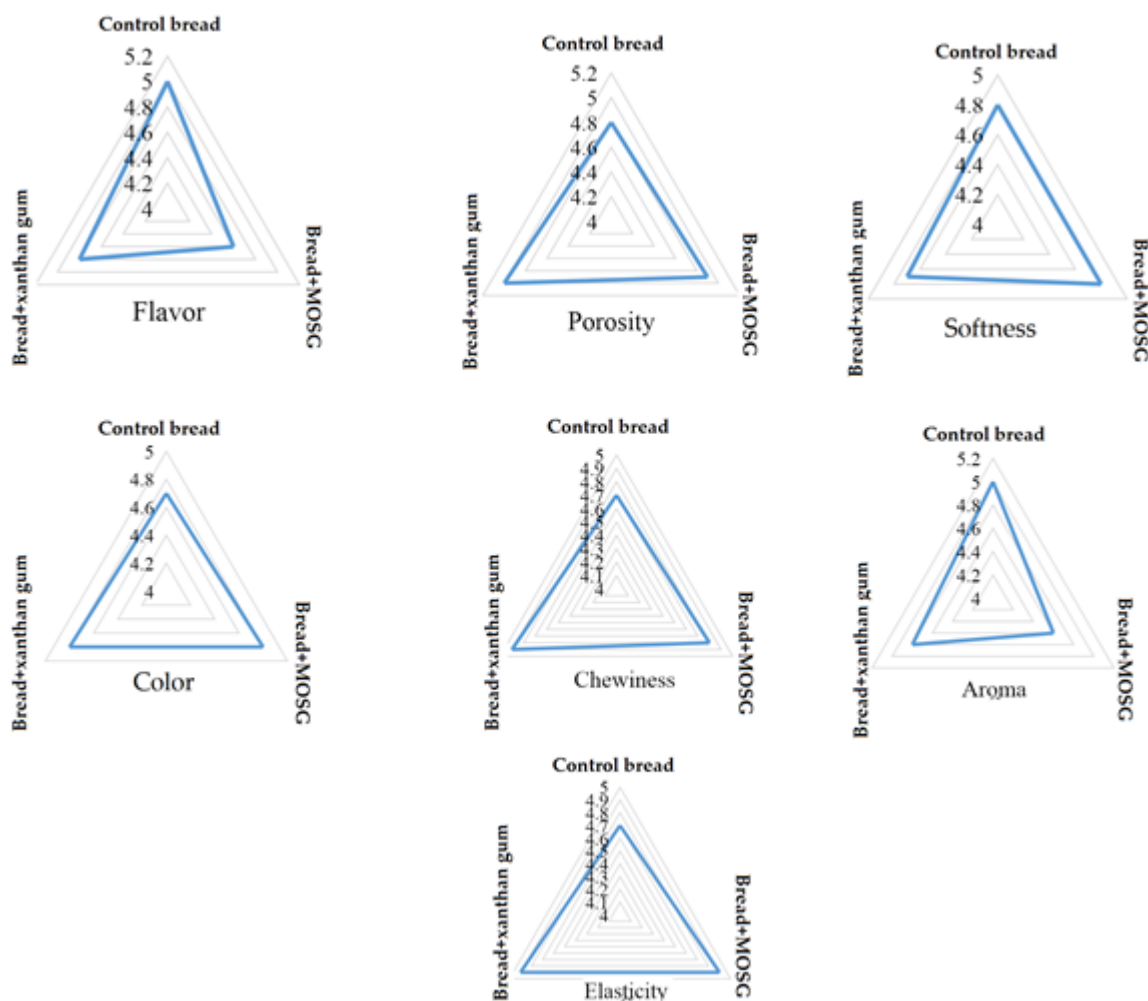


Fig. 5. Sensory properties of control bread and the sample containing xanthan gum and MOSG

#### 4- Conclusion

The findings of this paper demonstrated that MOSG addition in bread formulation is very effective in reducing acrylamide formation. However, the hydrocolloid addition did not inhibit the formation of this hazardous compound. The textural and sensory properties of the bread samples also improved with the

addition of MOSG. The addition of MOSG in the bread formulation increased the lightness and yellowness.

#### Authorship

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content.

Moreover, each author certifies that this material or similar material has not been and will not be submitted to or published in any other publication.

### Conflicts of interest

We declare that there is no conflict of interest.

### Data Availability

Data available on request due to privacy/ethical restrictions

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**Figures caption:**

Fig. 1. The moisture content of control bread and the samples containing MOSG and xanthan gum

Fig. 2. Acrylamide content of control bread and the samples containing MOSG and xanthan gum

Fig. 3. Hardness of control bread and the samples containing MOSG and xanthan gum

Fig. 4. The values of L\* (A), a\* (B) and b\* (C) of control bread and the samples containing MOSG and xanthan gum

Fig. 5. Sensory properties of control bread and the sample containing xanthan gum and MOSG



اثرات صمغ های زانتان و دانه بادرنجبویه بر ویژگی های فیزیکومکانیکی، تشکیل آکریل آمید و پذیرش نان باگت

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آکریل آمید به عنوان یک ترکیب سمی و سرطان زا در بسیاری از غذاهای پخته شده یا سرخ شده یافت می شود. در این کار، اثر صمغ دانه بادرنجبویه در غلظت ۱٪ (وزنی/وزنی) بر تشکیل آکریل آمید در نان بررسی و با نان باگت حاوی زانتان ۱٪ (وزنی/وزنی) و نمونه شاهد مقایسه شد. افزودن صمغ دانه بادرنجبویه سطح رطوبت نان ها را از ۲۲/۱ درصد به ۲۳/۶ درصد افزایش داد ( $p < 0/05$ ). میزان آکریل آمید نان فاقد صمغ و نان های که حاوی صمغ دانه بادرنجبویه و صمغ زانتان بودند به ترتیب ۱۱۸۰، ۸۳۶/۷۵، ۱۱۶۷/۶ نانوگرم در گرم بود. افزودن صمغ دانه بادرنجبویه سختی نمونه ها را از ۳۳۸۰ گرم به ۷۳۰ گرم کاهش داد و نان حاوی صمغ زانتان کمترین سختی (۴۱۰ گرم) را نشان داد. افزودن صمغ دانه بادرنجبویه به فرمول نان باعث افزایش مقادیر  $L^*$  (روشنایی) و  $b^*$  (آبی/زرد) و کاهش مقدار  $a^*$  (قرمز/سبز) نمونه ها شد. نتایج ارزیابی حسی نشان داد که افزودن صمغ دانه بادرنجبویه باعث بهبود تخلخل، نرمی، طعم و مزه، عطر، رنگ، جویدن و خاصیت ارتجاعی نمونه ها شد. در نتیجه، صمغ دانه بادرنجبویه می تواند به عنوان یک کاندید بالقوه با کارایی عالی برای کاهش آکریل آمید در نان باگت استفاده شود.