

**DETERMINATION OF HYDROXYMETHYLFURFURAL (HMF) CONTENT  
AND SOME PHYSICOCHEMICAL PROPERTIES IN GRANOLA BARS  
FORMULATED WITH VARIOUS TYPES OF HONEY AND MOLASSES**

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**ABSTRACT**

The rising prevalence of obesity-related health issues has increased the demand for healthier foods with reduced refined sugar content. This study evaluated granola bars formulated with natural sweeteners, including honey and molasses, as alternatives to refined sugar, analyzing color, pH, moisture, ash, fat, and hydroxymethylfurfural (HMF) content. Statistical analysis using One-Way ANOVA followed by Duncan's multiple range test revealed significant differences among samples ( $P<0.05$ ). The flower honey sample (CCK) exhibited the highest  $L^*$  (52.44) and  $b^*$  (27.53) values, whereas the carob molasses sample (KCB) showed the highest  $a^*$  (12.50). KCB had the highest pH and ash content, UZM the highest moisture (11.70%), and DUT the highest fat (26.30%). The lowest HMF was detected in pine honey (CAM, 15.25 mg/kg), and the highest in CCK (38.39 mg/kg). These findings indicate that the type of honey or molasses significantly influences HMF formation and physicochemical properties of granola bars.

**Keywords:** Granola bar, HMF, molasses, honey

**FARKLI BAL VE PEKMEZ TÜRLERİ İLE ÜRETİLEN GRANOLA BARLARDA  
HİDROKSİMETİLFURFURAL (HMF) DÜZEYLERİNİN VE BAZI  
FİZİKOKİMYASAL ÖZELLİKLERİN BELİRLENMESİ**

**ÖZ**

Obeziteyle ilişkili sağlık sorunlarının artması, sağlıklı beslenme ve rafine şeker tüketiminin azaltılmasını önemli hale getirmiştir. Bu çalışmada, rafine şeker yerine bal ve pekmez gibi doğal tatlandırıcılar kullanılarak üretilen granola barların renk, pH, nem, kül, yağ ve hidroksimetilfurfural (HMF) içerikleri belirlenmiş ve istatistiksel olarak değerlendirilmiştir. One-Way ANOVA ve Duncan

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çoklu karşılaştırma testleri sonucunda örnekler arasında anlamlı farklılıklar bulunmuştur ( $P<0.05$ ). En yüksek  $L^*$  (52.44) ve  $b^*$  (27.53) değerleri çiçek balı (CCK) örneğinde, en yüksek  $a^*$  (12.50) değeri keçiyoynuzu pekmezli (KCB) örnekte belirlenmiştir. En yüksek pH ve kül içeriği KCB’de, en yüksek nem üzüm pekmezli (UZM, %11.70) örnekte, en yüksek yağ dut pekmezli (DUT, %26.30) örnekte, en düşük HMF çam balı (CAM, 15.25 mg/kg) örneğinde saptanmıştır. En yüksek HMF içeriği CCK örneğinde (38.39 mg/kg) belirlenmiştir. Sonuçlar, şeker ikamesi olarak kullanılan bal ve pekmez türlerinin HMF oluşumuna etkisinin farklılık gösterdiğini ortaya koymaktadır.

**Anahtar kelimeler:** Granola bar, HMF, pekmez, bal

## INTRODUCTION

For many years, health professionals have emphasized the importance of limiting sugar consumption. It is recommended that refined sugar intake should be kept below 10% of total energy consumption for a healthy diet (WHO, 2015; EFSA, 2021). Additionally, reducing sugar intake to below 5% of total energy is advised (WHO, 2015). Excessive sugar consumption increases the risk of obesity, metabolic syndrome, and cardiovascular diseases (Johnson et al., 2009; Cottrell, 2012; WHO, 2015). Excessive sugar intake can lead to obesity and weight gain. It has been observed that refined sugar affects blood pressure and serum lipid levels, and reducing free sugar consumption can lower cardiovascular disease risks (Te Morenga et al., 2014). For these reasons, reducing refined sugar consumption has gained importance in recent years.

HMF is a compound with a molecular weight of 126.11 g/mol and a density of 1.29 g/cm<sup>3</sup>, with the chemical formula C<sub>6</sub>H<sub>6</sub>O<sub>3</sub>. It consists of an aromatic alcohol, an aromatic aldehyde, and a furan ring (Choudhary et al., 2021). HMF is highly soluble in solvents such as water, alcohol, and acetone, while its solubility in petroleum ether is low (Gökmen, 2014).

HMF formation occurs through two main processes: sugar caramelization and the Maillard reaction. In caramelization, sugars undergo enolization and dehydration to form fructofuranosyl cations. The Maillard reaction occurs when the carbonyl groups of reducing sugars combine with free amino groups. During this process, the Amadori rearrangement takes place, leading to HMF synthesis (Kroh, 1994; Choudhary et al., 2021).

The amount of HMF is directly related to the type of sugar used in products, as well as storage

temperature and duration. For instance, the HMF content in sucrose-containing cookies is lower than in glucose- and fructose-containing cookies (Metin, 2014). Basic amino acids (lysine, histidine, arginine) reduce HMF formation, whereas acidic amino acids (glutamic acid, aspartic acid) increase it (Li et al., 2019). Additionally, salt has been observed to accelerate HMF formation (Fiore et al., 2012).

While dietary intake of HMF within established limits (e.g., up to 2 mg/kg body weight per day) does not pose harmful effects, excessive consumption may have carcinogenic, mutagenic, and genotoxic effects (Janzowski et al., 2000). HMF and its byproducts have been associated with health issues such as colon cancer, skin papillomas, and kidney tumors (Rufian-Henares and De la Cueva, 2008; Durling et al., 2009). However, further in vivo studies are needed to confirm these effects (Şahiner et al., 2019). In rat and mouse experiments, the lethal dose of HMF was found to be 3.1 g/kg, and at high doses, it caused irritation to the skin, eyes, and respiratory tract (Windsor et al., 2013; Mihcioglu, 2023). On the other hand, no toxic effects were observed at low levels (80–100 mg/kg) (Abraham et al., 2011).

Granola bars are made by combining oats, cereal flakes, puffed rice, nuts (almonds, walnuts, hazelnuts, etc.), dried fruits, and spices with liquid binders (honey, molasses, water, oil) (Baş et al., 2011). Honey is particularly favored by consumers for its sweet taste and golden hue (LaGrange et al., 1988; 1991). The production steps include mixing dry ingredients, granulation with liquid binders, and baking at 150–220°C (Vengateson and Mohan et al., 2016).

Sugar is derived from natural sources or processed and plays a role in food as a

preservative, color enhancer, and flavor enhancer (EFSA, 2021; Akder, 2023). The Maillard reaction and caramelization processes contribute to sensory properties (Petisca et al., 2014; Van der Sman and Renzetti, 2021). The use of sugar alternatives in the food industry allows for the production of low-sugar and low-calorie products (Gao et al., 2016). According to the Turkish Food Codex, products with a 25% reduction in sugar, fat, and carbohydrates are classified as "low-calorie." Refined sugar plays a crucial role in taste, moisture control, texture enhancement, and starch gelatinization limitation (Nip, 2014). However, to reduce the negative health effects of sugar and develop functional products, various alternatives such as lavender honey, grape molasses, carob molasses, and liquid stevia are used (Palamutoğlu et al., 2018; Kazancı, 2021; Acun et al., 2024).

In recent years, sugar substitutes such as honey and molasses, which have high reducing sugar content, have been recommended to reduce sugar consumption (Kazancı, 2021). Honey is a natural sweetener produced by bees from plant nectar, consisting of approximately 80% glucose, fructose, and sucrose (Almasaudi, 2021). The Turkish Food Codex Honey Regulation specifies honey's properties, including moisture content (20%), acidity (50 meq/kg), diastase number (minimum 8), and maximum HMF content (40 mg/kg) (Karahan, 2017). During thermal processing, HMF formation must be controlled, as high temperatures and prolonged storage increase HMF levels (Godoy et al., 2022).

Molasses is a concentrated product obtained by boiling sugar-rich fruits such as grapes, mulberries, and carobs (Erbil, 2020). HMF formation should be controlled during molasses production, and according to the TS 3792 Standard, molasses should contain no more than 75 mg/L of HMF (Metin, 2014).

With the growing interest in healthy eating, granola has become popular, and sweeteners such as honey and molasses are used instead of sugar. However, processing these alternatives at high temperatures may increase HMF formation. In

granola bar production, the risks associated with HMF formation have not been sufficiently investigated. The aim of this study is to examine HMF levels in homemade granola bars, raise public awareness, and contribute to the literature. Studies comparing the effects of sugar substitutes on HMF formation are limited. In this context, the main objective of this study is to evaluate the impact of substitutes such as pine honey, flower honey, carob molasses, mulberry molasses, and grape molasses on the HMF levels and nutritional quality of granola bars.

## MATERIALS AND METHODS

### Materials

The ingredients used in granola production included rolled oats (Kelly's, Istanbul), raw hazelnuts (Simbat, Istanbul), raisins (Simbat, Izmir), butter (Karlıdağ, Malatya), and brown sugar (Migros, Istanbul). The sugar substitutes used were flower honey (Balkaşık, Istanbul), grape molasses (Serel, Istanbul), pine honey (Kozan Cooperative Filtered, Adana), mulberry molasses (Koska, Istanbul), and carob molasses (Koska, Istanbul), all of which were obtained from local markets in Aydın, Turkey. High-performance liquid chromatography (HPLC)-grade water, methanol, petroleum ether, and other chemical reagents of analytical purity were supplied by Sigma-Aldrich (Steinheim, Germany) and Merck (Darmstadt, Germany).

### Method

#### *Preparation of Granola Bars*

The granola bar formulations used in the study are presented in Table 1, while the visual representations of the sugar and sugar substitutes are shown in Figure 1. The essential ingredients for granola bar preparation were determined based on literature data, consisting of oats, dried nuts, dried fruits, honey, and sugar combinations (Wang et al., 2019). The control granola bar formulation (brown sugar-based) and its preparation process were adapted from the study conducted by Kiat et al. (2021).

Table 1. Formulations of granola bars produced in the study

| Granola Bar | Rolled oats | Raisins | Raw Hazelnuts | Ingredients (g) |                   |                 | Total Weight |
|-------------|-------------|---------|---------------|-----------------|-------------------|-----------------|--------------|
|             |             |         |               | Butter          | Sugar/ Substitute | Added Sweetener |              |
| SKR         | 200         | 100     | 100           | 100             | Brown sugar       | 150             | 650          |
| CAM         | 200         | 100     | 100           | 100             | Pine honey        | 150             | 650          |
| CCK         | 200         | 100     | 100           | 100             | Flower honey      | 150             | 650          |
| UZM         | 200         | 100     | 100           | 100             | Grape molasses    | 150             | 650          |
| DUT         | 200         | 100     | 100           | 100             | Mulberry molasses | 150             | 650          |
| KCB         | 200         | 100     | 100           | 100             | Carob molasses    | 150             | 650          |

A granola bar was prepared in nine steps using raisins, raw hazelnuts, butter, sugar substitutes (pine honey, flower honey, grape molasses, mulberry molasses, carob molasses), brown sugar, and oats. First, the oven was preheated to 150°C. Then, raisins and raw hazelnuts were broken into small pieces using a hand blender (Arzum AR1042). Butter was melted in a large pot, and sugar substitutes (pine honey, flower honey, grape molasses, mulberry molasses, carob molasses) were added along with brown sugar for the control group, ensuring all ingredients were well mixed. Next, oats, raw hazelnuts, and raisin pieces

were added to the mixture and stirred continuously.

In the following step, a 28.7 cm x 28.7 cm square borosilicate glass baking dish (Paşabahçe 59314 Premium 4100 cc) was lined with baking paper, and the mixture was poured into it. The mixture was then baked in the middle rack of a fanless oven (ARÇELİK AFC 340 S) preheated to 150°C for 30 minutes until it turned golden brown. Afterward, it was left to cool for approximately 15-20 minutes. Finally, the baked and cooled granola bars were cut into small squares.

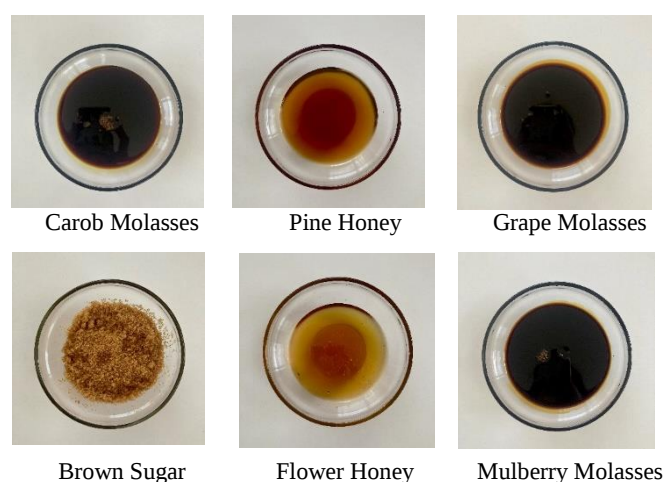


Figure 1. Sugars and sugar substitutes used in the study

### Analyses

The physicochemical properties (color, pH, moisture, ash, and fat content) and HMF levels of granola bar samples sweetened with various types of honey and molasses were analyzed. Color analysis was conducted using powdered samples with two repetitions and three parallels (Konica

Minolta, Osaka). The pH analysis was performed following the method of Torley et al. (2008). Moisture analysis was conducted according to the AOAC 925.10 standard (AOAC, 1990). Ash content was determined following the AOAC 923.03 standard, and fat analysis was performed

using the Soxhlet method in accordance with the AACC 30-25 standard (AACC, 2010).

HMF analysis was carried out using a High-Performance Liquid Chromatography (HPLC) system. First, 6.9 grams of the sample were diluted with 50 mL of distilled water. The diluted samples were then filtered through a 0.45  $\mu\text{m}$  syringe-tip membrane filter to remove impurities. Following filtration, the samples were injected into the HPLC system. The amount of HMF in the samples was quantitatively determined by constructing a calibration curve using HMF standards prepared at various concentrations.

The mobile phase consisted of 80% distilled water and 20% methanol, with a flow rate of 1 mL/min. Chromatographic separation was performed at room temperature using a diode array detector

(DAD) at a wavelength of 285 nm. The column used was a C18 column with a particle size of 3  $\mu\text{m}$  and dimensions of 150  $\times$  4.6 mm (Koç, 2015).

The identification of HMF in granola bar samples was based on the retention time, UV spectrum, and similarity to the standard HMF peak. Calculations were made using a calibration curve constructed from standard solutions of HMF (Sigma-Aldrich) prepared in distilled water to contain HMF concentrations ranging from 0.625 to 20 mg/kg. The peak areas of these standards were measured under the same HPLC conditions described above, and the relationship between peak area and HMF content was established. Figure 2 presents the HPLC chromatograms of the HMF standard and Figure 3 presents HPLC chromatograms of the honey-sweetened granola bar sample.

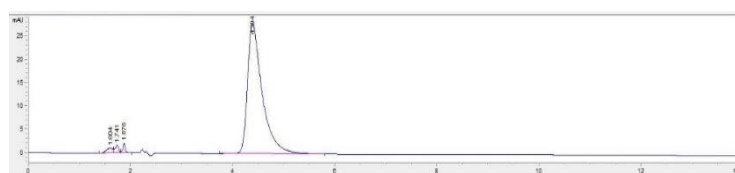


Figure 2. HPLC chromatograms of HMF standard

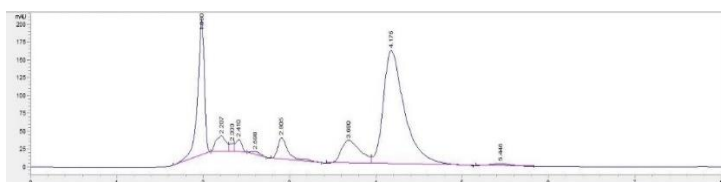


Figure 3. HPLC chromatograms of honey-sweetened granola bar sample

### Statistical Analysis

Data were analyzed using SPSS 25 software. A significance level of  $P < 0.05$  was considered, and differences between groups were evaluated using One-Way ANOVA and Duncan's test.

## RESULTS AND DISCUSSION

### Effect of Honey and Molasses Types on the Physicochemical Properties of Granola Bars

The physicochemical properties (color, pH, moisture, ash, and fat content) and HMF levels of

granola bar samples sweetened with various types of honey and molasses are presented in Table 2. Regarding color properties, the highest  $L^*$  result among the granola bar samples was recorded for CCK (52.44), whereas the lowest was observed in KCB (37.86) ( $P < 0.05$ ). The  $a^*$  result was highest in KCB (12.50), whereas the lowest result was detected in the sample SKR (9.35) ( $P < 0.05$ ). The  $b^*$  result was measured as the highest in CCK (27.53) and the lowest in KCB (20.89) ( $P < 0.05$ ).

Table 2. Physicochemical properties of granola bars produced in the study

| Parameter*   | SKR<br>(Sucrose,<br>Control) | CAM<br>(Pine<br>Honey) | CCK<br>(Flower<br>Honey) | UZM<br>(Grape<br>Molasses) | DUT<br>(Mulberry<br>Molasses) | KCB<br>(Carob<br>Molasses) |
|--------------|------------------------------|------------------------|--------------------------|----------------------------|-------------------------------|----------------------------|
| L            | 51.62 <sup>a</sup>           | 51.60 <sup>a</sup>     | 52.44 <sup>a</sup>       | 44.93 <sup>b</sup>         | 41.70 <sup>b</sup>            | 37.86 <sup>c</sup>         |
| a            | 9.35 <sup>c</sup>            | 10.40 <sup>cd</sup>    | 9.91 <sup>de</sup>       | 11.34 <sup>bc</sup>        | 12.16 <sup>ab</sup>           | 12.50 <sup>a</sup>         |
| b            | 24.83 <sup>bc</sup>          | 27.52 <sup>a</sup>     | 27.53 <sup>a</sup>       | 25.72 <sup>b</sup>         | 23.69 <sup>c</sup>            | 20.89 <sup>d</sup>         |
| pH           | 5.10 <sup>ab</sup>           | 4.97 <sup>ab</sup>     | 4.90 <sup>b</sup>        | 5.02 <sup>ab</sup>         | 5.13 <sup>ab</sup>            | 5.17 <sup>a</sup>          |
| Moisture (%) | 7.21 <sup>c</sup>            | 9.01 <sup>bc</sup>     | 9.54 <sup>abc</sup>      | 11.77 <sup>a</sup>         | 10.31 <sup>ab</sup>           | 10.16 <sup>ab</sup>        |
| Ash (%)      | 1.49 <sup>c</sup>            | 1.54 <sup>c</sup>      | 1.50 <sup>c</sup>        | 2.40 <sup>a</sup>          | 2.02 <sup>b</sup>             | 2.65 <sup>a</sup>          |
| Fat (%)      | 19.94 <sup>b</sup>           | 20.69 <sup>b</sup>     | 25.17 <sup>a</sup>       | 18.52 <sup>b</sup>         | 26.30 <sup>a</sup>            | 22.28 <sup>ab</sup>        |
| HMF (mg/kg)  | 30.76 <sup>ab</sup>          | 15.24 <sup>c</sup>     | 38.38 <sup>a</sup>       | 24.00 <sup>b</sup>         | 31.01 <sup>ab</sup>           | 33.00 <sup>a</sup>         |

\*The same superscript letters in the same row indicate no statistically significant difference between groups ( $P>0.05$ ). SKR: Sweetened with sucrose granola bars (brown sugar), CAM: Sweetened with pine honey granola bars, CCK: Sweetened with flower honey granola bars, UZM: Sweetened with grape molasses granola bars, DUT: Sweetened with mulberry molasses granola bars, KCB: Sweetened with carob molasses granola bars.

Color analysis was conducted to determine the physical properties of the granola bars. The  $L^*$  result represents brightness, where a result of 0 indicates black, and 100 indicates white. An  $L^*$  result between 0 and 50 signifies a darker sample, while a result between 51 and 100 indicates a lighter sample. The  $a^*$  and  $b^*$  color results range between -60 and +60. A negative  $a^*$  result represents green, whereas a positive  $a^*$  result indicates red. A positive  $b^*$  result represents yellow, while a negative  $b^*$  result corresponds to blue (Yıldız, 2023).

The  $L^*$  brightness results of the granola bars, ranked from highest to lowest, were as follows: CCK> SKR> CAM> UZM> DUT> KCB (Table 2). Bars containing honey exhibited higher brightness levels compared to those containing molasses. This result aligns with the findings of Kazancı (2021), Aşkin (2016), and Aigster et al. (2011). Kazancı (2021) reported that the addition of molasses in cake production (170°C for 30 min) decreased the  $L^*$  result. Similarly, Aşkin (2016) found that cereal bars (medium heat for 25–30 min) with honey had the highest  $L^*$  result (50.77), while those containing pomegranate molasses had the lowest (39.58). Additionally, Akder (2023) (180°C for 30 min) and Hedayati et al. (2022) (180°C for 40 min) reported that cakes sweetened with sucrose had higher  $L^*$  results. However, in the present study, sucrose was used

in the form of brown sugar, differing from previous research.

The  $a^*$  result, ranked from highest to lowest, was as follows: KCB> DUT> UZM> CAM> CCK> SKR (Table 2). Granola bars containing molasses exhibited a higher red color degree which is attributed to the natural color properties of molasses. In Aşkin's (2016) study, the highest  $a^*$  result (10.19) was observed in bars containing pomegranate molasses, whereas the lowest result (7.38) was found in those with honey. Yaman (2019) demonstrated that grape molasses has a high and positive  $a^*$  result. Kazancı (2021) also reported that increasing the amount of molasses in formulations led to an increase in the  $a^*$  result. The average  $a^*$  results in this study follow a similar ranking to previous research, but they are higher than the mean results reported by some researchers. This discrepancy may be due to the granola bars in this study being ground before measurement.

The  $b^*$  result, ranked from highest to lowest, was as follows: CCK> CAM> UZM> SKR> DUT> KCB (Table 2). The granola bars containing flower and pine honey exhibited the highest yellowness results. Aşkin (2016) reported that the  $b^*$  result in bars containing honey, apple molasses, and pomegranate molasses ranged between 19.54 and 27.02. Aigster et al. (2011) found the  $b^*$  result of granola bars (163 °C for 35 min) to be  $30.3 \pm$

0.76, while Zamora et al. (2014) reported a range of  $21.79 \pm 1.36$  to  $25.20 \pm 1.38$ . Additionally, Dikyokuş (2022) found that granola bars (the buckwheat grains were oven-baked at 160–200°C for 20 min, and the granola bars underwent a fan-assisted drying process at 30°C for 2 hours) enriched with bee products had  $b^*$  results ranging from  $17.88 \pm 0.16$  to  $33.86 \pm 0.70$ . The findings of this study show some differences in  $L^*$ ,  $a^*$ , and  $b^*$  values compared to previous research. For instance, the  $L^*$  values of the granola bars were generally higher than those reported for cereal bars and cakes containing molasses by Aşkin (2016) and Kazancı (2021); this difference may be attributed to the processing conditions in the present study, namely baking the granola bars at 150°C for 30 minutes and grinding them prior to measurement. The higher  $a^*$  values observed in granola bars containing molasses may be related to the natural pigment concentration of the molasses types used and the color development during baking (Yaman, 2019; Akder, 2023), while the higher  $b^*$  values in bars sweetened with honey are likely due to the intrinsic color of honey and Maillard reaction products formed during heat treatment (Aigster et al., 2011; Dikyokuş, 2022). Overall, these differences highlight the impact of sweetener type, baking parameters, and sample preparation methods on the color characteristics of granola bars.

pH is a crucial parameter that influences the taste, aroma, and texture of foods. The pH results of the granola bars, ranked from highest to lowest, were measured as follows: KCB > DUT > SKR > UZM > CAM > CCK (Table 2). The highest pH result was found in the KCB group (5.17), while the lowest was in the CCK group (4.90), and this difference was statistically significant ( $P < 0.05$ ). The pH results of the other samples were found to be similar to each other ( $P > 0.05$ ).

The pH results of honey typically range between 3.2–4.3 (Yılmaz and Küfrevioğlu, 2001) and 3.95–5.12 (Çınar, 2010), while for molasses, the pH results range between 5.0–6.0 in sweet varieties and 3.5–5.0 in sour varieties (Genç, 2017). According to the literature, Murat (2021) determined that the pH result of functional bars

containing date syrup and other ingredients was 4.06. Additionally, bars produced using papaya and banana were found to have pH results of 3.9 and 3.8, respectively (Megala and Hymavathi, 2011). This difference could be attributed to the lower pH results of the fruits used in these studies compared to the honey (Yılmaz and Küfrevioğlu, 2001; Çınar, 2010) and molasses types (Genç, 2017) used in our study.

Küçük and Veliöğlu (2022) reported the average pH result of carob molasses as 5.12. Kazancı (2021) examined the effect of molasses on pH in cake production and found the highest pH result in the control group (sucrose, 8.18) and the lowest pH result in cakes containing 100% grape molasses (5.74). The pH result of cake samples containing flower honey was reported as 7.01. The pH result of the granola bars sweetened with grape molasses (5.02) in this study was found to be consistent with the findings of Kazancı (2021).

The observed pH differences in granola bar samples can be attributed to the intrinsic properties of the sweeteners used and the processing conditions. In this study, bars sweetened with carob molasses (KCB) exhibited the highest pH value (5.17), whereas those containing pine honey (CCK) showed the lowest value (4.90). This finding is consistent with the reported pH ranges for honey (Yılmaz and Küfrevioğlu, 2001; Çınar, 2010) and aligns with the reported ranges for molasses (Genç, 2017). When compared to functional bars produced with fruit syrups such as date, papaya, or banana, which exhibited lower pH values (Murat, 2021; Megala and Hymavathi, 2011), the higher pH values of the granola bars in this study can be explained by the relatively higher pH of the honey and molasses used. Furthermore, the observed pH value of bars sweetened with grape molasses (5.02) is consistent with values reported by Kazancı (2021) for cake samples. These differences highlight the significant role of sweetener selection and their inherent acidity or alkalinity in determining the final pH of baked products, which may also influence HMF formation.

In our study, the highest moisture content was observed in the granola bar sweetened with grape molasses (11.77%), while the lowest moisture content was found in the granola bar sweetened with sucrose (brown sugar) (7.21%) ( $P<0.05$ ). The SKR, CAM, and CCK samples exhibited similar moisture content ( $P>0.05$ ) (see Table 2).

Moisture content is one of the key quality indicators in food products and is associated with desirable sensory attributes in bakery goods (Dadkhah et al., 2012). In the literature, Flynn et al. (2010) reported the moisture content of cereal bars as 11.8%, whereas Aşkin (2016) determined the moisture content of cereal bars produced with honey, apple molasses, and pomegranate molasses to range between 6.78% and 7.62%. Aigster et al. (2011) reported the moisture content of resistant starch-enriched cereal-based bars as 7.14%. Additionally, Akder (2023) found that cakes sweetened with sucrose had the lowest moisture content.

Demir and Kılınç (2019), in their study investigating the chemical and physical properties of cakes produced (160°C for 50 min) using the honey spraying method, stated that increasing the amount of honey powder in the formulation led to an increase in moisture content. Similarly, in our study, the addition of honey and molasses to granola bars resulted in an increase in moisture levels. This difference in moisture content can be attributed to the ability of sugars to form hydrogen bonds with water due to their hydroxyl groups. Since honey and molasses contain more functional groups than sucrose, they form more hydrogen bonds, reducing the mobility of free water and consequently increasing moisture retention (Ayoubi and Porabolghasem, 2017).

The highest ash content was observed in the sample sweetened with carob molasses (KCB) (2.65 g/100 g), while the lowest was found in the sucrose (brown sugar)-sweetened sample (SKR) (1.49 g/100 g) ( $P<0.05$ ). The SKR, CAM, and CCK samples exhibited statistically similar ash contents ( $P>0.05$ ), all of which were significantly lower than those observed in the other sample groups (see Table 2). Aşkin (2016) reported that

the ash content of bars prepared with honey, apple molasses, and pomegranate molasses ranged from 1.66% to 2.02%. The lowest ash content was found in the honey-sweetened bar (1.66%), while the highest was in the apple molasses-sweetened bar (2.02%).

Dutcosky et al. (2006) found the ash content of prebiotic cereal bars (added syrup heated 91-99 °C) to be 1.51%, whereas Sun-Waterhouse et al. (2010) reported that the ash content of fiber- and polyphenol-rich snack bars (130 °C for 15 min) ranged between 0.71% and 0.89%. In a study by Bilgiçli and Akbulut (2009), replacing refined sugar with different types of molasses (mulberry, apricot, andız, grape, watermelon) in cake formulations resulted in increased ash and mineral content. Similarly, Aşkin (2016) noted that the ash content of cereal bars containing molasses was higher than those containing honey. Consistent with these findings, our study also found that granola bars made with sucrose (brown sugar) had a lower ash content, which can be attributed to the lower mineral content of sucrose. Additionally, the higher ash content observed in granola bars containing molasses can be explained by the fact that molasses contains more minerals compared to honey and sucrose. The variations in ash content among different molasses types may be attributed to differences in the mineral composition of their raw materials.

The highest fat content was measured in DUT (26.30 g/100 g), while the lowest was observed in UZM (18.52 g/100 g) ( $P<0.05$ ). DUT, CCK and KCB samples have similar fat contents ( $P>0.05$ ) (see Table 2). The fat content of granola bars varies depending on the sugar substitutes and formulation used. In Aşkin's (2016) study, it was determined that the fat content of granola bars produced with the addition of sugar and sugar substitutes ranged from 18.52% to 26.30%. In the same study, the fat content of bars made with honey, apple molasses, and pomegranate molasses was reported to be between 12.07% and 12.18%. Flynn et al. (2010) determined the fat content of cereal bars to be 15.90%, while Aigster et al. (2011) found the fat content of bars produced with resistant starch to be 18.60%. The

average fat content obtained in our study is very close to the results reported by Aigster et al. (2011) and is consistent with the literature (Table 2). However, the fat results obtained in Akşin's (2016) study were found to be lower than those of our study. This difference is believed to be due to the use of butter in the formulation based on Kiat et al. (2021) used in our study.

### Effect of Honey and Molasses Type on HMF Content in Granola Bars

The average HMF results for granola bar samples produced with sugar and sugar substitute products are presented in Table 2. The highest HMF content was found in CCK (38.38 mg/kg), and the lowest in CAM (15.24 mg/kg) ( $P<0.05$ ). The CCK sample showed similarities with SKR, DUT, and KCB ( $P>0.05$ ). HMF is used as an indicator of thermal processing intensity, storage conditions, and shelf life in food products (Kuşçu and Bulantekin, 2021). Additionally, due to its potential toxic effects, it is also examined as a contaminant (Kowalski et al., 2013). When the HMF results reported in the literature for the raw materials used are examined, it is observed that the results range between 0.1–23.75 mg/kg for flower honey (Çiftçi, 2018; Ü Özgüven et al., 2020), 0.93–116.83 mg/kg for pine honey (Çapar, 2010; Uçak et al., 2017), 5.69–134.68 mg/kg for mulberry molasses (Şimşek and Artık, 2002; Tosun and Keleş, 2005; Karataş and Şengül, 2018), 0.79–50.25 mg/kg for carob molasses (Şimşek and Artık, 2002; Şengül et al., 2007; Turhan et al., 2007; Tetik et al., 2010; Yiğit, 2016; Yavuz and Duraklı, 2022), and 5.93–801.80 mg/kg for grape molasses (Türkben and Uylaşer, 2018; Üstün and Tosun, 1997; Kus et al., 2005; Koca et al., 2007; Türkben et al., 2016). In our study, the HMF levels in granola bars produced with sugar and sugar substitutes were determined in descending order as follows: CCK> KCB> DUT> SKR> UZM> CAM (Table 2). While high HMF levels are generally expected in molasses, the unexpectedly elevated HMF content observed in flower honey may be attributed to factors such as processing temperatures, storage conditions, and its fructose-to-glucose ratio (Yavuz and Duraklı, 2022). Furthermore, literature suggests that the high pH and rich phenolic compound

content of the fruits used in molasses production may contribute to a reduction in HMF formation. In addition, the high vitamin C content naturally present in these fruits has also been reported to exert an inhibitory effect on HMF formation (Erbil, 2020).

Mihçioğlu (2023) reported that the HMF content in fit bars ranged from 1.0 to 1295.2 mg/kg. In Coşkun's (2023) study, the HMF content in pine honey was found to be 10.19 mg/kg, while Çınar (2010) determined that HMF levels in pine honey from different regions varied between 0.17 and 6.64 mg/kg. The fact that the HMF content of granola bars containing pine honey in our study was higher than these reported results can be explained by the exposure of honey to heat treatment.

The granola bar sample sweetened with flower honey had the highest HMF content, while the sample sweetened with pine honey had the lowest. This suggests that the natural honeys used may have been adulterated with invert syrup produced by the acid hydrolysis of sucrose (Can, 2014; Wu et al., 2020) or that the differences may be related to factors such as the processing temperatures, storage conditions, and fructose-glucose content of the two types of honey (Yavuz and Duraklı, 2022).

According to Coşkun (2023), HMF results in grape molasses ranged from 17.40 to 113.29 mg/kg, in carob molasses from 1.39 to 23.70 mg/kg, and in mulberry molasses from 6.71 to 95.39 mg/kg. The HMF levels determined in our study were found to be consistent with these results.

The types and proportions of sugars found in natural sweeteners such as honey and molasses significantly influence both their nutritional and technological properties. According to literature reports, the fructose and glucose contents of these products vary considerably. For example, blossom honey contains approximately 38.2% fructose and 31.3% glucose, corresponding to a fructose/glucose (F/G) ratio of 1.22 (Bogdanov et al., 2008). In a study on pine honey, the fructose

content ranged from 25.9% to 39.2%, while glucose ranged from 14.4% to 33.2%, yielding an average F/G ratio of 1.26 (Tsava et al., 2022). For grape molasses, F/G ratios have been reported to range between 0.53 and 1.75 (Türkben and Uylaşer, 2018; Kaya et al., 2012; Erbil, 2020). In the case of mulberry molasses, this ratio varies between 0.68 and 1.41 (Erdem et al., 2024; Erbil, 2020). As for carob molasses, traditional samples were found to contain 9.00–20.80% fructose and 12.98–18.74% glucose, while industrial samples contained 16.44–32.64% fructose and 9.12–29.43% glucose, corresponding to an F/G ratio of 0.69–1.81 (Erbil, 2020).

Fructose is more reactive under acidic conditions than glucose and sucrose in terms of its contribution to the formation of HMF (Batu et al., 2014). Moreover, it has been reported that fructose is approximately 40 times more reactive than glucose as a precursor in HMF formation (Toker et al., 2013). The increasing contribution of fructose derived from sucrose to HMF formation can be explained by the hydrolysis of glycosidic bonds in sucrose under mildly acidic conditions at high temperatures, leading to the formation of the fructofuranosyl cation. However, it is more difficult for free fructose to form this intermediate (Lee and Nagy, 1990). Glucose does not convert to HMF through direct dehydration but must first be converted to 3-deoxyglucosone (3-DG) (Batu et al., 2014).

In this context, products with high fructose content are known to carry a higher risk of HMF accumulation when subjected to inappropriate storage conditions or excessive thermal treatment (Capuano and Fogliano, 2011). Therefore, to keep HMF levels under control, it is necessary to optimize production processes and consider the fructose content of the product during processing.

HMF formation occurs through the degradation of hexose and pentose sugars (such as fructose and glucose) under high temperatures and acidic conditions. In a study by Gökmen et al. (2008), it was also reported that a sudden drop in pH levels can lead to an increase in HMF concentration. In

our study, the granola bar sample sweetened with flower honey was found to have the lowest pH result and the highest HMF content among all samples analyzed. This result is consistent with the literature, which reports that high temperature and low pH conditions promote HMF formation (Toker, 2013; Gökmen et al., 2008). On the other hand, the granola bar sweetened with carob molasses exhibited a higher HMF content and a higher pH result compared to other molasses-containing samples. This difference is thought to stem from variations in the production processes of the molasses types used.

The relationship between moisture content and HMF formation is a critical parameter in terms of both quality and safety in food products. The Maillard reaction is promoted in foods that contain high levels of amino acids and reducing sugars, especially under intermediate moisture conditions, at temperatures above 50 °C, and within a pH range of 4–7. In contrast, caramelization requires more extreme conditions: temperatures above 120 °C, pH results below 3 or above 9, and low water activity (Gökmen et al., 2008; Capuano and Fogliano, 2011). In this study, although all samples were analyzed for moisture content, the CCK sample exhibited the highest HMF result despite having lower moisture content compared to other molasses-sweetened samples (UZM, KCB, DUT). This finding supports the notion that low moisture levels can contribute to increased HMF formation and highlights the need to control water activity and moisture during processing. On the other hand, the CAM and SKR samples, despite having low moisture levels, also showed low HMF content. These discrepancies are likely due to differences in chemical characteristics such as fructose/glucose ratios, pH results, and other compositional factors of the samples.

It is observed that the HMF levels reported in the literature for similar food products vary significantly. This variability can be attributed to numerous factors, including the analytical methods used for HMF determination (colorimetric analysis or HPLC), processing temperature, pH, water activity, sugar type, and

the presence of phenolic compounds in the medium (Tounsi et al., 2020).

## CONCLUSION

This study demonstrated that the type of sweeteners, including sugars and sugar substitutes, significantly influences the physicochemical properties and 5-hydroxymethylfurfural (HMF) content of granola bars. Among the tested formulations, HMF levels were highest in granola bars sweetened with flower honey, followed by carob molasses, date molasses, brown sugar, and grape molasses, with pine honey-sweetened granola bars exhibiting the lowest HMF content. Granola bars sweetened with grape molasses showed lower HMF content compared to those sweetened with carob or date molasses, highlighting the impact of Maillard reactions and caramelization during baking on HMF formation. Based on our findings, the use of pine honey in homemade granola production is recommended due to its relatively lower HMF content. The selected honey and molasses types were chosen considering practical availability, documented use in the literature, and local cultural context. Overall, sweetener selection plays a critical role in HMF formation, nutritional quality, and product characteristics, emphasizing the importance of optimizing processing conditions to minimize HMF formation for both consumer health and product quality.

## CONFLICT OF INTEREST

The authors declare that there are no personal or financial conflicts of interest related to this study.

## AUTHOR CONTRIBUTIONS

İclal Şahiner: Conducted the analyses and contributed to the writing of the article, Ali Göncü: Planned the methodology, contributed to the writing of the article, and evaluated the results, Aslı Yıldırım Vardin: Performed and interpreted the analyses, Serdal Ögüt: Determined the topic and critically reviewed the article.

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