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EFFECT OF THERMAL TREATMENT ON PHYSICOCHEMICAL QUALITY AND ANTIOXIDANT CAPACITY OF MULTIFLORAL HONEY

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Abstract: This study investigated the impact of accelerated thermal treatment on the physicochemical characteristics, Maillard reaction development, and antioxidant properties of multifloral honey. Honey samples were subjected to controlled thermal treatments at 45, 70, and 95 °C for 1, 2, and 3 h. Moisture content, pH, free acidity, color intensity, hydroxymethylfurfural (HMF), total phenolic content, total flavonoid content, radical scavenging activity, and reducing power were evaluated. The initial honey exhibited satisfactory quality parameters, including a moisture content of 17.47%, pH 4.07, free acidity of 8.45 meq/kg, and HMF concentration of 22.46 mg/kg. Thermal treatment at 45 and 70 °C did not significantly affect moisture content or pH. However, incubation at 95 °C significantly increased free acidity and induced marked browning. HMF formation was strongly temperature-dependent: values remained stable at 45 °C and during the first two hours at 70 °C, whereas severe accumulation occurred at 95 °C, exceeding the regulatory limit (40 mg/kg) after 1 h and reaching 127.41 mg/kg after 3 h. Regarding antioxidant-related parameters, moderate heating (45–70 °C) preserved total phenolic and flavonoid contents, while 95 °C significantly increased their measured levels, likely due to enhanced extractability and the formation of Maillard reaction products (MRPs). Similarly, reducing power and antiradical activity increased progressively at high temperature, reflecting both phenolic transformation and non-enzymatic browning reactions. The results indicate that moderate thermal treatment (≤70 °C for up to 2 h) preserves honey quality without excessive HMF formation, whereas exposure to 95 °C accelerates chemical degradation and browning reactions. Therefore, temperatures above 70 °C should be avoided during processing or storage to maintain honey quality and comply with international standards.

Keywords: multifloral honey, thermal treatment, quality parameter, Maillard reaction, antioxidant characteristic.

INTRODUCTION

Honey is a natural sweetener widely appreciated for its nutritional, sensory, and bioactive properties. Its composition, mainly consisting of sugars, organic acids, enzymes, minerals, and phenolic compounds, determines both its physicochemical quality and antioxidant potential. However, these constituents are sensitive to processing and storage conditions, particularly temperature. Thermal exposure may alter acidity, color, and the stability of bioactive compounds, thereby affecting honey quality and commercial value (Rababah *et al.*, 2024).

In commercial practice, honey is frequently heated to reduce viscosity, delay crystallization, and facilitate filtration. Nevertheless, excessive heating can accelerate chemical degradation reactions. Among the most recognized indicators of heat damage is hydroxymethylfurfural (HMF), formed through acid-catalyzed dehydration of hexoses and Maillard-type reactions. HMF accumulation is widely used as a marker of overheating and prolonged storage and is regulated by international standards (Zarei *et al.*, 2019; Ojha *et al.*, 2025).

Thermal processing also promotes non-enzymatic browning reactions that generate Maillard reaction products (MRPs), including melanoidin-like compounds. These reactions contribute to increased color intensity and may influence antioxidant measurements. Although MRPs can enhance measured antioxidant activity due to their electron-donating capacity, their formation also reflects advanced thermal stress and potential quality deterioration (Yan *et al.*, 2023; Qu *et al.*, 2023).

Heating can additionally affect phenolic and flavonoid profiles. Depending on temperature and exposure time, thermal treatment may increase measured phenolic content through improved extractability and

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matrix disruption, or decrease antioxidant compounds due to degradation. Recent investigations emphasize the complex balance between phenolic transformation, Maillard reactions, and antioxidant responses under thermal stress (Hempattarasuwan *et al.*, 2019; Islam *et al.*, 2022).

Therefore, the objective of the present study was to evaluate the effects of controlled thermal treatment at 45, 70, and 95 °C for different durations (1–3 h) on the physicochemical properties (moisture content, pH, free acidity, color, and HMF content) and antioxidant characteristics (total phenolics, total flavonoids, radical scavenging activity, and reducing power) of multifloral honey. This study aims to identify thermal conditions that preserve honey quality while limiting heat-induced deterioration and ensuring compliance with international standards.

MATERIALS AND METHODS

Honey sample

A multifloral honey sample (2 kg) was obtained directly from a local beekeeper in the Akfadou region (Bejaia province, Algeria; DMS coordinates 36°37′59.99″ N, 4°35′59.99″ E). This honey is a nectar-derived multifloral honey collected during the summer season. The botanical origin of the honey was confirmed by melissopalynological analysis carried out at the Laboratory of Applied Biochemistry, University of Bejaia. The sample was freshly harvested and stored in opaque glass containers at ambient temperature, protected from light until use. Prior to analysis, the honey was homogenized to ensure uniformity. All experiments were performed using the same batch of honey.

Accelerated thermal aging

Accelerated thermal aging was applied to simulate the effects of long-term storage under controlled laboratory conditions. Honey aliquots (30 g) were placed in sealed glass containers and subjected to thermal treatment using a thermostatically controlled water bath (WB 22, Memmert, Schwabach, Germany). Three temperatures were selected: 45, 70, and 95 °C. For each temperature, heating was applied for 1, 2, and 3 h. Untreated honey maintained at room temperature served as the control. After heating, samples were immediately cooled to ambient temperature before analyses.

Physicochemical analyses

Moisture content

Moisture content was determined by refractometry using a digital refractometer (ABBE type refractometer AR 12, SCHMIDT and HAENSCH, Germany) at 20 °C. The refractive index values obtained were converted into moisture percentages using standard reference tables established for honey analysis. Results were expressed as percentage (%).

pH

The pH was measured in a 10% (w/v) honey solution prepared by dissolving 10 g of honey in 75 mL of distilled water than adjusted to 100 mL with distilled water. Measurements were carried out at room temperature using a calibrated digital pH meter (Bante 920, Bante Instruments Ltd., China).

Free acidity

Free acidity was determined by titration according to international standard methods. A 10% (w/v) honey solution was titrated with 0.05 M sodium hydroxide (NaOH) until a pH value of 8.3 was reached (Mouhoubi-Tafinine *et al.*, 2024). Free acidity was expressed as milliequivalents per kilogram of honey (meq/kg) and calculated using the following equation:

Free acidity (meq/kg) = $(V \times M \times 1000)/m$

where V is the volume of NaOH used for titration (mL), M is the normality of the NaOH solution, and m is the mass of honey (g).

Color intensity

Honey color was evaluated spectrophotometrically as an indicator of thermal-induced browning. Honey samples were diluted with distilled water, and absorbance was measured at 430 nm using a UV–Vis spectrophotometer (Uvline 9400, Secomam, France). Results were expressed as absorbance units (Nugraha *et al.*, 2022).

Hydroxymethylfurfural content

Hydroxymethylfurfural (HMF) content was determined using the UV spectrophotometric method after clarification with Carrez I (potassium hexacyanoferrate(II) solution, 15%, w/v) and Carrez II (zinc acetate solution, 30%, w/v) (Bogdanov *et al.*, 1999). Absorbance was measured at 284 and 336 nm. HMF concentration was calculated according to the following equation: HMF (mg/kg) = $[(A284 - A336) \times 149.7 \times 5]/m$

where A_{284} and A_{336} correspond to the absorbance values measured at 284 and 336 nm, respectively; *149.7* is the conversion factor, *5* is the dilution factor, and m is the mass of honey (g).

Antioxidant-related analyses

Preparation of honey extracts

Honey extracts were prepared by dissolving honey in 80% (v/v) ethanol, followed by centrifugation (Nuve NF 200, Ankara, Turkey) at 4000 rpm for 10 min. The supernatants were collected and used for subsequent antioxidant analyses.

Total phenolic content

Total phenolic content was determined using the Folin–Ciocalteu colorimetric method. Briefly, 200 µL of honey extract was mixed with 1.5 mL of diluted Folin–Ciocalteu reagent (previously diluted 10-fold with distilled water) and 400 µL of 6% (w/v) sodium

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carbonate solution. The mixture was incubated in the dark at room temperature for 30 min. Absorbance was measured at 765 nm using a UV–Vis spectrophotometer (Mouhoubi-Tafinine et al., 2024). Results were expressed as mg gallic acid equivalents (GAE)/100 g of honey.

Total flavonoid content

Total flavonoid content was determined using the aluminum chloride colorimetric method. Briefly, 1.5 mL of honey extract was mixed with 1.5 mL of aluminum chloride solution (2% w/v in methanol). The reaction mixture was incubated at room temperature for 15 min. Absorbance was then measured at 430 nm using a UV–Vis spectrophotometer (Khamtache-Abderrahim et al., 2021). Results were expressed as mg quercetin equivalents (QE)/100 g of honey.

Radical scavenging activity

The DPPH radical scavenging activity was determined according to the method described by Benchikh et al. (2019). A volume of 3.9 mL of freshly prepared methanolic DPPH solution (60 µM) was mixed with 100 µL of honey extract. The mixture was incubated in the dark at room temperature for 30 min. The control was prepared by replacing the extract with 100 µL of 80% (v/v) ethanol. The decrease in absorbance was measured at 517 nm using a UV–Vis spectrophotometer. Radical scavenging activity was calculated using the following equation:

$$\text{DPPH inhibition (\%)} = [(Ac - As)/Ac] \times 100$$

where Ac is the absorbance of the control and As is the absorbance of the sample.

Reducing power

Reducing power was determined according to the method described by Mouhoubi-Tafinine et al. (2024). Briefly, 1 mL of honey extract was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide solution (1% w/v). The mixture was incubated at 50 °C for 20 min. After incubation, 2.5 mL of trichloroacetic acid (10% w/v) was added to stop the reaction, and the mixture was centrifuged at 4000 rpm for 10 min. Then, 2.5 mL of the supernatant was mixed with 2.5 mL of distilled water and 0.5 mL of ferric chloride solution (0.1% w/v). Absorbance was measured at 700 nm using a UV–Vis spectrophotometer. Results were expressed as mg gallic acid equivalents (GAE)/100 g of honey.

Statistical analysis

Descriptive statistics (mean ± standard deviation) were calculated from triplicate measurements using Microsoft Excel 2007. Titratable acidity curves were plotted using OriginLatis-Pro software. Analysis of variance (ANOVA) was performed with STATISTICA 5.5. Where significant effects were identified, means were compared using the Least Significant Difference (LSD) post-hoc test at a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

Physicochemical parameters

Moisture content

Moisture content is a fundamental quality parameter used to estimate the degree of honey maturity and provides insight into product stability against fermentation during storage. The moisture contents of the untreated (control) honey sample and those incubated at 45, 70, and 95 °C are shown in Fig. 1. In the present study, the honey exhibited an initial moisture content of 17.47%, indicating satisfactory maturity and conformity with international quality standards, which generally specify a maximum moisture content of 20% (Codex Alimentarius, 2001; Kartalovic *et al.*, 2014).

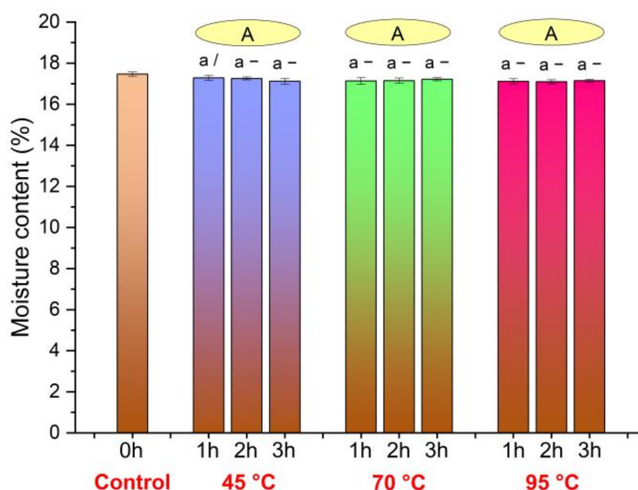

Fig. 1. Effect of temperature (45, 70, and 95 °C) and time (1, 2, and 3 h) on honey moisture content.
Data were analyzed using two-way ANOVA followed by Tukey's HSD post hoc test (p < 0.05). Mean comparisons with the control were performed using Student's t-test (p < 0.05). Different lowercase letters indicate significant differences among treatment times within each temperature, whereas different uppercase letters indicate significant differences among temperatures. The symbols "+" and "/" denote values significantly higher than or not significantly different from the control (untreated honey), respectively.

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Moisture content in honey is influenced by numerous factors including seasonal harvest conditions, initial moisture of nectar and honeydew, level of maturity reached before extraction, and geographic origin (Kartalovic *et al.*, 2014; Albu *et al.*, 2025). In our study, honey samples subjected to thermal treatment showed a slight decrease in moisture content after incubation at 45, 70, and 95 °C, with an average value of 17.17%. Statistical analysis revealed that moisture content remained stable between 1 and 3 h of incubation for all three temperature levels.

These observations are consistent with recent studies indicating that short-term thermal exposure does not significantly alter moisture content in honey (Rababah *et al.*, 2024), while moisture remains a relatively stable parameter under moderate heat, due to the strong hydrogen bonding between water molecules and sugars in honey (Salama & Chennaoui, 2024). Moreover, studies on honey stored at different temperatures for extended periods have also reported negligible changes in moisture content, supporting the present findings (Kartalovic *et al.*, 2014).

Maintaining moisture below 20% is critical because increased water content elevates the risk of fermentation by osmophilic yeasts, which can lead to quality deterioration through increased acidity and off-flavors (Albu *et al.*, 2025). Therefore, the moisture stability observed in this study suggests that the thermal treatments applied did not compromise the fermentative stability of the honey within the experimental conditions.

pH

Fig. 2 presents the pH values of honey samples before and after thermal treatment. The control (untreated) honey exhibited a pH of 4.07, indicating that the honey originates from nectar sources, as nectar honeys typically have lower pH values than honeydew honeys due to differences in mineral and organic acid composition. In contrast, honeydew honeys often exhibit higher pH values owing to their elevated mineral content (Homrani *et al.*, 2020).

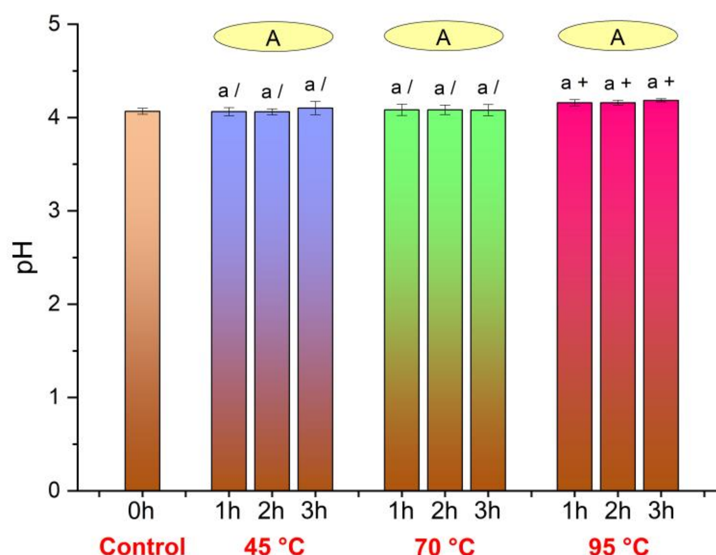

Fig. 2. Effect of temperature (45, 70, and 95 °C) and time (1, 2, and 3 h) on honey pH.
Data were analyzed using two-way ANOVA followed by Tukey's HSD post hoc test (p < 0.05). Mean comparisons with the control were performed using Student's t-test (p < 0.05). Different lowercase letters indicate significant differences among treatment times within each temperature, whereas different uppercase letters indicate significant differences among temperatures. The symbols "+" and "/" denote values significantly higher than or not significantly different from the control (untreated honey), respectively.

Thermal incubation at 45 and 70 °C did not significantly affect the pH of the honey. However, treatment at 95 °C induced a slight increase in pH after 1 h of incubation, reaching 4.16, and this level remained stable after 2 and 3 h of incubation at the same temperature. Despite this increase, all treated and untreated samples retained an acidic character. The slight increase in pH observed at 95 °C may result from heat-induced modifications of organic acid equilibria or the release of bound acidic compounds that alter the buffering capacity of honey. However, the overall pH changes remain within the acidic range that inhibits the growth of many spoilage microorganisms, thus preserving honey stability even under severe thermal conditions.

Honey generally exhibits an acidic pH, primarily due to the presence of organic acids such as gluconic, acetic, citric, and lactic acids, which arise from enzymatic activity during nectar transformation and contribute to its microbial stability (Kiran *et al.*, 2026). Typical pH values in nectar honeys range from approximately 3.4 to 5.5, depending on botanical origin and geographical factors. Studies on Algerian honeys corroborate this range, with

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pH values between 3.61 and 4.86 reported for different floral types (Derrar *et al.*, 2024).

Acidity

Acidity is an essential quality parameter in honey, significantly influencing texture, stability, and resistance to microbial spoilage during processing and storage (da Silva *et al.*, 2016; Kartalovic *et al.*, 2014). The acidity of honey arises from organic acids, some of which exist in free form while others are present as lactones. While certain organic acids can be traced to nectar or honeydew, the majority are generated through the enzymatic activity of bees during nectar transformation (Alvarez-Suarez *et al.*, 2014; Homrani *et al.*, 2020). Gluconic acid, formed by the oxidation of glucose via glucose oxidase, is the predominant acid in honey and the main contributor to its free acidity. Inorganic acids such as phosphoric, sulfuric, and hydrochloric acids may also contribute to the overall acidity profile, although to a lesser extent (Rahman *et al.*, 2023).

In the present study, the free acidity of the untreated honey sample was 8.45 meq/kg. Thermal treatment at 45 and 70 °C did not significantly alter free acidity over three hours of incubation. However, incubation at 95 °C induced a clear increase in free acidity, with values rising progressively from 10.55 to 12.63 meq/kg after 1 to and 3 h of treatment, respectively (Fig. 3). Statistical analysis confirmed that incubation time exerted a significant increase ($p < 0.05$) on free acidity at 95 °C.

These findings are in line with other studies reporting minimal changes in acidity at intermediate thermal conditions, whereas high temperatures tend to promote acid formation via enhanced degradation of sugars and non-enzymatic browning reactions (Zarei *et al.*, 2019). The observed increase in free acidity under severe thermal stress can also be partially attributed to the hydrolysis of organic acid lactones into their free acid forms. Lactonic acidity represents a reserve of acidity that can be converted during chemical breakdown, thereby contributing to increased free acidity under heat exposure (Apriceno *et al.*, 2018).

Despite the observed increases at 95 °C, all free acidity values, both untreated and thermally treated, remained well below the maximum limit of 50 meq/kg established by the Codex Alimentarius, indicating that the honey maintained acceptable quality with respect to this parameter (Codex Alimentarius, 2001).

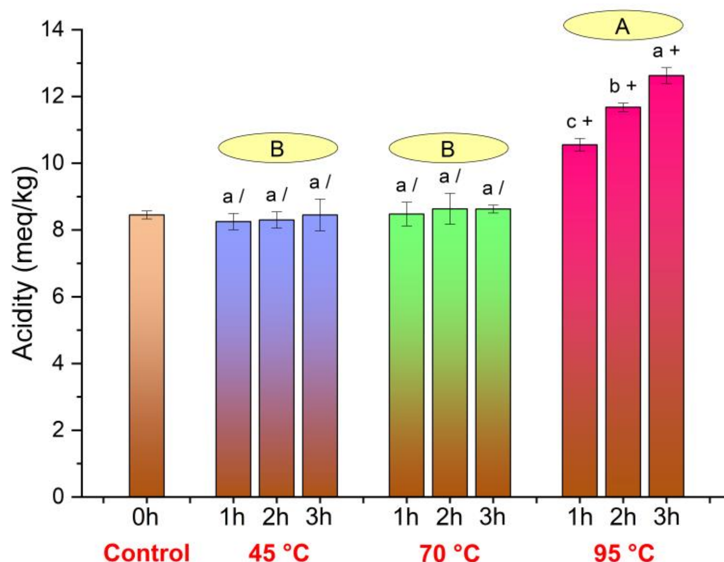

Fig. 3. Effect of temperature (45, 70, and 95 °C) and time (1, 2, and 3 h) on free acidity of honey.
Data were analyzed using two-way ANOVA followed by Tukey's HSD post hoc test ($p < 0.05$). Mean comparisons with the control were performed using Student's t-test ($p < 0.05$). Different lowercase letters indicate significant differences among treatment times within each temperature, whereas different uppercase letters indicate significant differences among temperatures. The symbols "+" and "/" denote values significantly higher than or not significantly different from the control, respectively.

Hydroxymethylfurfural content

The evolution of HMF content in untreated (control) honey and honey samples incubated at 45, 70, and 95 °C is presented in Fig. 4. The initial HMF concentration of the control honey was 22.46 mg/kg, which is well below the maximum limit of 40 mg/kg established by international standards. This low value indicates that the honey had not been subjected to thermal treatment and had been stored under appropriate conditions during its storage (Codex Alimentarius, 2001; Zarei *et al.*, 2019).

Thermal treatment at a low temperature (45 °C) for three hours and at a moderate temperature (70 °C) during the first two hours did not affect HMF content. These limited variations suggest that mild heating does not substantially accelerate HMF formation. In contrast, a significant increase was observed after 3 h of treatment at 70 °C. These findings are consistent with the results

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reported by Seyyedi-Mansour et al. (2023), who demonstrated that HMF formation is significantly influenced by both thermal processing intensity and storage duration. Their study showed that HMF levels increased progressively with higher heating temperatures and prolonged storage, exceeding regulatory limits under severe conditions (75 °C for 20 min followed by storage at 40 °C for 90 days), whereas mild treatment (55 °C for 10 min with storage at 25 °C for 45 days) maintained optimal HMF concentrations.

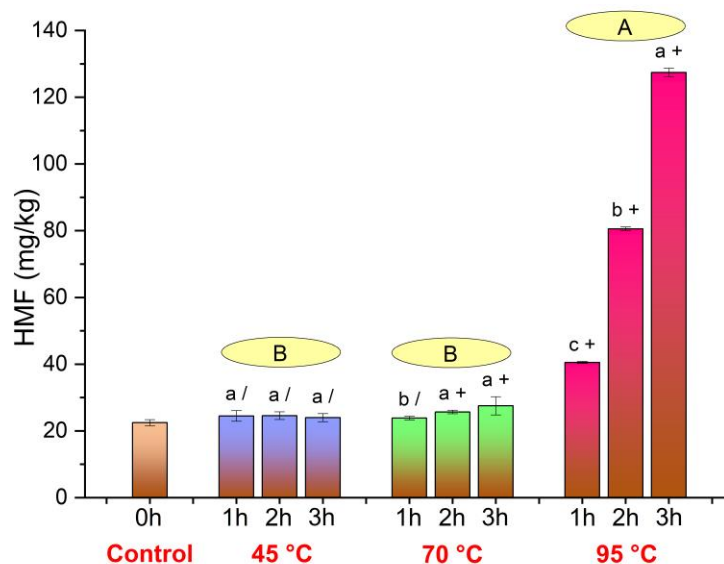

Fig. 4. Effect of temperature (45, 70, and 95 °C) and time (1, 2, and 3 h) on hydroxymethylfurfural (HMF) content of honey. *Data were analyzed using two-way ANOVA followed by Tukey's HSD post hoc test (p < 0.05). Mean comparisons with the control were performed using Student's t-test (p < 0.05). Different lowercase letters indicate significant differences among treatment times within each temperature, whereas different uppercase letters indicate significant differences among temperatures. The symbols "+" and "/" denote values significantly higher than or not significantly different from the control, respectively.*

In contrast, incubation at 95 °C induced a pronounced and progressive increase in HMF concentration throughout the incubation period. After 1, 2, and 3 h of treatment, HMF levels increased sharply to 40.52, 80.54, and 127.41 mg/kg, respectively. These values clearly exceeded the regulatory threshold after one hour of heating, highlighting the strong sensitivity of HMF formation to high-temperature exposure. Similar trends have been reported in other investigations, where severe thermal stress markedly accelerated HMF accumulation due to enhanced sugar degradation reactions (Zarei *et al.*, 2019; Mouhoubi-Tafinine *et al.*, 2018).

The marked increase in HMF at elevated temperatures can be attributed to two main chemical pathways: the dehydration of hexoses under acidic conditions and the progression of Maillard reactions between reducing sugars and amino compounds. Both mechanisms are known to intensify with increasing temperature and heating duration, leading to rapid HMF formation and quality deterioration (da Silva *et al.*, 2016; Alvarez-Suarez *et al.*, 2014). Therefore, HMF remains a reliable indicator of honey freshness and thermal damage, particularly under extreme processing conditions.

Color

The evolution of color in control honey and honey samples incubated at three temperatures (45, 70, and 95 °C) is illustrated in Fig. 5. The results indicate that honey color remained relatively stable under moderate thermal conditions. Statistical analysis showed no significant changes in color intensity for samples heated at 45 and 70 °C throughout the incubation period, suggesting that mild heating does not substantially affect the visual appearance of honey.

In contrast, incubation at 95 °C resulted in a marked and progressive increase in color intensity. After 1, 2, and 3 h of heating, color values increased by approximately 25, 38, and 49%, respectively. This pronounced darkening became particularly evident after prolonged exposure to high temperature, highlighting the strong sensitivity of honey color to severe thermal treatment.

The observed color intensification can be mainly attributed to the caramelization of sugars and the formation of brown pigments associated with non-enzymatic browning reactions. These reactions include Maillard pathways involving reducing sugars and amino compounds, which are strongly accelerated at elevated temperatures and extended heating times (da Silva *et al.*, 2016; Zarei *et al.*, 2019). The extent of color development is known to depend on the concentration and nature of sugars and amino acids, as well as on their thermal stability and the acidic environment of honey (Alvarez-Suarez *et al.*, 2014; Kartalovic *et al.*, 2014).

Several studies have reported a close relationship between thermal stress and honey darkening, confirming that high-temperature treatments promote the

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accumulation of brown pigments and melanoidin-like compounds, which significantly alter the sensory and commercial quality of honey (Starowicz *et al.*, 2021). Therefore, color remains a sensitive indicator of heat-induced deterioration in honey, particularly under severe thermal treatments or prolonged exposure to high temperatures.

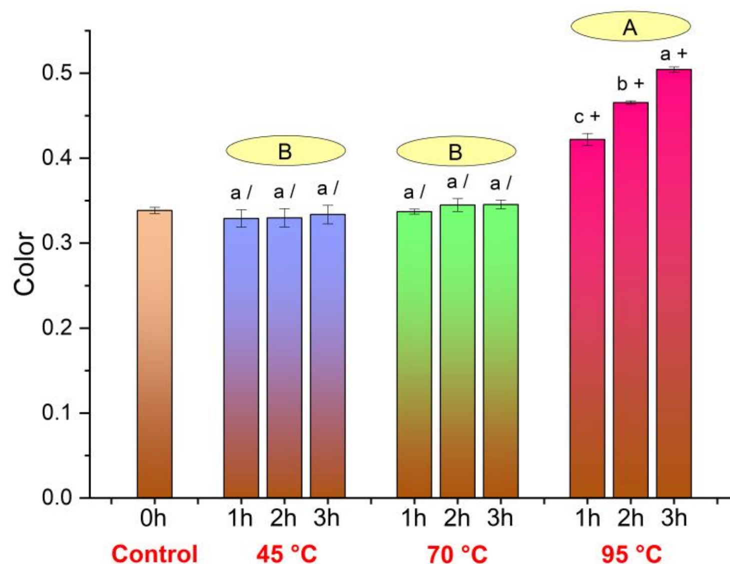

Fig. 5. Effect of temperature (45, 70, and 95 °C) and time (1, 2, and 3 h) on honey color.
Data were analyzed using two-way ANOVA followed by Tukey's HSD post hoc test ($p < 0.05$). Mean comparisons with the control were performed using Student's t-test ($p < 0.05$). Different lowercase letters indicate significant differences among treatment times within each temperature, whereas different uppercase letters indicate significant differences among temperatures. The symbols "+" and "/" denote values significantly higher than or not significantly different from the control, respectively.

Antioxidant parameters

Total phenolic content

The evolution of total phenolic content in untreated honey and incubated honey samples is presented in Fig. 6. The control honey exhibited a total phenolic content of 66.81 mg GAE/100 g. This value is within the range commonly reported for nectar honeys and reflects a moderate phenolic richness, which contributes to the antioxidant potential of the product (Alvarez-Suarez *et al.*, 2014; Homrani *et al.*, 2020).

Thermal treatment at 45 and 70 °C did not induce significant changes in total phenolic content throughout the incubation period, indicating good thermal stability of phenolic compounds under moderate heating conditions. Similar observations have been reported in recent studies, where mild thermal exposure preserved phenolic integrity by limiting oxidative degradation and enzymatic activity (Kartalovic *et al.*, 2014; Becerril-Sánchez *et al.*, 2021).

In contrast, incubation at 95 °C resulted in a marked increase in total phenolic content after three hours, reaching 77, 92, and 99 mg GAE/100 g after 1, 2, and 3 h of treatment, respectively. This apparent increase in phenolic content under severe thermal conditions has been previously documented and may be explained by several mechanisms. High temperatures can promote the hydrolysis of glycosylated phenolic compounds, leading to the release of free phenolic aglycones with higher reactivity toward the Folin-Ciocalteu reagent. Additionally, severe heating may inactivate oxidative enzymes such as polyphenol oxidase, thereby reducing phenolic degradation and contributing to the observed increase (Mouhoubi-Tafinine *et al.*, 2018; Alvarez-Suarez *et al.*, 2014).

Furthermore, thermal disruption of the honey matrix may enhance the extractability of bound phenolic compounds and increase the number of free hydroxyl groups available for detection. This phenomenon explains why severe thermal treatments can lead to higher measured phenolic contents despite potential degradation processes, particularly when exposure time is extended (Kartalovic *et al.*, 2014; Becerril-Sánchez *et al.*, 2021).

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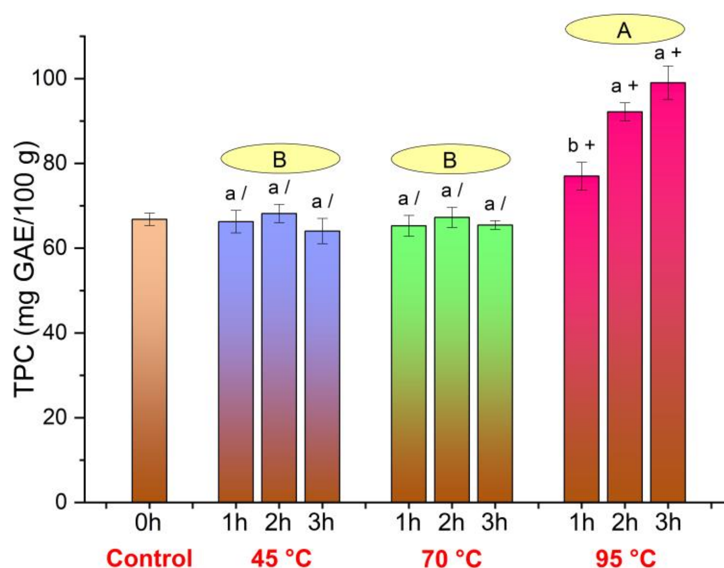

Fig. 6. Effect of temperature (45, 70, and 95 °C) and time (1, 2, and 3 h) on total phenolic content of honey.
Data were analyzed using two-way ANOVA followed by Tukey's HSD post hoc test (p < 0.05). Mean comparisons with the control were performed using Student's t-test (p < 0.05). Different lowercase letters indicate significant differences among treatment times within each temperature, whereas different uppercase letters indicate significant differences among temperatures. The symbols "+" and "/" denote values significantly higher than or not significantly different from the control, respectively.

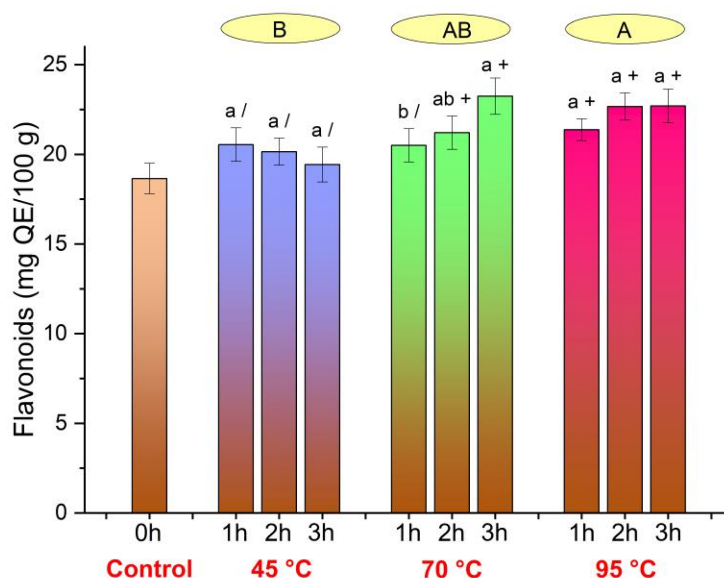

Fig. 7. Effect of temperature (45, 70, and 95 °C) and time (1, 2, and 3 h) on total flavonoid content of honey.
Data were analyzed using two-way ANOVA followed by Tukey's HSD post hoc test (p < 0.05). Mean comparisons with the control were performed using Student's t-test (p < 0.05). Different lowercase letters indicate significant differences among treatment times within each temperature, whereas different uppercase letters indicate significant differences among temperatures. The symbols "+" and "/" denote values significantly higher than or not significantly different from the control, respectively.

Flavonoid content

The flavonoid contents of untreated honey (control) and honey samples incubated at three different temperatures are presented in Fig. 7. The raw honey used in this study showed an initial flavonoid content of 18.65 mg QE/100 g. Heating at 45 °C did not result in a significant change in flavonoid content throughout the 3 h treatment period. At 70 °C, no significant effect was observed after 1 h of treatment; however, a significant increase was recorded after 2 and 3 h of incubation, with the highest value obtained at 3 h. At 95 °C, a moderate but significant increase in flavonoid content was observed after 1 h, followed by a slight increase at 2 h and stabilization between 2 and 3 h of treatment.

The initial increase in flavonoid content at moderate temperatures may be related to improved release of

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bound flavonoids from the honey matrix due to reduced viscosity and disruption of weak molecular interactions (Gül and Pehlivan, 2018). The increase at 70 °C indicates that flavonoid release predominates over degradation at this temperature, as previously reported for thermally processed honeys (Mahani *et al.*, 2024). At 95 °C, stabilization after prolonged heating reflects a balance between thermal degradation and compound liberation, confirming the relative thermal stability of flavonoids in honey matrices (Turkmen *et al.*, 2006).

Antioxidant activity

Antiradical activity

The evolution of antiradical activity of control honey and treated honey samples is illustrated in Fig. 8. The antiradical activity of the untreated sample was 22.58 mg GAE/100 g. Heating at 45 and 70 °C resulted in a relatively stable antiradical activity throughout the three hours of incubation. In contrast, at 95 °C, DPPH radical activity showed a noticeable evolution during the three heating hours.

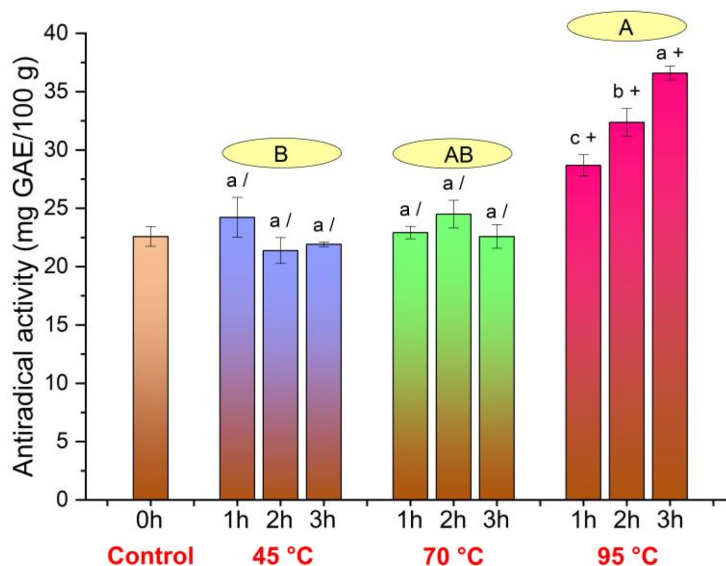

Fig. 8. Effect of temperature (45, 70, and 95 °C) and time (1, 2, and 3 h) on radical scavenging activity of honey.
Data were analyzed using two-way ANOVA followed by Tukey's HSD post hoc test ($p < 0.05$). Mean comparisons with the control were performed using Student's t-test ($p < 0.05$). Different lowercase letters indicate significant differences among treatment times within each temperature, whereas different uppercase letters indicate significant differences among temperatures. The symbols "+" and "/" denote values significantly higher than or not significantly different from the control, respectively.

The stability observed at 45 and 70 °C suggests that the antioxidant compounds naturally present in honey, including phenolics and flavonoids, retain their radical scavenging capacity under moderate thermal conditions (da Silva *et al.*, 2016). The variation observed at 95 °C can be explained by the formation of new antioxidant compounds during heating, particularly melanoidins generated through non-enzymatic browning reactions (Khaled Khodja *et al.*, 2020; Gül and Pehlivan, 2018). These Maillard reaction products are known to exhibit strong radical scavenging properties and can compensate for the partial degradation of native antioxidants at high temperatures (Mahani *et al.*, 2024). Similar thermal-induced increases in antiradical activity have been reported in honey subjected to high-temperature treatments, confirming the contribution of Maillard-derived compounds to overall antioxidant activity (Turkmen *et al.*, 2006).

Reducing power

The reducing power of control honey and heat-treated samples as a function of temperature and time is presented in Fig. 9. The untreated honey exhibited an initial reducing power of 34.76 mg GAE/100 g. At 45 °C, a significant and progressive increase in reducing power was observed throughout the heating period (1–3 h). At 70 °C, the reducing power significantly increased after 1 h and remained relatively stable during the second and third hours of treatment, with no significant differences among these time points. In contrast, heating at 95 °C resulted in a marked and continuous increase in reducing power over time, with the highest value recorded after 3 h of treatment.

The enhancement in reducing power may be attributed to several thermal-induced mechanisms. Moderate heating can promote the release of bound phenolic compounds and increase the availability of hydroxyl groups capable of donating electrons (da Silva *et al.*, 2016), thereby enhancing antioxidant capacity. Moreover, at elevated temperatures, the formation of Maillard reaction products (MRPs), including intermediate and advanced browning compounds such as melanoidins, may contribute significantly to the reducing activity. These compounds are known to possess strong electron-donating properties and antioxidant potential, which increases as thermal processing intensifies (Gül

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and Pehlivan, 2018). Therefore, the progressive increase observed particularly at 95 °C may be associated with both phenolic transformation and Maillard-driven browning reactions.

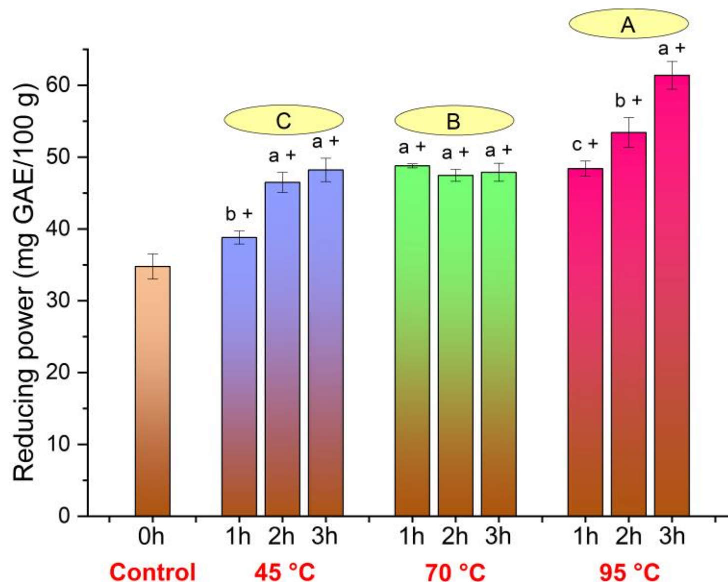

Fig. 9. Effect of temperature (45, 70, and 95 °C) and time (1, 2, and 3 h) on reducing power of honey.
Data were analyzed using two-way ANOVA followed by Tukey's HSD post hoc test ($p < 0.05$). Mean comparisons with the control were performed using Student's t-test ($p < 0.05$). Different lowercase letters indicate significant differences among treatment times within each temperature, whereas different uppercase letters indicate significant differences among temperatures. The symbols "+" and "/" denote values significantly higher than or not significantly different from the control, respectively.

CONCLUSION

Thermal treatment significantly affected the physicochemical and antioxidant properties of multifloral honey in a temperature- and time-dependent manner. Moderate heating at 45 °C and short exposure at 70 °C preserved quality parameters and maintained HMF levels within regulatory limits. In contrast, treatment at 95 °C led to marked increases in HMF, free acidity, and browning, indicating accelerated thermal degradation. Although antioxidant activity increased at high temperature, this effect was likely associated with phenolic transformations and Maillard reaction products. Therefore, honey processing should not exceed 70 °C and heating duration should be limited to ensure product quality and compliance with international standards.

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AUTHORS CONTRIBUTIONS

Conceptualization: A.B. and S.O.; methodology: M.B.B. and S.H.; data collection: A.B. and S.H.; data validation: T.K., A.B. and S.O.; data processing: M.B.B. Y.K.K., A.C. and S.O.; writing—original draft preparation: A.C., S.H., T.K. and Y.K.K.; writing—review and editing, A.B. and M.B.B.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

ETHICAL APPROVAL

This study did not involve human participants or animals, and therefore ethical approval was not required.

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