Response surface approach to optimize temperature, pH and time on antioxidant properties of wild bush *(Plectranthus rugosus)* honey from high altitude region (Kashmir Valley) of India

Gulzar Ahmad Nayik, Vikas Nanda, Beenish Zohra, B.N. Dar, Mohammad Javed Ansari, Sami Al Obaid, Otilia Bobis

*Department of Food Science & Technology, Govt. Degree College Shopian, 192303, J&K, India*
*Department of Food Engineering and Technology, Sant Longowal Institute of Engineering & Technology, Longowal-148106, Distt. Sangrur, Punjab, India*
*Department of Nutrition & Dietetics, Sharda University, Greater Noida, UP 201310, India*
*Department of Food Technology, Islamic University of Science & Technology, Awantipora, Pulwama 192122, J&K, India*
*Department of Botany, Hindu College Moradabad (Mahatma Jyotiba Phule Rohilkhand University, Bareilly, UP 244001, India*
*Department of Botany & Microbiology, College of Science, King Saud University, PO Box-2455, Riyadh 11451, Saudi Arabia*
*Department of Beekeeping and Sericulture, University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, 400372 Cluj-Napoca, Romania*

**Abstract**

In this study, the combined effect of temperature (60 to 80 °C) time (10 to 15 min.) and pH (3 to 6) was employed on the anti-oxidant potential (1,1-diphenyl-2-picrylhydrazyl-radical scavenging activity-DPPH-RSA, total phenolic content-TPC, and total flavonoid content-TFC) of wild bush Indian honey from high altitudes of Kashmir Valley by using response surface methodology (RSM). The statistical analysis showed that all the process variables had a substantial effect on the responses related to DPPH-RSA, TFC, and TPC, all of which increased as temperature and time increased. With an increase in pH, the antioxidant activity of wild bush honey was significantly decreased. The heat treatment of honey at high temperature (80 °C) was found to be more efficacious than at 70 and 60 °C, respectively. The findings showed that at higher temperature, browning pigments were formed which enhanced considerably the antioxidant activity of honey.

© 2021 The Author(s). Published by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

**1. Introduction**

Honey, a naturally delightful alternative to sugar, is a product made by honeybees (*Apis mellifera*) from plant nectars (blossom) or secretions of plant sap (honeydew). Bees pick up nectar, modify it by integrating with their particular enzymes, and deposit it in the comb to reach maturity (Nayik et al., 2018; Kamboj et al., 2020). Carbohydrates are the most abundant molecules in honey, consisting of a complex combination of monosaccharides accounting for 70% (primarily glucose and fructose) and disaccharides (10%–15%) and a low percentage of trisaccharides (melezitose and raffinoses), which are in abundance proportion in honeydew honey (Nayik et al., 2019). The sugar content of honey affects its physicochemical characteristics like viscosity, hygroscopicity, and crystallization (Kamboj et al., 2020). It is often reported that sugar profile and other chemical compositions of specific honey is frequently found to be significantly dependent on the flower type sucked by the bees and other geographical factors (Warui et al., 2019; Fechner et al., 2016; Escuredo et al., 2014). Enzymes, proteins, organic acids, minerals, vitamins, and other volatile chemicals, as well as polyphenolic compounds, are among the minor but significant components of honey (De-Melo et al., 2018; Santos-Buelga and González-Paramás 2017; da Silva et al., 2016). Composition of honey is mostly determined by the species of plant flower visited by honeybees along with the habitat, processing, and storage factors (Nayik and Nanda, 2015). Due to the availability of diastase and invertase enzymes together with polyphenolic compounds and amino acids, honey is considered as a beneficial food, rich in antioxidants (Brudzynski and Maldonado-Alvarez, 2015;
Belay et al., 2017). The presence of principal polyphenolic compounds including phenolic acids (ferrulic acid, chlorogenic acid, caffeic acid, vanillic acid, ellagic acid, cinnamic acid, benzoic acid, and coumaric acid) along with flavonoids (apigenin, pinocembrin, quercetin, chrysin, pinobanksin, myricetin, luteolin, and hesperitin) in honey presents protective effects against atherosclerosis, cancer, thrombosis, and inflammation (Nayik and Nanda, 2016). The differences in antioxidant capabilities across honeys from various sources are related to differences in polyphenol content. Honey’s color varies from light yellow to dark red and light black, depending on the temperature and total time in storage, where darker honey contains higher phenolic content and thus exhibits stronger antioxidant activity (Bodó et al., 2021).

India is one of the six world’s leading honey producers, with an estimated production of 1.2 lakh MT from almost 16 lakh registered bee colonies (Press Information Bureau, 2021). Taking into consideration, the necessity to promote beekeeping for the integrated farming system in India, the government for National Beekeeping & Honey Mission has authorized Rs. 500 crores allocation for the period of three years (2020–21 to 2022–23) (Press Information Bureau, 2021).

Kashmir valley, located at 32°44’N and 74°54’E in union territory of Jammu and Kashmir of India, is the most phytogeographically diversified zone with moderate weather and diverse geographical circumstances making it ideal for growing a variety of fruits and spices (Nayik et al., 2018). There are thousands of honey-manufacturing units in Jammu and Kashmir, with honey output totaling over 7200 quintals (Press Information Bureau, 2021).

During the months of August and September, pollen and nectar from Zinnia elegans, Thymus vulgaris, Cyanooglossum officinale, Plectranthus rugosus, and Impatiens glandulifera help to boost bee activity. Crocus sativus (saffron), which blooms from late October to mid-November, is extremely valuable to bees as a source of brood, which is also a part of the natural vegetation of the valley (Nayik et al., 2019).

Raw honey is usually processed before being sold because it includes extraneous materials (bee pollen and wax) that can cause early crystallization. As a result, raw honey is heated to avoid or delay crystallization, as well as to inactivate osmo-tolerant yeasts that might induce undesired fermentation (da Silva et al., 2016; Yan et al., 2019). Few researches have studied the effect of heat treatment on honey antioxidant properties (Zarei et al., 2019; Al-Ghamdi et al., 2019). However, this is the first report in which the combined effect of temperature, pH, and time on the antioxidant properties of wild bush honey (Plectranthus rugosus) using response surface methodology (RSM) has been developed.

RSM is a statistical method that has been proven to be a useful tool for evaluating the effect of responses and the interactions among them. RSM economizes experiments by reducing the time and resources required to optimize processing conditions. Therefore, the major goal of this research was to investigate the influence of temperature, pH, and time on the antioxidant properties of wild bush honey produced in India’s Kashmir Valley.

2. Materials and methods

2.1. Material collection and testing

Wild bush (Plectranthus rugosus) raw honey samples (10) were gathered from regional apiculturist, sealed and packed in glass bottles, and kept at a temperature of 4 °C until analysis. Mellissoponology was used to demonstrate the botanical origin of wild bush (Plectranthus rugosus) honey samples using the method adopted by Von der Ohe et al. (2004). Laboratory chemicals including Folin–Ciocalteu reagents, methanol, acetic acid, sodium acetate, aluminum chloride, sodium carbonate, and DPPH of analytical grade were acquired from Fluka Goldie, Mumbai, India.

2.2. Thermal conditions

The heating of honey samples was executed at diverse temperatures ranging from 60 °C to 80 °C for varied time durations (10–15 min) at pH levels ranging from 3 to 6 by using the solutions of acetate buffer (0.1 mol/L acetic acid and 0.1 mol/L sodium acetate), followed by cooling to 20 °C.

2.3. Determination of total phenolic and flavonoid contents

The Folin–Ciocalteu reagent was used in the estimation of the total phenolic content of all honey samples by using the method adopted by Pauliuc, Dranca and Orioian (2020). For the construction of the calibration curve, gallic acid (0–100 mg/ml) was utilized as a standard, and methanol was used as blank. The values were measured in milligrams of gallic acid equivalent (mg GAE) per 100 g honey. TFC was determined with the help of the Dowd technique (Pontis et al., 2014) in honey samples. By comparing the total flavonoid content to a standard curve of quercetin (0–100 g/ml), the total flavonoid concentration was calculated. The results were reported as mg quercetin/100 g honey (mg QE/100 g) based on the average of triplicate readings.

2.4. Determination of DPPH radical scavenging activity (DPPH-RSA)

The honey solution was prepared by dissolving honey (0.6 g) in methanol (4 ml), after which 1.5 ml of DPPH reagent solution (0.02 mg/mL) was added to the honey solution (0.75 ml) and the subsequent samples were held for 15 min in the dark at room temperature. A spectrophotometer (Hach Lange DR6000 UV–VIS Dusseldorf Germany) was employed to measure the honey mixture’s absorbance at 517 nm against a methanol blank. The DPPH-RSA reported as percent inhibition was determined based on the equation (1) as recently reported by Nayik et al. (2016).

\[
\text{Inhibition(\%)} = \frac{\text{ControlsAbs} - \text{SampleAbs}}{\text{ControlsAbs}} \times 100
\]

where Abs control represents the absorbance of the control (0.75 ml methanol and 1.50 ml DPPH) and sample Abs represents the absorbance of the sample.

2.5. Experiment design

The combined impact of variables: pH, temperature and time having the values between 3 and 6, 60 to 80 °C, and 10 to 15 min on three responses, TPC, DPPH-RSA, and TFC respectively, of wild bush honey was evaluated using RSM employing Design-Expert version 9.0.4 (Statease Inc., Minneapolis, MN, USA) (Tekindal et al., 2012). At the centre point, a three-level Box–Behken design including a three-factor, with seventeen experimental runs and five replicates was utilized. Multiple regressions utilizing the least-squares approach were used to analyse the data. The data were fitted with the following second-order polynomial Eq. (2):

\[
Y_k = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{22} X_2^2 + \beta_{33} X_3^2
\]

where \(Y_k\) denotes the response variable and \(X_1, X_2, X_3\) denotes TPC and DPPH activity Y2 denotes TPC and Y3 denotes TFC while X1, X2, and X3 are fitted with the following second-order polynomial Eq. (2):
were considered to be coded independent variables for time (min), pH, and temperature (°C), respectively. \( \beta_9 \) was used to represent the fitted response value at the design’s central point, i.e. \((0,0,0)\). The quadratic and linear regression coefficients were \( \beta_{11-33} \) and \( \beta_{1-3} \), respectively, whereas the cross-product regression coefficients were \( \beta_{12}, \beta_{13}, \) and \( \beta_{23} \). The test of statistical significance was performed on the total error criteria with a confidence level of 95%. Analysis of variance (ANOVA) were utilized to determine the important terms in the model for each response. Model adequacy was tested by calculating the coefficient of determination \( R^2 \) and \( \text{adj-R}^2 \). The data were presented using three-dimensional surface profilers.

### 3. Results

The pollen spectra percentages were associated with nectar plant pollens. The high frequency (42–54%) of pollen grains from *Plectranthus species* dominated the wild bush honey (Table 1). Table 2 shows the antioxidant activity response (DPPH-RSA, TFC, and TPC) of wild bush (*P. rugosus*) honey as a result of the experiment. Table 3 summarizes the experimental data utilized in the model, a lower quadratic equation for wild bush honey. For many of the components, calculation of the significant coefficients of the second-order polynomial regression coefficients were \( \beta_{12}, \beta_{13}, \) and \( \beta_{23} \). The test of statistical significance was performed on the total error criteria with a confidence level of 95%. Analysis of variance (ANOVA) were utilized to determine the important terms in the model for each response. Model adequacy was tested by calculating the coefficient of determination \( R^2 \) and \( \text{adj-R}^2 \). The data were presented using three-dimensional surface profilers.

#### 3.1. Response surface analysis of total phenolic and flavonoid contents

#### 3.1.1. Total phenolic contents (TPC)

Temperature, time, and pH had a significant impact on TPC. \((p < 0.0001)\), with a fairly high model adequacy level \((R^2 = 0.99)\) (Table 3). On the TPC, time and temperature had a negative quadratic and a positive linear impact \((p < 0.0001)\). Fig. 2b and c demonstrate the collective effect of pH-time and pH-temperature on TPC of wild bush honey.

#### 3.1.2. Total flavonoid contents (TFC)

The process variables (temperature, time, and pH) were seen to significantly predict the total flavonoid contents (Table 3) with a very strong coefficient determination \((R^2 = 0.9971)\) and its adjusted form should be reported, which ranged between 0.9993 and 0.9998 (Table 3). Table 4 shows the second order (quadratic) regression coefficients for the responses (DPPH-RSA, TFC, and TPC) of wild bush honey. ANOVA also described that the second order polynomial model significantly explained the dependence of the responses (DPPH-RSA, TFC, and TPC) on the predictor variables (heating temperature, time, and pH).

#### 3.2. Response surface analysis of antioxidant activity (DPPH-RSA)

Table 3 shows that the relationship between temperature and independent variables has a quadratic RSA with a decent regression coefficient \((R^2 = 0.99)\). The collective impact of time, temperature, and pH on the antioxidant activity of wild bush honey is presented in Fig. 4a-c. The enhancement in antioxidant activity of wild bush honey was higher at 80 °C than at 60 and 70 °C, indicating that when honey is heated at a higher temperature, the antioxidant activity increases and becomes more obvious.

The antioxidant activity of honey was also impacted by its pH. Temperature and pH had a positive quadratic \((p = 0.0001)\) and negative linear \((p = 0.0382)\) influence on the activity of DPPH (Fig. 4b).

---

### Table 1

| Frequency classes. | Pollen grains detected |
|-------------------|------------------------|
| Predominant pollen| >45%                   |
| Secondary pollen  | 16–45%                 |
| Important minor pollen | 3–15%            |
| Minor pollen      | 3%                     |

### Table 2

| Experiment No. | Temperature (°C) \( X_1 \) | Time (min) \( X_2 \) | pH \( X_3 \) | DPPH-RSA (%) | TFC (mg GAE/100 g) | TPC (mg QE/100 g) |
|---------------|-----------------------------|----------------------|-------------|--------------|-------------------|------------------|
| 1             | 60.00                       | 12.50                | 3.00        | 61.29        | 58.22             | 19.21            |
| 2             | 70.00                       | 15.00                | 3.00        | 61.06        | 58.23             | 17.22            |
| 3             | 70.00                       | 12.50                | 4.50        | 59.48        | 55.34             | 16.73            |
| 4             | 80.00                       | 10.00                | 4.50        | 65.75        | 52.98             | 19.27            |
| 5             | 80.00                       | 15.00                | 4.50        | 65.81        | 56.62             | 20.63            |
| 6             | 70.00                       | 12.50                | 4.50        | 59.52        | 56.00             | 16.67            |
| 7             | 70.00                       | 12.50                | 4.50        | 60.96        | 56.00             | 16.73            |
| 8             | 70.00                       | 15.00                | 6.00        | 59.52        | 50.34             | 16.92            |
| 9             | 70.00                       | 12.50                | 4.50        | 60.96        | 56.32             | 16.66            |
| 10            | 70.00                       | 12.50                | 4.50        | 59.53        | 55.72             | 16.91            |
| 11            | 60.00                       | 10.00                | 4.50        | 61.33        | 47.79             | 16.34            |
| 12            | 60.00                       | 12.50                | 6.00        | 61.13        | 47.26             | 13.59            |
| 13            | 70.00                       | 10.00                | 3.00        | 61.23        | 50.23             | 13.42            |
| 14            | 80.00                       | 12.50                | 3.00        | 65.72        | 54.86             | 16.92            |
| 15            | 70.00                       | 10.00                | 6.00        | 61.23        | 53.15             | 16.91            |
| 16            | 80.00                       | 12.50                | 6.00        | 65.82        | 60.75             | 23.62            |
| 17            | 60.00                       | 15.00                | 4.50        | 61.00        | 49.51             | 17.01            |

All response values are mean values of duplicates.
activity (Fig. 2a), which might be attributed to the production of non-enzymatic antioxidant-rich products (MRPs) in honey, as honey's antioxidant action is mostly attributed to polyphenols (Nayik et al., 2016). TPC increased with temperature (60 to 80 °C) and acidic pH (pH, 6 to 3), which was likely owed to phenolic compounds, as these reduced susceptibility to oxidation at a relatively decreased pH by the protection of hydroxyl groups through deprotonation (Nayik et al., 2016).

The pH level of honey influences the antioxidant characteristics of its phenolic compounds in addition to the heating temperature and time combinations. This implies that the quality and nutritional as well as nutraceutical functionalities of honey from different localities could be optimized to a desired end-use requirements. The current research presented the honey's potential as a source of natural antioxidant compounds and their radical scavenging properties under different thermal processing time–temperature combinations and pH, in an effort to optimize the combination of three important predictive factors.

pH and temperature had a negative quadratic impact and a positive linear influence, respectively, but time had both positive linear and quadratic effects. Because honey's antioxidant characteristics is mostly attributable to the availability of polyphenols, the impact of temperature was similar to that of DPPH-RSA activity, i.e., as temperature and time increased, there was an increase in TFC as well. The TFC was higher at acidic pH because polyphenol antioxidant activity is higher at acidic pH and decreases monotonically with increasing pH from 3 to 6 owing to deprotonation of hydroxy groups (Nayik et al., 2016).

On the antioxidant activity, the temperature had a positive linear and quadratic impact (p < 0.0001, p < 0.0001) which might be attributed to the formation of Maillard reaction products (MRPs) that could have improved the antioxidant potential of wild bush honey as time and temperature increased (Fig. 4a). MRPs have been found to have substantial antioxidant effects in a variety of foods (Wagner et al., 2002; Xiang et al., 2008). The high-temperature heating and storage conditions trigger the production of hydroxymethylfurfural (HMF), which is internationally often used to determine whether honey is fresh or not. Commercial honey suitable for household usage should not contain more than 40 mg of HMF per kg of honey, according to the relevant regulation (Shapla et al., 2018). However, thermal treatment of honey (close to 80 °C) results in the production of distinct compounds in non-enzymatic browning reactions that proceed via distinctive chemical pathways, contributing to a logarithmic increase in the antioxidant activity of honey. Turkmen et al. (2006) reported equivalent results for antioxidant activity in honey after extended heating. The findings were similarly congruent with those of Fauzi et al. (2014), who found that high-pressure processing with thermal treatment increased the antioxidant activity of Manuka honey from New Zealand significantly. The results from the present study are also in association with that from a previous study on cherry honey (Nayik and Nanda, 2016).

Table 3
Significant levels of wild bush (P. rugosus) honey responses using RSM.

| P > F   | DPPH-RSA(%) | TPC (mg GAE/100 g) | TFC (mg QE/100 g) |
|---------|-------------|--------------------|--------------------|
| Model   | < 0.0001    | < 0.0001           | < 0.0001           |
| A: Temperature (°C) | < 0.0001 | < 0.0001           | < 0.0001           |
| B: Time (min)    | < 0.0001    | < 0.0001           | < 0.0001           |
| C: pH          | < 0.0001    | < 0.0001           | < 0.0001           |
| A²         | < 0.0001    | 0.0021             | < 0.0001           |
| B²         | < 0.0001    | < 0.0001           | 0.4397             |
| C²         | < 0.0001    | 0.1367             | 0.5903             |
| AB         | 0.0004      | 0.0484             | 0.0983             |
| AC         | 0.0043      | < 0.0001           | < 0.0001           |
| BC         | 0.1539      | < 0.0001           | 0.0016             |
| R²         | 0.9999      | 0.9953             | 0.9971             |
| Adjusted R² | 0.9998    | 0.9892             | 0.9933             |
| Pred. R²   | 0.9991      | 0.9570             | 0.9613             |
| Adeq. Precision | 263.95   | 45.47              | 70.112             |
| Lack of Fit | 0.0853     | 0.3501             | 0.0641             |

It was indicated that the DPPH-RSA increases with increasing acidity (from pH 6 to 3) and increasing range of temperature (from 60 to 80 °C).

4. Discussion

The analysis of pollen in honey samples demonstrated that all honey samples could be characterized as unifloral honey since the significant proportion of pollen (greater than 45%) was derived from the respective plant species (Table 1). The Fig. 1 shows the microscopic view of pollen found in honey of Plectranthus rugosus.

An increase in TPC corresponded to an increase in antioxidant activity (Fig. 2a), which might be attributed to the production of non-enzymatic antioxidant-rich products (MRPs) in honey, as honey's antioxidant action is mostly attributed to polyphenols (Nayik and Nanda, 2015). TPC increased with temperature (60 to 80 °C) and acidic pH (pH, 6 to 3), which was likely owed to phenolic compounds, as these reduced susceptibility to oxidation at a relatively decreased pH by the protection of hydroxyl groups through deprotonation (Nayik et al., 2016).

The pH level of honey influences the antioxidant characteristics of its phenolic compounds in addition to the heating temperature and time combinations. This implies that the quality and nutritional as well as nutraceutical functionalities of honey from different localities could be optimized to a desired end-use requirements. The current research presented the honey's potential as a source of natural antioxidant compounds and their radical scavenging properties under different thermal processing time–temperature combinations and pH, in an effort to optimize the combination of three important predictive factors.

Table 4
Second order regression coefficients for responses (DPPH-RSA, TPC and TFC) wild bush honey.

| Response | Intercept | A   | B   | C   | AB  | AC  | BC  | A²  | B²  | C²  |
|----------|-----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| DPPH-RSA | 59.61     | 2.29| −0.089| −0.020| 0.097| 0.065| −0.025| 3.16| 0.79| 0.81|
| TPC      | 55.88     | 2.80| 1.32| −1.25| −0.48| 4.21| −2.70| −0.93| −3.22| 0.33|
| TFC      | 16.74     | 1.79| 0.48| 0.29| 0.17| 3.08| −0.45| 1.64| −0.07| −0.05|

Where A = Temperature; B = time; C = pH.
The gain of H⁺ ions caused by a decrease in pH might be the cause for the observed shift. Due to the decrease of H⁺ ions with an increase in pH, the joint effect of pH (3–6) and time (10 to 15 min) also reduced antioxidant activity (Fig. 4c).

The overall implication is that the antioxidant activity as radical scavenging potential of honey can be optimized to meet specific product and consumer demands. This presents a scenario-specific potential for honey thermal treatments and pH adjustment to maximize the health functionality of honey, in addition to the need for just sweetening foods.

5. Conclusion

The reduction in antioxidant activity caused by heat treatment of honey is offset by the Maillard reaction products. By adjusting all three process variables (pH, temperature, and time), it is feasible to optimize the flavonoid and total phenolic contents as well as the DPPH scavenging activity of honey. The low pH, high temperature (80 °C), and prolonged time were found to be the most desired choices for the optimal thermal treatment of wild bush honey. As
a result of the combined heat treatment and pH adjustment, the antioxidant activity in honey samples increased, presenting some new and beneficial health effects for consumers under the perspectives of regular consumption.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The project was supported by researchers supporting project number (RSP-2021/315) King Saud University Riyadh, Saudi Arabia

References

Al-Ghamdi, A., Mohammed, S.E.A., Ansari, M.J., Adgaha, N., 2019. Comparison of physicochemical properties and effects of heating regimes on stored Apis mellifera and Apis florea honey. Saudi J. Biol. Sci. 26 (4), 845–848. https://doi.org/10.1016/j.sjbs.2017.06.002.

Belay, A., Haki, C.D., Birringer, M., Borck, H., Lee, Y.C., Kim, K.T., Baye, K., Meláku, S., 2017. Enzyme activity, amino acid profiles and hydroxymethylfurfural content in Ethiopian monofloral honey. J. Food Sci. Technol. 54 (9), 2769–2778. https://doi.org/10.1007/s13197-017-2713-6.

Bodă, A., Radvăniții, L., Köszegi, T., Cziepregi, R., Nagy, D.J., Farkas, Á., Kocis, M., 2021. Quality Evaluation of Light-and Dark-Colored Hungarian Honeys, Focusing on Botanical Origin, Antioxidant Capacity and Mineral Content. Molecules 26 (9), 2825. https://doi.org/10.3390/molecules26092825.

Brudzynski, K., Maldonado-Alvarez, L., 2015. Polyphenol-protein complexes and their consequences for the redox activity, structure and function of honey. A current view and new hypothesis—a review. Polish J. Food Nutr. Sci. 65 (2), 71–80. https://doi.org/10.1515/pjfn-2015-0030.

da Silva, P.M., Gauche, C., Gongaza, L.V., Costa, A.C.O., Fett, R., 2016. Honey: Chemical composition, stability and authenticity. Food Chem. 196, 309–323. https://doi.org/10.1016/j.foodchem.2015.09.051.

De-Melo, A.A.M., de Almeida-Muradian, L.B., Sancho, M.T., Pascual-Maté, A., 2018. Composición y propiedades de la miel de Apis mellifera: una revisión. J. Apic. Res. 57 (1), 5–37. https://doi.org/10.1080/00218839.2017.1338444.

Escuredo, O., Dobre, I., Fernández-González, M., Seijo, M.C., 2014. Contribution of botanical origin and sugar composition of honeys on the crystallization phenomenon. Food Chem. 149, 84–90. https://doi.org/10.1016/j.foodchem.2013.10.097.

Fauzi, N.A., Farid, M.M., Silva, F.V., 2014. High-pressure processing of manuka honey: Improvement of antioxidant activity, preservation of colour and flow behaviour. Food Bioprocess Technol. 7 (8), 2299–2307. https://doi.org/10.1007/s11947-013-1204-7.

Fechner, D.C., Moreisi, A.L., Díaz, J.D.R., Pellerano, R.G., Vazquez, F.A., 2016. Multivariate classification of honeys from Corrientes (Argentina) according to geographical origin based on physicochemical properties. Food Biosci. 15, 49–54. https://doi.org/10.1016/j.fbio.2016.03.002.

Kamboj, R., Nayik, G.A., Bera, M.B., Nanda, V., 2020. Sugar profile and rheological behaviour of four different Indian honey varieties. J. Food Sci. Technol. 57 (8), 2985–2993. https://doi.org/10.1007/s13197-020-04331-7.

Kovács, D., Kirdy, P., Tóth, G., 2021. Sample-size dependence of validation parameters in linear regression models and in QSAR SAR QSAR Environ. Res. 32 (4), 247–268. https://doi.org/10.1080/1062936X.2021.1900208.

Nayik, G.A., Nanda, V., 2015. Physico-chemical, enzymatic, mineral and colour characterization of three different varieties of honeys from Kashmir valley of India with a multivariate approach. Polish J. Food Nutr. Sci. 65 (2), 101–108. https://doi.org/10.1515/pjfn-2015-0022.

Nayik, G.A., Nanda, V., 2016. Application of response surface methodology to study the combined effect of temperature, time and pH on antioxidant activity of cherry (Prunus avium) honey. Polish J. Food Nutr. Sci. 66 (4), 287–293. https://doi.org/10.1515/pjfn-2015-0655.

Nayik, G.A., Dar, B.N., Nanda, V., 2016. Optimization of the process parameters to establish the quality attributes of DPPH radical scavenging activity, total phenolic content, and total flavonoid content of apple (Malus domestica) honey using response surface methodology. Int. J. Food Prog. 19 (8), 1738–1748. https://doi.org/10.1080/109492129.2015.1107733.

Nayik, G.A., Suhag, Y., Majid, I., Nanda, V., 2018. Discrimination of high altitude Indian honey by chemometric approach according to their antioxidant properties and macro minerals. J. Saudi Soc. Agric. Sci. 17 (2), 200–207. https://doi.org/10.1016/j.jssas.2016.04.004.

Nayik, G.A., Dar, B.N., Nanda, V., 2019. Physico-chemical, rheological and sugar profile of different unifloral honeys from Kashmir valley of India. Arab. J. Chem. 12 (8), 3151–3162. https://doi.org/10.1016/j.arabjc.2015.08.017.

Pauluc, D., Dranca, T., Oroian, M., 2020. Antioxidant activity, total phenolic content, individual phenolics and physicochemical parameters suitability for Romanian honey authentication. Foods 9 (3), 306. https://doi.org/10.3390/foods9030306.

Pontis, J.A., Costa, L.A.M.A.D., Silva, S.J.R.D., Flach, A., 2014. Color, phenolic and flavonoid content, and antioxidant activity of honey from Roraima, Brazil. Food Sci. Technol. 34 (1), 69–73. https://doi.org/10.1590/S0101-20612014000100015.

Press Information Bureau, 2021. National Beekeeping & Honey Mission (NBHM) aims to achieve the goal of ‘Sweet Revolution’ as part of Atmanirbhar Bharat Abhiyan. https://pib.gov.in/PressReleaseDetail.aspx?PRID=1697113 (accessed 13 June 2021).

Santos-Buelga, C., González-Paramás, A.M., 2017. Chemical Composition of Honey. In: Alvarez-Suarez, J. (Ed.), Bee Products - Chemical and Biological Properties. Springer, Cham, pp. 43–82. https://doi.org/10.1007/978-3-319-59689-1_3.
Shapla, U.M., Solayman, M., Alam, N., Khalil, M.I., Gan, S.H., 2018. 5-Hydroxymethylfurfural (HMF) levels in honey and other food products: effects on bees and human health. Chem. Cent. J. 12 (1), 1–18. https://doi.org/10.1186/s13065-018-0408-3.

Tekindal, M.A., Bayrak, H., Ozkaya, B., Genc, Y., 2012. Box- Behnken experimental design in factorial experiments: the importance of bread for nutrition and health. Turkish J. Field Crops 17, 115–123.

Turkmen, N., Sari, F., Poyrazoglu, E.S., Velioğlu, Y.S., 2006. Effects of prolonged heating on antioxidant activity and colour of honey. Food Chem. 95 (4), 653–657. https://doi.org/10.1016/j.foodchem.2005.02.004.

Von Der Ohe, W., Persano Oddo, L., Piana, M.L., Morlot, M., Martin, P., 2004. Harmonized Methods of melissopalynology. Apidologie 35 (Suppl. 1), S18–S25. https://doi.org/10.1051/apido:2004050.

Wagner, K.H., Derkits, S., Herr, M., Schuh, W., Elmadiâ, L., 2002. Antioxidative potential of melanoids isolated from a roasted glucose–glycine model. Food Chem. 78 (3), 375–382. https://doi.org/10.1016/S0308-8146(02)00200-5.

Warui, M.W., Hansted, L., Gikungu, M., Mburu, J., Kironchi, G., Bosselmann, A.S., 2019. Characterization of Kenyan honeys based on their physicochemical properties, botanical and geographical origin. Int. J. Food Sci. 2019, 1–10. https://doi.org/10.1155/2019/2912509.

Yan, S., Sun, M., Zhao, L., Wang, K., Fang, X., Wu, L., Xue, X., 2019. Comparison of differences of α-dicarbonyl compounds between naturally matured and artificially heated Acacia honey: their application to determine honey quality. J. Agric. Food Chem. 67 (46), 12885–12894. https://doi.org/10.1021/acs.jafc.9b0548410.1021/acs.jafc.9b05484001.

Xiang, H.D., Xu, S.Y., Wang, Z., 2008. Antioxidant Activities of Maillard Reaction Products of Protein and Reducing Sugar. J. Food Sci https://en.cnki.com.cn/Article_en/CJFDTotal-SPKX2008007006.htm. (accessed: 28-May-2021).

Zarei, M., Fazlara, A., Tulabifard, N., 2019. Effect of thermal treatment on physicochemical and antioxidant properties of honey. Heliyon 5, http://doi.org/10.1016/j.heliyon.2019.e01894 e01894.