Shelf life determination of *Apis mellifera* honey

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Abstract
This study predicted the shelf life of honey using an accelerated method, setting the hydroxymethylfurfural (HMF) content at 60 mg/kg as the perishability limit. HMF content, water activity, free acidity, pH, and color were evaluated over time at storage temperatures of 45 °C, 55 °C, and 65 °C. A kinetic study was conducted to determine zero- and first-order reaction rate constants for HMF formation, as well as the activation energy (E_a) and temperature acceleration factor (Q_{10}). Artificial Neural Networks (ANNs) were employed to estimate honey shelf life (output layer) from temperature data (input layer), using the K-fold and Holdback validation methods. A significant correlation was observed between honey color change and HMF formation during heating ($P < 0.05$). HMF formation followed a zero-order kinetic model ($0.9856 \leq R^2 \leq 0.9949$), with an E_a of 29.349 cal/mol and a Q_{10} of 3.92. The ANN model using the Holdback method and 10 neurons in the hidden layer showed excellent predictive performance ($R^2 = 1$; RMSE = 1.07×10^{-5}), estimating a shelf life of 344 days at 25 °C. Colorimetry results also demonstrated potential for the indirect evaluation of HMF content, supporting its applicability in honey quality and preservation assessments. These findings highlight the effectiveness of combining kinetic modeling with ANN for honey shelf life prediction.
Keywords: *Apis mellifera* honey; Arrhenius model; Artificial Neural Networks; heating; reaction kinetics.

Determinação do prazo de validade do mel da abelha Apis mellifera

Resumo
Este estudo previu a vida de prateleira de mel utilizando um método acelerado, estabelecendo o teor de hidroximetilfurfural (HMF) em 60 mg/kg como limite de perecibilidade. Foram avaliados, ao longo do tempo, o teor de HMF, a atividade de água, a acidez livre, o pH e a cor em temperaturas de armazenamento de 45 °C, 55 °C e 65 °C. Um estudo cinético foi realizado para determinar as constantes de velocidade de reação de ordem zero e primeira ordem para a formação de HMF, além da energia de ativação (E_a) e do fator de aceleração da temperatura (Q_{10}). Redes Neurais Artificiais (RNAs) foram utilizadas para estimar a vida útil do mel (camada de saída) a partir dos dados de temperatura (camada de entrada), aplicando os métodos de validação K-fold e Holdback. Observou-se uma correlação significativa entre a mudança de cor do mel e a formação de HMF durante o aquecimento ($P < 0,05$). A formação de HMF seguiu um modelo cinético de ordem zero ($0,9856 \leq R^2 \leq 0,9949$), com E_a de 29,349 cal/mol e Q_{10} de 3,92. O modelo de RNA utilizando o método Holdback e 10 neurônios na camada oculta apresentou excelente desempenho preditivo ($R^2 = 1$; RMSE = $1,07 \times 10^{-5}$), estimando uma vida útil de 344 dias a 25 °C. Os resultados da colorimetria também demonstraram potencial para a avaliação indireta do teor de HMF, apoiando sua aplicabilidade na análise da qualidade e conservação do mel. Esses resultados demonstram a eficácia da combinação entre modelagem cinética e inteligência artificial na predição da vida de prateleira de mel.
Palavras-chave: *aquecimento; cinética de reação; mel da abelha* Apis mellifera*; modelo de Arrhenius*; *Redes Neurais Artificiais.*

1 Introduction

Shelf life refers to the maximum duration during which food products can be stored without undergoing unacceptable deterioration in sensory attributes, nutritional value, or safety for consumption (Giménez; Ares; Ares, 2012; Franklin *et al*., 2017). In the case of honey, its shelf life can be significantly reduced when stored under conditions that accelerate the formation of 5-hydroxymethylfurfural (HMF), a compound whose formation is mainly influenced by temperature (Erbakan *et al*., 2021; Shapla *et al*., 2018). HMF is a furanic compound that results from the dehydration of hexose sugars in acidic environments (pH ≤ 5), or it may also appear as an intermediate during the Maillard reaction, which involves reducing sugars and amino acids (Nagai *et al*., 2018; Shapla *et al*., 2018). According to Brazilian regulations, the maximum acceptable HMF concentration in honey is set at 60 mg/kg (Brasil, 2000), while the Codex Alimentarius (FAO, 2001) allows up to 80 mg/kg for honeys produced in tropical regions. Although the complete toxicity profile of HMF has not been fully elucidated, animal studies indicate that it can be metabolized into 5-sulfoxymethylfurfural, a compound with genotoxic and potentially carcinogenic properties (Bakhiya *et al*., 2009).

Accelerated shelf life testing methods are designed to minimize the time and cost associated with traditional long-term storage experiments (Franklin *et al*., 2017; Rothkopf *et al*., 2017). These methods expose food products to intensified conditions, such as higher temperatures, in order to accelerate chemical degradation processes (Martins *et al*., 2016). Various tools, including kinetic models and Artificial Neural Networks (ANNs), have been successfully applied to analyze data from these accelerated tests and to predict shelf life more efficiently (Bai *et al*., 2022; Chutia *et al*., 2024; Feng *et al*., 2024; Laksanawati *et al*., 2024). Fallico, Arena e Zappala (2009) observed that five honey samples surpassed the 60 mg/kg HMF threshold in less than 18 months under conventional storage, reinforcing the importance of faster shelf life determination techniques for producers.

Therefore, this study aimed to estimate the shelf life of honey by employing an accelerated methodology, combining the Arrhenius equation and Artificial Neural Networks, using the HMF limit of 60 mg/kg as a criterion for quality degradation. In addition, selected physicochemical characteristics are evaluated to assess the changes that occur in honey when subjected to storage temperatures of 45 °C, 55 °C, and 65 °C, and to analyze their correlation with HMF formation.

To achieve the proposed objectives, this study was conducted in several stages. Initially, honey samples from the species *Apis mellifera* were subjected to controlled heating conditions at temperatures of 45 °C, 55 °C, and 65 °C to accelerate the degradation process and predict shelf life based on the formation of HMF. Physicochemical and colorimetric analyses were periodically performed to evaluate water activity, free acidity, pH, and color parameters, along with the quantification of HMF over time. Subsequently, a kinetic study was carried out to model HMF formation using zero- and first-order reaction equations, allowing for the calculation of activation energy and temperature acceleration factor. ANNs were also employed to predict honey shelf life based on temperature, testing different architectures and validation methods. The results are discussed to highlight the influence of temperature on the physicochemical behavior of honey and HMF formation, and to validate the applicability of kinetic modeling and artificial intelligence as effective tools for estimating the shelf life of apicultural products.

2 Research method

Honey samples from the species *Apis mellifera* were carefully collected from the apiary located at the Federal University of Viçosa Florestal Campus (19° 50' 52.8" S, 44° 26' 16.8" O). The experiments were carried out at the Food Chemistry Laboratory of the Federal University of Viçosa Florestal Campus.

2.1 Experimental design

The experimental design used was completely randomized, employing a factorial scheme of 3 × *n*, where three different water bath temperatures (45 ºC, 55 ºC, and 65 °C) were combined with storage times (*n*) that extended until the honey reached a HMF concentration of 60 mg/kg.

Preliminary tests were carried out to verify whether there were significant differences in the time required to reach this critical HMF level at each temperature, which supported the selection of the temperature conditions used in the study. These temperatures were intentionally chosen to enable the subsequent calculation of the Q_{10} coefficient and to allow the application of an accelerated shelf-life testing approach. The accelerated method was based on temperatures higher than the typical storage condition for honey (room temperature, approximately 25 °C), aiming to increase the reaction rate and thereby shorten the experimental time.

Each treatment condition was conducted with three replicates and analyzed in triplicate to ensure statistical reliability, resulting in 9 samples and 27 experimental units. Physicochemical and colorimetric analyses were systematically conducted at intervals specific to each temperature: every 96 or 72 hours for samples kept at 45 °C, every 24 hours for those at 55 °C, and every 12 hours for samples at 65 °C. To further validate the kinetic model, the HMF content was also measured after a long-term storage period of 13 months at room temperature, which ranged between 9 ºC and 37 ºC.

2.2 Physicochemical and colorimetric analyses

The analyses of HMF content and free acidity were conducted following the standardized methodologies recommended by the Adolfo Lutz Institute (IAL, 2008), ensuring accuracy and comparability of results. To determine the HMF content, approximately 5 g of the sample were dissolved in distilled water, clarified with 0.5 mL of Carrez I and II solutions, and the volume was adjusted to 50 mL. After filtration through a qualitative filter, 5 mL of the filtrate were mixed with 5 mL of distilled water (samples) or 0.2% sodium bisulfite solution (reference). Absorbance readings were performed using a UV–Vis spectrophotometer at 284 and 336 nm, employing a 1 cm quartz cuvette. When necessary, the solutions were diluted to ensure that absorbance values remained below 0.6. The HMF content was calculated using Equation 1 and expressed as mg/kg of honey.

$$\frac{(A\,284 \times A\,336) \times 149{,}7 \times 5}{P} = HMF\ (mg/Kg) \qquad (1)$$

Where:

- $A_{(284)}$ = absorbance reading at 284 nm
- $A_{(336)}$ = absorbance reading at 336 nm
- P = sample mass (g)
- 126 = molecular weight of HMF
- 16,830 = molar absorptivity of HMF at 284 nm
- 1,000 = conversion factor from g to mg
- 149.7 = (126/16,830) × (1,000/10) × (1,000/5)
- 10 = dilution factor of 5 g of honey to 50 mL
- 5 = nominal sample mass
- 1,000 = conversion factor from g to kg

The pH levels of the honey samples were determined in accordance with the procedures described by Abu-Jdayil *et al*. (2002), employing a HANNA HI 99163 digital pH meter (HANNA Instruments, Barueri, Brazil) for precise measurement. To assess the color characteristics of the samples, a Delta Vista 650G colorimeter (Delta Color, São Leopoldo, Brazil) was used, allowing objective quantification of honey coloration. Additionally, the water activity of the samples was measured at a controlled temperature of 25 °C using a Testo 650 analyzer (TEquipment, Sparta, New Jersey, United States).

2.3 Kinetic study of HMF formation

The shelf life of honey was estimated based on HMF formation kinetics by determining the reaction order (n), rate constant (k), activation energy (E_a), and temperature acceleration factor (Q_{10}) for both zero- and first-order reactions (Fonseca *et al*., 2023). The influence of temperature on k was mathematically modeled using the Arrhenius equation, which allowed for the calculation of the

activation energy (Ea) and the temperature acceleration factor (Q10) according to the formulas presented in Equations 2 and 3, respectively (Arabshahi; Lund, 1985; Fonseca *et al*., 2023).

$$lnk = -\frac{E_a}{R}\left(\frac{1}{T} - \frac{1}{T_0}\right) + lnk_0 \quad (2)$$

where R is the universal gas constant (1.987×10^{-3} kcal·mol^{-1}·K^{-1}); T is the final absolute temperature (K); T_0 is the reference absolute temperature, and k_0 is the reaction constant.

$$Q_{10} = 10^{\frac{E_a}{0,46*T^2}} \quad (3)$$

where T is the average studied temperature (K), which in this case is 55 °C (328.15 K).

The shelf life estimation was based on the time required for the HMF concentration in honey to reach the maximum permissible limit of 60 mg HMF/kg honey, as established by Normative Instruction n° 11 of October 20, 2000 (Brasil, 2000).

2.4 Modeling using ANNs

ANNs were employed to predict the shelf life of honey, which was defined as the duration required for the honey to reach a HMF concentration limit of 60 mg/kg, represented as the output layer of the network. The prediction was based primarily on the storage temperature, which served as the input layer. Various network topologies were tested, featuring between 2 and 20 neurons in the hidden layers to determine the most effective architecture. Two distinct methods for training and validation were applied: the Holdback method, which utilized 75% of the dataset for training and the remaining 25% for validation, and the K-fold cross-validation method, where the dataset was divided into five equal subsets, with four subsets used for training and one subset reserved for validation in a rotating manner. The learning algorithm used during the training process was backpropagation with momentum, while the activation function chosen for the neurons was the hyperbolic tangent function (Abbasi-Tarighat; Shahbazi; Niknam, 2013). The optimal neural network architecture was selected through a trial-and-error approach, using the coefficient of determination (R^2) as the primary measure of performance and accuracy. The development and analysis of the ANNs were carried out using JMP 17 software (SAS Institute Inc., Cary, North Carolina, United States).

2.5 Statistical analyses

The effect of time on the physicochemical and colorimetric characteristics of honey at each temperature was evaluated using analysis of variance and linear regression in SPSS (International Business Machines Corporation, Armonk, New York, United States) software, with a significance level of 5%. This approach enabled a assessment of the impact of heating duration on key parameters such as pH, water activity, free acidity, and color, facilitating the identification of trends and their association with the degradation process in honey during thermal exposure.

3 Results and discussion

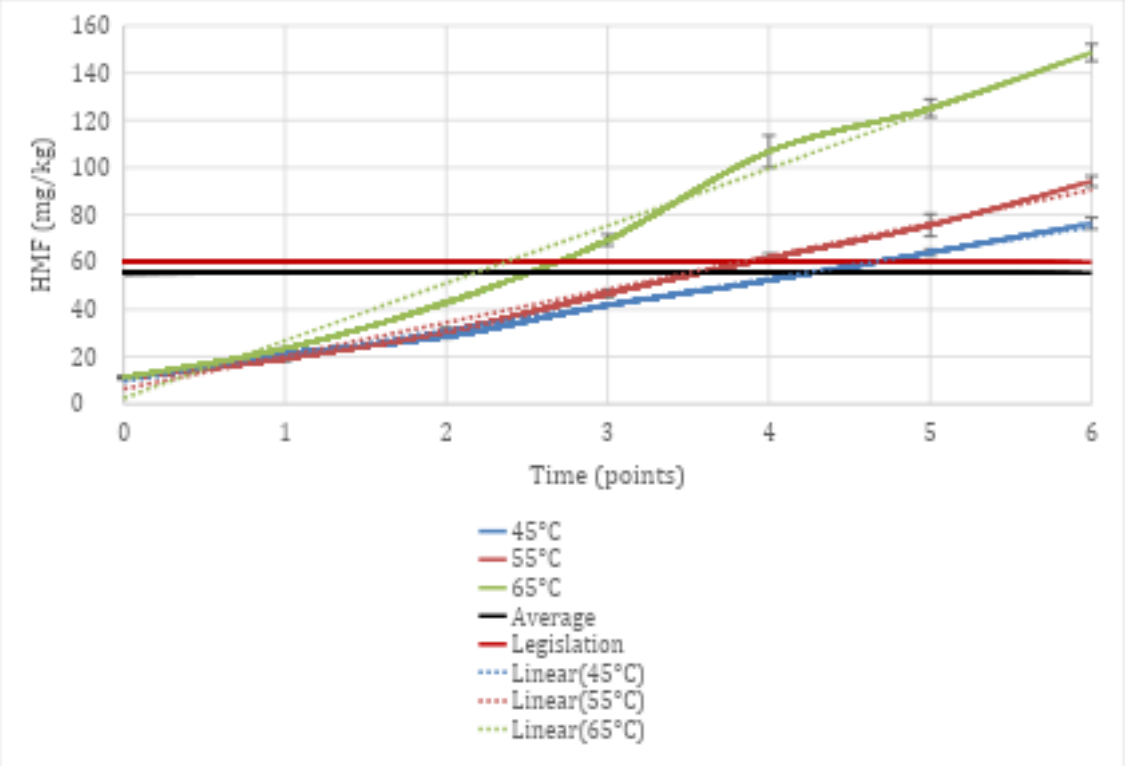
The results obtained in this study are presented and discussed below, with a focus on predicting the shelf life of the evaluated honey. The data are discussed based on theoretical principles and on studies previously reported in the literature.

3.1 Physicochemical analyses

The initial HMF content in the honey samples immediately after harvest was 11.13 mg/kg and increased linearly ($P < 0.05$) over time, with a more pronounced increase at higher temperatures. HMF can be formed as an intermediate in the Maillard reaction, which is promoted by increasing the reaction temperature. Additionally, HMF formation can occur due to prolonged heating, facilitating the

dehydration of hexoses present in honey, catalyzed by its acidic environment (pH = 4.31), resulting in this furanic compound. In sugar-rich matrices such as honey, temperature acts as the main driving force for dehydration reactions and Maillard pathways, leading to the progressive accumulation of HMF (Chua *et al*., 2014; Nagai *et al*., 2018). The samples subjected to 45 °C, 55 °C, and 65 °C took approximately 394.4 (16.4 days, between points 4 and 5), 91.7 (3.8 d, near point 4), and 28.4 h (1.2 d, between points 2 and 3), respectively, to reach the limit of 60 mg HMF/kg of honey, as established by legislation (Figure 1).

Figure 1 – HMF content over time



Source: research data

Water activity values were very similar (P > 0.05), varying close to the average (0.64 ± 0.01) and between 0.62 and 0.66, regardless of time or temperature (45 ºC, 55 ºC, or 65 °C). Acidity values were also similar (P > 0.05), fluctuating around the average (38.60 ± 1.72 mEq/kg) and between 34.87 and 42.13 mEq/kg, independent of storage temperature. No consistent variation pattern was observed for pH (P > 0.05) at each temperature. The pH values occasionally fluctuated around the average (4.31 ± 0.08), ranging from 4.18 to 4.53, irrespective of the temperature.

Determination of free acidity is crucial for assessing the conservation status of honey, as an appropriate pH reduces the risk of microbial development in honey. The acidity and pH values complied with the limits established by Brazilian legislation (Brasil, 2000) and Codex Alimentarius (FAO, 2001).

3.2 Colorimetric analysis

All color indices (red/green index [*a**], yellow/blue index [*b**], saturation index [*C**], hue angle [*h*], and lightness [*L**]) showed significant changes (P < 0.05) in honey color over time at 45 ºC, 55 ºC, and 65 °C. The *a** index (Figure 2) increased linearly (P < 0.05) over time, with a more pronounced increase (P < 0.05) at higher temperatures to produce a reddish honey hue. The *b**, *C**, *h*, and *L** indices (Figures 3 to 6) decreased linearly over time (P < 0.05), with a more pronounced reduction (P < 0.05) at higher temperatures, indicating a less yellow and saturated, darker, and more orange-toned honey color.

Although the linear model adequately described most color parameters, the behavior of color saturation (C*) at higher temperatures suggests a more complex degradation pattern. Therefore, the linear model may not fully capture the behavior of C* under severe thermal conditions. Studies focusing on honey browning kinetics have reported nonlinear responses for certain color attributes, particularly at elevated temperatures, due to the overlapping of pigment degradation and polymerization reactions (Cavaco *et al*., 2021). In food quality modeling, linear kinetic approaches represent useful simplifications but may not completely describe complex quality changes under extreme processing or storage conditions (Van Boekel, 2008).

Figure 2 – Red/green index (a^*) over time

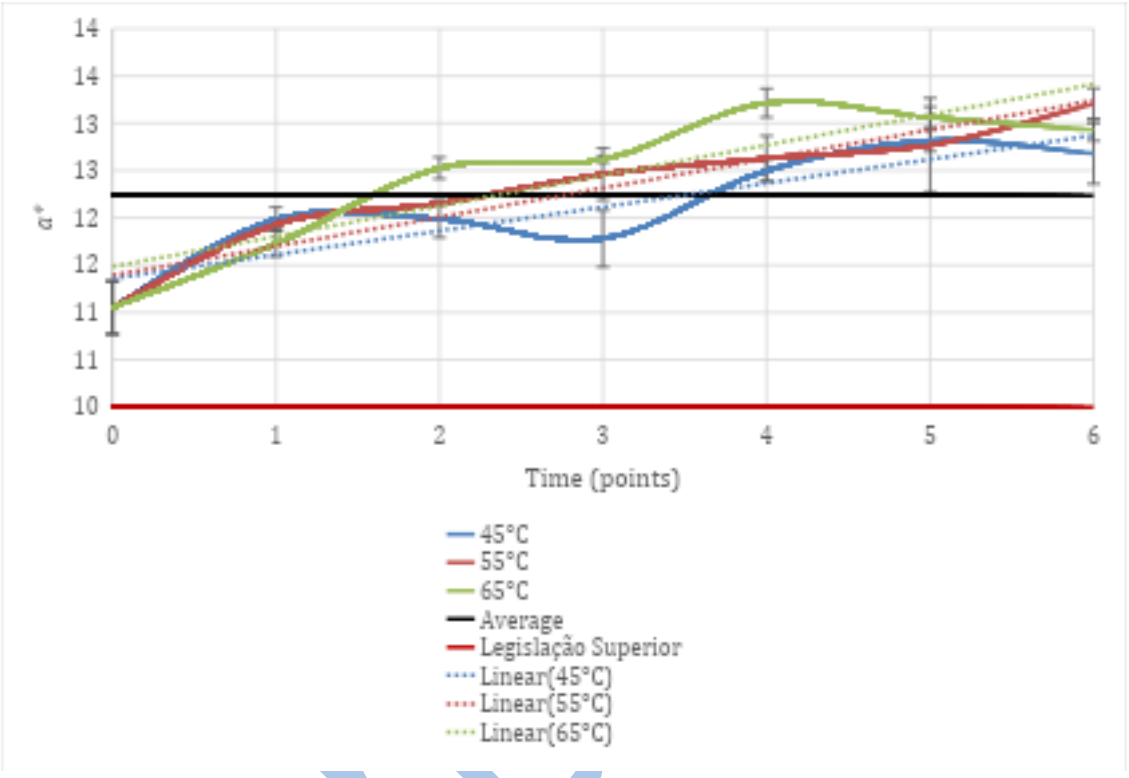


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Figure 3 – Lightness (L^*) over time

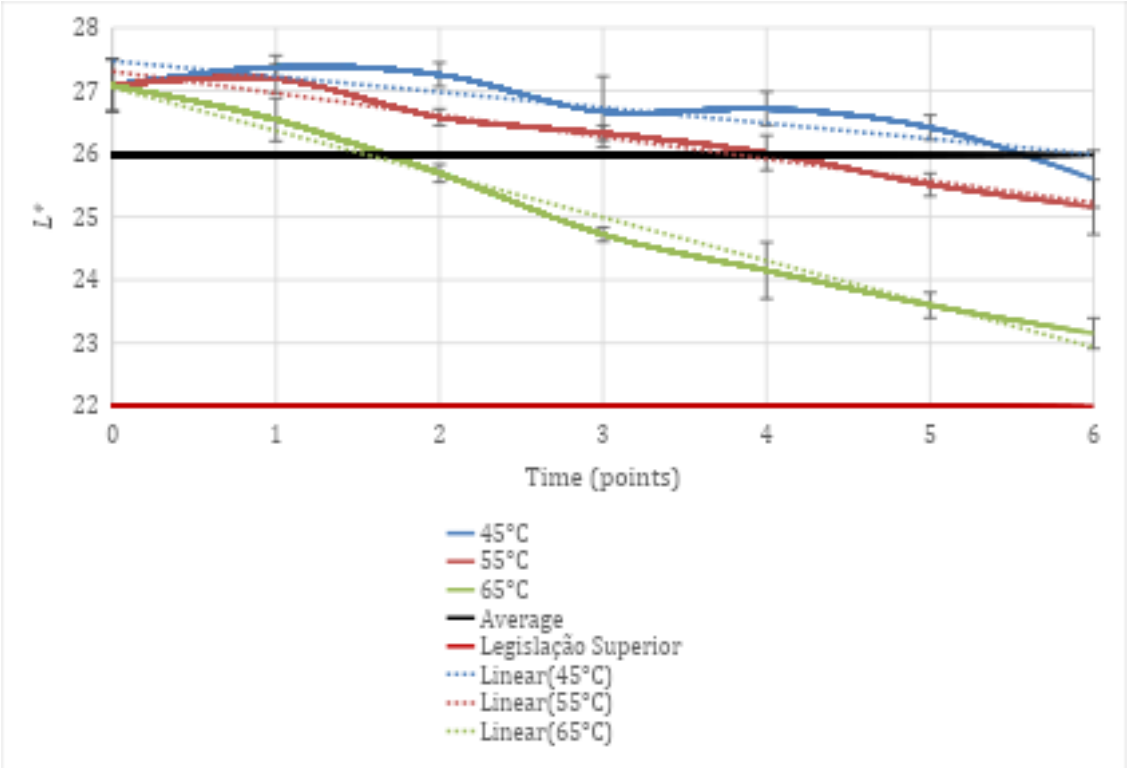

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Figure 4 – Yellow/blue index (*b**) over time

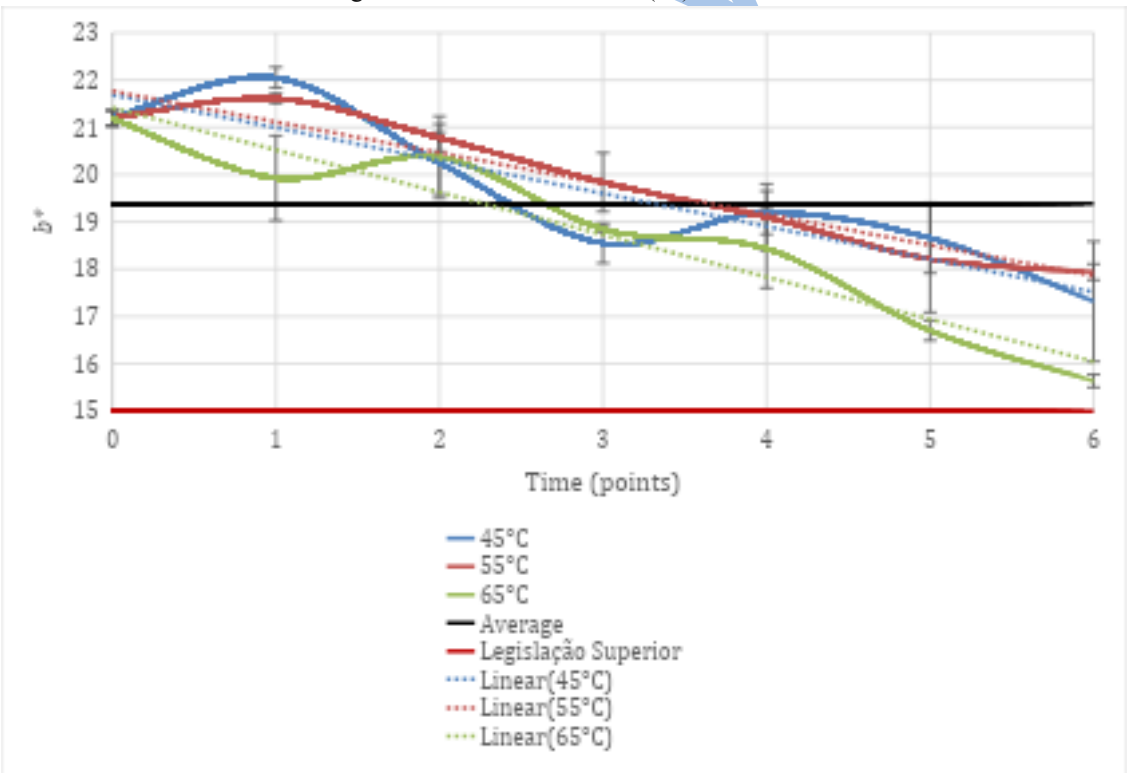

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Figure 5 – Saturation index (*C**) over time

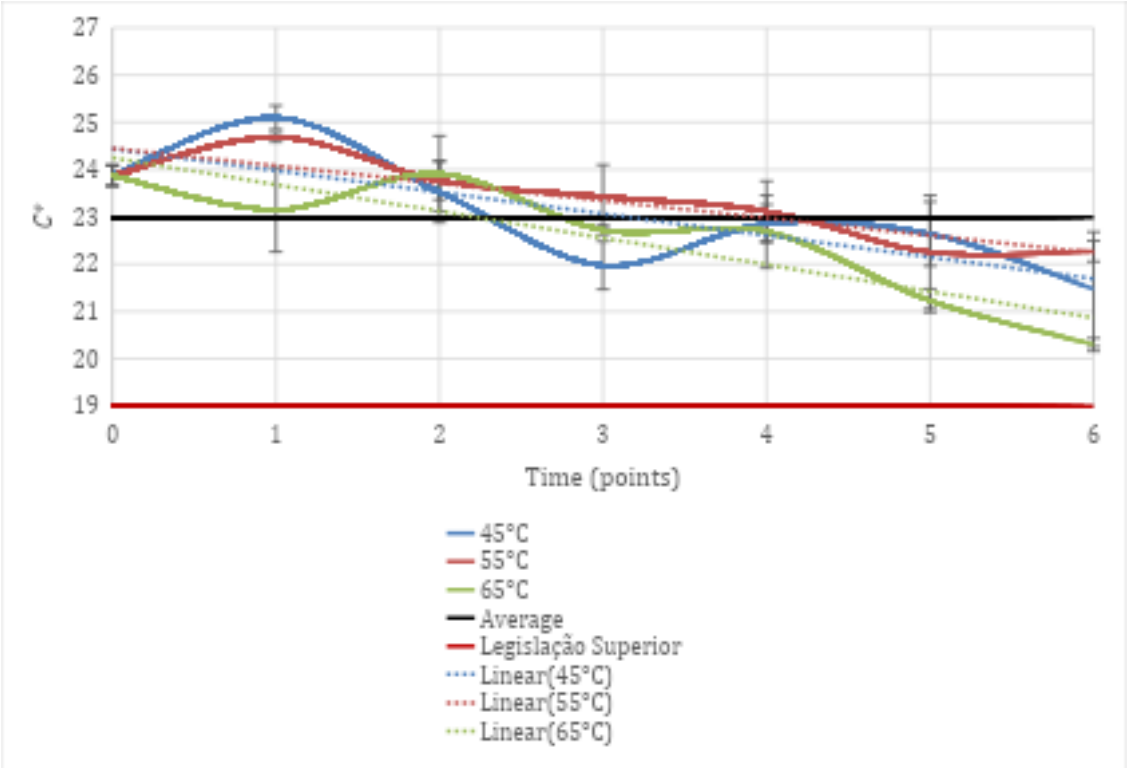

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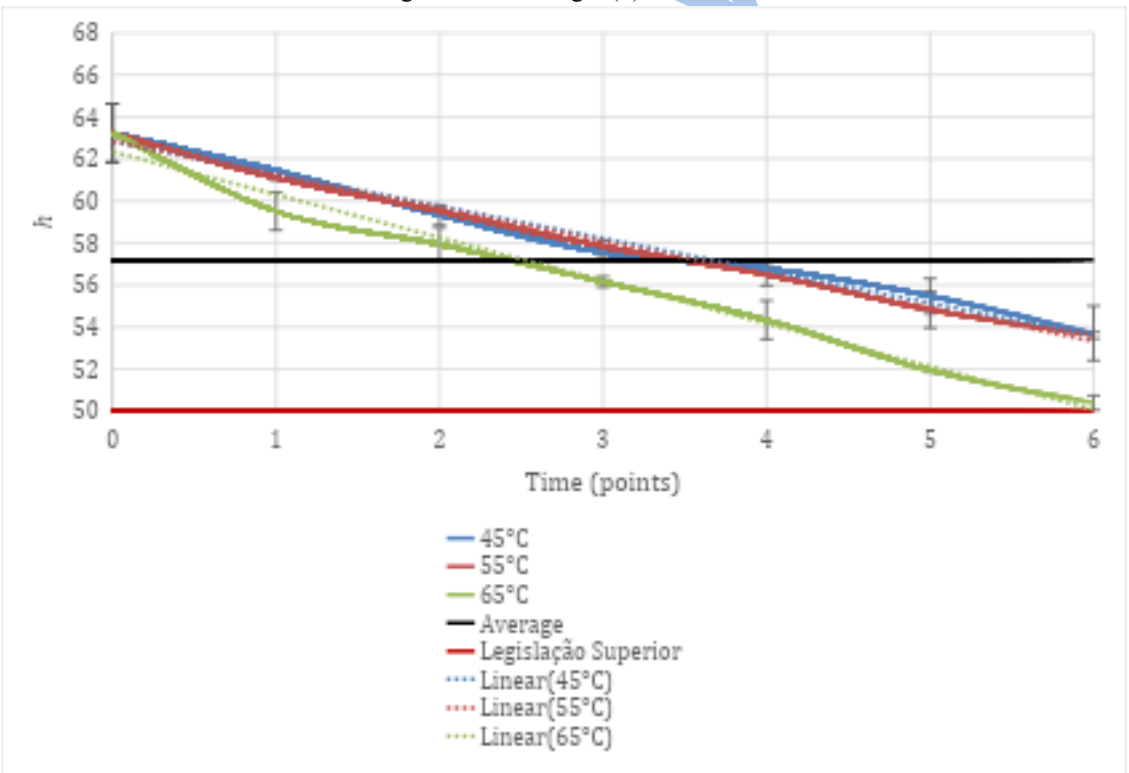
Figure 6 – Hue angle (*h*) over time



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The HMF content was significantly ($P < 0.05$) and strongly correlated with the color parameters. Correlations were negative for hue angle (*h*: r = -0.954), lightness (*L**: r = -0.945), yellow index (*b**: r = -0.925), and color saturation (*C**: r = -0.850), and positive for the red index (*a**: r =

0.845). Therefore, the higher HMF content in honey was associated with a color that was more orange-toned, less yellow and saturated, darker, and more reddish. These changes in honey color may be attributed to dark pigments generated by non-enzymatic browning reactions, primarily the Maillard reaction and caramelization, which are intensified at elevated temperatures (Ajlouni; Sujirapinyokul, 2010; Nagai *et al*., 2018). Reviews on honey quality have highlighted that color changes are not merely visual attributes but are closely associated with chemical degradation processes, including the formation of brown polymeric compounds and HMF (Bogdanov *et al*., 2008). Therefore, color parameters may serve as complementary indicators of the thermal deterioration of honey.

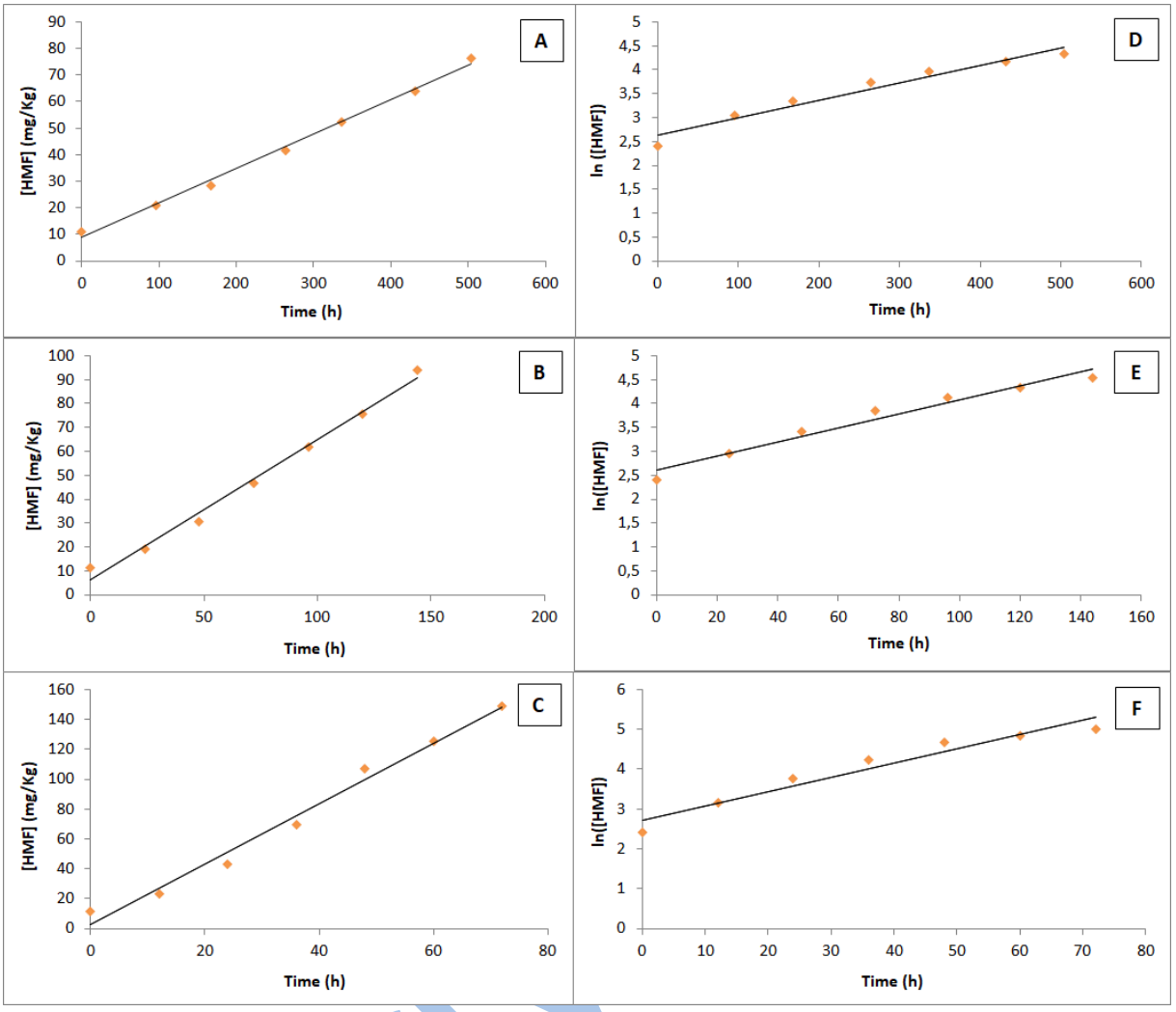
Physicochemical and colorimetric analyses revealed significant quality changes in honey under the storage conditions, especially temperature, which accelerated these alterations. Color changes driven by the Maillard reaction and caramelization highlight the importance of studying the effects of prolonged heating on honey quality. The findings are crucial for consumer safety and guiding proper honey handling by beekeepers.

3.3 Shelf life determination using the Arrhenius model

The kinetics of HMF formation in honey were best described by a zero-order reaction, with high coefficients of determination ($0.9856 \leq R^2 \leq 0.9949$), whereas the first-order model showed lower fitting accuracy ($0.9409 \leq R^2 \leq 0.9645$) (Figure 7). The superiority of the zero-order model suggests that HMF accumulation under the evaluated conditions was primarily governed by temperature and time, rather than by the concentration of reactants, which is consistent with the sugar-rich nature of honey. Accordingly, the reaction rate constant (k) increased markedly with temperature, from 0.1298 $mg \cdot kg^{-1} \cdot h^{-1}$ at 45 °C to 2.0232 $mg \cdot kg^{-1} \cdot h^{-1}$ at 65 °C, reflecting the strong thermal sensitivity of HMF formation.

Based on the zero-order kinetics, the estimated shelf life, defined as the time required for honey to reach the regulatory limit of 60 mg HMF·kg^{-1}, decreased substantially with increasing temperature, reaching 394.4, 91.7, and 28.4 h at 45 °C, 55 °C, and 65 °C, respectively. These results highlight the importance of controlling heating time during honey processing operations. In this context, technological steps such as liquefaction (~ 45 °C) and pasteurization (~ 65 °C) may be applied without compromising honey quality, provided that exposure times are carefully managed to limit HMF accumulation.

Figure 7 – Kinetic models for HMF formation during storage. (A–C) Zero-order model at 45 °C (A), 55 °C (B), and 65 °C (C). (D–F) First-order model at 45 °C (D), 55 °C (E), and 65 °C (F)



Source: research data

The zero-order behavior observed in the present study is in agreement with previous investigations on thermally treated honeys. Turkut *et al*. (2018) heated pine, chestnut, and multifloral honey samples at 50 °C, 70 °C, and 80 °C for up to 48 h, also finding that HMF formation followed a zero-order reaction. Similarly, Chua *et al.* (2014) treated tropical honey samples at 90 °C for 60 min, observing a zero-order reaction for HMF formation. These consistent findings across different honey types and temperature ranges reinforce the suitability of zero-order modeling for describing HMF formation under thermal stress.

The calculated activation energy (E_a = 29.349 cal·mol^{-1}) and Q_{10} value (3.92) further confirm the strong influence of temperature on HMF formation. Using the Arrhenius model, the predicted shelf life of honey was 1924 d (5.3 years) at 15 °C, 802 d (2.2 years) at 20 °C, 344 d (0.94 years) at 25 °C, and 151 d (0.41 years) at 30 °C, emphasizing the critical role of storage temperature in preserving honey quality. These predictions provide practical insights into the combined effects of processing and storage conditions on honey stability.

Model validity was further supported by experimental storage data, as honey samples stored at room temperature for 13 months reached an HMF content of 67.10 mg·kg^{-1}, which closely matched the predicted shelf-life estimate of 12.6 months at 25 °C. This agreement between experimental and predicted values demonstrates the effectiveness of the Arrhenius-based approach for estimating honey shelf life. Similar applications of the Arrhenius equation have successfully predicted shelf life in other food products, including beef meatballs (Laksanawati *et al*., 2024), apple juice (Bai *et al*., 2022), and fresh-cut potatoes (Zhao *et al*., 2022), further supporting the reliability and broad applicability of this method.

3.4 Shelf life determination using ANNs

A dataset of 500 values was generated using the aforementioned kinetic models, varying storage temperatures between 15 °C and 65 °C. The ANNs were trained using the Holdback and K-fold methods with varying numbers of neurons in the hidden layer. The ANN performance during validation is summarized in Table 1.

Table 1 – Validation results of Artificial Neural Networks using Holdback and K-fold methods with different topologies

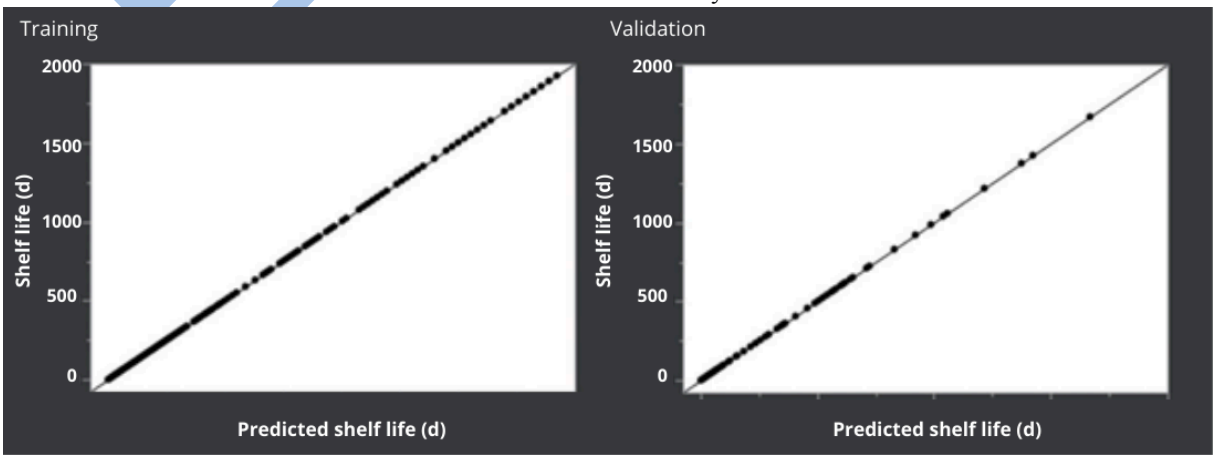
Neurons in Hidden Layer	Holdback		K-fold	
	R^2	RMSE	R^2	RMSE
2	0.956	70.809	0.999	0.267
4	0.999	0.059	1	0.018
6	0.999	2.162	0.999	1.539
8	0.999	1.073	0.999	0.285
10	1	1.07×10^{-5}	1	2.21×10^{-4}
12	1	0.013	1	0.009
14	0.999	0.0254	1	9.88×10^{-4}
16	1	0.001	1	7.45×10^{-4}
18	1	0.006	1	1.47×10^{-4}
20	1	2.28×10^{-4}	1	4.40×10^{-4}

R^2: R-squared; RMSE: root mean square error.
Source: research data

During ANN validation, the Holdback method with a 10-neuron hidden layer topology exhibited the best predictive performance, as indicated by the coefficient of determination, low root mean square error, and random error distribution. The close agreement between predicted and experimental shelf-life values, evidenced by their alignment with the 1:1 line, suggests that the network was able to effectively capture the temperature-dependent patterns governing HMF formation (Figure 8).

The high predictive accuracy observed is consistent with the ability of artificial neural networks to model complex and nonlinear relationships without requiring explicit mechanistic assumptions. Similar ANN-based approaches have been successfully applied to shelf-life prediction in different food matrices, including shrimp (Feng *et al*., 2024), passion fruit juice (Chutia *et al*., 2024), and potatoes (Khorramifar *et al*., 2023), reinforcing the robustness and versatility of this modeling strategy. In this context, the present results highlight the potential of ANN models as complementary tools to conventional kinetic approaches for improving shelf-life prediction and process control in honey.

Figure 8 – Observed vs predicted shelf life during training and validation with the Holdback method and 10 neurons in the hidden layer



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Despite the robustness of the proposed kinetic and predictive models, some limitations of this study should be acknowledged. Accelerated shelf-life tests rely on elevated temperatures to reduce experimental time, which may intensify degradation pathways that do not occur to the same extent under ambient storage conditions (Van Boekel, 2008). In addition, the physicochemical characteristics, thermal behavior, and shelf life of honey may vary depending on its botanical origin, geographical region, and centesimal composition (Bogdanov *et al*., 2008). Therefore, the extrapolation of the present results should be interpreted with caution, and future studies should evaluate different honey types and incorporate additional quality parameters to improve the generalizability of the model.

4 Conclusions

Honey color becomes less yellow, darker, redder, and more orange when heated, with higher temperatures causing more significant changes. Instrumental color analysis is a quick and cost-effective method for assessing HMF levels in honey. The shelf life of honey, in terms of HMF formation, varies with temperature. The use of ANNs to predict shelf life based on temperature shows promise, allowing better control over honey processing, such as liquefaction, pasteurization, and storage, to prevent HMF levels from exceeding regulatory limits, thus ensuring a safer and higher-quality product. Nevertheless, as accelerated shelf-life testing relies on elevated temperatures and honey properties vary with botanical origin and composition, the extrapolation of these results to different honey types and ambient storage conditions should be interpreted with caution.

Acknowledgements

The authors gratefully acknowledge FAPEMIG for funding this research (grant number: APQ-03935-22) and for supporting the scientific initiation scholarships. We also thank the Association of Meliponiculturists and Beekeepers of Médio Paraopeba for their technological contributions and the valuable exchange of knowledge.

Funding

Research funded by FAPEMIG (grant number: APQ-03935-22).

Conflict of interest

The authors declare no conflict of interest.

Author contributions

VIEIRA, D. A. F.: study conception and design; data analysis and/or interpretation. **DAMASCENO, A. E. M.:** study conception and design; data analysis and/or interpretation. **MENEZES, A. P. L.:** study conception and design; data analysis and/or interpretation. **SILVA, V. M.:** study conception and design; final critical and intellectual review of the manuscript. **TORRES FILHO, R. A. T.:** study conception and design; final critical and intellectual review of the manuscript. All authors contributed to the writing, discussion, review, and approval of the final version of the manuscript.

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