ReGeo ISSN: 2177-3246

QUALITY OF HONEY MARKETED IN UNAÍ/MG: A PHYSICOCHEMICAL APPROACH

QUALIDADE DE MÉIS COMERCIALIZADOS EM UNAÍ/MG: UMA ABORDAGEM FÍSICO-QUÍMICA

CALIDAD DE MIELES COMERCIALIZADAS EN UNAÍ/MG: UN ENFOQUE FISICOQUÍMICO

https://doi.org/10.56238/revgeov17n10-216

Data de submissão: 29/09/2026 **Data de publicação:** 29/10/2026

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ABSTRACT

Honey quality is an important food-safety concern, since adulteration with commercial sugar syrups, excessive heating, and inadequate storage can compromise its nutritional and therapeutic value. In this study, the quality of eight honey samples sold in the municipality of Unaí-MG was evaluated through the determination of pH, free, lactonic, and total acidity, all performed in triplicate, together with qualitative tests for hydroxymethylfurfural (HMF) – the Fiehe and Jagerschmidt reactions. The results showed that pH ranged from 3.85 to 4.69 and that all samples presented total acidity values below the maximum limit of 50 meq/kg established by MAPA's Normative Instruction No. 11/2000, indicating compliance with current Brazilian legislation. The Fiehe Reaction was positive in seven of the eight samples, while the Jagerschmidt Reaction was negative in all of them. These findings suggest the presence of moderate HMF levels, possibly associated with storage or heating of the product, with no conclusive evidence of adulteration. The results reinforce the relevance of continuous physicochemical monitoring of honey marketed to consumers and point to the need for complementary quantitative analyses to confirm HMF levels and further characterize product authenticity.

Keywords: Honey. Acidity. Quality. Hydroxymethylfurfural.

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RESUMO
A qualidade do mel é uma preocupação importante de segurança alimentar, uma vez que a adulteração com xaropes de açúcar comercial, aquecimento excessivo e armazenamento inadequado podem comprometer seu valor nutricional e terapêutico. Neste estudo, a qualidade de oito amostras de mel comercializadas no município de Unaí-MG foi avaliada através da determinação de pH, acidez livre, lactonada e total, todas realizadas em triplicata, juntamente com testes qualitativos para hidroximetilfurfural (HMF) – as reações de Fiehe e Jagerschmidt. Os resultados mostraram que o pH variou de 3,85 a 4,69 e que todas as amostras apresentaram valores de acidez total abaixo do limite máximo de 50 meq/kg estabelecido pela Instrução Normativa nº 11/2000 do MAPA, indicando conformidade com a legislação brasileira vigente. A Reação de Fiehe foi positiva em sete das oito amostras, enquanto a Reação de Jagerschmidt foi negativa em todas elas. Esses resultados sugerem a presença de níveis moderados de HMF, possivelmente associados ao armazenamento ou aquecimento do produto, sem evidências conclusivas de adulteração. Os resultados reforçam a relevância do monitoramento físico-químico contínuo do mel comercializado aos consumidores e apontam para a necessidade de análises quantitativas complementares para confirmar os níveis de HMF e caracterizar melhor a autenticidade do produto.

Palavras-chave: Mel. Acidez. Qualidade. Hidroximetilfurfural.

RESUMEN
La calidad de la miel es una preocupación importante de seguridad alimentaria, ya que la adulteración con jarabes de azúcar comercial, calentamiento excesivo y almacenamiento inadecuado pueden comprometer su valor nutricional y terapéutico. En este estudio, la calidad de ocho muestras de miel comercializadas en el municipio de Unaí-MG fue evaluada mediante la determinación de pH, acidez libre, lactonada y total, todas realizadas por triplicado, junto con pruebas cualitativas para hidroximetilfurfural (HMF) – las reacciones de Fiehe y Jagerschmidt. Los resultados mostraron que el pH osciló entre 3,85 y 4,69 y que todas las muestras presentaron valores de acidez total por debajo del límite máximo de 50 meq/kg establecido por la Instrucción Normativa nº 11/2000 del MAPA, indicando conformidad con la legislación brasileña vigente. La Reacción de Fiehe fue positiva en siete de las ocho muestras, mientras que la Reacción de Jagerschmidt fue negativa en todas ellas. Estos resultados sugieren la presencia de niveles moderados de HMF, posiblemente asociados con el almacenamiento o calentamiento del producto, sin evidencia concluyente de adulteración. Los resultados refuerzan la relevancia del monitoreo físico-químico continuo de la miel comercializada a los consumidores e indican la necesidad de análisis cuantitativos complementarios para confirmar los niveles de HMF y caracterizar mejor la autenticidad del producto.

Palabras clave: Miel. Acidez. Calidad. Hidroximetilfurfural.

1 INTRODUCTION

Common adulterations of honey involve the addition of commercial sugar syrups, such as cane sugar or corn syrup, aiming to increase volume and reduce production costs. Such practices not only compromise the nutritional and medicinal value of honey but also represent a risk to public health (Carvalho *et al.*, 2023). In addition to intentional adulteration, the quality of honey can be compromised by excessive heating, which causes the formation of hydroxymethylfurfural (HMF) and enzymatic inactivation, as well as by inadequate storage conditions, such as exposure to humidity and temperature variations (Silva *et al.*, 2022).

Based on this, obtaining physicochemical parameters of honey is essential to ensure the quality of this product on the market (Welke *et al.*, 2008). Its quality ensures consumer food safety, protecting against adulterated or contaminated products that may be harmful to health (Silva; De Marchi, 2010; Silva *et al.*, 2022). Quality can be altered based on physicochemical parameters of honey that are established by Brazilian legislation, taking into account factors such as moisture, acidity, mineral content, and HMF levels, among others (Brasil, 2000).

Among the physicochemical parameters used for honey quality control, acidity and hydroxymethylfurfural (HMF) content stand out, both provided for in MAPA's Normative Instruction No. 11/2000 (Brasil, 2000). Honey acidity results from the presence of natural organic acids and directly influences its microbiological stability and consumer acceptability, with values above the legal limit potentially indicating undesirable fermentation (Sousa, 2008). HMF, in turn, is a compound formed by sugar degradation under conditions of heat and acidity, and its presence in high concentrations may indicate overheating, prolonged storage, or the addition of commercial syrups to the product (Faleiros-Quevedo *et al.*, 2024). Thus, the determination of these parameters constitutes an indispensable tool for evaluating the authenticity and compliance of honey with current quality standards.

Given the above, this study aimed to evaluate the quality of eight honey samples marketed in the municipality of Unaí/MG, by determining total acidity and performing qualitative tests for hydroxymethylfurfural (HMF), in order to verify their compliance with the standards established by current Brazilian legislation.

2 THEORETICAL FRAMEWORK

Honey is a natural product made by bees from the nectar of flowers or from secretions of living parts of plants, which are collected, transformed, combined with substances specific

to the bees, stored, and left to mature in the combs of the hive (Silva; De Marchi, 2010; Sousa, 2008). From a chemical standpoint, honey is a supersaturated solution composed mainly of sugars (fructose and glucose), but it also harbors a wide range of minor compounds, such as amino acids, vitamins, minerals, lipids, enzymes (such as diastase and invertase), and various phytochemicals, including flavonoids and phenolic acids (Carvalho *et al.*, 2023). Its composition and chemical characteristics depend on the types of floral sources visited by bees, environmental factors, storage conditions, and species (Silva *et al.*, 2022).

Historically, honey transcends its value as a food, being recognized for its therapeutic and biological properties. It assists in the production of blood cells, the purification of the circulatory system, and offers protection against arteriosclerosis (Carvalho *et al.*, 2024). Such benefits are attributed to the presence of antioxidant compounds, such as organic acids, aromatic compounds, ascorbic acid, catalases, and carotenoids, which act against free radicals in the human body (Carvalho *et al.*, 2024). In addition to these properties, honey exhibits antimicrobial, anti-inflammatory, and wound-healing activity, characteristics widely explored both in traditional medicine and in contemporary scientific research (Welke *et al.*, 2008). Furthermore, the enzymatic activity present in fresh honey is one of the main indicators of its biological integrity, being sensitive to heat and to the aging of the product.

The antioxidant activity of honey is closely related to its content of phenolic compounds, which includes phenolic acids and flavonoids in proportions that vary according to the botanical and geographic origin of the product. These compounds act synergistically in neutralizing reactive oxygen species, contributing to cell protection and the prevention of chronic non-communicable diseases (Alvarez-Suarez *et al.*, 2010). The phytochemical diversity of honey, therefore, not only underlies its functional properties but also serves as an important marker of authenticity and floral origin, being used in characterization and quality control studies.

3 METHODOLOGY

Eight honey samples sold in different markets in the municipality of Unaí/MG were acquired during January. The samples were stored on shelves, at room temperature, without refrigeration and without direct exposure to heat sources. All analyses were carried out in triplicate, totaling 24 determinations, with the aim of ensuring greater precision and reproducibility of the results.

The analyses were conducted at the Chemistry Laboratory of the Institute of Agricultural Sciences of UFVJM, Unaí Campus. The determination of free, lactonic, and total acidity was carried out according to the analytical methods described in the Manual of Physicochemical Methods for Food Analysis of the Adolfo Lutz Institute (IAL, 2008).

For the determination of free acidity, 10 g of honey were weighed and subsequently dissolved in 75 mL of distilled water. Potentiometric titration was then performed with 0.05 N sodium hydroxide (NaOH) solution until pH 8.5 was reached. Lactonic acidity was determined by back-titration with 0.05 N hydrochloric acid (HCl) solution until pH 8.3, after titration with NaOH. Total acidity was obtained by summing the free acidity and lactonic acidity values.

The Fiehe Reaction was also performed according to IAL recommendations. For this, approximately 5 g of honey were weighed in a beaker, 5 mL of ethyl ether were added, and the mixture was stirred. The ether layer was then transferred to a test tube, 0.5 mL of hydrochloric resorcinol solution was added, and the mixture was left to stand for 10 minutes. The appearance of intense red coloration was considered a positive result.

The Jagerschmidt Reaction was conducted based on the same methodological recommendations. Approximately 10 g of honey were ground with 10 mL of acetone in a porcelain mortar. After the solvent settled, 3 mL of the liquid phase were transferred to a test tube containing an equal volume of concentrated HCl. The mixture was cooled under running water, and the appearance of red coloration was interpreted as a positive result.

Results were expressed as the mean and standard deviation of the triplicates. Compliance was assessed based on the limits established by MAPA's Normative Instruction No. 11/2000 (BRASIL, 2000), which defines a maximum limit of 50 meq/kg for total acidity and 60 mg/kg for HMF content in honey from tropical regions.

4 RESULTS AND DISCUSSION

The results obtained from the pH, free acidity, lactonic acidity, and total acidity analyses of the eight honey samples, performed in triplicate, are presented in Tables 1 and 2, which summarize the means and standard deviations per honey sample. According to Table 1, the pH values of the honey samples analyzed ranged from 3.85 to 4.69, with most samples showing pH in the range of 4.15 to 4.61. These results are consistent with the literature, which reports honey pH values generally between 3.5 and 4.5, characterizing it as a naturally acidic food. A similar result was observed by Carvalho *et al.* (2023) when analyzing honey marketed in Araguaína-TO, with pH values ranging from 3.60 to 4.43, a range

attributed to the typical composition of organic acids present in honey. Similarly, Welke *et al.* (2008) reported pH values between 3.72 and 4.64 in *Apis mellifera* honey from the northwest region of Rio Grande do Sul, corroborating the results obtained in the present study.

Table 1

Mean and Standard Deviation of Free Acidity Parameters by honey sample

Sample	pH (Mean)	pH (SD)	Free Acidity (meq/kg) (Mean)	Free Acidity (meq/kg) (SD)
1	4.61	0.02	29.50	0.87
2	4.69	0.02	23.00	0.50
3	3.92	0.02	24.17	0.58
4	4.43	0.11	34.83	1.76
5	4.17	0.02	26.50	0.50
6	4.15	0.03	26.17	1.04
7	4.20	0.03	29.50	0.50
8	3.85	0.01	18.67	1.04

SD = Standard Deviation; Mean = Mean.
Source: Prepared by the authors, 2026.

SD = Standard Deviation; Mean = Mean. Source: Prepared by the authors (2026).

Regarding total acidity (Table 2), the mean values observed ranged from 23.50 meq/kg to 42.67 meq/kg. Normative Instruction No. 11, of October 20, 2000, of the Ministry of Agriculture, Livestock, and Food Supply, establishes a maximum limit of 50 meq/kg for this parameter (BRASIL, 2000). Thus, all samples analyzed presented values below the permitted limit, demonstrating compliance with current Brazilian legislation. These results suggest that the honey evaluated presented satisfactory physicochemical quality regarding acidity, with no indication of pronounced fermentation processes or alterations capable of significantly compromising product quality. Furthermore, while examining honey commercialized in the same municipality of Unaí, Minas Gerais, Deckers *et al.* (2025) also reported adequate general parameters in compliant samples, contrasting with irregularities occasionally found in informal market products.

Table 2

Mean and Standard Deviation of Lactonic Acidity and Total Acidity Parameters by honey sample

Sample	Lactonic Acidity (meq/kg) (Mean)	Lactonic Acidity (meq/kg) (SD)	Total Acidity (meq/kg) (Mean)	Total Acidity (meq/kg) (SD)
1	5.33	1.26	34.83	1.61
2	4.83	1.53	27.83	1.26
3	6.33	1.04	30.50	0.50
4	7.83	1.89	42.67	0.58
5	6.50	0.00	33.00	0.50
6	8.50	0.50	34.67	1.53
7	9.67	0.76	39.17	1.04
8	4.83	1.26	23.50	2.29

SD = Standard Deviation; Mean = Mean.
Source: Prepared by the authors, 2026.

The results found are also similar to those reported by Silva *et al.* (2022), who evaluated honey produced in the Rio Bois Valley, in Goiás, and recorded total acidity between 16.50 and 46.30 meq/kg, all within the legal limit. Carvalho *et al.* (2023) also verified compliance with legislation in industrialized and artisanal honey marketed in Araguaína-TO, with total acidity values ranging from 19.40 to 43.00 meq/kg, close to those observed in the present study. Similarly, Deckers *et al.* (2025) evaluated honey samples commercialized formally and informally in Unaí, Minas Gerais, observing that compliant samples also maintained appropriate acidity parameters. According to Sousa (2008), total acidity values above the established limit may indicate the occurrence of fermentation, a process associated, among other factors, with high moisture content and yeast development, which can compromise the stability, shelf life, and acceptability of honey.

By analyzing Tables 1 and 2, it can be observed that free acidity ranged from 18.67 meq/kg to 34.83 meq/kg, while lactonic acidity presented values between 4.83 meq/kg and 9.67 meq/kg. The relationship between these two fractions contributes directly to total acidity and constitutes a relevant parameter for evaluating honey quality. Free acidity is mainly associated with the presence of organic acids in the product, while lactonic acidity corresponds to the fraction related to lactones, compounds that can be converted into acids during the analytical process. Welke *et al.* (2008) highlight that lactonic acidity, although generally present in a smaller proportion, is important for the stability of honey, since it contributes to the acid-base balance and the buffering effect of the product. In the present study, lactonic acidity values remained relatively low, in agreement with the results of

Carvalho *et al.* (2023) and Deckers *et al.* (2025), who observed consistent acidity parameters in commercial Brazilian honey.

The low standard deviations observed for most of the parameters analyzed indicate good reproducibility of the determinations performed in triplicate. This behavior suggests consistency in the analytical procedures adopted and low variability among the repetitions of each sample. Furthermore, the low dispersion of the data reinforces the reliability of the results obtained for the parameters of pH, free acidity, lactonic acidity, and total acidity.

The Fiehe Reaction is a qualitative test used to indicate the possible presence of hydroxymethylfurfural (HMF) in honey, a compound associated with aging, overheating, or the addition of invert sugar syrups. The reaction occurs through a colorimetric assay with hydrochloric resorcinol solution, with the appearance of red coloration indicating a positive result (FALEIROS-QUEVEDO *et al.*, 2024).

In the present study, samples 1, 3, 4, and 6 showed positive coloration for the Fiehe Reaction (Figure 1). Similar results were observed in studies by Deckers *et al.* (2025). Among them, samples 4 and 6 (Figure 1) stood out for the greater intensity of coloration, suggesting a higher relative presence of compounds associated with HMF formation. Samples 2, 5, and 7 also showed coloration, but with lower intensity, indicating a less pronounced positive reaction. On the other hand, sample 8 (Figure 1) showed no characteristic coloration, being the only sample with a negative result for this test, as observed in Figure 1.

Figure 1

Samples 4, 5, and 8 subjected to the Fiehe Reaction



Source: Prepared by the authors, 2026.

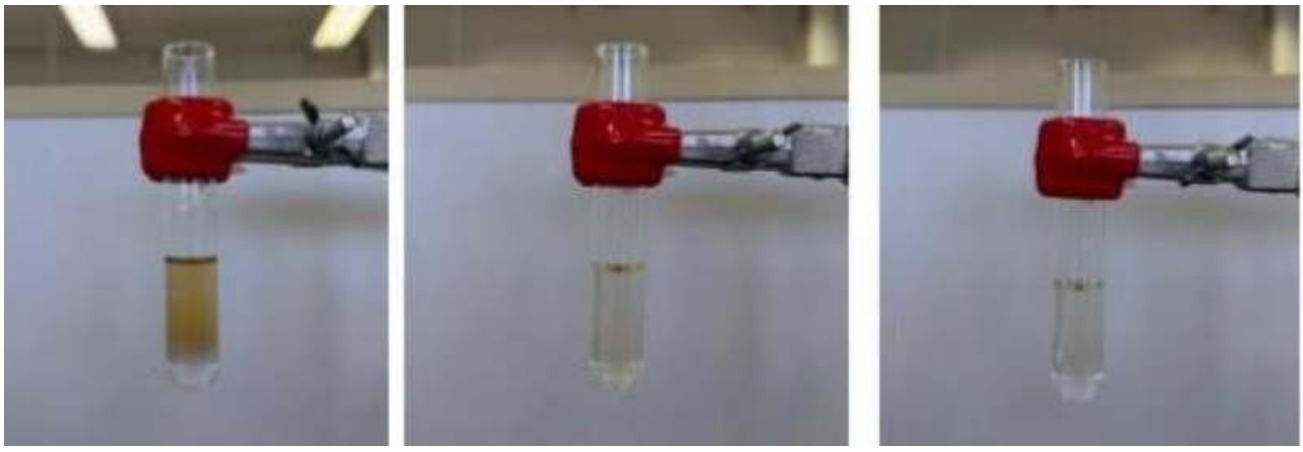
The presence of red coloration, albeit at different intensities, in most of the samples analyzed suggests that part of the honey marketed in Unaí/MG may have been subjected to storage or processing conditions capable of favoring HMF formation. Among these conditions, exposure to heat, prolonged storage, or inadequate thermal processing stand out. However, since it is a qualitative test, the Fiehe Reaction does not, by itself, allow quantification of HMF content or confirmation of adulteration. Thus, positive results should be interpreted as indicative of the possible presence of this compound, requiring complementary quantitative methods for confirmation.

The Jagerschmidt Reaction was performed as a complementary qualitative test, with the aim of verifying the presence of HMF or evidence of adulteration by commercial sugars. A positive result is characterized by the appearance of intense red or violet coloration at the reaction interface, as described in laboratory protocols applied to honey analysis (UFPR, [n.d.]).

All eight samples analyzed showed negative results in the Jagerschmidt Reaction. The colorations observed ranged from light yellow to clear amber, with no formation of a reddish ring or violet coloration characteristic of a positive result. Thus, the data obtained by this method did not indicate the presence of significant HMF levels or qualitative evidence of adulteration by commercial sugar, as shown in Figure 2.

Figure 2

Samples 2, 5, and 7 subjected to the Jagerschmidt Reaction



Source: Prepared by the authors, 2026.

The divergence between the results of the Fiehe Reaction, positive in seven of the eight samples, and the Jagerschmidt Reaction, negative in all samples, suggests that HMF may be present at levels detectable by the first test, but without evidence of sufficient intensity

to produce a positive response in the second method. This interpretation indicates that the honey analyzed may have undergone some degree of aging, heating, or exposure to inadequate storage conditions, but does not show consistent qualitative evidence of gross adulteration through the addition of commercial sugars.

Similar results were described by Carvalho *et al.* (2023), who, when analyzing commercial honey in Araguaína-TO, observed positivity in the Fiehe Reaction in some samples, associating this behavior mainly with storage and processing conditions, and not necessarily with intentional adulteration. Silva *et al.* (2022) also reported that honey subjected to high temperatures during storage may show a positive Fiehe Reaction without, however, showing evidence of fraud by commercial sugars in complementary tests. Similarly, Welke *et al.* (2008) emphasize that the joint interpretation of qualitative tests is essential to avoid premature conclusions, since each method has its own sensitivity and specificity.

Therefore, the results obtained indicate that most samples showed a positive reaction for Fiehe, suggesting possible HMF formation to some degree. However, the negativity of the Jagerschmidt Reaction in all samples reduces the possibility of evident adulteration by commercial sugars. It is recommended, however, that future studies include the quantification of HMF by instrumental methods, such as spectrophotometry or high-performance liquid chromatography, in order to confirm the qualitative results and more precisely establish the freshness and quality condition of the honey evaluated.

5 CONCLUSION

It is concluded that the eight honey samples analyzed presented satisfactory quality regarding the acidity parameters, since all total acidity values remained below the maximum limit of 50 meq/kg established by Brazilian legislation. The variations observed in pH, free acidity, and lactonic acidity may be related to floral origin, processing, and storage conditions of the honey.

The positive Fiehe Reaction in most samples suggests a possible presence of HMF to some degree, indicating possible aging, heating, or inadequate storage. However, the Jagerschmidt Reaction, negative in all samples, did not show elevated HMF levels or consistent evidence of adulteration by commercial sugars. Thus, although the honey evaluated is in compliance regarding acidity, additional quantitative analyses are recommended for a better characterization of its quality and authenticity.

ACKNOWLEDGMENTS

The authors thank the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) for granting a scholarship linked to PIBIC/FAPEMIG (Brazil). We also acknowledge the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for granting a scholarship through PIBIC-EM/CNPq (Brazil).

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