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Propolis extracts in Juiz de Fora, Minas Gerais: Unveiling quality and identity

Extratos de própolis em Juiz de Fora, Minas Gerais: Revelando qualidade e identidade

Extractos de propóleo en Juiz de Fora, Minas Gerais: Revelando calidad e identidad

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Abstract
This research aims to analyze the quality of propolis extracts sold in Juiz de Fora, Minas Gerais, verifying the compliance of these products with the standards established in Normative Instruction N° 3, of January 19, 2001, of the Ministry of Agriculture, Livestock and Food Supply (MAPA). Physicochemical parameters were analyzed, including alcohol content, dry extract, phenolic compounds, flavonoids, wax content and antioxidant activity. The results revealed that two samples presented dry extract below the regulatory limit, while three demonstrated low antioxidant activity, indicating a lower concentration of bioactive compounds. Despite the adequacy of the alcohol and wax contents, the variation in the chemical composition reinforces the influence of factors such as botanical origin and processing on the final quality of the product. These findings highlight the need for more rigorous quality control, ensuring the standardization and safety of the extracts marketed.
Keywords: Quality; Bioactive Compounds; Antioxidants; Standardization; Quality Control.

Resumo
Esta pesquisa tem como objetivo analisar a qualidade dos extratos de própolis comercializados em Juiz de Fora, Minas Gerais, verificando a conformidade desses produtos com os padrões estabelecidos na Instrução Normativa nº 3, de 19 de janeiro de 2001, do Ministério da Agricultura, Pecuária e Abastecimento (MAPA). Foram analisados parâmetros físico-químicos, incluindo teor alcoólico, extrato seco, compostos fenólicos, flavonoides, teor de cera e atividade antioxidante. Os resultados revelaram que duas amostras apresentaram extrato seco abaixo do limite regulamentar, enquanto três demonstraram baixa atividade antioxidante, indicando menor concentração de compostos bioativos. Apesar da adequação dos teores alcoólicos e de cera, a variação na composição química reforça a influência de fatores como origem botânica e processamento na qualidade final do produto. Esses achados evidenciam a necessidade de um controle de qualidade mais rigoroso, garantindo a padronização e segurança dos extratos comercializados.
Palavras-chave: Qualidade; Compostos Bioativos; Antioxidantes; Padronização; Controle de Qualidade.

Resumen
Esta investigación tiene como objetivo analizar la calidad de los extractos de propóleos comercializados en Juiz de Fora, Minas Gerais, verificando la conformidad de estos productos con las normas establecidas en la Instrucción Normativa N° 3, de 19 de enero de 2001, del Ministerio de Agricultura, Ganadería y Abastecimiento (MAPA). Se analizaron parámetros fisicoquímicos, incluyendo contenido de alcohol, extracto seco, compuestos fenólicos, flavonoides, contenido de ceras y actividad antioxidante. Los resultados revelaron que dos muestras presentaron extracto seco por debajo del límite regulatorio, mientras que tres demostraron baja actividad antioxidante, lo que indica una menor concentración de compuestos bioactivos. A pesar de la idoneidad de los contenidos de alcohol y cera, la variación en la composición química refuerza la influencia de factores como el origen botánico y el procesamiento en la calidad final del producto. Estos hallazgos resaltan la necesidad de un control de calidad más riguroso, que garantice la estandarización y seguridad de los extractos comercializados.
Palabras clave: Calidad; Compuestos Bioactivos; Antioxidantes; Estandarización; Control de Calidad.

1. Introduction

Propolis is a resinous substance produced by bees that mix wax with plant exudates collected mainly from leaves, floral buds, and stems of plants and trees (Barrientos-Lezcano et al., 2023; Silva, Carvalho, and Mendes, 2023). The word propolis is

derived from the Greek *pro* (defense) and *polis* (city), meaning defense of the hive (Laaroussi et al., 2021).

Propolis must be purified through solvent extraction due to its low solubility in water and high content of contaminants, mainly resins, waxes, and other substances. The extraction of propolis extracts can be performed using various methods, each suitable for different needs (Mello and Hubinger, 2012). Among the most common are maceration, which uses solvents such as ethanol, glycerol, and/or water; it is simple and economical but less efficient for certain compounds. Organic solvent extraction offers good efficacy in obtaining liposoluble substances. The choice of the ideal method depends on the types of desired compounds, the intended use of the extract, and the available resources, aiming to balance efficiency, cost, and quality (Pobiega, Kraśniewska, and Gniewosz, 2019; Bankova, Trusheva, and Popova, 2021).

Therefore, propolis extract is the product obtained by extracting the soluble components of propolis in neutral (food-grade) alcohol through an appropriate technological process (Brasil, 2001). The variety of brands available on the market can raise doubts about the authenticity and quality of propolis extracts. Therefore, this research aims to analyze the quality of propolis extracts sold in Juiz de Fora, Minas Gerais, verifying the compliance of these products with the standards established in Normative Instruction N°. 3, of January 19, 2001, of the Ministry of Agriculture, Livestock and Food Supply (MAPA).

2. Methodology

This study is characterized as an experimental, lab research of a quantitative nature (Pereira et al., 2020) and that used descriptive statistics with mean values and standard deviation (Shitsuka et al., 2014), and that was based on the acquisition of experimental data on the physicochemical parameters of propolis extracts. The methodological design was structured to ensure the reproducibility and reliability of the results, aligning with the criteria established by Normative Instruction N° 3, of January 19, 2001, of the Ministry of Agriculture, Livestock, and Supply (Brasil, 2001).

Five samples of alcoholic propolis extracts were analyzed, purchased from local commerce in Juiz de Fora - MG. Each sample was processed in three independent repetitions, totaling 15 individual analyses.

The analyzed parameters included phenolic compounds (method 3.6), flavonoid compounds (method 3.8), dry extract (method 3.14), and alcohol content (method 3.19) according to official methods for analyzing animal origin products (Brasil, 2022). Lead acetate and sodium hydroxide tests were conducted following Kawakita (2015).

The wax content was evaluated using the methodology of Barreto et al. (2020) with adaptations: 10 g of each sample was maintained at a temperature of 4ºC for 24 hours. After this period, each sample was placed in a beaker and filtered through pre-dried filter paper in an oven at 105°C for 2 hours. The beaker and filter paper were then washed three times with 5 mL of refrigerated ethyl alcohol. After filtration, the filter paper was dried at room temperature for 12 hours and then placed in an oven at 50ºC for 15 minutes. After removal from the oven, the filter paper was placed in a desiccator and weighed, repeating the process until a constant weight was obtained.

Oxidation activity was evaluated using the methodology of Barreto et al. (2020) with adaptations: a 100 mL beaker was used to prepare a solution with 4 mL of the sample and 46 mL of distilled water. In a test tube, 0,5 mL of this solution, 0,5 mL of distilled water, and 1 mL of 20% sulfuric acid were added. After mixing and cooling, 5 µL of 0,1 N potassium permanganate was added. The time required for the disappearance of the red color against a white background was recorded.

Quantitative results were subjected to mean and standard deviation calculations using the R statistical program (R Development Core Team, 2021).

3. Results and Discussion

The results of the physicochemical analyses of the propolis extract samples acquired in Juiz de Fora - MG are presented

in Table 1. The dry extract content varied between 10,7% and 12,6%, with two samples (C and D) falling below the minimum required limit (≥ 11%). Wieczorek et al. (2022), Campoccia et al. (2021), Ferreira, Machado, and Chagas (2023), and Salatino et al. (2011) indicate that propolis composition varies according to collection region, phenological variation of the main plant resin sources, and genetic differences among bee species, directly influencing its physicochemical parameters.

Table 1 - Physicochemical parameters of the propolis extracts analyzed and the parameters established by legislation.

Parameters	**Samples**					**BRASIL (2001)**
	A	**B**	**C**	**D**	**E**	
Dry extract (m%v)	11,1±0,1	11,0±0,1	10,7±0,1	10,8±0,2	12,6±0,2	Minimum of 11%
Alcohol content G (v/v)	55,3±0,3	52,5±0,2	54,4±0,4	53,4±0,4	53,5±0,3	Maximum of 70° GL
Wax (m/m)	0,87±0,04	0,87±0,07	0,71±0,09	0,64±0,05	0,86±0,05	Maximum 1% of the dry extract
Flavonoid compounds (m/m)	0,32±0,06	0,26±0,02	0,22±0,03	0,20±0,01	0,47±0,05	Minimum of 0,25%
Phenolic compounds (m/m)	0,69±0,08	0,46±0,04	0,38±0,04	0,41±0,02	0,91±0,05	Minimum of 0,50%
Oxidation activity (seg.)	9±1	25±2	27±2	25±2	11±1	Maximum of 22 seg.
Sodium Hydroxide	Positive	Positive	Positive	Positive	Positive	Positive
Lead Acetate	Positive	Positive	Positive	Positive	Positive	Positive

Mean ± standard deviation of three determinations. Source: Authors (2025).

The alcohol content ranged from 52,5° to 55,3° GL, below the maximum limit of 70° GL. The ethanol concentration used in extraction affects the efficiency of bioactive compound recovery, with moderate alcohol concentrations favoring extraction without compromising extract stability (Ma et al., 2016; Sun et al., 2015; Bankova, Trusheva, and Popova, 2021).

Wax content remained below 1,0%, indicating proper processing. According to current legislation, the maximum wax limit in propolis extract is 1,0% of the dry extract value. High wax content may interfere with extract quality, affecting its physicochemical and biological properties, and can result from improper handling during collection or extraction failures (Buitrago et al., 2024; Aboulghazi et al., 2022).

Regarding bioactive compounds, flavonoid levels ranged from 0,20% to 0,47%, and phenolic compounds from 0,38% to 0,91%. Samples C and D had values below the minimum recommended limits (≥ 0,25% and ≥ 0,50%, respectively), while sample B was below the minimum only for phenolic compounds. Salgueiro and Castro (2016) found average results for flavonoid and phenolic compounds of 0,50% and 0,89% for green propolis extracts. Bioactive compound variations are linked to the botanical origins of propolis, geographic origins, environmental conditions, and seasonal variations (López et al., 2014; Gargouri et al., 2019).

Antioxidant activity testing showed oxidation times between 9 and 27 seconds, with three samples (B, C, and D) exceeding the recommended maximum limit of 22 seconds. Antioxidant capacity is associated with phenolic and flavonoid compounds, whose concentrations vary with propolis's geographic and botanical origins (Wieczorek et al., 2022; Campoccia et al., 2021).

Antioxidant activity is a parameter frequently used to suggest the time elapsed since propolis harvest, the type of storage and the antioxidant activity of the sample (Melo et al., 2012). This was demonstrated in samples B, C and D, which presented low levels of bioactive compounds and consequently longer oxidation times, respectively, 25, 27 and 25 seconds.

Qualitative tests with lead acetate and sodium hydroxide showed positive results for all samples, indicating compliance with current legislation. The sodium hydroxide analysis verifies the homogeneity of the propolis extract particles in a base and the lead acetate analysis verifies the homogeneity of the propolis extract particles in a salt (Lima et al., 2020).

4. Final Considerations

The results showed variations among the analyzed samples, particularly in dry extract content, bioactive compounds, and antioxidant activity. Two samples had dry extract levels below the required minimum, while three exceeded the recommended oxidation time, suggesting differences in stability and antioxidant potential.

Future research should focus on expanding the sample size to assess a broader range of commercial extracts and investigate the influence of different extraction methods on the chemical profile of propolis. Additionally, studies correlating bioactive compound levels with antioxidant activity and biological efficacy could provide a more comprehensive evaluation of product quality and functional potential. The development of standardized analytical techniques for quality control could also contribute to greater regulatory compliance and consumer confidence in propolis-based products.

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