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Isotopic Characterization of Cumaru Essential Oil (Dipteryx odorata) for Quality and Authenticity Validation

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Abstract: Cumaru oil (Dipteryx odorata) has chemical properties that make it a sought-after input in the cosmetics, perfumery, alternative medicine and food industries. The appreciation of these essential oils, as well as other native plants, not only reinforces Brazil's position as a leader in the production and export of natural products, but also strengthens its identity on the international scene, promoting sustainability and environmental preservation. For the analysis of carbon-13 ($\delta^{13}C$), oxygen-18 ($\delta^{18}O$) and deuterium (δD) of the essential oil extracted from Cumaru seeds, isotope ratio mass spectrometry (IRMS) was used to determine the authenticity and quality of Cumaru oil. The results obtained allowed validating the methodology used both in the analysis and in the extraction of the essential oil, which used solvents such as ethanol and ethyl acetate, and demonstrated its ability to distinguish genuine products from fraud.

Keywords: Essential oils; Cumaru; Isotopes; IRMS

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Caracterização isotópica do Óleo Essencial de Cumaru (Dipteryx odorata) para validação da autenticidade e qualidade

Resumo: O óleo de cumaru (*Dipteryx odorata*) possui propriedades químicas que o torna insumo desejado nas indústrias de cosméticos, perfumaria, medicina alternativa e alimentos. A valorização desses óleos essenciais, assim como de outras plantas nativas, não apenas reforça a posição do Brasil como líder na produção e exportação de produtos naturais, mas também fortalece sua identidade no cenário internacional, promovendo sustentabilidade e preservação ambiental. Para as análises de carbono-13 ($\delta^{13}C$), oxigênio-18 ($\delta^{18}O$) e deutério (δD) do óleo essencial extraído de sementes de cumaru (*Dipteryx odorata*), foi utilizado espectrometria de massa de razão isotópica (IRMS) com o objetivo de determinar autenticidade e qualidade de óleo de Cumaru. Os resultados obtidos permitiram validar a metodologia empregada tanto na análise como na extração do óleo essencial, que utilizou solventes como etanol e acetato de etila, e demonstraram sua capacidade em distinguir produtos genuínos de fraudes.

Palavras-chave: Óleos essenciais; Cumaru; Isótopos; IRMS

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1. INTRODUCTION

Cumaru, known as "tonka bean", is a seed that comes from trees of the Dipteryx genus, widely found in the Amazon and other tropical regions of South America. In Brazil, Cumaru has great economic and cultural importance due to its use in perfumery, cosmetics, food and even in traditional medicine. Its seeds contain coumarin, a chemical compound responsible for the sweet and striking smell reminiscent of vanilla and almonds, highly valued in the international market. Cumaru essential oil, extracted from these seeds, has aromatic and bioactive properties that make this product very interesting, both for industries and for scientific studies. The growing demand for natural and high-quality products places Brazil in a strategic position as a supplier of this resource, but it also brings the challenge of guaranteeing the authenticity and quality of the essential oil, especially given the risk of adulteration and falsification [1; 2].

In this context, isotopic analysis emerges as an essential tool for the characterization and authentication of Cumaru essential oil. This technique is based on the study of the proportions of stable isotopes of carbon-13 (δ13C), oxygen-18 (δ18O) and deuterium (δ2H) present in the chemical compounds of this oils. Stable isotopes do not undergo radioactive decay and are present in natural proportions in the biosphere, varying according to environmental factors such as climate, soil type, altitude and even the metabolic processes of plants. Thus, the isotopic profile of a sample can be used to identify its geographic origin, purity and the extraction methods used. [3].

Applying isotopic analysis to Cumaru seeds is particularly relevant because these seeds are the raw material for essential oil production. The proportions of stable isotopes in the chemical compounds of the seeds reflect the specific environmental and geographic conditions of the region where the plant was grown, allowing the differentiation between seed lots of different origins or between genuine and adulterated samples. In the case of

Cumaru, isotopic analysis can help determine whether the essential oil was extracted from pure seeds or whether it was adulterated with oils of lower commercial value or synthetic compounds. In addition, isotopic values can be used to assess the quality of the essential oil, since specific aromatic compounds, such as coumarin, have distinct isotopic signatures that can be monitored to identify variations in the production process or adulteration [4].

The analysis of δ13C in the compounds of Cumaru essential oil, for example, can provide information about the origin of carbon in coumarin and other aromatic compounds, revealing variations between natural and synthetic sources. The analysis of δ18O and δ2H is useful for investigating the environmental conditions to which the plants were exposed, such as water availability and the climatic characteristics of the growing region. These parameters are particularly important for establishing geographical traceability and ensuring that the essential oil meets the authenticity standards required by the international market.

The use of isotopic analysis adds value to the product as it allows the development of reference standards that guarantee the quality and authenticity of an authentic, high-quality product.

2. MATERIALS AND METHODS

The essential oil samples extracted from Cumaru (Tonka Bean) seeds were obtained from a dealer in Belém, Pará state, Brazil (Figure 1). The preparation of these seed samples began with the grinding of the seeds using a blade mill to reduce the particle size. The objective of this step was to increase the contact surface, facilitating the efficiency of the extraction of chemical compounds during the next steps of the process.

Figure 1: Cumaru Seeds - Dipteryx odorata [1]

The extraction process was performed using the Soxhlet method, where approximately 20 g of crushed seeds were subjected to extraction cycles for 24 hours. The choice of solvents followed European regulatory guidelines, using a mixture of solvents to maximize the extraction. The main solvent used was cyclohexane, a nonpolar solvent, initially applied pure for extraction. Then, mixtures of cyclohexane (80%) with other solvents (20%) were performed, including: Ethyl Acetate and Ethanol.

After oil extraction, isotope analysis was performed by Isotope Ratio Mass Spectrometry (EA-IRMS), which allowed the investigation of oxygen-18 ($\delta^{18}O$), deuterium (δD) and carbon-13 ($\delta^{13}C$) isotopes, chosen for their relevance in the chemical characterization of natural compounds and in verifying the authenticity of the product.

The reference samples used for comparison was seeds samples from Brazil, validated and stored in the Institut des Sciences Analytiques (ISA) of the Université Claude Bernard Lyon 1. The isotopic analyses were performed using a Flash HT elemental analyzer coupled to a Delta V Advantage mass spectrometer (Thermo Fisher, Bremen, Germany) through a Conflo V interface. The equipment was located at ISA in Lyon (France).

The analytical conditions followed a rigorous methodology to ensure accuracy and reliability in isotope ratio mass spectrometry (IRMS) analysis. For each analysis, approximately 200 µg were used. To guarantee consistency, each sample was analyzed in duplicate for 13C and triplicate for 2H e 18O, with verification conducted every six analyses.

High-purity helium (99.999%, Messer) was used as the carrier gas throughout the process. The combustion chamber was maintained at a temperature of 950°C, with an oxygen pulse ensuring the complete oxidation of carbon. Subsequently, the gas mixture passed through a secondary reduction chamber held at 650°C, where excess oxygen was removed.

For calibration and verification, international reference standards were utilized, including IAEA CH7 (polyethylene film, δ13C -32.15‰, δ2H -100‰), USGS40 (L-glutamic acid, δ13C -26.39‰), and IAEA-601 (benzoic acid, δ18O +23.14‰). These standards ensured the stability and precision of the instrument.

The isotopic ratios were expressed in δ notation relative to internationally recognized references: Vienna Pee Dee Belemnite (VPDB) for δ13C, and Standard Mean Ocean Water (SMOW) for δ18O and δ2H. The analytical precision was estimated at ±0.2‰ for δ13C, ±5‰ for δ2H, and ±0.6‰ for δ18O, reinforcing the robustness of the measurements.

Although there are no other analyses for direct comparison, the results obtained provide important preliminary data: $\delta^{13}C$ may indicate the presence of synthetic coumarin, which presents a distinct isotopic profile, while the values of $\delta^{18}O$ and δD help to infer the environmental cultivation conditions. With the analyzed sample, it was possible to obtain relevant information to validate the origin and purity of Cumaru essential oil, establishing a basis for future investigations and quality control.

Isotope Ratio Mass Spectrometry is a precise analytical technique used to determine the relative abundance of isotopes in organic and inorganic materials. In the context of food and natural products, such as essential oils, IRMS allowed the detection of small variations in the isotopic ratio of the analyzed elements: carbon (13C/12C), hydrogen (2H/1H) and oxygen

(18O/16O), which show intrinsic characteristics such as production method and geographic origin. This technique is particularly effective in identifying fraud or adulteration, since isotopic elements exhibit unique patterns based on environmental and biological factors.

The procedure involves converting samples into simple gases, followed by ionization and analysis in spectrometers designed to detect isotopic variations with high precision. The approach has proven effective in identifying natural origins and regional differences, being essential in quality control and product traceability [5].

3. RESULTS AND DISCUSSIONS

The isotopic data obtained (Table 1) provide essential information about the essential oil of Cumaru extracted from the seeds, allowing a detailed evaluation of its chemical composition, authenticity and quality. The samples identified as "Cristal-1" are reference samples, providing a consistent point of comparison for analyzing the other samples. By observing the values of carbon-13 (δ13C), oxygen-18 (δ18O) and deuterium (δ2H), it is possible to understand how the stable isotopes represent the intrinsic characteristics of the essential oil and the effects of the extraction methods employed.

Table 1: Cumaru seed isotope results

Samples	δ13C (‰)					
	Date of analysis	**Measure 1**	**Measure 2**	**Average**	**Standard Deviation**	
Cumarine Cristal-1 ETOH	16/11/2023	-33.41	-33.59	-33.50	0.13	
Cumarine Cristal-1 AE	16/11/2023	-33.74	-33.50	-33.62	0.17	
Cumarine ETOH	16/11/2023	-33.54	-33.42	-33.48	0.08	
Cumarine AE	16/11/2023	-33.47	-33.50	-33.49	0.02	
Samples	**δ2H (‰)**					
	Date of analysis	**Measure 1**	**Measure 2**	**Measure 3**	**Average**	**Standard Deviation**
Cumarine Cristal-1 ETOH	01/12/2023	-115	-114	-116	-115	1
Cumarine Cristal-1 AE	01/12/2023	-112	-112	-113	-112	1
Cumarine ETOH	01/12/2023	-113	-114	-113	-113	1
Cumarine AE	01/12/2023	-114	-116	-116	-115	1

Samples	δ18O (‰)					
	Date of analysis	**Measure 1**	**Measure 2**	**Measure 3**	**Average**	**Standard Deviation**
Cumarine Cristal-1 ETOH	01/12/2023	21.8	21.9	22.0	21.9	0.1
Cumarine Cristal-1 AE	01/12/2023	21.8	21.8	21.6	21.7	0.1
Cumarine ETOH	01/12/2023	22.3	22.2	22.2	22.2	0.1
Cumarine AE	01/12/2023	22.1	22.1	22.1	22.1	0.0

The δ13C values, which range from -33.48‰ to -33.62‰, demonstrate high consistency between samples. The small variation observed (approximately 0.4%) indicates that the isotopic carbon present in coumarin, the main component of tonka bean essential oil, is characteristic of a natural source and is in line with the 'crystal' reference sample. This stability in δ13C values reinforces the authenticity of the oil, since adulteration or contamination with synthetic sources or other compounds would result in greater deviations.

In the case of δ18O, the average values ranged from 21.7‰ to 22.2‰, showing the isotopic signature of oxygen present in the essential oil. The stability of the standard deviations indicates accurate measurements. The "crystal" references show measurements that are maintained in the oil samples, suggesting that the extraction process preserves the natural compounds enriched in oxygen-18. The δ18O values are also related to environmental factors, such as the water and climate conditions of the region where the seeds were grown, highlighting the role of this isotope in geographic traceability and characterization of origin, since the samples in the present study originate from Brazil, as does the reference.

The δ2H values, in turn, show average variations of -112‰ to -115‰ (approximately 2.7% variation). Although they show greater variation than the other isotopes, these values still remain within a statistically consistent range. Variations of less than 5% are in line with expectations for natural samples and do not necessarily indicate adulteration or contamination, in addition to being frequently interpreted as indicative of stability or consistency. The fact that the values of the analyzed samples agree with the "crystal" references reinforces the integrity of the material.

Deuterium is associated with the isotopic signature of hydrogens present in the context where Cumaru is found, in this case, influenced by both the climatic conditions of the region, such as precipitation regime, and the method of oil extraction. In addition, the isotopic composition of plants is influenced by factors such as soil type, which can interfere with water availability, for example. Thus, plants grown in regions with greater humidity tend to have more negative δ2H values due to the incorporation of lighter hydrogen isotopes from water. In this study, the δ2H values (-112‰ to -115‰) suggest that the seeds were probably grown in a tropical climate with consistent rainfall, which is typical of the Amazon region where Cumaru is native.

By integrating the three isotopic parameters, δ13C, δ18O and δ2H, an agreement is observed between the analyzed samples and the reference samples. This consistency indicates that the Cumaru essential oil extracted from the seeds shows the natural characteristics of the plant and does not show signs of adulteration. The combination of isotopic values provides a detailed view of the authenticity and quality of the oil, allowing such analysis to be performed on commercialized oils, with the establishment of rigorous criteria for their validation and traceability.

Finally, although the differences between extractions performed with ethanol and ethyl acetate are small and statistically acceptable (<5%), they are noticeable, particularly in the δ18O and δ2H values. The ethanol extracts present slightly higher δ18O values, as well as more negative values for δ2H compared to those obtained with ethyl acetate. This can be attributed to the polarity of ethanol, which influences the extraction of specific compounds, enriching the oil with molecules that carry these isotopic signatures. These differences highlight the importance of carefully choosing the extraction method, especially when isotopic analysis is used to assess the authenticity and quality of the essential oil.

To assess the consistency of the results, they were compared with data obtained in the article entitled "Authentication of Tonka beans extracts (Dipteryx odorata) using LC-UV/MS, GC-MS and multi-element (13C, 2H and 18O) bulk specific isotope analysis" by CUCHET et al., who used isotopic analyses of carbon-13, oxygen-18 and deuterium to investigate the authenticity and quality of tonka bean extracts obtained by different extraction methods. The comparison between the results allows to assess the consistency of the isotopic methods, identify patterns of variation and reinforce the importance of these analyses for the validation of natural essential oils. Table 2 presents a side-by-side comparison of the isotopic values obtained in this study with those reported by Cuchet et al. (2024).

Table 2: Comparison of isotopic results between this study and Cuchet et al. (2024)

Parameter	This Study	Cuchet et al. (2024)	Comments
δ13C (‰)	-33.62 to -33.48	-32.72 and -32.94	Slightly more negative values in this study, likely due to regional growing conditions.
δ18O (‰)	21.7 to 22.2	25.1 and 25.7	Lower δ18O values in this study, possibly due to differences in extraction methods or environmental factors.
δ2H (‰)	-115 to -112	-100 and -104	More negative proposed values in this study, probably due to variations in regional growing conditions.

Comparing the data and considering the same solvents used, it is possible to observe relevant similarities and differences. Although the δ13C values are slightly more negative in the present study, they remain very close with statistically acceptable variations. The δ18O values show a more significant difference, which can be attributed to variations in environmental conditions and extraction methods. The δ2H values are consistent with those reported by Cuchet et al. (2024), further confirming the origin of the samples.

The present study emphasizes that the solvents used for extraction do not significantly affect the isotopic values, since the variations in these values are less than 5%. Both ethanol and ethyl acetate resulted in similar isotopic signatures, although with small differences. The study by Cuchet et al. also did not show large variations considering these two solvents, while other solvents showed greater variations. The convergence between studies validates the use of isotopic analysis as a robust approach for the characterization and quality control of Cumaru essential oils, provided that the oil extraction methods are well defined.

4. CONCLUSIONS

Isotopic analysis of Cumaru essential oil has proven to be a robust and effective tool for ensuring the authenticity, quality and geographical traceability of the product. The results obtained for the δ13C, δ18O and δ2H isotopes demonstrated consistency with reference standards and reinforced the natural origin of the samples analyzed, evidencing the absence of significant adulteration. Furthermore, the small variations related to the extraction methods, mainly with the use of ethanol, highlighted the importance of optimizing the processes to preserve the characteristic isotopic signatures of the essential oil.

These results not only contribute to the quality control and valorization of Cumaru oil in the global market, but also establish a solid foundation for future studies exploring the application of isotopic techniques in natural products. The convergence of the isotopic data obtained with those of studies already carried out shows the relevance of this type of analysis for the differentiation of genuine products and the detection of fraud, consolidating Brazil's role as a strategic supplier of authentic and high-quality essential oils.

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