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Extração, quantificação e detecção da cafeína em alimentos como estratégias de ensino

Extraction, quantification and detection of caffeine in foods as teaching strategies

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ABSTRACT

This study aims to determine the caffeine content in different food samples through liquid-liquid extraction, which can be carried out in laboratory practices in Science, Biology, Chemistry, Pharmacy classes and related areas. Caffeine, an alkaloid present in coffee beans, was chosen due to its relevance in global consumer culture. The methodology involves extracting caffeine from green coffee, roasted coffee, cocoa powder and energy drinks. The results indicate that the extraction was effective, with varying concentrations of caffeine in the samples analyzed. Roasted coffee had the highest concentration (101mg), followed by green coffee (56mg), cocoa (15mg) and energy drink (64mg). Thin layer chromatography qualitatively confirmed the presence of caffeine in the samples. The methodology used in this research provides an interdisciplinary approach, integrating concepts from biology and chemistry. Furthermore, it highlights the importance of experimentation in science teaching, encouraging practical investigation. The experiment is simple to execute, allowing students to take an active role in the steps necessary to carry it out. The work also points to possible expansions, such as investigating the influence of the coffee roasting process on caffeine concentration and exploring regional variations in caffeine concentrations in green coffee beans. It is concluded that this practical approach contributes significantly to students' scientific knowledge, promoting understanding of the separation of substances and the presence of caffeine in foods and drinks.

RESUMO

Este estudo tem como objetivo a determinação do teor de cafeína em diferentes amostras alimentícias por meio da extração líquido-líquido, que possa ser realizado em práticas laboratoriais em aulas de Ciências, Biologia, Química, Farmácia e áreas afins. A cafeína, um alcaloide presente em grãos de café, foi escolhida devido à sua relevância na cultura global de consumo. A metodologia envolve a extração de cafeína de café verde, café torrado, cacau em pó e bebida energética. Os resultados indicam que a extração foi eficaz, com concentrações variadas de cafeína nas amostras analisadas. O café torrado apresentou a maior concentração (101mg), seguido pelo café verde (56mg), cacau (15mg) e bebida energética (64mg). A cromatografia em camada delgada confirmou qualitativamente a presença de cafeína nas amostras. A metodologia utilizada nesta pesquisa proporciona uma abordagem interdisciplinar, integrando conceitos de biologia e química. Além disso, destaca a importância da experimentação no ensino de ciências, promovendo o estímulo à investigação prática. O experimento é de execução simples, permitindo que os alunos assumam um papel ativo nas etapas necessárias para sua realização. O trabalho também aponta para possíveis ampliações, como investigar a influência do processo de torrefação do café na concentração de cafeína e explorar variações regionais nas concentrações de cafeína em grãos de café verde. Conclui-se que esta abordagem prática contribui significativamente para o conhecimento científico dos alunos, promovendo a compreensão da separação de substâncias e a presença de cafeína em alimentos e bebidas.

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Introduction

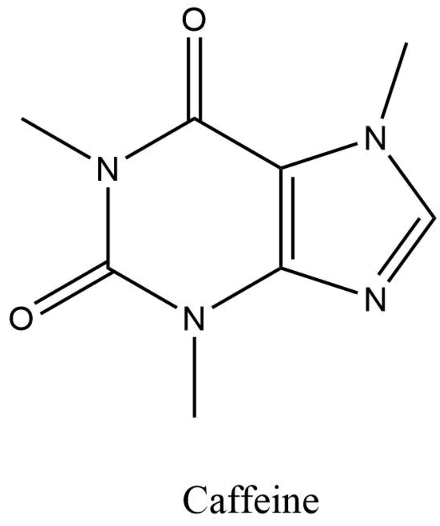
Scientific education is one of the fundamental pillars in developing students' critical and analytical skills. Science teaching plays a crucial role in shaping future citizens, enabling them to understand and contribute to scientific advancements in a society increasingly oriented towards science and technology (Interaminense, 2019). Educational research has shown that practical learning, involving students in experimentation, observation, and active analysis, is essential for knowledge retention and the development of critical skills (Braga et al., 2021; Albuquerque, 2008). Practical experimental classes allow students to apply theoretical concepts to real-world situations, stimulating critical thinking and problem-solving. Furthermore, the interaction between theory and practice helps to spark students' interest, making learning more engaging and memorable (Miranda, Leda & Peixoto, 2013).

In this context, liquid-liquid extraction (LLE) emerges as a key technique, incorporating both biological and chemical theory and practical applications in biomolecule analysis. It is an analytical technique capable of selectively transferring a solute from one liquid phase to another based on the difference in solute affinity between the two phases. Typically, one phase is aqueous, and the other is organic, necessitating the addition of an organic solvent in solution preparation (Facchin & Pasquini, 1997). In the classical methodology of the technique, a separation funnel containing the mixture to be separated is used, with the addition of a solvent that will dissolve the solute. This technique is commonly used in the separation and purification of compounds in foods, such as in the removal of flavors and unwanted substances, or in the extraction of aromatic compounds, dyes, and antioxidants (Zuñiga, 2003).

In order to apply the liquid-liquid extraction technique in practical science teaching, the chosen approach aims to explore a compound commonly found in everyday life: caffeine (Figure 1). Caffeine is a natural product classified as an alkaloid of the methylxanthine family, which is primarily metabolized in the liver to produce 3,7-dimethylxanthine, 1,7-dimethylxanthine, and 1,3-dimethylxanthine that act as stimulants in the body (Weng, 2021; Al-Mssallem, 2022; Fan, 2023). It occurs naturally in various plants but has become more notable due to its prominent presence in coffee beans, which led to the origin of the term "caffeine," named after its association with these fruits where it was first discovered and became an integral part of the global caffeine consumption culture, most commonly in beverages such as energy drinks, coffee, and tea (Associação Brasileira das Empresas do Setor Fitoterápico, Suplemento Alimentar e de Promoção da Saúde [ABIFISA], 2022). It is estimated that, in Brazil, adults consume 300mg of caffeine per day, a high number due to the country's dietary traditions (Heckman, Weil & Mejia, 2010).

Figure 1.

Structural formula of caffeine



Note: Authors (ChemDraw, 2023)

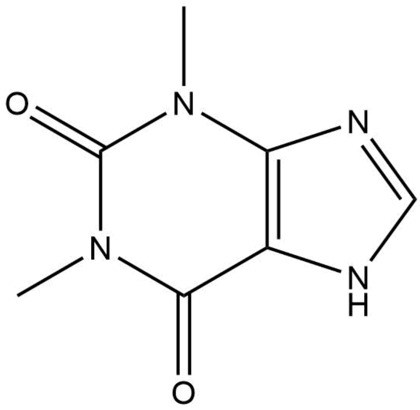
The effect of caffeine on the body is well-studied due to its diverse actions. It positively impacts the human body by improving mood, enhancing physical performance in exercises, preventing certain types of cancer (Abel et al., 2007), having anti-inflammatory properties (Andersen, Jacobs, Carlsen, & Blomhoff, 2006), and increasing alertness. However, it can also have negative aspects, such as disrupting sleep, influencing obesity and dental caries due to its presence in sugary drinks, and potentially affecting fetal development during pregnancy (Heckman et al., 2010).

Other stimulant compounds belonging to the same group as caffeine are theophylline and theobromine. The similarities also extend to their structural chemical formulae (Figures 2 and 3), the foods in which they are found, and many of their actions in the body (Alves & Bragagnolo, 2002). Regarding theophylline, studies have observed its use in treating various airway diseases, such as asthma and chronic obstructive pulmonary disease (COPD), as it promotes bronchodilation and improves the performance of respiratory and cardiac muscles, reducing dyspnea (Bueno, 2003; Barnes, 2010). Similarly, theobromine acts as a vasodilator, aiding in blood flow and blood pressure, as well as providing a sense of relaxation and calm by enhancing brain circulation and increasing oxygen supply (Peres, Brandão & Rezende, 2018).

In cocoa, both alkaloids are found in its composition, which explains the increased desire for consumption. In general foods, theobromine, along with caffeine and theophylline, constitutes the most prevalent methylxanthines in nature. They are widely consumed and exhibit a variety of pharmacological effects in humans (Zoumas, Kreiser & Martin, 1980).

Figure 2.

Structural formula of theophylline

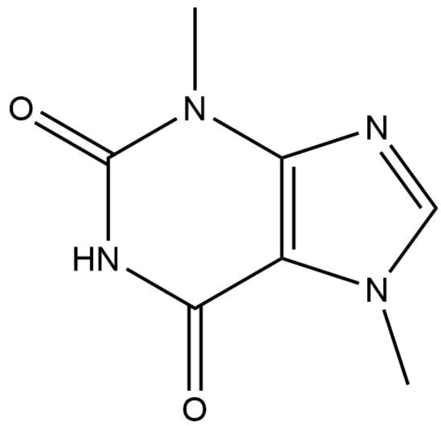


Theophylline

Note: Authors (ChemDraw, 2023)

Figure 3.

Structural formula of theobromine



Theobromine

Note: Authors (ChemDraw, 2023)

Since caffeine is widely consumed worldwide, it is subject to regulations regarding permitted limits in certain foods, making the determination of its concentration in products relevant. Therefore, the separation and quantification of this compound in a variety of common foods is justified, contributing to a better understanding of food composition and dietary habits among populations.

The objective of this work is to determine the caffeine content in different food samples using liquid-liquid extraction, which can be performed in laboratory practices in Science, Biology, Chemistry, Pharmacy, and related fields.

Materials and Method

Obtaining the samples

Samples of green coffee beans, roasted coffee, cocoa powder, and energy drink were obtained from local markets in the city of Montes Claros, northern Minas Gerais, Brazil.

Preparation of the standard and extractre

The caffeine and theophylline standards were prepared with 1 mg of each substance in 1 mL of 95% ethanol.

Solid samples

Green and roasted coffee beans (10 grams) were ground into a fine and homogeneous powder using a knife mill, achieving a consistency similar to cocoa powder. A 1.5% hydrochloric acid solution was prepared, and 125 mL of this solution was added to each Erlenmeyer flask containing the respective samples. The mixture was then boiled for 5-10 minutes. After boiling, the solutions were allowed to cool to room temperature.

The boiled mixture was filtered through filter paper, diluted to a volume of 250 mL with distilled water, and the pH was adjusted to 10 using a 10% sodium hydroxide solution. The filtrate was then reserved for further analysis.

Liquid sample

Due to the liquid state of the energy drink, there was no need for extraction. The liquid was transferred into an Erlenmeyer flask, degassed, and stabilized to pH 10 using 10% sodium hydroxide.

Liquid-liquid extraction

In a separation funnel, the extract and 20 mL of dichloromethane were added. The flask was agitated, degassed during agitation, and after decantation, the organic phase was collected. This extraction process was repeated three times consecutively. The same procedure was repeated for the other samples.

Drying and yield of the extract

The organic phase was dried using a heating plate in a fume hood, and subsequently, the yield of caffeine in milligrams was calculated for each sample.

Thin-layer chromatography

On a silica gel chromatography plate with UV detection at 254 nm, standards and 1 mg of each extract suspended in 95% ethanol were spotted using a capillary, 1 cm above the bottom edge of the plate.

Elution

The plate was eluted in a tank for planar chromatography, using a mobile phase composed of ethyl acetate: methanol: water (100:13.5:10), until 0.5 cm from the top edge of the plate.

Chromatography detection

After elution, the plate was visualized under UV light at 254 nm and developed using the iodine-hydrochloric acid reagent (Wagner; Bladt, 1996), aimed at purine detection. The developer consists of 2 solutions: a) 1 g of potassium iodide and 1 g of iodine dissolved in 100 mL of 96% ethanol, and b) 25 mL of 25% hydrochloric acid and 25 mL of 96% ethanol. The plate was sprayed with approximately 5 mL of solution "a," allowed to dry, followed by spraying with approximately 5 mL of solution "b." This process resulted in the formation of spots on the plate.

Results and Discussion

In the study, a methodology for caffeine extraction from various sources was proposed, including green coffee beans, roasted coffee, energy drink, and cocoa powder. Additionally, thin-layer chromatography was applied for qualitative visualization of the extraction.
The obtained results provide data on the concentration and presence of caffeine and other compounds in the analyzed samples (Table 1), which positively validate the extraction technique used. The extraction process was effective in obtaining caffeine from the different sources. In the roasted coffee sample, the caffeine concentration was the highest (101 mg) compared to green coffee (56 mg), a difference that may be related to the type of bean and cultivar variety, as Arabica coffee beans typically contain about half the caffeine content found in Robusta coffee beans (ABIFISA, 2022).

Table 1.

Mass of caffeine extracted from the samples

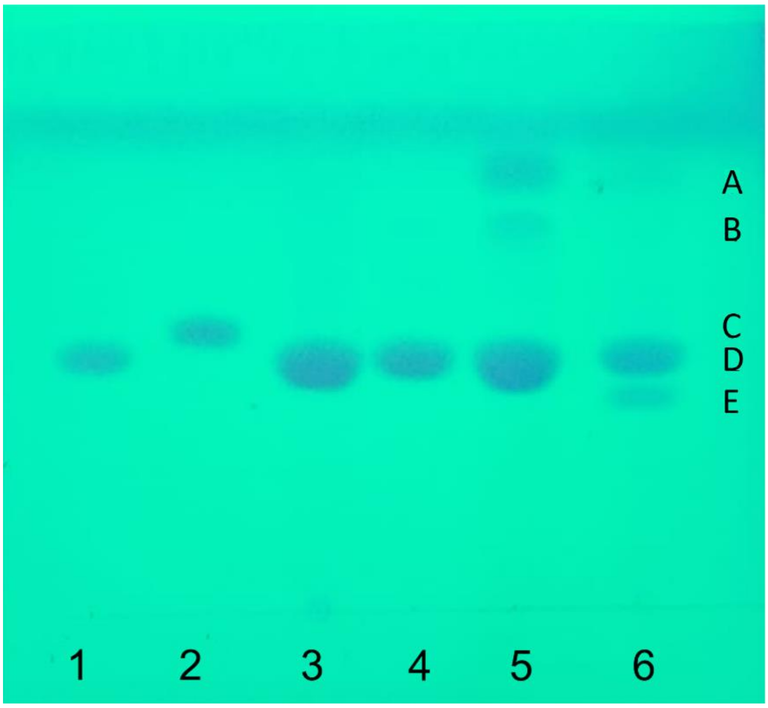
Sample	Initial mass or volume	Mass of extracted caffeine (mg)
Roasted coffee	10g	101
Green coffee	10g	56
Cocoa	10g	15
Energy drink	269 mL	64

The energy drink showed an extracted alkaloid concentration close to that of green coffee, at 64 mg. Considering caffeine predominance, the value found was close to the manufacturer's claim (80 mg). The presence of caffeine in cocoa powder was confirmed, although in smaller quantities compared to coffee sources, totaling 15 mg along with traces of another substance.

A thin-layer chromatography is a useful tool for the separation and qualitative detection of compounds in samples, as it can compare a substance with a standard based on the course of the chemical reaction, as well as separate or define the purity of a sample by its affinity with the mobile phase (Zuben & Souza, 2022). It proved its efficiency in this experiment, allowing the identification of caffeine based on relative mobility. In the figure below, the chromatographic plate revealed under UV light at 254 nm.

Figure 4.

Chromatography plate with samples revealed under UV light



(1) Caffeine sample. (2) Theophylline sample. (3) Roasted coffee. (4) Green coffee. (5) Energy drink. (6) Cocoa. (A, B) Unknown substances. (C) Theophylline. (D) Caffeine. (E) Theobromine.

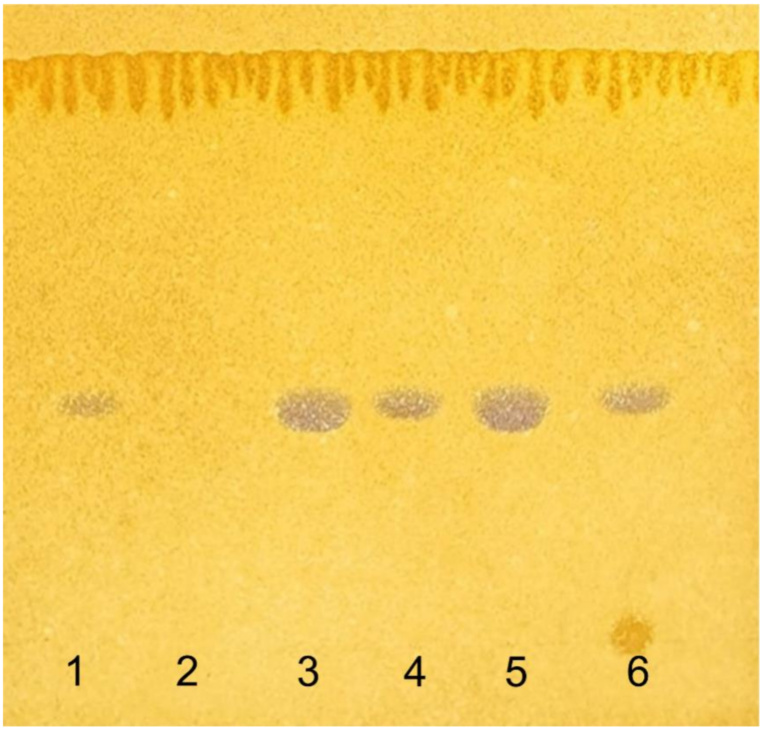
Note: Authors

On the right side of the image, vertically identified are the distances traveled by eluted substances. The alignment of the samples with the caffeine standard substance identified as D can be observed, validating the presence of caffeine. Despite the extraction method targeting caffeine, theobromine, the more abundant alkaloid in cocoa (Bartella et al., 2019), was detected as a residue in thin-layer chromatography (E). Additionally, the visualization shows two other compounds in the energy drink sample, identified as A and B, which we believe to be taurine, a synthetic amino acid present in the drink's composition (Souza et al., 2023), also extracted in residual amounts, and another unidentified substance.

A chemical revelation with iodine-hydrochloric acid detected caffeine on the plate aligned with the standard sample of the substance (1), as can be observed in the image below:

Figure 5.

Chromatography plate with iodine-hydrochloric acid revelation



Note: Authors

It is important to highlight that this study used a simplified methodology. The extraction and qualitative visualization of caffeine are just the first step in the detailed characterization of compounds present in the samples. Acidification of solutions containing caffeine is necessary to increase the alkaloid's solubility in water.

Os alcaloides têm um pH elevado, e a adição de um ácido faz com que o composto se torne um sal, aumentando sua solubilidade em água e removendo impurezas lipídicas. A alcalinização subsequente é necessária para converter o alcaloide de sua forma salina para a forma de base livre, solúvel em solventes orgânicos (Brenelli, 2003). Esse processo aumenta a eficiência da extração final.

Furthermore, this study provides an opportunity to investigate the influence of coffee roasting on caffeine concentration (ABIFISA, 2022), as well as explore regional variations in caffeine concentrations in green coffee beans. These investigations can provide insights into the relationship between lipophilic compounds and the presence of caffeine in an inversely proportional manner.

Final considerations

The proposed experiment facilitates the approach to interdisciplinary questions encompassing both biology and chemistry, introducing concepts and methods that promote practical investigation. It's important to emphasize that the activity is straightforward to execute, allowing students to take a central role in the necessary steps for completion. Numerous themes can and should be explored, such as molecule interactions, polarity, the influence of gravity, chemistry of natural substances, molecules with biological activity, different concentrations of the same compound in various foods, and other issues that intersect with the fields of physics, chemistry, and biology.

The primary strategy to promote experimentation in science education is through didactic practices, which can be conducted both within and outside the school environment, adapting to the specific needs of each subject matter. However, it's crucial to emphasize that laboratory classes play an irreplaceable role in biology courses. This is because such classes provide direct contact and observation of phenomena, while also enabling the analysis of biological processes and the confrontation of unexpected results, challenging students' imagination and reasoning abilities. Engaging students with everyday materials in these processes effectively and contextually brings them closer to science.

The study serves as an example of a practical class to effectively demonstrate the separation of substances and the understanding of caffeine in foods and beverages, contributing to the scientific knowledge of students who are learning separation and purification techniques as part of their curriculum or course plan.

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