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Chromatographic evaluation of curcumin content in commercial samples

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Abstract

Turmeric, also known as ground saffron, is a widely used spice that colours and flavours traditional dishes. It has antioxidant, antimicrobial, and coloring properties due to the presence of curcumin in its composition. Studies indicate that its daily consumption promotes a reduction in the symptoms of chronic non-communicable diseases such as obesity, diabetes, cancer, Alzheimer's, and Parkinson's, among others, and can be used in therapies for longevity. Thus, this study aimed to quantify the curcumin present in commercial turmeric samples using the High-Performance Liquid Chromatography method. Curcumin extraction was performed using the Soxhlet system, with the samples previously defatted with hexane. The samples of turmeric powder showed a wide variation in curcumin content (0.06% to 2.06% w/w), highlighting the need for stricter quality control, not compromising its application as a food input and its bioactivity.

Keywords: Açafrão-da-terra; Turmeric; Quality; High-Performance Liquid Chromatography – HPLC; Natural bioactive additive; Soxhlet.

Resumo

A cúrcuma, ou açafrão-da terra, é muito utilizada como especiaria para colorir e dar sabor a pratos tradicionais. Possui características antioxidantes, antimicrobianas e corantes, devido à presença da curcumina em sua composição. Estudos indicam que seu consumo diário promove redução nos sintomas de doenças crônicas não transmissíveis como obesidade, diabetes, câncer, Alzheimer e Parkinson, dentre outras, podendo ser utilizada em terapias para longevidade. Assim, o objetivo deste trabalho foi quantificar a curcumina presente em amostras comerciais de cúrcuma, utilizando método por Cromatografia Líquida de Alta Eficiência. A extração da curcumina foi realizada por sistema de Soxhlet, com as amostras previamente desengorduradas com hexano. As amostras de cúrcuma em pó apresentaram uma grande variação no teor de curcumina (0,06% a 2,06% m/m) evidenciando a necessidade de um controle de qualidade mais rígido, de modo a não comprometer sua aplicação como insumo alimentício e sua bioatividade.

Palavras-chave: Açafrão-da terra; Cúrcuma; Qualidade; Cromatografia Líquida de Alta Eficiência – HPLC; Aditivo natural bioativo; Soxhlet.

Highlights

- Turmeric is widely used for its antioxidant and coloring properties
- Quality control is essential to ensure the bioactivity of curcumin in turmeric
- Studies indicate that the daily consumption of turmeric can delay the symptoms of chronic diseases

1 Introduction

Turmeric (*Curcuma longa L.*), a rhizome belonging to the Zingiberaceae family, is widely used in oriental gastronomy due to its sensory characteristics and functional compounds. Its therapeutic potential comprises the suppression of pro-inflammatory cytokines, with antioxidant, anti-inflammatory, antifungal, bactericidal, anticancer, neuroprotective power that delay the onset of chronic pathologies such as metabolic syndrome, hypertension, liver disease, rheumatoid arthritis, neurological diseases, among others due to the presence of the yellow-orange polyphenolic pigment, dominant in turmeric, called curcumin (Hewlings & Kalman, 2017; Jantawong et al., 2021; Khan et al., 2023; Sadeghian et al., 2023).

Turmeric's composition has two main classes of active metabolites: curcuminoids (curcumin, demethoxycurcumin, and bis-demethoxycurcumin) and essential oil, extremely relevant for research. Curcumin structurally presents a lipophilic molecule with a molecular mass of 368.38 g/mol ($C_{21}H_{20}O_6$), which can pass easily through the cell membrane, but with low absorption and bioavailability in the body when consumed in isolation. It is low efficiency for therapeutic effects, but co-administered with the piperine alkaloid, derived from *Piper nigrum* (black pepper) and other *Piper* species, plasma levels of curcumin showed a significant increase in plasma absorption (Luber et al., 2019; Di Pierro & Settembre, 2013; Racz et al., 2022).

In Brazil, it is used as a food condiment and as a natural colorant to replace synthetic colorants. JECFA established an ADI of 3 mg/kg body weight per day (bw/d) for curcumin, based on a NOEL of 250–320 mg/kg bw/d, through a study in rats using a safety factor of 100 (Brasil, 2016, 2017, 2019).

According to Genchi et al. (2024), there is a lack of scientific data demonstrating the toxicity of this compound in high amounts of consumption, which suggests its non-toxic profile. According to current international regulations, it is a safe food additive. The assessment of the World Health Organization (WHO) (World Health Organization, 2007) suggests an acceptable daily intake of 0 to 3 mg/kg. However, if adulterated with lead chromate, metanil yellow, or tartrazine yellow, its consumption can cause toxicity. Lead chromate is highly toxic and carcinogenic, while tartrazine yellow is an artificial dye with an allergenic degree in sensitive people, which can affect human body functions when consumed frequently (Behera et al., 2020; Brasil, 2016; Erasmus et al., 2021; Jahanbakhshi et al., 2021; Prasad & Aggarwal, 2011; Prasad et al., 2021).

Curcumin can be extracted using the Soxhlet apparatus, associated with solvents such as ethanol, dichloromethane, ethyl acetate, isopropanol, methanol, n-butanol, and acetone, or by microwave-assisted extraction, ultrasound, supercritical fluid, pressurized fluid, or enzyme-assisted extraction – each with its advantages and disadvantages. The extraction methodology influences the mass yield of the process and eventually the stability of the analyte of interest, and some require sophisticated equipment. The resulting extracts can be analyzed by UV-Vis spectrophotometry (Majumder et al., 2020), high-performance liquid chromatography (HPLC) with UV detection (Jadhav et al., 2007), mass (Ciuca & Racovita, 2023; Satyavert et al., 2022), and high-performance thin layer chromatography (HPTLC) (Kushwaha et al., 2021), among other techniques. The result is expressed as a percentage of curcuminoids or curcumin.

The Brazilian Pharmacopoeia 6th edition presents the monograph of the *Curcuma longa* L. (roots) plant drug with at least 2.5% essential oil and at least 2.5% di-cinnamoyl methane derivatives expressed in curcumin, the latter quantified by spectrophotometric method (Brasil, 2019).

Several researchers have used turmeric in food matrices due to its health benefits and vibrant yellow color. This supplementation with bioactive natural products is a current trend. Its application in bread is an example of this supplementation. Our group addressed this topic, highlighting the improvement in the healthiness of the product without the loss of sensory characteristics. Turmeric is a raw material whose standardization in curcumin is affected by agricultural factors, and is susceptible to fraud (Mélo et al., 2021), which affects its quality. Thus, this study aimed to evaluate the curcumin content in commercial samples of turmeric to assess the suitability for food application, without losing its functionality.

2 Materials and methods

We analyzed 13 commercial samples of turmeric (*Curcuma longa*), purchased at open markets, markets, natural products stores, or obtained through donations. All samples of turmeric powder analyzed were found in transparent packaging. Brands 9 to 13, sold in bulk, were kept in plastic containers in the establishments and were often handled during the selling period. These samples were sold in transparent plastic bags, except for brand 9, which was packaged in a rigid, disposable plastic packaging. The samples' price per kilogram ranged from R$ 40.00 to R$ 100.00, with an average of 50 g purchased for each brand. On the other hand, the commercial samples had individual packaging at the points of sale, with significant variations. The packages included transparent plastic (brands 1, 4, and 5 with 50 g of product and brand 7 with 15 g of product), a plastic container (brand 8 with 130 g of product), and a glass container (brand 6 with 60 g of product). These samples' price per kilogram ranged widely, from R$ 96.00 to R$ 593.33.

2.1 Reagents, solvents, and analytical standards

Standard curcumin (purity ≥ 95%) was purchased from SIGMA. All solvents used were of PA grade or HPLC grade.

2.2 HPLC analysis

Curcumin analysis on the samples was performed on the LC-DAD Alliance Waters chromatographic system composed of a separation module 2695, diode array detector 2996, column oven, and Empower software. Separation of components was carried on a 250 x 4.6 mm Shim-Pack CLC-ODS (M) column with 5 µm particle, maintained at 35 °C and isocratic elution with a ternary mixture containing 2% acetic acid in water, acetonitrile and methanol in the ratio of 59:36:5 (v/v/v) at a flow rate of 0.8 mL/min. The injection volume was 10 µL. The chromatograms were obtained at 425 nm, and the area of the peaks was used in the quantification. The methodology was based on the method described by Jayaprakasha et al. (2002), with modifications.

2.3 Sample extraction

The extraction was performed in triplicate in a Soxhlet apparatus. Its methodology was based on the method described by Jayaprakasha et al. (2002). About 2.0 g of the sample was extracted with 200 mL of hexane for 30 min to eliminate fatty substances from the sample before the extraction of curcumin with methanol. After the elapsed time, the obtained hexane phase was discarded, and 200 mL of methanol were added for Soxhlet extraction of curcumin for 2 h. After this time, the heating was removed and the sample was allowed to cool. After the samples reached room temperature, the extract volume was increased to 250 mL with methanol in a volumetric flask and filtered on a 0.45 µm membrane before injection into the HPLC.

2.4 Statistical analysis

Curcumin concentration was calculated based on the linear calibration functions and the dilution factor. The curcumin content found was expressed as mass percent. All data were expressed as mean ± standard deviation, with decimal places according to the amplitude of the standard deviation.

3 Results and discussion

Given the importance of quantifying turmeric samples with curcumin values that meet recommended levels in the literature for food applications, this study evaluated the quality of various samples available on the market.

The analyzed samples presented peaks of curcumin, demethoxycurcumin and bisdemethoxycurcumin (Figure 1), using an analytical curve of curcumin ranging from 17 to 340 µg/mL.

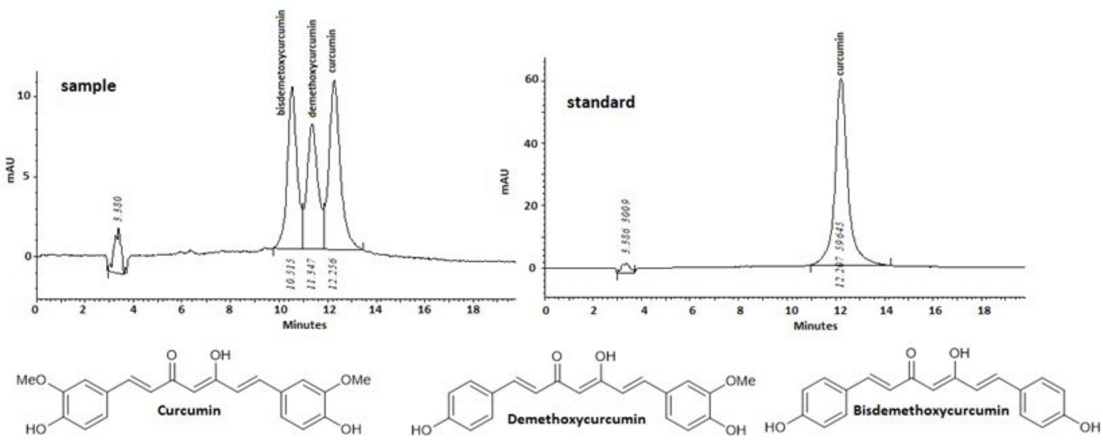

Figure 1. Chromatograms obtained at 425 nm of the turmeric sample 1 and the curcumin standard at 27.8 µg/mL.

The results found for the curcumin content in the commercial samples are presented in Figure 2.

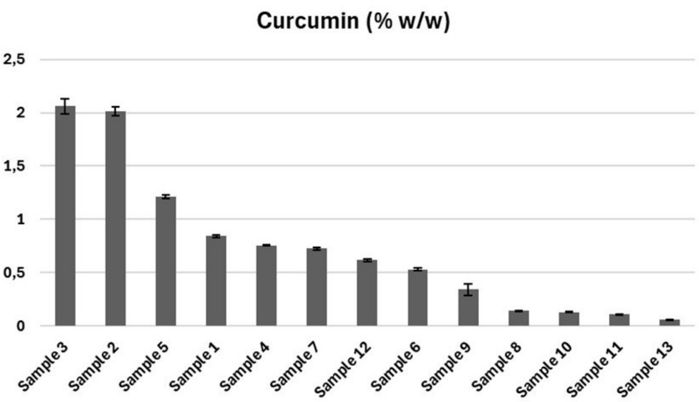

Figure 2. Curcumin concentration in the different samples analyzed. *Packaging type of the samples: Sample 1 – transparent flexible plastic bag, 50 g; Sample 2 – transparent flexible plastic bag, 500 g; Sample 3 – transparent flexible plastic bag, 500 g; Sample 4 – transparent flexible plastic bag, 50 g; Sample 5 – transparent flexible plastic bag, 50 g; Sample 6 – transparent glass jar, 60 g; Sample 7 – transparent flexible plastic bag, 15 g; Sample 8 – transparent rigid plastic container, 130 g; Sample 9 – transparent rigid plastic container, 100 g; Sample 10 – transparent flexible plastic bag, 52 g; Sample 11 – transparent flexible plastic bag, 42 g; Sample 12 – transparent flexible plastic bag, 56 g; Sample 13 – transparent flexible plastic bag, 58 g.* Source: Prepared by the authors.

Curcumin content ranged from 0.057% to 2.06% w/w, with most samples (77% of the analyzed samples) having a content below 1% w/w. Among the 13 samples studied, only two (coded 2 and 3) had a content in the range of 2% w/w. The low content found in most samples indicates the poor quality of the raw material available on the market, as curcumin is considered a quality standard due to being the main curcuminoid of *C. longa*. This low quality affects the applicability of the product, since a minimum value is required for its functionality.

An evaluation of commercial samples carried out in India showed that curcumin contents in commercial samples ranged from 2.2 to 3.7% w/w (Dixit et al., 2009), values much higher than those found in this study.

The low curcumin content of the samples analyzed in this study may result from poor-quality plant raw material, adulteration with starch, for example, or insufficient sample extraction during quantification, among others. Therefore, a preliminary test was carried out to verify the suitability of the method employed, more precisely on the turmeric extraction step.

In this study, the sample was submitted to 2 consecutive extractions with 200 mL of methanol for 2 h and analyzed separately to guarantee depletion of the drug, to ensure that all curcumin present in the sample was extracted. The results obtained in this test confirmed that 99% of the curcumin present in the sample was extracted in the first 2 h; therefore, the analytical procedure adopted in the extraction guarantees the depletion of the sample.

The analytical method used was linear from 17 to 340 µg/mL. The regression was statistically significant and presented a correlation coefficient of 0.9999, experimentally proving the linearity of the method in the studied range. Linearity and high correlation are critical parameters in calibration studies, as they ensure the accuracy and reliability of the analytical methods used, as established in guidelines such as those of the International Conference on Harmonisation (2005) and the National Health Surveillance Agency (Brasil, 2017).

A factor that should be considered is the packaging of the samples under evaluation. Curcumin is susceptible to degradation by several processes, which can compromise its stability and effectiveness. One of the main factors contributing to this degradation is exposure to light. Studies demonstrate that curcumin degrades rapidly under ultraviolet (UV) light, resulting in the formation of degradation products that may have reduced biological activity (Zhang et al., 2023). In addition, the presence of oxygen and moisture can intensify these degradation processes, especially in products sold in bulk, food, and pharmaceutical formulations (Pardeshi et al., 2023). This can be proven by the results found in our study, mainly for samples sold in bulk (samples 9 to 13), since they were purchased in transparent packages, with no filter to protect this photosensitive compound.

The products of brands 2 and 3 were packaged in sealed transparent plastic packages and labeled as organic products. The companies prioritized sustainable agricultural practices, which may have contributed to the higher concentration of the active compound. Palaniappan & Karthikeyan (2023) corroborate this perspective, noting that the variability in curcumin concentrations can be attributed to several factors, including extraction methods, raw material origin, and manufacturing processes. Brands that adopt sustainable agricultural practices and processing techniques that minimize curcumin degradation tend to have higher and more consistent concentrations.

Studies demonstrate that the proper choice of packaging materials can delay the degradation of the active compounds in turmeric, directly influencing the quality and value of the final product. Gómez-Estaca et al. (2014) and Qu et al. (2024) report in their studies that degradation caused by external factors, such as light, oxygen, moisture, and temperature, can be prevented through packaging, in addition to ensuring the integrity of the compound during storage, transport and display at the point of sale, prolonging the stability and effectiveness of curcumin over time (Bellary et al., 2017; Roy et al., 2022).

Packaging quality is also closely linked to the perception of product quality and consumer trust in the brand. Packaging that is practical, informative, and aesthetically pleasing can be a differentiating factor for a condiment at the point of sale and contribute to a satisfying consumer experience. Brands that adopt organic cultivation methods tend to have higher prices, justified by the superior quality and therapeutic efficacy of the products (Tayyem et al., 2006). This variability suggests the need for consumer awareness of all quality parameters involved.

These findings reinforce the need for a meticulous approach in the production chain, from harvest to final processing, to ensure the quality and effectiveness of turmeric products available on the market. It should be considered that insufficient curcumin contents compromise potential technological and health benefits, limiting the effectiveness in the prevention and treatment of various pathological conditions due to reduced absorption by the body, limiting its bioactivity and therapeutic efficacy, as demonstrated by the reports of

Hewlings & Kalman (2017), Nelson et al. (2017), and Lopresti (2018). In addition to the compromised therapeutic efficacy of the products, turmeric quality variation leads to distrust among consumers, who seek to ensure that purchased products meet their expectations of quality and efficacy (Kebede et al., 2021). Therefore, clear directives and certification processes in the industry are essential to mitigate these issues and ensure the reliability of commercially available products.

The acquired turmeric powder samples showed considerably discrepant sensory characteristics, such as color and odor. Figure 3 shows the color variation of the analyzed samples, while in Figure 4 we can see the variation in their price.

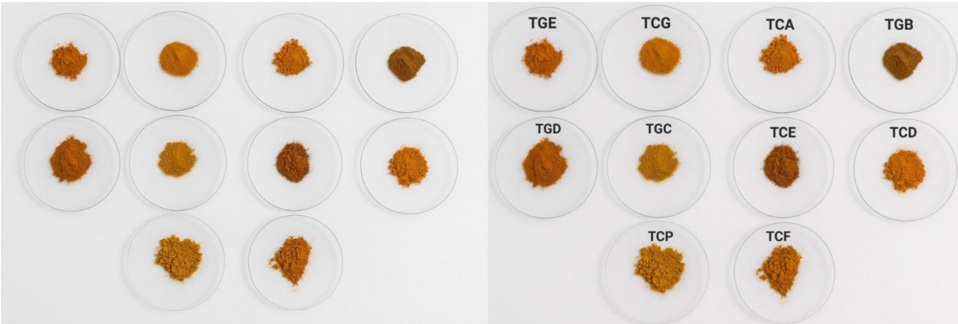

Figure 3. Color variation between the different brands analyzed, where the letters correspond to: TC - Commercial Turmeric; TG - Bulk Turmeric and A – Sample 1, B – Sample 2, C – Sample 3, D – Sample 4, E – Sample 5, F – Sample 6, G – Sample 7 and P – Sample 8. Source: Prepared by the authors.

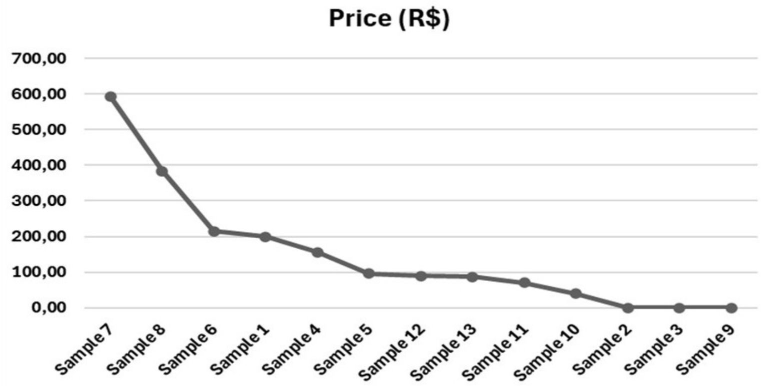

Figure 4. Price variation of the turmeric samples analyzed. Source: Prepared by the authors.

The commercial samples showed a significant price variation, which does not reflect their quality in terms of curcumin content. Samples 2 and 3, which showed higher curcumin contents (Figure 2), were obtained by donation and are excluded in Figure 3, as well as sample 9. The results indicate that the form of sale and the type of packaging directly influence the value of the product. This disparity suggests that factors such as sale practices, packaging type, and market positioning can significantly impact price, regardless of the intrinsic quality of the product.

Curcumin, recognized for its health benefits, is susceptible to degradation, which compromises its stability and effectiveness. Exposure to light, especially ultraviolet (UV) light, is one of the main factors that speed up this process, catalyzing chemical reactions that result in the formation of degradation products with reduced biological activity (Zhang et al., 2023). In addition, the presence of oxygen and moisture can intensify these degradation processes, especially in products sold in bulk, food and pharmaceutical formulations (Pardeshi et al., 2023). This can be proven by the results found in our study, mainly for samples sold in bulk (samples 9 to 13), since they were purchased in transparent packages, with no filter to protect this photosensitive compound.

The proper choice of packaging materials is critical to slowing the degradation of active compounds found in turmeric, directly impacting the quality and value of the final product. Studies indicated that packaging can prevent degradation caused by external factors such as light, oxygen, moisture, and temperature, ensuring the curcumin content during storage and transport (Gómez-Estaca et al., 2014; Qu et al., 2024; Roy et al., 2022). In addition, packaging quality influences the consumers' perception of the product and trust in the brand. Practical and attractive packaging can be a differentiating factor for products in the market, while brands that adopt organic cultivation methods tend to have higher prices, reflecting their superior quality (Tayyem et al., 2006). This variability demonstrates the importance of consumer awareness of quality parameters when choosing products.

Another important factor affecting the stability of curcumin is the pH conditions in which it is present. Curcumin is more stable in acidic conditions. However, its degradation increases at neutral or alkaline pH levels, leading to the formation of unwanted degradation products (Kharat et al., 2017). The application of turmeric to gluten-free breads shows significant impacts on the sensory and technofunctional properties of the products. In addition to affording a vibrant color, turmeric can enhance the antioxidant and anti-inflammatory profile of the product (Tayyem et al., 2006). Studies indicate that the addition of turmeric provides better results concerning texture and sensory acceptance, exhibiting a balance between softness and elasticity, which is the major issue in gluten-free baking (Qazi et al., 2022). Thus, the use of turmeric in gluten-free breads not only makes the products more visually attractive but also meets the growing consumer demand for functional and healthy foods. Moreover, high temperatures play a critical role in curcumin degradation, since excessive heat can accelerate oxidation and thermal degradation reactions, resulting in a significantly decreased compound concentration (Pardeshi et al., 2023). Therefore, understanding these factors is essential for the development of strategies that ensure the stability of curcumin in commercial products, ensuring its therapeutic efficacy and quality (Kebede et al., 2021).

4 Conclusion

The evaluation of the different brands of turmeric powder showed a significant variation in curcumin concentrations: 0.0569% to 2.0587% w/w. This variation shows the need for improved standardization and quality control in turmeric production, to provide the beneficial characteristics of the products offered on the market and promote a significant effect when applied as an ingredient. Curcumin degradation under different environmental conditions, such as light and temperature, further limits the stability of the analyzed products, showing the need for a review of the methods adopted in selling the product. To address these issues, we suggest that future research further examine the impacts of agricultural practices and production processes on curcumin content. Furthermore, developing methods for preservation and stabilization of curcumin in food and herbal products, by analyzing the synergy between curcumin and other functional ingredients, would be valuable; this could provide new prospects for formulating more effective, higher value-added products. These approaches will help ensure the quality and reliability of turmeric products on the market.

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Data Available Upon Request

The data supporting this study are not publicly available due to [e.g., confidentiality agreements, contractual terms], but can be requested from the corresponding author upon reasonable request.

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