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Authenticity Control of Turmeric and Cinnamon Samples Through the Determination of Fraud Indicators Using HPLC-UV and LC-MS/MS

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Abstract: Food adulteration is a significant challenge for the quality and authenticity of traditional Greek products. This study, conducted within the framework of the National Recovery and Resilience Plan "Greece 2.0", aimed to develop and validate reliable analytical methods using High-Performance Liquid Chromatography with Ultraviolet detection (HPLC-UV) and Liquid Chromatography with Tandem Mass Spectrometry (LC-MS/MS) to detect fraud indicators in turmeric and cinnamon. Curcumin and coumarin were selected as target analytes. A total of 82 turmeric samples (primarily from official import controls at the Port of Rotterdam) and 112 cinnamon samples from defined origins were analyzed. Method validation confirmed high sensitivity and accuracy. Statistical analysis (Grubb's and Hampel's tests) identified four turmeric samples (4.9%) as potential adulterants due to abnormally low curcumin content. For cinnamon, coumarin content proved to be a strong species marker, with C. *verum* (Sri Lanka) showing the lowest levels (46.5 mg/kg) and C. *cassia* (China) the highest (5,267.8 mg/kg). Three C. *verum* samples were statistical outliers, indicating potential adulteration or mislabeling. The methods proved highly effective for authenticity control in both commercial and official monitoring contexts.

Key words: Food Authenticity, Adulteration, Turmeric, Cinnamon, Curcumin, Coumarin, LC-MS/MS, HPLC-UV

1. Introduction

Food adulteration represents a widespread, economically motivated malpractice that directly compromises product quality, consumer safety, and market fairness [1]. This issue is particularly acute for traditional Greek products, whose reputation and commercial value are intrinsically linked to their authenticity and specific quality characteristics [2, 5]. The project "Development of Modern Analytical Methods for the

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Determination of Adulteration Indicators in Greek Traditional Products," implemented under the National Recovery and Resilience Fund "Greece 2.0", focuses on addressing this challenge.

Turmeric, a key spice in Greek cuisine (e.g., in pies, cheeses, spreads, and sausages), is often adulterated through dilution with inferior materials or the addition of synthetic dyes like lead chromate, which poses serious health risks [3]. Cinnamon, essential in baked goods and desserts, is subject to a different fraud type. The prized Ceylon cinnamon (Cinnamomum *verum*) is frequently substituted with cheaper *cassia* varieties (C. *cassia*, C. *burmannii*, C. *loureiroi*) [4]. This substitution constitutes economic fraud and a health concern, as *cassia* species contain high levels of coumarin, a hepatotoxic compound in high doses [1].

Robust analytical methods are crucial for verifying the authenticity of these spices. This study aimed to develop, validate, and apply specific HPLC-UV and LC-MS/MS methods for determining coumarin in cinnamon and curcumin in turmeric, respectively. These methods were rigorously applied to a significant number of samples, including those from official import controls, providing a realistic assessment of adulteration prevalence in the market and validating their use for regulatory compliance (EU Regulations 1334/2008 and 1333/2008).

2. Materials and Methods

2.1. Chemicals and Reagents

Analytical standards of curcumin (⩾99.5%) and coumarin (⩾99%) were purchased from Sigma-Aldrich, while HPLC-grade solutes (methanol, acetonitrile, acetic acid and ammonium acetate) were purchased from Merck. All aqueous solutions were prepared using ultrapure water (Milli-Q system).

2.2. Sample Preparation

A total of 194 samples were analyzed: 82 turmeric powders (mostly from official controls at Rotterdam port) and 112 cinnamon samples from various species and origins (47 samples C. *verum* from Sri Lanka, 25 samples C. *loureiroi* from Vietnam, 20 samples C. *burmannii* from Indonesia, 20 samples C. *cassia* from China). Samples were homogenized to a fine powder. For curcumin, 1.0 ± 0.01 g of turmeric was extracted with 10 mL methanol via sonication for 30 minutes followed by centrifugation for 5 minutes (4000 rpm). For coumarin, 0.5 ± 0.005 g of cinnamon was extracted with 10 mL methanol using the same procedure while sonication lasted 5 minutes. All extracts were filtered (0.45 μm PTFE syringe filter) before analysis.

2.3. Instrumentation and Chromatographic Conditions

Coumarin Determination in Cinnamon (HPLC-UV): Analysis used an Agilent 1200 HPLC with a UV detector. Separation used a Phenomenex Luna C18 column (4.6 mm x 250 mm, 5 µm). The mobile phase system used consisted of: (A) water with 0.15% ammonium acetate & 0.15% acetic acid and (B) methanol. The flow rate was 1.0 mL/min using the following gradient elution program: 0.0 min (90% A, 10% B), 17.0 min (30% A, 70% B), 18.0 min (0% A, 100% B), hold until 25.0 min, return to initial conditions at 26.0 min, and re-equilibrate until 34.0 min. The detection of coumarin was performed at 280 nm and the injection volume was 10 µL.

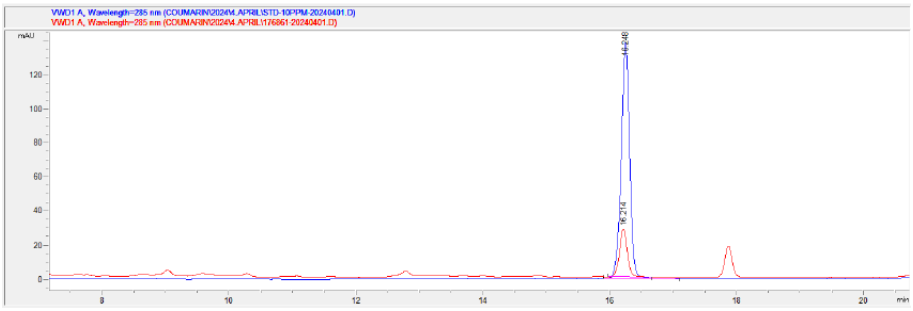

Fig. 1 : HPLC-UV detection of coumarin in cinnamon

Curcumin Determination in Turmeric (LC-MS/MS): Analysis used an Agilent 1200 HPLC coupled to a triple quadrupole mass spectrometer. Separation used a C18 column (4.6 mm x 250 mm, 5 µm) with the following mobile phase system: (A) 5% methanol in water and (B) methanol. The flow rate was 1.0 mL/min with the following gradient elution program: 0 min (100% A, 0% B), 10 min (0% A, 100% B), hold until 16 min, return to initial conditions at 17 min, and re-equilibrate until 25 min. Curcumin detection was performed on ESI+ using MRM transition m/z 369.1 → 285.0.

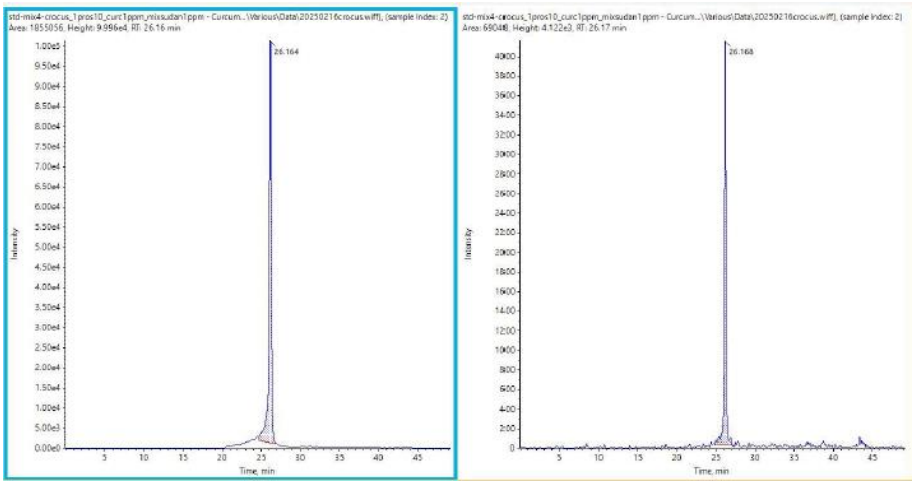

Fig. 2 : LC-MS/MS detection of curcumin standard compound in turmeric

2.4. Preparation of Standard Solutions

Stock Solution (10,000 mg/L): 250 mg of pure reference standard was dissolved in methanol in a 25 mL volumetric flask. Stored at -20°C for up to 3 years.

Working Standards: Prepared by appropriate dilutions of the stock solution with methanol to concentrations of 10, 5, 1, 0.5, and 0.2 mg/L.

2.5. Method Validation

Methods were validated per ICH guidelines. Calibration curves for both analytes showed excellent linearity ($R^2 > 0.999$). LOD and LOQ were 0.03 mg/kg and 0.1 mg/kg for coumarin, and 0.1 mg/kg and 0.5 mg/kg for curcumin, respectively. Precision and accuracy were within acceptable limits (<10% RSD).

2.6. Statistical Analysis

Statistical analysis was performed using Minitab 19. Grubb's test and Hampel's test ($p < 0.05$) were employed to identify statistically significant outliers.

3. Results and Discussion

3.1. Analysis of Turmeric Samples

Table 1 provides a statistical summary of the curcumin content found in the 82 turmeric samples analyzed. The data reveals an exceptionally wide concentration range**,** spanning from 156.4 mg/kg to 82,869 mg/kg**.** The mean curcumin concentration across all samples was calculated to be 22,394±15,363 mg/kg**.** This high variability aligns with known factors like genetic cultivar, geographical origin, cultivation practices, and post-harvest processing [6].

Table 1: Summary of analytical results for curcumin concentration in turmeric samples (n=82).

Statistic parameter	Curcumin concentration (mg/kg)
Mean	22,394
Standard Deviation	15,363
Minimum	156.4
Maximum	82,869
Number of Outliers	4 (4.9%)

Statistical analysis using both Grubb's test and Hampel's test ($p < 0.05$) identified four samples (4.9% of the total) as significant outliers. These outliers were characterized by curcumin concentrations drastically lower than the population mean. Specifically, their values fell below 15% of the mean value and below 20% of the standard deviation of the entire dataset. This low curcumin content provides strong evidence of adulteration with non-curcuminoid fillers (such as starch, chalk, or other cheaper turmeric varieties) or, less likely, represents spice material of exceptionally poor quality. It is particularly noteworthy that these outliers were identified within a batch of samples sourced from official import controls at the Port of Rotterdam, highlighting that adulteration remains a tangible challenge within international spice supply chains, even under regulatory scrutiny.

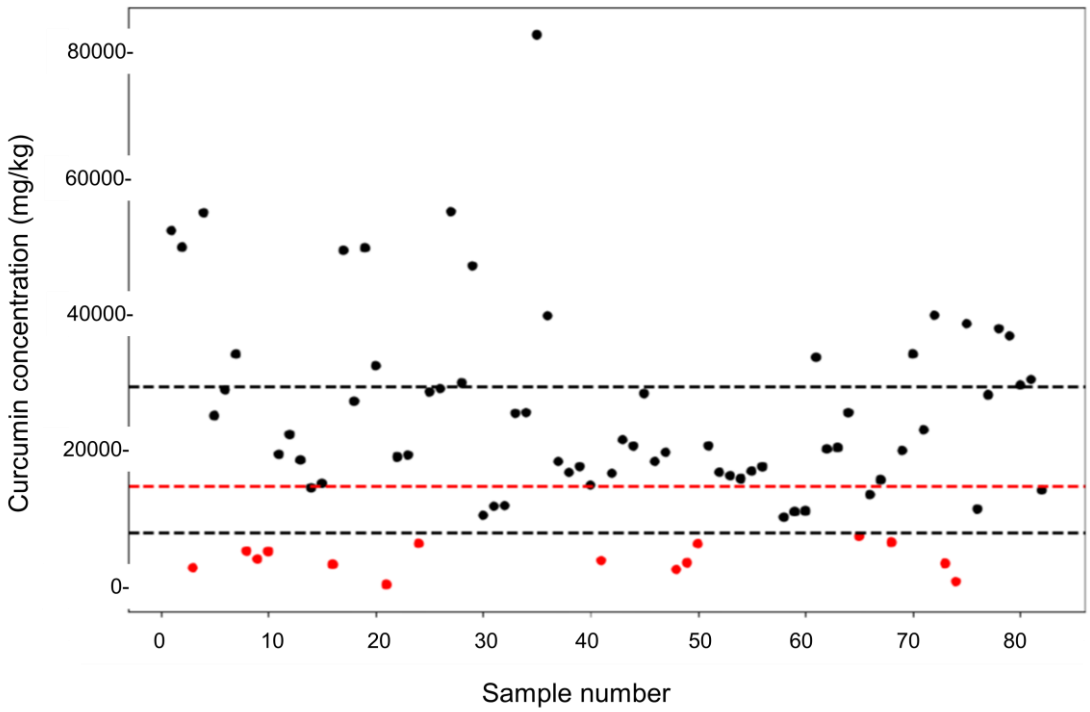

Fig. 3 : Distribution of curcumin concentration among turmeric samples

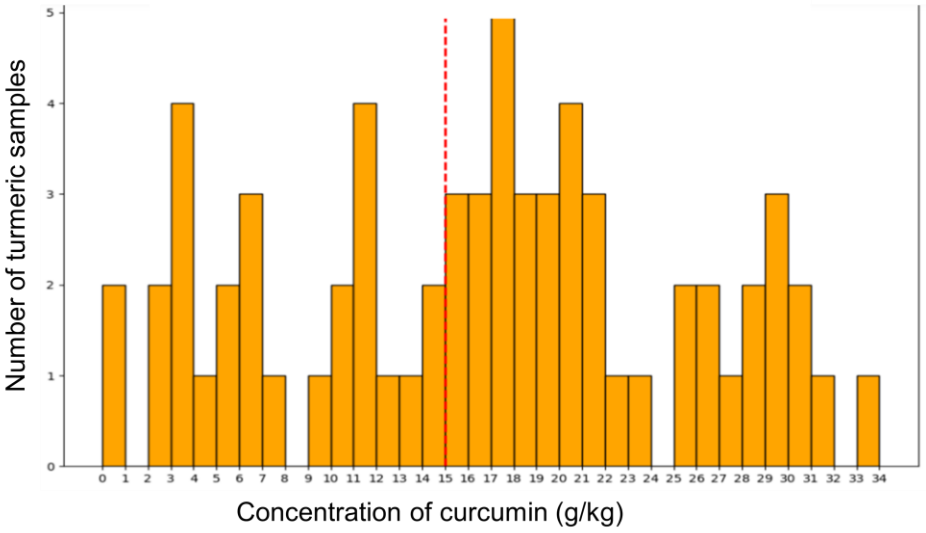

Fig. 4 : Cluster analysis of curcumin content among turmeric samples

3.2. Analysis of Cinnamon Samples

Table 2 presents the results of cinnamon analysis, including origin, species and coumarin content. As expected, Cinnamomum *verum* from Sri Lanka, renowned as "true" or Ceylon cinnamon, exhibited the lowest mean coumarin concentration at 46.5 mg/kg. The concentration range for this species was reported from 2.2 mg/kg to 615.8 mg/kg. On the contrary, the various cassia-type cinnamons showed significantly higher levels. C. *loureiroi* from Vietnam had a mean coumarin content of 2,803.1 mg/kg, while C. *burmannii* from Indonesia averaged 2,547.1 mg/kg. The highest levels were found in C. *cassia* from China, with a remarkably high mean concentration of 5,267.8 mg/kg and a broad range extending up to 13,691.0 mg/kg.

Table 2: Coumarin content in cinnamon samples by species and origin.

Cinnamon Species	Origin	Number of Samples	Mean Coumarin concentration (mg/kg)	Concentration Range (mg/kg)
C. verum	Sri Lanka	47	46.5	2.2 - 615.8
C. loureiroi	Vietnam	25	2,803.1	890.7 - 6,236.0
C. burmannii	Indonesia	20	2,547.1	405.0 - 6,416.0
C. cassia	China	20	5,267.8	2,287.4 - 13,691.0

When Grubb's test was applied specifically to the dataset of the 47 declared C. *verum* samples, it identified three samples as significant statistical outliers ($p < 0.05$). These samples had coumarin concentrations of 415.0 mg/kg, 424.0 mg/kg, and 615.8 mg/kg. These values are orders of magnitude higher than the group's mean of 46.5 mg/kg and are far more consistent with the coumarin profile of cassia varieties. This strongly indicates that these three samples, despite being labeled as C. *verum*, were adulterated with cheaper, high-coumarin cassia species. This finding translates to a non-compliance rate of 6.4% within the subset of samples declared as authentic Sri Lankan cinnamon.

Figure 5, which depicts the coumarin concentration distribution by geographical origin, visually reinforces this conclusion. It shows a tight, low-level cluster for Sri Lankan (C. *verum*) samples, with clear separation from the higher and more dispersed clusters for the cassia-type cinnamons from Vietnam, Indonesia, and China. The three outlier samples appear as obvious anomalies within the C. verum group, graphically supporting the case of mislabeling or adulteration.

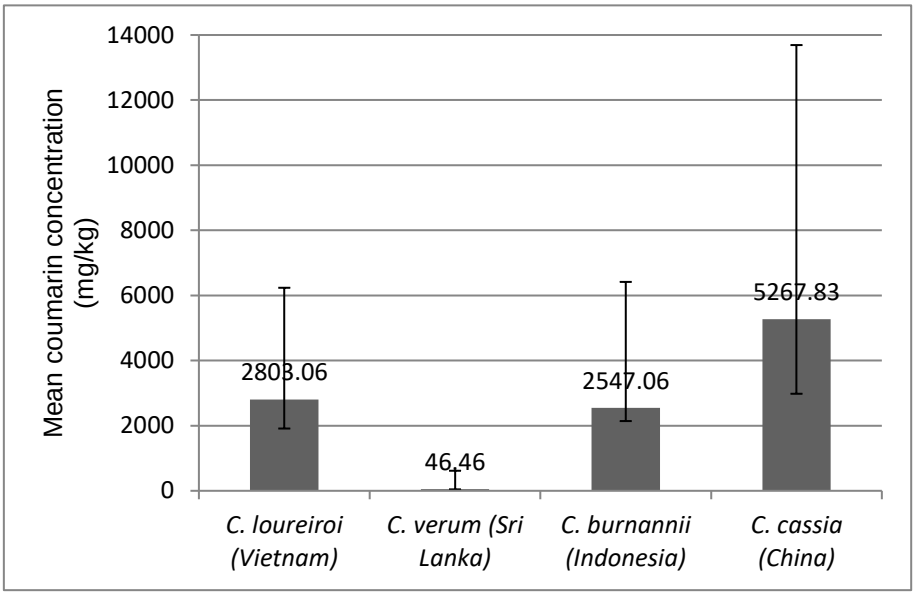

Fig. 5 : Coumarin concentration distribution by cinnamon origin region

4. Conclusion

This study successfully developed, validated, and applied specific HPLC-UV and LC-MS/MS methods for the authenticity control of turmeric and cinnamon. The methods proved to be sensitive, reliable, and suitable for application in both quality control laboratories and official regulatory settings, as demonstrated by analyses of samples obtained through port authorities. The findings reveal a tangible prevalence of adulteration, approximately 4.9% for turmeric and 6.4% for declared C. *verum* cinnamon. The strong correlation between coumarin content and cinnamon species was confirmed, providing a solid basis for detecting mislabeling and economic fraud. These results underscore the critical need for continuous, rigorous monitoring of the spice supply chain using robust analytical techniques to protect consumers, ensure fair trade, and safeguard the integrity and reputation of traditional food products in the global market.

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