BEYOND CAFFEINE QUANTIFICATION: FINGERPRINTING RED BULL ENERGY DRINK VARIANTS BY UV-VIS SPECTROSCOPY AND PCA/HCA

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Abstract

Despite its inadequacy for qualifying coffeine in complex energy drinks due to profound matrix effects, direct UV-Vis spectroscopy proves highly valuable as rapid tool for qualitative discrimination. In this study, UV–Vis spectroscopral fingerprints combined with chemometric tools were used to discriminate among Red Bull variants. Absorbance spectra (200-500 nm) showed consistent peaks at 200-230 nm and 270-280 nm, associated with caffeine and other aromatic compounds, with Peach and Red exhibiting higher intensities than Blue or Summer. PCA of the raw spectra explained 97% of the variance, while HCA grouped Blue-Summer, Coconut-Red, and Yellow-Normal, consistently identifying Sugarfree as distinct. First derivative preprocessing enhanced subtle differences, improving separation in PCA (PC1 = 79.8%, PC2 = 11.6%) and reinforcing Sugarfree's unique profile. Second derivative preprocessing sharpened spectral features but introduced noise, reducing explained variance (PC1 = 17.9%, PC2 = 13.1%) while distinguishing Coconut and Green. Overall, Sugarfree emerged as the most divergent variant, while recurring clusters highlighted formulation similarities. The results demonstrate that UV–Vis spectroscopy with PCA and HCA offers a rapid, non-destructive approach for quality control and authentication of energy drinks.

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Keywords: UV–Vis, chemometrics, principal component analysis, hierarchical cluster analysis, energy drinks, quality control.
Received: October 3, 2025 Accepted: October 20, 2025 Revision Received: October 24, 2025 Published: October 27, 2025

Introduction

The global energy drink market has experienced exponential growth over the past two decades, becoming a multi-billion dollar industry fueled by demand from adolescents, young adults, and professionals seeking enhanced cognitive performance and physical endurance.[1] Among these beverages, Red Bull® is a pioneering and dominant brand, consistently ranking as one of the most consumed energy drinks worldwide.[2] The purported efficacy of these drinks is primarily attributed to their functional ingredients, which commonly include caffeine, taurine, B-vitamins, and glucuronolactone, often in a high-glycemic matrix.

Caffeine (1,3,7-trimethylxanthine) is the central psychoactive ingredient in most energy drinks. As a non-selective adenosine receptor antagonist, it promotes neuronal excitation, leading to increased alertness, improved concentration, and delayed onset of fatigue.[3] The caffeine content in a standard 8.4 fl oz (250 mL) can of Red Bull is approximately 80 mg, an amount designed to provide a stimulant effect while remaining within generally recognized safe limits for most adults.[4] Given its critical role, accurate quantification of caffeine is essential for both regulatory compliance and consumer information.

Ultraviolet-visible (UV-Vis) spectroscopy is a widely employed technique for the quantitative analysis of caffeine due to its simplicity, cost-effectiveness, and rapid turnaround time.[5] Caffeine exhibits a characteristic absorption maximum near 273 nm in aqueous solutions. However, a significant analytical challenge arises from the complex and often opaque matrix of commercial energy drinks. Other constituents, such as artificial colorants (e.g., Brilliant Blue FCF, Allura Red AC), preservatives like benzoic acid, and various flavoring compounds, can also absorb light in the UV region, leading to spectral overlap and potential interference.[6] This matrix effect can cause inaccuracies in direct spectrophotometric measurements, yielding values that may not reflect the true caffeine concentration.

To deconvolute these complex spectral signals and extract more robust chemical information, chemometric techniques are increasingly applied. Derivative spectroscopy is a powerful method that enhances spectral resolution by eliminating baseline drift and separating overlapping absorption peaks.[7] By converting a standard absorbance spectrum into its first or second derivative, the contributions of broad, background absorption from interferents can be minimized, allowing for more accurate quantification of the target analyte.

In addition, chemometric techniques have become indispensable tools for extracting meaningful information from spectroscopic data with principal component analysis (PCA) and hierarchical cluster analysis (HCA) among the most widely used methods. PCA reduces complex, high-dimensional datasets into a few uncorrelated components that capture the main sources of variation, allowing visualization of patterns, clusters, and outliers.[8,9] This approach has been applied in food and beverage research to authenticate products and distinguish varieties.[10] HCA complements PCA by grouping samples based on spectral similarity into dendrograms, providing an intuitive representation of their relationships.[11] Together, PCA and HCA offer a powerful strategy for identifying natural groupings and have been effectively used to differentiate products such as tea and honey.[12,13]

This study has two main objectives: (1) to demonstrate the limitation of direct UV-Vis specrsocpy for quantifying caffeine in complex energy drink marices by comparing the results with the manufaacturer's product labeling, highlighting the significant matrix effects; and (2)to apply derivative spectroscopy combined with PCA and HCA to differentiate samples based on their full UV absorption profiles. This shift in approach—from failed targeted quantification to successful non-targeted fingerprinting establishes UV-Vis spectroscopy as a potent,

rapid, and cost-effective tool for the qualitative authentication and quality control of beverages.

Materials and Methods

Reagents and Samples.

An analytical standard of caffeine (≥99.0% purity) was purchased from Sigma-Aldrich (St. Louis, MO, USA). High-performance liquid chromatography (HPLC) grade water was used for all dilutions to minimize UV-absorbing impurities. Nine distinct flavors of Red Bull energy drink were purchased from local retail stores in New York, NY. The Red Bull variants analyzed (Figure 1) included: Original (Normal)**,** Sugarfree**,** Red (Watermelon)**,** Yellow (Tropical)**,** Blue (Blueberry)**,** Green (Dragon Fruit)**,** Coconut**,** Peach and Summer (Curuba Fruit)**.** The samples were stored in sealed containers at room temperature prior to analysis to maintain their original composition.

Sample Preparation.

A 0.1 mL aliquot of each sample was accurately pipetted into a 10 mL volumetric flask and diluted to the mark with HPLC grade water, resulting in a 100-fold dilution. This dilution factor was determined to bring the caffeine concentration within the linear range of the calibration curve while reducing the absorbance of interferents to a manageable level.

Calibration Curve.

A primary stock solution of caffeine (100 ppm) was prepared by dissolving 10.0 mg of the analytical standard in HPLC water in a 100 mL volumetric flask. A series of working standard solutions were prepared by appropriate dilution of the stock solution to concentrations of 0.5, 1.0, 2.5, 5.0, 7.5, and 10.0 ppm**.**

Absorbance Collection.

Each sample solution was then scanned using a UV-Vis spectrophotometer (JASCO V600) over the range of 200-500 nm. Three replicates were obtained for each sample (3 containers for each tea variant) and each reading was saved in csv file and then exported to Excel file.

Derivative Spectroscopy.

The average of the absorbance data collected in Excel was obtained and then transferred to Igor software to generate the first and second derivative spectra using Igor software. The different spectra (zero, first, and second derivative) were plotted, and comparisons were made between the absorbance and derivative spectra to identify key chemical differences among the samples.

Multivariate Analysis.

OriginPro was used to conduct multivariate analyses, specifically PCA and HCA. Both absorbance from different containers of each sample and averaged derivative data were exported from Excel into Origin, where PCA and HCA were performed following the procedures described by Grabato et al. (2022).[14]

Figure 1. Red Bull samples used in this study.

Results and Discussion

The caffeine content in the nine Red Bull variants was determined using a validated external calibration curve ($y = 0.0312x + 0.0241$, $R^2 = 0.9903$), where y is absorbance and x is the concentration in ppm. he non-zero y-intercept (0.0241 AU) is a minor systematic bias, but the high R^2 value confirms a strong linear relationship across the concentration range studied

According to the manufacturer, all flavors are standardized to contain approximately 80 mg of caffeine per 8.4 fl oz can. The direct UV-Vis quantification method failed to accurately determine the caffeine content, with calculated concentrations (Table 1) deviating substantially from the manufacturer's label declaration ranging from values close to 79 mg for Sugarfree and Green up to more than 200 mg per can for Peach. Such overestimation is a well-documented pitfall of direct UV spectrophotometry in complex matrices and is not indicative of labeling inaccuracy.[5,15]These results confirm that univariate UV-Vis analysis is unsuitable for targeted caffeine quantification in these complex matrices due to insurmountable spectral interference.

The high relative standard deviations observed (e.g., exceeding 50% for several variants) indicate poor precision in the direct spectrophotometric measurements. This variability is likely attributed to the heterogeneous nature of the beverage matrix, which includes suspended components and micelles that may not be uniformly distributed across aliquots, especially after a 100-fold dilution. This effect is compounded by the spectral overlap from interferents, making the measurement highly sensitive to minor pipetting and dilution inconsistencies

These significant discrepancies are not indicative of inaccurate product labeling but are a direct consequence of spectral interference from the complex beverage matrix. Energy drinks contain multiple UV-absorbing compounds, including synthetic colorants (e.g., Allura Red AC in the Red variant, Brilliant Blue FCF in the Blue variant), preservatives like benzoic acid, and various phenolic flavoring compounds.[6] As shown in the raw absorbance spectra (Figure 2), all samples exhibited strong, overlapping absorption bands between 200–230 nm and 270–280 nm. While caffeine contributes significantly to the peak at ~273 nm,[5] other matrix components absorb strongly in this same region,

Table 1: Calculated caffeine content of various Red Bull samples

Sample Flavor	Measured Concentration (mg/8.4 fl oz)	Deviation from Label Claim (%)
Blue	83.18 ± 40.64	3.98
Coconut	94.65 ± 51.08	18.31
Green	78.73 ± 14.25	-1.59
Normal	174.67 ± 45.98	118.33
Peach	201.70 ± 48.36	152.12
Red	152.09 ± 25.73	90.11
Sugarfree	78.54 ± 9.52	-1.82
Summer	149.00 ± 29.42	86.25
Yellow	144.48 ± 32.46	80.60

inflating the apparent absorbance and leading to an overestimation of caffeine concentration via the univariate calibration model. The greatest overestimations were observed in darker, more intensely colored variants such as Peach and Red, underscoring the pronounced contribution of their specific dye and additive profiles.

The inherent complexity of energy drinks, which function as multicomponent mixtures, precludes accurate univariate analysis. The raw absorbance spectra (Figure 2) confirm this complexity, with all variants showing intense, overlapping absorption bands in the 200–230 nm and 270–280 nm regions. The characteristic caffeine peak at ~273 nm is obscured by the concomitant absorption of synthetic colorants (e.g., Allura Red AC, Brilliant Blue FCF), preservatives like benzoic and sorbic acids, and flavor compounds.[6,16] This spectral overlap leads to a confounding matrix effect, where the measured absorbance is a composite signal. Consequently, the calibration curve, which is specific to pure caffeine in solvent, fails to accurately reflect the caffeine concentration in the drink, a challenge also reported in the analysis of soft drinks and other fortified beverages.[17] The most pronounced overestimations occurred in darker, more pigmented variants (Peach and Red), directly implicating their specific cocktail of high-intensity colorants as major interferents.

Given the limitations of univariate analysis, we treated the full UV-Vis spectrum (200-500 nm) as a unique chemical fingerprint for each variant. Although all samples shared a similar spectral shape, reflecting a common base formulation, systematic variations in absorbance intensity were evident (Figure 2). Peach and Red exhibited the highest overall absorption, while Blue and Summer showed the lowest, providing a visual basis for differentiation. Principal component analysis (PCA) of this raw spectral data yielded a highly effective discrimination model. The first two principal components (PCs) captured 90.8% and 6.4% of the total spectral variance (Figure 3), indicating that the major differences between the drinks are largely contained in PC1. The scores plot showed partial overlap among flavors, reflecting broad compositional similarities, but the distribution still suggests that PC1 is likely influenced by variations in the total concentration of strong UV-absorbing compounds, which are predominantly artificial dyes (e.g., Allura Red AC, Brilliant Blue FCF) given their high molar absorptivity. PC2 appears to capture more subtle variations, potentially from other composition differences such as preservatives (e.g., benzoic acid) or flavor compounds. However, without further targeted analysis, these assignments remain speculative and

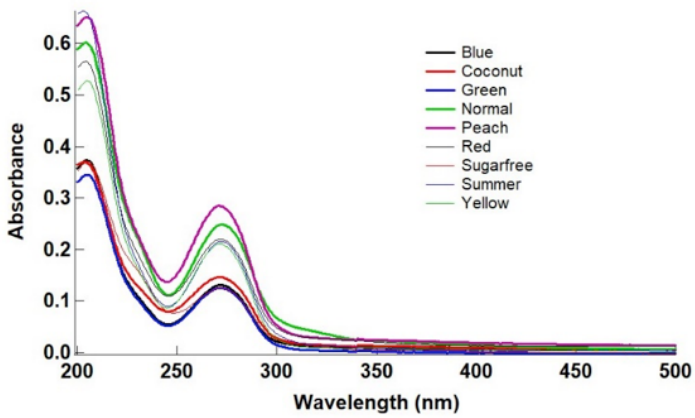

Figure 2. Absorbance of different Red Bull samples.

are based on the known composition of such beverages.[10,18] This approach aligns with the growing use of spectroscopic fingerprinting combined with PCA for the quality control of food and beverages, such as juices and wines.[19]

Hierarchical cluster analysis (HCA) on the raw data produced a dendrogram that partially supported the PCA findings (Figure 4). The algorithm grouped variants with spectral similarities, such as Blue with Summer and Coconut with Red, while the Sugarfree variant consistently appeared as the most distinct, likely due to the absence of sucrose and the presence of artificial sweeteners like aspartame and acesulfame K, which impart distinct UV characteristics.[20]

To enhance spectral resolution and suppress broad-band baseline effects, first-derivative transformation was applied. This

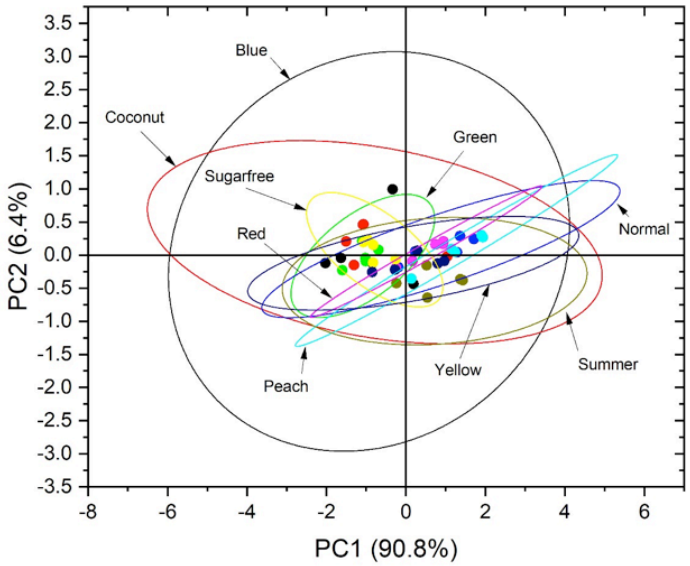

Figure 3: PCA plot of the different Red Bull samples.

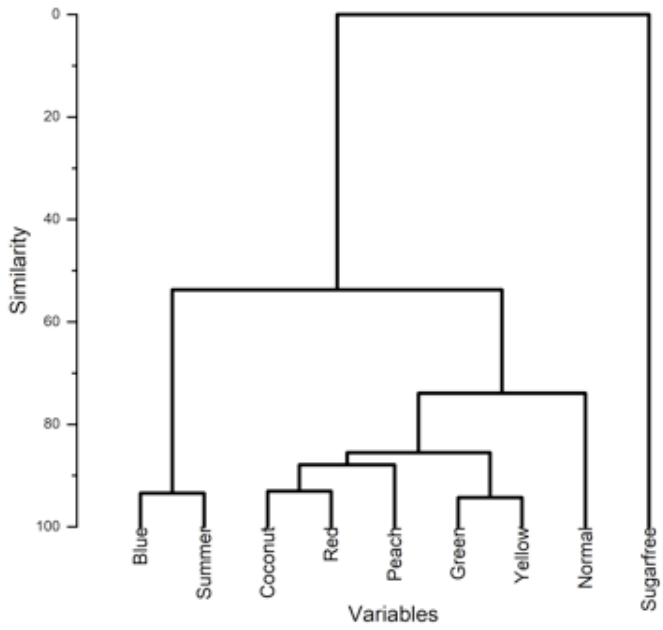

Figure 4. HCA dendrogram of the absorbance different Red Bull samples.

mathematical technique amplifies the visibility of shoulder peaks and sharp spectral features.[7,21] As shown in Figure 5, the first-derivative spectra revealed clearer features, particularly in the 250–350 nm region, where the contributions of individual compounds become more discernible.

Principal Component Analysis of the first-derivative data yielded a model with different variance distribution (PC1 = 79.8%, PC2 = 11.6%) compared to the raw data (Figure 6). While the total variance explained by the first two PCs was lower, the separation between clusters improved, as Peach, Red, and Yellow appeared more distinct in the scores plot. The reduced contribution of PC1 suggests that the dominant, broad-scale intensity differences (likely from colorant concentration) were mitigated, allowing more subtle, shape-based spectral features to contribute significantly to the model.10 The unique position of the Sugarfree variant was further reinforced, solidifying its status as an outlier.

Hierarchical Cluster Analysis on the first-derivative data (Figure 7) produced a dendrogram with new grouping patterns compared to the raw data. Blue was now clustered with Coconut and Red, while Green grouped more closely with Peach and Yellow. These shifts reflect the refined measure of spectral similarity based on spectral shape rather than absolute intensity. The effectiveness of first-derivative preprocessing in improving classification models for complex mixtures is well documented in food science, as it minimizes baseline offsets and highlights the contributions of minor components[13].

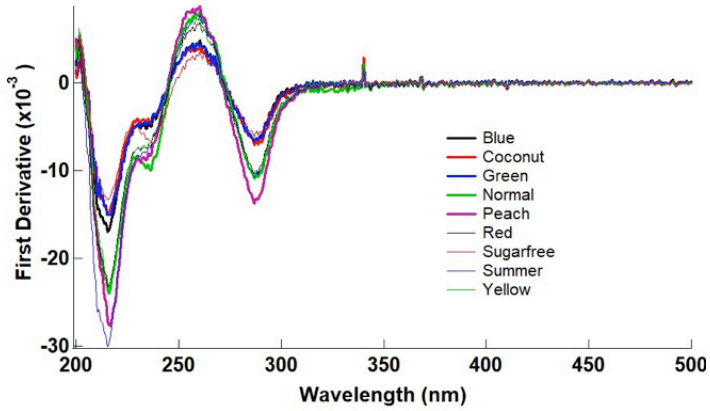

Figure 5: 1st derivatives absorbance of different Red Bull samples.

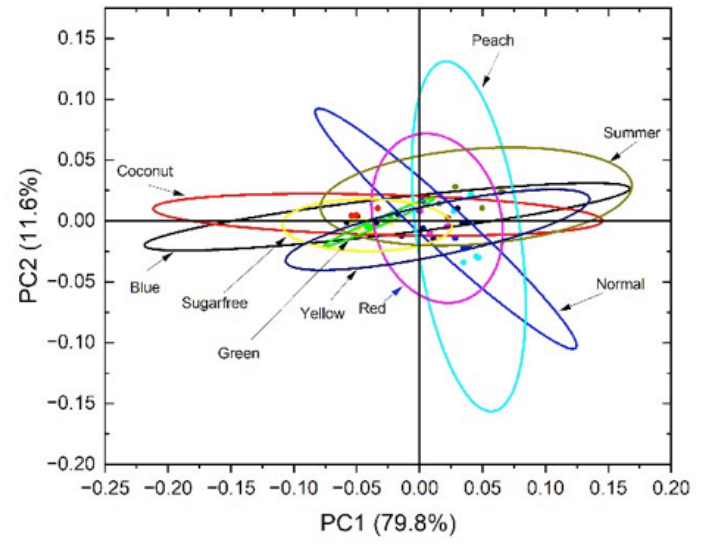

Figure 6: PCA plot of the different Red Bull samples absorbance (1st derivative).

The second derivative transformation was applied to further sharpen spectral features by resolving hidden inflection points and emphasizing narrower peaks. The second-derivative spectra (Figure 8) amplified the fine structure, particularly around the caffeine peak region (~273 nm), potentially revealing contributions from other specific compounds like benzoic acid or individual colorants. However, this enhancement came at a cost. The second derivative is inherently sensitive to high-frequency noise, which was amplified alongside the spectral signals.[8] This effect is clearly reflected in the PCA model derived from the second-derivative data (Figure 9), where the explained variance was distributed much more evenly across numerous components (PC1 = 17.9%, PC2 = 13.1%). Unlike the raw data, no single dominant trend was observed, indicating that the dataset became more complex and noisy.[9] Despite this, the second-derivative PCA provided unique insights, offering improved distinction for the Coconut and Green variants, which emerged as outliers not evident in the raw or first-derivative models. This suggests that these two variants possess unique, sharp spectral features that are only highlighted when the broadest spectral patterns are suppressed.

The HCA dendrogram for the second-derivative data (Figure

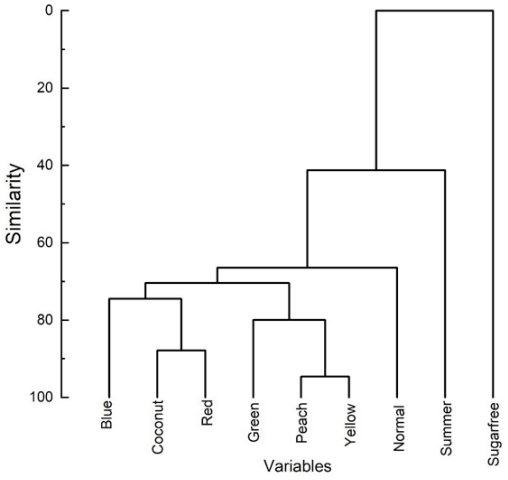

Figure 7: HCA dendrogram of the first derivative of absorbance of different Red Bull samples.

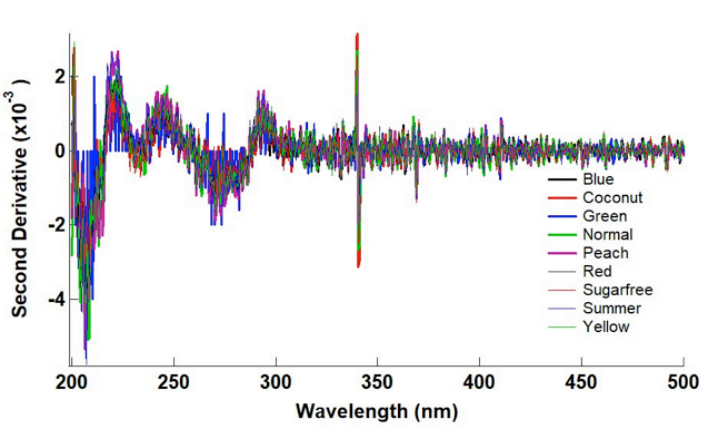

Figure 8: Second derivative absorbance of different Red Bull samples.

10) showed a markedly different clustering pattern compared to both raw and first-derivative results. While some expected clusters persisted, Blue, Red, and Sugarfree were grouped together, and Green separated as the most distinct variant. This demonstrates that the second derivative probes a different level of spectral information. The increased sensitivity to noise can sometimes lead to less stable clustering in HCA; however, the emergence of unique groups can also reveal subtle formulation differences not detected by other methods, a phenomenon observed in other spectroscopic fingerprinting studies.[14]

Conclusion

This study demonstrates that direct UV-Vis quantification fails to accurately determine caffeine in Red Bull due to profound matrix effects, as evidenced by significant deviations from the manufacturer's product labeling and high measurement variability. However, this limitation serves to highlight the strength of an alternative approach: using the full spectral fingerprint combined with chemometrics for robust product discrimination. Principal component analysis (PCA) and hierarchical cluster analysis (HCA) of raw and derivative spectra successfully differentiated all nine variants, consistently identifying Sugarfree as an outlier and grouping others by formulation similarities. This approach establishes UV-Vis spectroscopy with PCA/HCA as a rapid, non-destructive, and effective method for the quality control and authentication of energy drinks, moving beyond targeted quantification to holistic product fingerprinting.

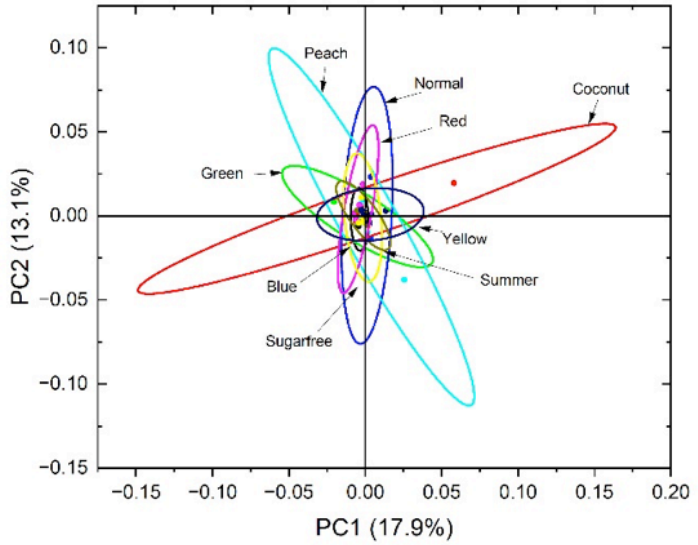

Figure 9: PCA plot of the different Red Bull samples absorbance (2nd derivative).

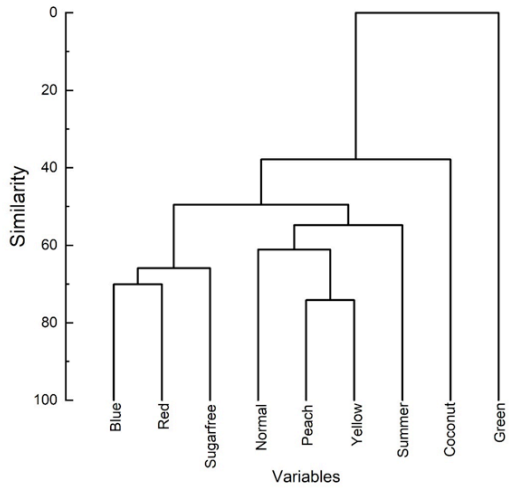

Figure 10: HCA dendrogram of the second derivative absorbance of different Red Bull samples.

A key limitation of this study is the use of averaged spectra for model development without a blind validation test. Future work should focus on building a robust classification model (e.g., using Linear Discriminant Analysis or SIMCA) with a larger sample set and validating its predictive ability by correctly identifying the flavor of unknown, single-shot spectra. This would be a crucial step towards implementing this technique in a practical quality control setting. Ultimately, while techniques like HPLC remain the gold standard for precise quantification, the method presented here offers a complementary, rapid, and cost-effective tool for qualitative discrimination and brand authentication.

Acknowledgements

This work was supported by the Pace University Course-Based Undergraduate Research Experiences (CURE) Award. We would also like to thank the reviewers for their valuable comments and suggestions, which helped improve the quality of this paper.

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