ABSORBANCE DISCRIMINATION OF READY-TO-DRINK TEA SAMPLES BY DERIVATIVE SPECTROSCOPY AND MULTIVARIATE ANALYSIS

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Abstract

Ready-to-drink (RTD) teas are a popular beverage choice due to their convenience and perceived health benefits. In this study, ultraviolet-visible (UV-Vis) spectroscopy was used to obtain absorbance of different commercially available and popular RTD tea samples (Lipton, AriZona and Snapple). Derivative spectroscopy that enhances the resolution of overlapping absorbance bands by providing more detailed spectral information was applied to the obtained absorbance to further distinguish subtle differences in their chemical profiles. In addition, multivariate analysis, including principal component analysis (PCA) and hierarchical clustering analysis (HCA), was also employed to classify and differentiate the tea samples based on their absorbance spectra. Results show that Lipton tea samples has the highest absorbance and the most complex derivative features which could be due to high polyphenol content. It also demonstrated that derivative spectroscopy combined with multivariate analysis is an effective method for distinguishing between different tea samples and detecting variations in composition.

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Introduction

Ready-to-drink (RTD) teas have become increasingly popular worldwide due to their convenience, refreshing taste, and associated health benefits. These beverages are widely consumed for their rich polyphenolic content, which includes catechins, flavonoids, and theaflavins, known for their antioxidant properties.^{1,2} The presence of these compounds has been linked to numerous health benefits, including reduced risks of cardiovascular diseases, cancer, and neurodegenerative disorders.³ With a growing market for RTD teas, distinguishing between brands and formulations has become crucial for manufacturers, regulators, and consumers. Such differentiation is essential for quality control, ensuring product consistency, and meeting consumer expectations.

The composition of RTD teas can vary significantly depending on several factors, such as tea type, brewing method, additives (e.g., sweeteners, flavorings), and processing conditions. Commercial RTD teas, particularly diet, decaffeinated, and flavored variants, often include additives like artificial sweeteners and flavor enhancers that can influence their chemical and nutritional profiles. While studies have extensively characterized brewed teas,⁴⁻⁵ limited research has focused on commercial formulations containing these additives.⁶ Furthermore, few studies explicitly correlate spectral features with manufacturers' ingredient claims, leaving gaps in understanding how these additives affect the chemical profiles and compositional distinctions of RTD teas.⁷

Traditional UV-Vis spectroscopy is a widely used technique for characterizing tea products due to its ability to detect compounds that absorb in the ultraviolet and visible ranges, such as polyphenols and other aromatic compounds.⁸⁻⁹ However, closely related products, such as diet and regular formulations, often exhibit overlapping absorbance features, making it challenging to identify subtle compositional differences using absorbance data alone. This limitation underscores the need for advanced spectroscopic techniques to improve resolution and sensitivity.

Derivative spectroscopy (DS) addresses this problem by enhancing spectral resolution. By calculating the first and second derivatives of the absorbance spectra, this method highlights inflection points and subtle variations, allowing for better discrimination between similar samples.¹¹ It is an advanced technique that generates derivative spectra from the original zero-order spectra. This process helps resolve overlapping signals and removes background interference from other compounds in the sample. For example, DS has been used to determine caffeine content in cola, coffee, and tea without the need for separation techniques or reagents12 and a very good technique for the elimination of interfering matrices and enable to determine the contents of CAF and CGA in defective and non-defective coffee beans without any background correction or reagent.¹³ It can also be applied to the various spectra as a qualitative method to further distinguish the small variations among different samples.14

Combined in addition with multivariate techniques such as principal component analysis (PCA) and hierarchical cluster analysis (HCA), absorbance spectroscopy provides an advanced approach to explore patterns and relationships in complex spectral data. PCA reduces the dimensionality of the data while retaining the maximum variance, enabling the visualization of sample groupings and differences.¹⁵ HCA further classifies the samples into hierarchical clusters based on similarity, complementing PCA by providing a clear representation of compositional relationships between samples.¹⁶ Together, these techniques offer a comprehensive toolkit for analyzing complex datasets and distinguishing between similar products.

Recent studies have employed advanced spectroscopic techniques, including derivative spectroscopy and multivariate analysis, to differentiate tea products based on their chemical composition. ¹⁷⁻¹⁸ These methods have proven effective in identifying subtle differences in polyphenolic content and other UV-absorbing compounds, which are critical for quality control and product differentiation in the beverage industry. However, there is a need for further research to explore the application of these techniques in distinguishing between commercially available RTD teas, particularly in the context of varying formulations and additives.

This study aims to demonstrate the application of UV-Vis spectroscopy, derivative analysis, PCA, and HCA to characterize and differentiate RTD tea samples from major commercial brands: Arizona, Lipton, and Snapple. The absorbance data and its derivatives are used to explore the impact of different formulations, such as regular, diet, decaffeinated, and flavored variants, on the chemical composition of these teas. These findings provide valuable insights into the use of advanced spectral and statistical techniques for product differentiation and quality control in the beverage industry. Additionally, this study highlights the potential of these methods for applications in food fraud detection, where identifying unique spectral signatures associated with specific ingredients or processing methods could help detect economically motivated adulteration in RTD teas and other beverages. The ingredients for each tea sample, as listed by the manufacturers, are provided in Table 1.

Experimental Method

Sample Preparation.

A total of nine RTD tea samples from three different popular brands (Arizona, Lipton, and Snapple) were used for analysis. The samples included diet and non-diet formulations, as well as variants with additional flavorings such as lemon. The tea samples analyzed included the following: AriZona (decaf diet tea, diet tea, tea and lemon tea), Lipton (tea and diet tea) and Snapple (tea

Table 1. Ingredients of RTD Tea Samples

Brand	Variant	Ingredients	
Arizona	Decaf Diet Tea	Water, tea extract, citric acid, natural flavors, sucralose, potassium sorbate, acesulfame K	
Arizona	Diet Tea	Water, tea extract, citric acid, natural flavors, sucralose, potassium sorbate, acesulfame K	
Arizona	Tea	Water, tea extract, cane sugar, citric acid, natural flavors	
Arizona	Lemon Tea	Water, tea extract, cane sugar, lemon juice concentrate, citric acid, natural flavors	
Lipton	Tea	Water, tea extract, citric acid, natural flavors, acesulfame K, sugar	
Lipton	Diet Tea	Water, tea extract, citric acid, natural flavors, acesulfame K, aspartame	
Snapple	Tea Lemon	Water, tea extract, citric acid, natural flavors, sugar	
Snapple	Zero Sugar Tea Lemon	Water, tea extract, citric acid, natural flavors, aspartame	



Figure 1. Absorbance of RTD tea samples.

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lemon and zero sugar tea lemon). A volume (100 uL) of each tea was diluted to 10 mL using a volumetric flask. There are at least 3 containers of each tea samples that was used and serve as sample sources. The ingredients for each tea sample, as listed by the manufacturers, are provided in Table 1.

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 samples (3 containers for each tea variant) and each reading were save in csv file and then exported to Excel file.

Derivative Spectroscopy.

The average of the absorbance data collected in Excel was obtained and then transfer to Igor software to generate the first and second derivative spectra using Igor software. To improve the clarity of the derivative spectra, the first derivative plots were smoothed using a Savitzky-Golay algorithm to reduce noise before generating the second derivative spectra. The different spectra (zero, first and second derivative) were plotted, and comparisons were made between the absorbance and derivative spectra to identify key differences in the chemical composition of the samples.

Multivariate Analysis.

Origin Pro was used to do the multivariate analyses namely PCA and HCA. The raw data of the absorbance obtained from different containers of each sample was utilized from the Excel file and exported to Origin for PCA and HCA analysis. The multivariate analyses were done similar to what is reported by Grabato et al (2022).¹⁶

Results and Discussion

The absorbance spectral measurement was done between 200-500 nm since this is the region where most phenolic compounds generally exhibit an absorption peak in the ultraviolet light range of 250-350 nm.19 The UV-Vis absorbance spectra of the nine ready-to-drink (RTD) tea samples revealed notable variations in their UV-absorbing properties (Figure 1). A prominent peak near 270 nm, characteristic of polyphenolic compounds²⁰ such as catechins and theaflavins, was observed across all samples. Among the teas analyzed, Lipton Diet Tea exhibited the highest absorbance, with values exceeding 0.6 at 270 nm, suggesting a higher concentration of UV-absorbing compounds compared to other samples. In contrast, Arizona Lemon Tea displayed the lowest absorbance at approximately 0.07, likely due to dilution effects from lemon juice or other non-UV-absorbing additives. Snapple Tea and Snapple Zero Sugar Tea showed moderate absorbance values of around 0.12 to 0.14, indicating similar polyphenolic content between these formulations. The Arizona teas, including the regular, diet, and decaffeinated variants, demonstrated relatively consistent absorbance patterns, forming a tight group with values ranging from 0.07 to 0.11. This consistency suggests a similar chemical composition across the Arizona tea variants.

The first derivative spectra (Figure 2) provided enhanced resolution of the absorbance data, emphasizing inflection points and subtle differences between the samples. Lipton Diet Tea and Lipton Tea displayed the most pronounced peaks and troughs near 250

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nm, reflecting a high concentration of UV-absorbing compounds and a unique polyphenolic profile compared to other brands. In contrast, Arizona Decaf Diet Tea exhibited moderate derivative features, with less pronounced peaks, likely due to the impact of decaffeination on the polyphenolic content. Arizona Lemon Tea consistently showed the least pronounced derivative features, supporting the hypothesis that its lower absorbance is due to a reduced concentration of UV-absorbing compounds. Snapple Tea and Snapple Zero Sugar Tea exhibited similar first derivative patterns, with minor variations indicating compositional differences between the regular and sugar-free variants. These first derivative analyses effectively distinguished Lipton samples fron non-Lipton samples (Arizona and Snapples teas), which appeared similar in their raw absorbance spectra.

The second derivative spectra (Figure 3) provided further detail by emphasizing changes in slope and inflection points, reducing baseline noise and enhancing the resolution of compositional differences. To improve the clarity of the second derivative spectra, the first derivative plots were smoothed using a Savitzky-Golay algorithm before generating the second derivative. Lipton teas again stood out, displaying the most complex and intense second derivative features, with pronounced peaks and troughs in the 200–350 nm range, confirming their enriched polyphenolic content. Arizona teas formed a relatively homogeneous group with less pronounced second derivative features, reflecting consistent but lower concentrations of UV-absorbing compounds across all



Figure 2. First derivative of the absorbance of RTD tea samples.



Figure 3. Second derivative of the absorbance of RTD tea samples.

variants. Snapple teas exhibited slightly greater variability in their second derivative spectra than Arizona teas, likely due to the influence of sweeteners and flavoring agents. Arizona Lemon Tea consistently showed the simplest second derivative spectra, highlighting its diluted polyphenolic content due to the addition of flavor enhancers like lemon juice.

The combined analysis of absorbance and derivative spectra demonstrated the ability to differentiate RTD tea samples based on their chemical composition. Lipton teas consistently exhibited the highest absorbance and the most complex derivative features, underscoring their distinct and rich polyphenolic composition. Arizona and Snapple teas showed similar absorbance profiles, with derivative analyses revealing slight differences, particularly in Snapple's formulations. Arizona Lemon Tea was consistently characterized by the lowest absorbance and simplest derivative spectra, likely due to dilution from flavor additives. These findings highlight the effectiveness of combining absorbance and derivative spectroscopy for detailed compositional analysis and product differentiation in RTD teas.

The absorbance data obtained from the UV-Vis spectra provided critical insights into the compositional differences among the RTD tea samples. However, to better interpret the relationships between different brands and formulations, multivariate analyses such as PCA and HCA were applied to the raw absorbance data. These multivariate statistical tools have been widely used in food and beverage research to distinguish products based on chemical composition and to explore patterns in complex datasets.²¹ By integrating PCA and HCA with the absorbance results, a more comprehensive understanding of the samples' compositional characteristics was achieved. PCA was performed on the raw absorbance data, reducing the high-dimensional dataset into two principal components (PCs) that accounted for 96.7% of the total variance (Figure 4). PC1 explained 87.9% of the variance, capturing the major compositional differences among the tea samples, while PC2 contributed an additional 8.8%, highlighting secondary variations.



Figure 4. PCA plot of the absorbance of RTD tea samples.

The PCA scatter plot (Figure 4) showed distinct clustering patterns that reflect significant compositional differences among the samples. Lipton teas (L tea and L dtea) were clearly separated from the Arizona teas (A tea, A dtea, A dtea, A ltea) and Snapple teas (S tea, S ztea) along PC1. This separation indicates that Lipton teas have a unique absorbance profile, which can be attributed to a higher concentration of UV-absorbing compounds, such as polyphenols. Studies have shown that variations in tea polyphenolic content are influenced by factors such as tea type, processing methods, and fortification with bioactive compounds.²²⁻²³ The higher absorbance values observed in Lipton teas align with reports of fortified or enriched RTD teas designed to enhance their health benefits.²⁴

In contrast, Arizona teas formed a tightly grouped cluster near the origin, reflecting their compositional uniformity across variants (regular, diet, decaf, and lemon). This result is consistent with findings from other studies where similar clustering patterns were observed in products with standardized manufacturing processes.²⁵ Snapple teas, while positioned closer to Arizona teas, displayed minor separation along PC2, likely due to variations in sweeteners or flavor additives. Research on beverage formulations has shown that sweeteners, such as artificial or zero-calorie options, can subtly influence chemical interactions and UV-Vis spectral profiles.^{6,26}

The spread along PC1 underscores the importance of polyphenolic content in differentiating RTD teas, with Lipton teas showing significantly higher values. PC2 captured secondary variations, including potential contributions from additives, sweeteners, or minor processing differences between Snapple and Arizona teas. The clear distinction of Lipton teas from the other brands suggests differences in tea blend selection or processing, as Lipton teas may use higher-quality or specifically enriched tea extracts.³

To complement the PCA findings, HCA was applied to the absorbance data using Ward's method and Euclidean distance as a measure of similarity. The resulting dendrogram (Figure 5) divided the tea samples into two major clusters with distinct subclusters. The first major cluster included the Arizona teas (A tea, A dtea, A dtea, A ltea) and Snapple teas (S tea, S ztea), reflecting their shared compositional characteristics. Within this cluster, Arizona teas formed a tightly grouped subcluster, indicating consistent formulation across their product line. Such uniformity is often observed in industrially manufactured beverages, where standardization is crucial for maintaining product consistency.¹⁶ Snapple teas formed a separate subcluster, suggesting compositional similarities to Arizona teas but with slight differences likely due to flavoring agents or sweeteners unique to Snapple's formulations.

The second major cluster contained the Lipton teas (L tea and L dtea), which were distinctly separated from Arizona and Snapple teas. This distinct clustering supports the PCA results, highlighting Lipton's unique compositional profile. The close grouping of Lipton regular and diet teas indicates that, despite differences in sugar content, their polyphenolic profiles remain largely similar. Such clustering has been reported in studies where diet and regular versions of beverages exhibited minor chemical differences beyond sugar replacement.

The similarity scale in the dendrogram further emphasizes the

high similarity between Arizona and Snapple teas (greater than 98%), while Lipton teas demonstrate a lower similarity to the other brands. This separation reflects Lipton's distinct processing or ingredient choices, which may prioritize polyphenol retention or enrichment to enhance perceived health benefits.^{1,3}

These findings underscore the critical role of additives, such as sweeteners and flavoring agents, in influencing the spectral characteristics of RTD teas. The absorbance and derivative spectral features observed can be directly correlated with the ingredient compositions provided by manufacturers (Table 1). Notably, acesulfame potassium is present in both Lipton tea samples and Arizona diet tea variants, while aspartame is found in diet tea formulations from Lipton and Snapple, with Arizona diet teas instead containing sucralose. Additionally, potassium sorbate is an ingredient unique to Lipton teas.

Each of these additives exhibits distinct UV absorption properties that contribute to the spectral differences observed among the samples. Acesulfame K has a characteristic absorbance peak at 226 nm, while aspartame absorbs at 191 nm.⁶ Sucralose is typically detected at 200 nm,²⁷ whereas potassium sorbate has an absor-



Figure 5. HCA dendrogram of the absorbance of RTD tea samples. Figure below shows blow-up of the dendrogram.

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bance peak at 254 nm, even when mixed with acesulfame K.²⁷ The presence of these compounds, particularly in Lipton samples, likely enhances absorbance in the 250 nm region, leading to a higher overall signal compared to other tea samples. This increased absorbance aligns with the distinctive peaks observed in Lipton teas, supporting the hypothesis that additive composition plays a significant role in their spectral profile.

Furthermore, certain ingredients may contribute to reduced absorbance and simpler derivative spectra, as observed in Arizona Lemon Tea. The presence of lemon juice - a unique ingredient among the tested samples - could dilute polyphenolic compounds, leading to lower UV absorbance. This dilution effect explains the lower intensity and simpler derivative features of Arizona Lemon Tea compared to other samples. Similarly, the minor spectral differences between the other RTD tea samples may be attributed to variations in additional ingredients, such as preservatives or stabilizers, that influence light absorption and spectral behavior. These results highlight the necessity of considering ingredient compositions when interpreting UV-Vis spectral data for RTD teas. By accounting for the impact of additives, researchers can more accurately differentiate between formulations and assess the role of specific compounds in shaping spectral characteristics.

The findings of this study have significant implications for product differentiation and quality control in the RTD tea market. The consistent clustering of Arizona teas across variants (regular, diet, decaf, and lemon) suggests a standardized manufacturing process, which is crucial for maintaining product consistency in industrially produced beverages. Similarly, the distinct profile of Lipton teas highlights the potential for using higher-quality tea extracts or fortification with bioactive compounds to enhance the perceived health benefits of RTD teas. By identifying unique spectral signatures associated with specific ingredients or processing methods, this approach could be used to detect economically motivated adulteration in RTD teas and other beverages. Future research could explore the sensitivity and specificity of this method in detecting common adulterants or substitutions in tea products.

While derivative spectroscopy and multivariate analysis provide valuable insights, there are limitations to consider. Derivative spectroscopy can amplify noise, particularly in the second derivative spectra, which may complicate the interpretation of subtle differences. Additionally, the preprocessing of spectral data, including smoothing and baseline correction, can influence the results. Future studies should explore methods to mitigate these challenges and improve the robustness of the analysis.

Conclusion

This study demonstrates the effectiveness of UV-Vis absorbance spectroscopy, derivative analysis, and multivariate statistical methods in characterizing and differentiating ready-to-drink (RTD) tea samples. The combination of these techniques provided a robust framework for understanding the chemical composition of RTD teas, offering valuable insights for manufacturers in quality control and product differentiation.

Lipton teas consistently showed the highest absorbance and most complex derivative features, indicative of a richer polyphenolic composition. This unique profile was further confirmed through PCA and HCA, which distinctly separated Lipton teas from Arizona and Snapple teas. Arizona and Snapple teas exhibited compositional similarities, with minor differences attributable to sweeteners or flavoring agents. Among the Arizona samples, Arizona Lemon Tea stood out for its reduced absorbance and simpler derivative features, reflecting the impact of lemon juice as a flavor additive.

The combination of spectroscopic and multivariate techniques provides a robust framework for understanding the chemical composition of RTD teas, offering valuable insights for manufacturers in quality control and product differentiation. The findings underscore the role of polyphenols in determining the antioxidant properties and potential health benefits of these beverages. Future research could explore the relationship between compositional differences and sensory attributes, as well as the impact of consumer preferences on product development in the RTD tea market.

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