INTERNATIONAL JOURNAL OF RESEARCH AND INNOVATION IN APPLIED SCIENCE (IJRIAS)
ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue V May 2025

Development of a Traceability Model for Geographical Origin of Kenya's Black Teas Based on Principal Component Analysis of FTIR and UV-Vis Spectral Data

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DOI: https://doi.org/10.51584/IJRIAS.2025.100500059

Received: 30 April 2025; Accepted: 09 May 2025; Published: 11 June 2025

ABSTRACT

Authentic tracing of the origins of black tea is important in the tea market as this helps to extrapolate the quality and thus prices. Tasting on its own can be a misleading test for tea quality and origin tracing since the method can be compromised by subjectivity of the taster and adulteration. Therefore, there is a need to develop an analytical chemical method for tracing tea origin that is void of subjectivity. This study presents a traceability model based on chemical fingerprinting techniques, specifically FTIR and UV-Vis spectroscopy combined with principal component analysis PCA and heat map analyses that can be applied in distinguishing the geographical origins of Kenyan black tea. In this study, processed black tea leave samples were collected from seven Kenya Tea development Authority KTDA, tea factories in tea growing geographical regions in Kenya. The dry black tea samples were characterized by Fourier transform infrared spectroscopy (FT-IR) at a wavelength range of 400-4000cm^{-1} whereas the tea aqueous extracts were analysed within 190-600nm range using Ultra violet visible spectroscopy (UV-vis). The resulting spectral data was subjected to PCA and the heat maps analyses and the clustering used in model development. The output showed clustering patterns among tea samples based on similarities in their chemical constituents. The spectral data revealed the presence of phenolic O-H and other O-H stretching vibration(3100-3400cm^{-1}), C-H peak associated with alkane (2850-2950cm^{-1}, with the asymmetric and symmetric stretching vibration at 2919cm^{-1} and 2851cm^{-1}). The peaks in the range 1590-1620cm^{-1} were identified as vibration frequencies of C=O of catechins and flavonoids. The stretching vibration of C-O associated with carboxylic acids, esters and alcohol was observed at 1032cm^{-1}. The PCA score plot for the FT-IR spectrum showed 82.45% with PC1 (Principal Component 1) contributing 56.15% and PC2 (Principal Component 2) contributing 26.30%. The tea samples from Kericho, Nyeri, Meru and Vihiga had positive score in the PC showing greater variation in characteristics. However, teas from Kericho, Meru, Nyeri and Vihiga exhibited similar characteristics. which were clearly distinct from samples from Bomet, Murang'a, and Kiambu teas. The intra group variance of samples from Murang'a was low indicating uniformity in the quality. This was also confirmed using UV-vis spectral data that showed variability index of 0.09 in PC output indicating relatively low variability compared to neighboring counties. From UV Vis data the samples from Kericho were predominantly situated at the centre of PC2 axis showing less distinct differences in the chemical properties. Overall the findings revealed that black tea samples from the same geographical origin clustered together thus enhancing distinction. The Heat maps from both UV Vis and FT IR data revealed that teas from Vihiga, Nyeri and Bomet had the lowest levels of phytochemicals and macro elements while those from Meru MERKA, had the highest levels followed by samples from Kericho, KERTOP and one from Nyeri NYRGA. From the findings, Meru tea MERKA is a potential standard since has the highest levels of the desired phytochemicals and macronutrients key to quality and origin tracing

INTRODUCTION

Tea, a highly consumed beverage in the world has origins in China and with a history that dates back to 2737 BC [1]. *Camelia Sinesis* belong to the genus *Camila* of the family of *Theaceae* which is the only family of tea.

Tea trees are globally distributed, and there are teas with different tastes depending on geographical regions of origin, and techniques used for processing. Tea is largely classified as black, green, white, yellow, dark and oolong. As of 2023 the global production of tea was approximately 6.6 million metric tons with China contributing 49%, India 20.5%, Kenya 8.2%, Sri Lanka 3.8%, Vietnam and Turkey had 3.2%, Indonesia 2.2%, Iran 2.45%, Argentina 1.6% and Japan 1.3 % [2]. From recent reports black tea and green tea are the most consumed types of tea. The health effects and quality of tea is greatly influence by the type of metabolites or natural products it contains [3]. The quantity and quality of metabolites present in tea are influenced by the region 's climatic conditions that include the, light intensity, temperature of the regions and the varying photoperiod. The type of metabolites mainly includes polyphenols (TPP), total amino acids (TAA), alkaloids and aroma substances [4]. The color and aroma of tea is influenced by the oxidation of tea polyphenols while TPP, TAA and alkaloids contribute to the quality of taste.

In Kenya tea is grown in different zones or regions with varying climatic conditions, soil type and altitudes, among others which causes thus each region produces tea with varying amount of metabolites. In Kenya tea is grown in the highlands and each region produce tea with unique levels of metabolites hence different aromas.

A growing demand for geographical traceability of the origin of tea and quality evaluation of tea to eliminate counterfeits in the market and to ensure that the products getting to the market are authentic. Counterfeits in tea affects branding thus giving misleading information to both the producers and consumers. Mislabeling of tea with prestigious origin labels despite not being produced from that regions, affects the reputation of genuine tea and misleads consumers. To combat such issues, reliable analytical methods are essential for verifying the authenticity and geographical origin of tea [6].

Fourier Transform Infrared (FTIR) spectroscopy and Ultraviolet-Visible (UV-Vis) spectroscopy are non-destructive techniques that provide valuable insights into the chemical makeup of samples. When coupled with multivariate statistical tools such as Principal Component Analysis (PCA), these methods allow for the classification of tea based on its chemical fingerprint [7].

Principal component analysis is a multivariate data analysis method that has the ability to visualize data information based on projection techniques [8] and PCA has been employed successfully in statistical analysis of spectra data of tea samples [9]. FT-IR spectra have high dimensional data with unneeded information. To remove this redundant information and reduce the dimensionality of FT-IR spectra PCA is used [5].

Another tool of spectral data analysis are the Heatmaps which aid to visualize matrix-like data based on variation in color [10], they are categorized into two. These categories are spatial heatmap and grid heatmap Spatial heatmap visualize spatially distributed patterns such as global temperature distribution, on the other hand grid heatmaps is a rectangular layout with two dimension that corresponds to two variable and it has colored grids. In previous studies heat maps have been used to analyze FTIR spectral data of teas and plant materials with the aim of ascertaining origin or similarity or identity [12].

The primary objective of this study was to characterize black tea from selected tea growing regions in Kenya using FT-IR and UV-vis spectroscopy, and to apply Principal Component Analysis (PCA) to cluster different varieties based on their distinct chemical patterns that can associated with geographical origin. This approach seeks to provide scientific basis for tea authentication and help curb the prevalence of counterfeit products in the Kenya tea market.

MATERIALS AND METHODS

A total of twenty-one samples were collected from Kenya Tea Development Agency factories located in geographical diverse tea growing regions in Kenya as shown in details in Table 1 and figures 1 and 2. The processing of tea leaves is standardized across all the KTDA tea factories hence a guarantee for reliable/ unbiased data. Further representation of the sampling regions is indicated on the map of Kenya in Figure 1.

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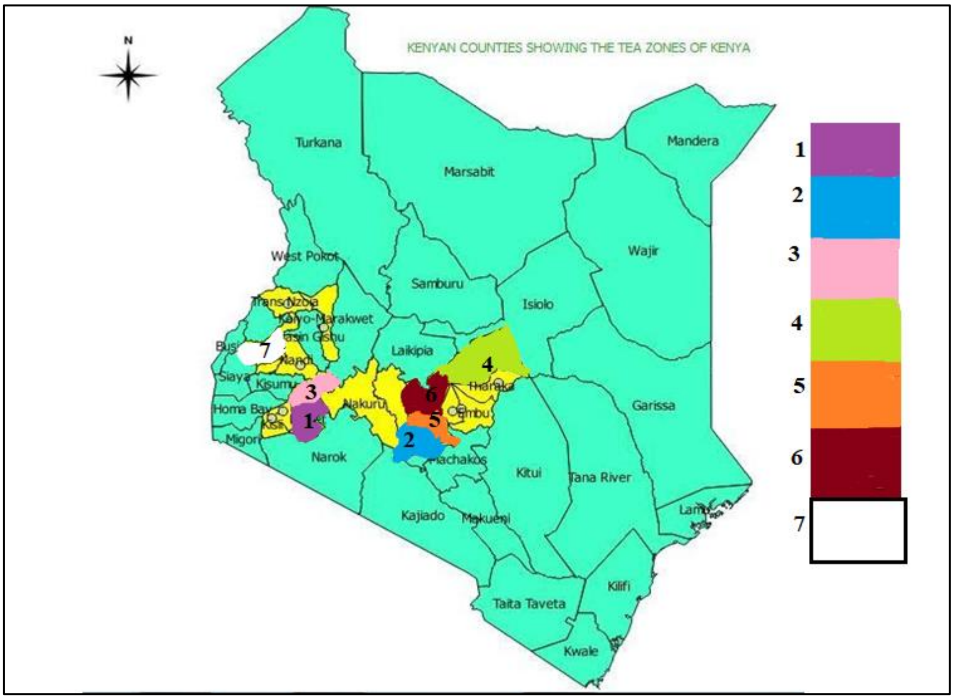

Figure 1. Tea growing zone under study

Tea is grown over the highlands west and east of the rift valley regions in Kenya which are cooler and wetter. These regions are within the altitude range of 1000m-2500m a.s.l (meters above sea level) and with red volcanic soils conditions that are favorable for tea production. Tea being a highland crop grows well where the soils is red volcanic and alluvial have proper drainage as in the sloppy areas. These areas are located mainly in the highlands of Kenya which are above 1,500m.a.s. l [13]. Kericho, Bomet vihiga and Kisii in the Highlands west of the Rift valley and Nyambene hills Meru, Nyeri, Murang'a and Kiambu, in the Highlands east of the Rift valley make the seven tea growing regions in Kenya according to KTDA. Figure 2 below shows a climate map of Kenya

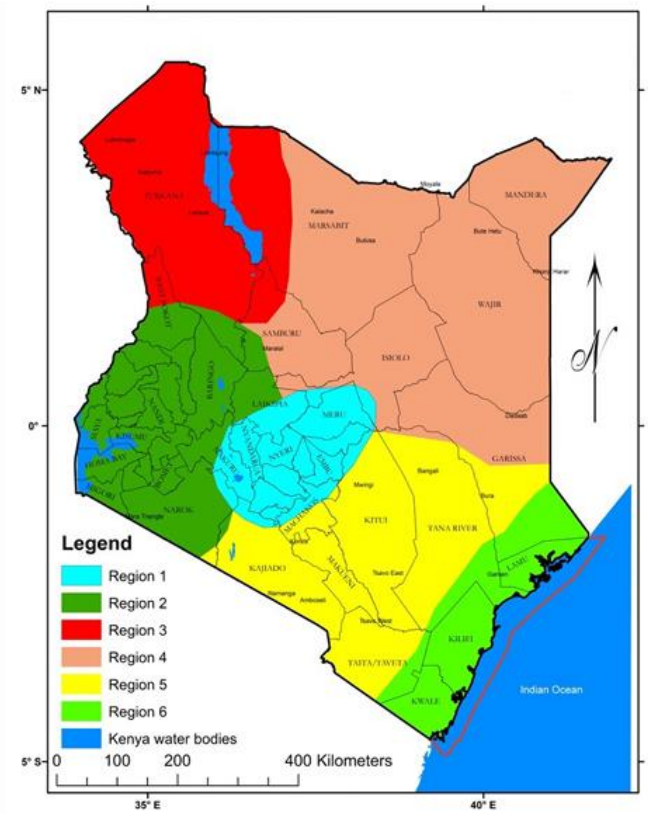

Figure 2: Climatological map of Kenya

Region 1 is in the central highlands and region 2 is Highlands of West Rift Valley and Lake Victoria Basin where the climate Conditions are suitable for tea cultivation [14]

Table 1. Shows the climatic conditions of the Tea growing areas under study

Tea growing region	Soil type	KTDA tea factory	Code 1	Altitude
Nyeri	Red volcanic soil	Iriani	NYRIR	800-2000
		Gathuthi	NYRGA	
		Chinga	NYRGI	
		Karatina	NYRKA	
Murang'a	Red volcanic soil	Gacharage	MURGACH	1500-2700
		Kanyenyaine	MURKAN	
		Gatunguru	MURGAT	
		Makomboki	MURMA	
Meru	Red volcanic soil	Kappa	MERKA	1500-2700
		Imenti	MERMA	
		Weru	MERWE	
Kericho	Deep Alluvial soil	Kerichotop	KERTOP	1800-2500
		Kapkatet	KERKAP	
		Litein	KERLIT	
Bomet	Red volcanic soil	Motigo	BOMO	1500-2700
		Tirgaga	BOTIRG	
Kiambu	Red volcanic soil	Mataara	KIMA	1500-2500
		Theta	KITHE	
		Kagwe	KIKA	
Vihiga	Deep Alluvial soil	Mundete	VIHMU	1500-2000
Kisii		Nyansiongo	KINYA	

METHODS

Preparation of samples

2.0g of each sample was crushed separately using mortar and pestle to obtain a fine powder. The tea samples were sieved to give a homogenous powder as a representative analytical sample for FTIR spectroscopy. For UV-vis analysis 10g of black tea was extracted with 150ml of deionized water. 0.5 ml of extract obtained was further diluted with 10ml of deionized water to lower the concentration to enhance sample transparency to UV-Vis light.

Characterization techniques

Fourier transform infrared spectroscopy (FT-IR) Shimadzu model was used to determine the functional groups present in the tea samples at a wavelength range of 400- 4000cm^{-1}. Ultra violet visible spectroscopy Thermo Scientififc Evolution One Plus model was used at a wavelength range of 200-800 nm and a resolution of 1 nm to generate the UV-Vis spectra of the samples.

To clearly visualize the differences between the tea samples obtained from different tea growing regions in Kenya, FT-IR spectra data was submitted to PCA. Variance contributor causes major differences in PCA score plots, corresponding to the direction of the highest variance. PCA has the ability to reduce the dimensionality of the FTIR data, making it easier to identify patterns and correlations among samples [14].

RESULT AND DISCUSSION

FT-IR spectra of dry tea samples

Figure 2 shows the FT-IR spectral obtained from the analysis of tea leaves collected from the various tea growing regions in Kenya. The spectral data revealed the presence of phenolic O-H and other O-H stretching vibration was observed in the region between 3100-3400cm^{-1} [15], While C-H peak of alkane was observed at 2850-2950cm^{-1}, with the asymmetric and symmetric stretching vibration at 2919cm^{-1} and 2851cm^{-1}. The absorption peaks in the region at 1590-1620cm^{-1} were identified as vibration frequencies of C=O of catechins and flavonoids as observed at 1619cm^{-1}. The stretching vibration of C-O peak associated with presence of carboxylic acids, esters and alcohol was observed at 1032cm^{-}. This peak is normally observed in the region between 1000-1300cm^{-1} [16]

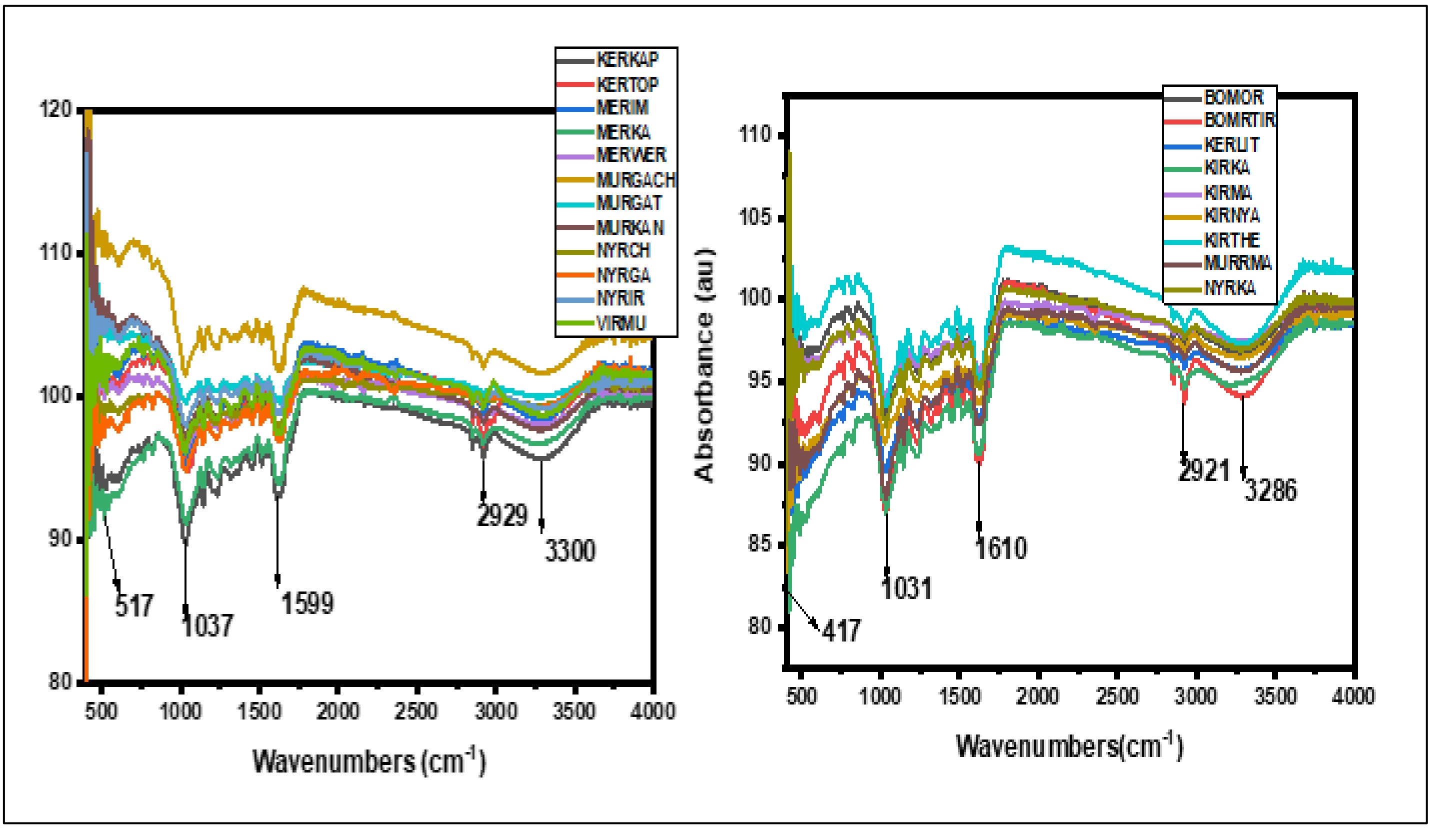

Figure 3. FT-IR spectra of dry tea samples from tea growing regions in Kenya.

PCA analysis of FTIR spectra of dry samples

Figure 3 shows the PCA score plot for the FTIR spectrum of all the tea samples. The two principal components (PC1, and PC2) constructs a two-dimensional score plot which account for 82.45% variance (PC1= 56.15% and PC2= 26.30%). The tea obtained from Kiambu, Murang'a and Bomet region had negative scores in PC1 and those obtained from Kericho, Nyeri, Meru and Vihiga had positive score in the same PC. This suggest that PC1 was associated with greater variance between Kiambu, Kericho, Bomet, Nyeri, Meru, Murang'a and Vihiga. The teas from Kericho, Meru, Nyeri and Vihiga tea had similar characteristics, which is as well distinct from Bomet, Murang'a, and Kiambu teas.

The tea sample from Kiambu and Nyeri regions had both positive and negative PC1 scores. Tealeaves obtained from Kagwe in Kiambu had positive score as compared to other samples from the same region which had negative scores. Similarly, the sample from Iriani in Nyeri had a negative score and the rest of samples from Nyeri region had positive PC1 score. Samples from Kericho were predominantly situated at the centre of PC2 axis showing less distinct differences in the chemical properties. In PC2, samples from central region of Kenya such as Meru, Nyeri and Murang'a had a positive score whereas those from Bomet, Kericho, Kiambu, Kisii and Vihiga had negative PC2 scores. To be noted the intra group variance of samples from Murang'a was lower than from any other region, this means that the quality of tea from Murang'a region is fairly uniform. Intra group separation of samples was easier since the samples did not overlap, thus making PCA analysis technique useful in distinguishing teas from different growing regions in Kenya. The distance between circles imply the difference between and within groups. Closer circles indicate higher similarity.

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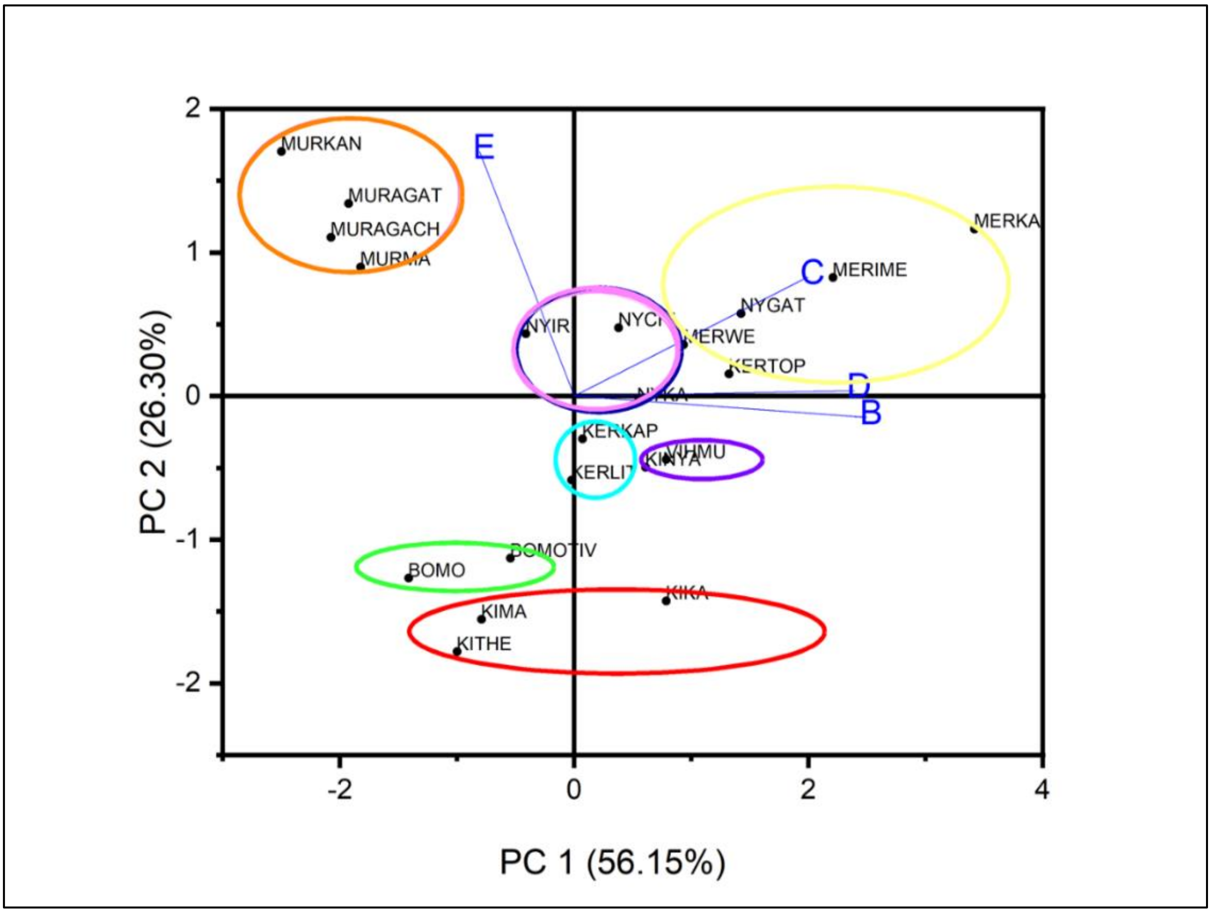

Figure 4: PCA score plot of FT-IR spectra of dry tea samples

Table 2 shows the eigenvalues, which represent the number of variance captured by PCA. The first two PCs account for 82.45% of the total variation. The eigenvalue of each PC revealed that only few variables contributed towards the position of each PC.

Table 2. Show the statistical data for the PCA used to cluster the data in PCA Biplot for FTIR Data.

Principal component Number	**Eigenvalues**	**PC 1**	**PC2**	
		56.15%	**26.30%**	
Eigenvalue	**Eigenvalue**	**Loading Plot Coefficients of PC1**	**Loading Plot, Coefficients of PC1**	**Loading Plot**
1	2.24616	0.613334	-0.07839	B
2	1.05188	0.48979	0.43647	C
3	0.47261	0.58722	0.011956	D
4	022934	-0.19773	0.896609	E

Heatmaps from FTIR spectra of dry tea samples

Figure 4 shows the heatmap of tea samples obtained from 7 geographical regions in Kenya. The column shows the functional groups present in the sample and the row indicates the region from which the samples were obtained. Samples from Kappa in Meru and Kanyenyaine in Murang'a had the highest level of macro elements. These regions have volcanic soil due to their proximities to Mt Kenya, thus contains high levels of macro elements such as copper, manganese, potassium and zinc. Tea samples from Kappa in Meru (MERKA) contain the highest level of carbonyl group C=O, the carbonyl group is associated with polyphenols and flavonoids as seen from the absorptions in the region between 1615-1680cm^{-1} in the FT-IR spectra [17]. The amount of flavonoids and polyphenols in tea extract is affected by factors such as plant maturity stage, extraction mode and post-harvest and processing stage [18]. For samples obtained from Theta, Motigo and Gacharage these factors

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might have contributed to low level of carbonyl group. The relationship between the carboxylate group and carbonyl compounds in all the samples were directly proportional. Sample obtained from Kappa had the highest levels of alkaloids whereas, the sample from Gacharage in Murang'a had the lowest level of alkaloids. The level of O-H absorbed at a region between 3100-3400cm^{-1} were high in most tea samples. The hydroxyl group in tea comes from polyphenols, flavonoids and water contents, these factors are affected by leaf maturity and harvesting season [19]. The soil components and climate in the Meru regions favor the presence of high polyphenols and flavonoids, therefore the tea from this region can be distinguish from tea obtained from other regions. Kappa tea from Meru region has the highest levels of metabolites as observed from the heat maps and thus can be easily distinguished from tea samples from other regions of Kenya.

Teas with the highest levels of macronutrients were from KERITOP, MERKA, MURGACH, MURKAN and NYRGA (Fig. 4). The Murang'a samples i.e. MURGACH and MURKAN are unique since they have lowest levels in all but highest in Macro elements. The other clearly unique tea is the one from Meru, MERKA which has the highest levels of phytochemicals and macro elements, followed by KERTOP and NYRGA. The MERKA tea has potential to be used as the standard for quality assessment of teas produced in Kenya. With confidence we can tease out samples that are poor in both macronutrients and phytochemicals. In general, the teas from Vihiga, Nyeri and Bomet have lowest levels of phytochemicals and macro elements.

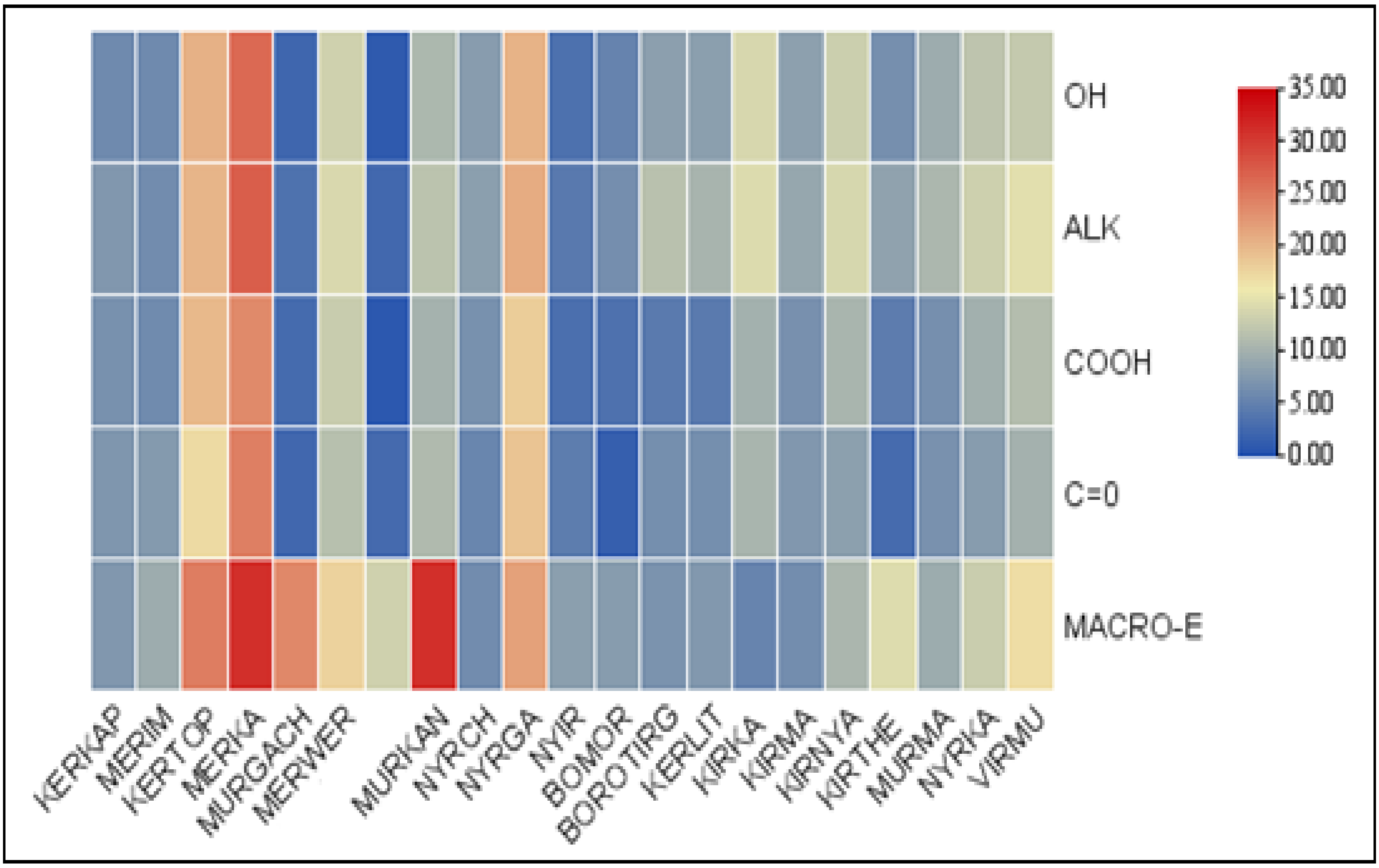

Figure 5. Heatmap plot of FT-IR data of dry black tea samples for major functional groups

Ultra violet visible spectroscopy of aqueous tea extracts

Figure 5, shows the UV-vis spectra of tea leaves obtained from seven tea growing regions in Kenya. The absorbance spectra were obtained at a wavelength of 190-600nm. The absorbance observed in the range of 200-360nm is associated with electronic transition from non-bonding orbital to antibonding orbital in C=O bond. Peaks related to caffeine which is among the main component of black tea is normally observed at a range of 270-280nm. The peaks at a wavelength range of 400 to 800 were almost flat thus lacks useful information. From the UV spectra one can deduce that all the teas generally contain reasonable levels of phytochemicals with carboxylates and carbonyl [20].

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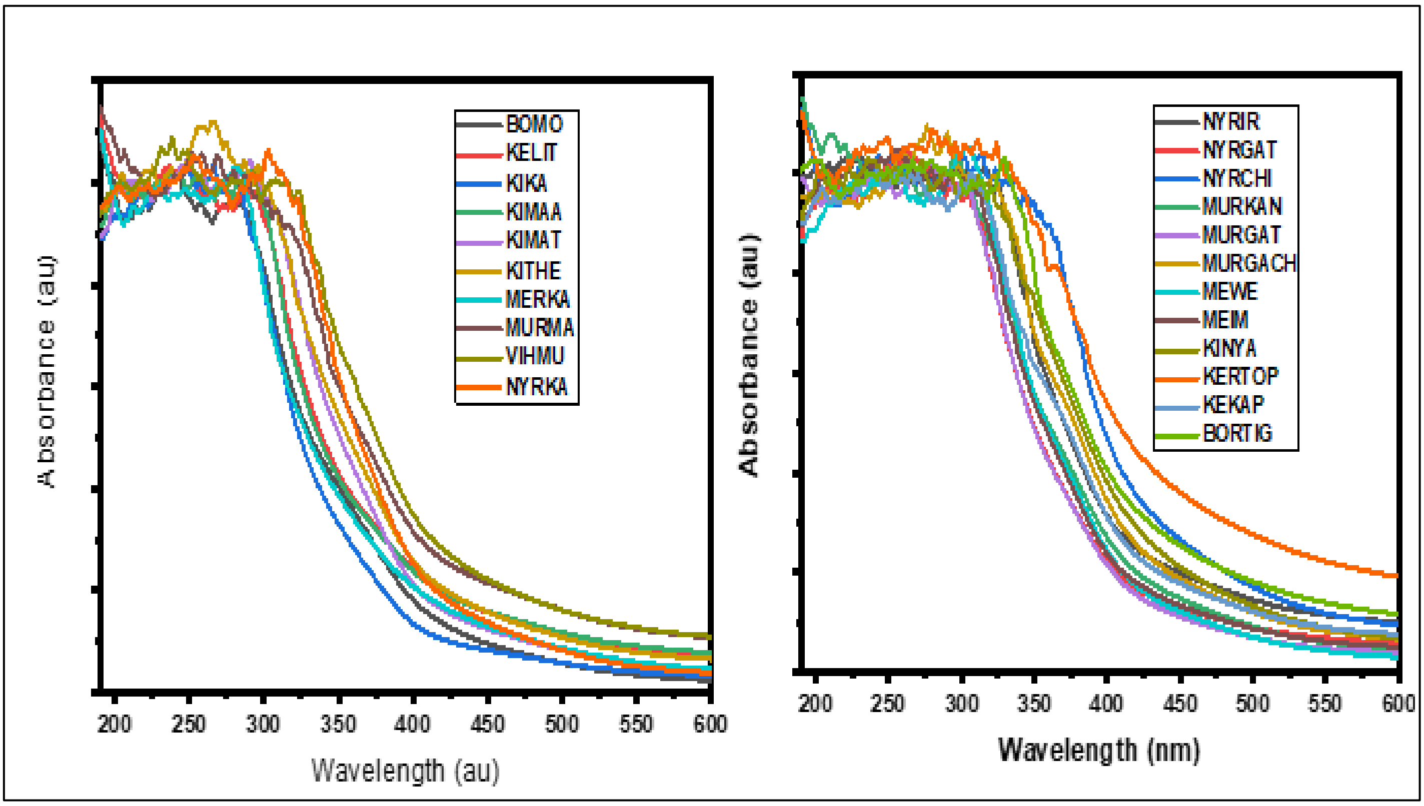

Figure 6. UV-vis spectra of aqueous tea samples

PCA analysis of UV-Vis spectral data of aqueous tea extracts

The UV spectroscopic data in the region between 190-370nm were subjected to PCA after mean centering the data. In order to obtain linear combination of the original variable also referred to as principal components (PC) dimensional data had to be auto-scaled by PCA. Each successive principal component captures the maximum possible amount of residual variance. Therefore, the first principal component (PC1 has the highest variability of data). Figure 6 shows the PCA score plot of UV-vis data, which successfully categorized the tested samples into two clusters PC1 and PC2 computed for 58.39% and 20.09% respectively.

Table 3 Show the statistical data for the PCA used to cluster the data in PCA Biplot for Uv-Vis data

Principal component Number	**Eigenvalues**	**PC 1**	**PC2**	
		58.39%	**20.09%**	
Eigenvalue	**Eigenvalue**	**Loading Plot, Coefficients of PC1**	**Loading Plot, Coefficients of PC1**	**Loading Plot**
1	2.33577	-0.4218	0.69334	B
2	0.80379	0.42154	0.66908	C
3	0.63133	0.57103	-0.17864	D
4	0.22911	0.56419	0.19926	E

	Eigenvalue	**Percentage of Variance**	**Cumulative**
1	2.33577	58.39%	58.39%
2	0.80379	20.09%	78.49%
3	0.63133	15.78%	94.27
4	0.22911	5.73%	100.00%

Tea samples from Murang'a and Vihiga region separates well from other tea samples from other regions, these samples had a positive PC1 score whereas, the samples from Kiambu, Nyeri, Kericho, Bomet and Meru had negative PC1 score. Samples from Murang'a were located at the far centre of PC1 positive score. Samples from Nyeri, Bomet and Kericho were all clustered in the same region. In PC2 score plot, samples from Kiambu, Meru and Murang'a had a positive score whereas those from Vihiga, Kericho, Bomet and Nyeri had negative PC2 scores. The intra-group uniformity of samples from Kiambu and Bomet were very low since the distance between the samples from the two regions is greater, this is possible since the region experience different amount of rainfall, soil fertility and the method used to process tea might have interfere with tea properties. In comparison, the samples from Murang'a had very close similarity confirming that the variability index of precipitation in Murang'a is 0.09, indicating relatively low variability compared to neighboring counties [21]

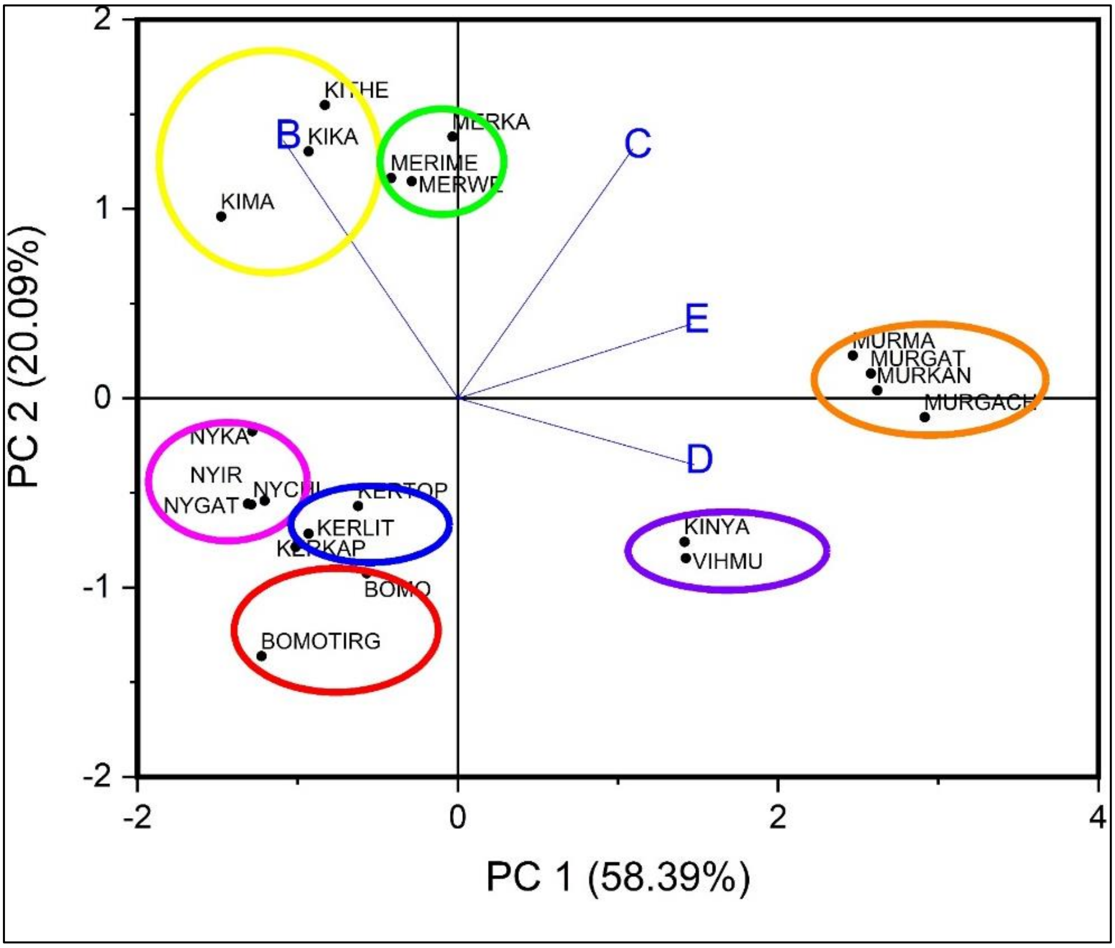

Figure 7. PCA score plot of UV-vis data of aqueous tea extracts

Heat map of UV-vis data of aqueous tea extracts

Figure 5 shows a heatmap of UV-vis data, the absorbance level or intensities is shown by different colors on the grid. Each sample had different absorption rate for the different compounds present in the sample depending on the region where the sample was obtained. The UV-vis absorbance spectra of O-H sample from Imenti (MERIM) and Weru (MERWER) both from Meru region had the highest absorbance of O-H, this is due to presence of volcanic soil and high altitude in the region and this implies that the region had high level of polyphenols and flavonoids contents [22]. Samples from Gacharage (MURGA), Gatunguru (MURGAT) and Makomboki (MURMAK) all from Murang'a region had low level of O-H. For carboxylate content, all samples were found to contain moderate levels; this shows that tea samples were rich in organic acids or glycoside which is associated with the soil in regions where tea leaves are grown. The presence of carbonyl group was uniform for samples obtained from Nyeri region thus heatmap data for C=O could be used easily to distinguish teas from Nyeri from the others regions. Tea samples from Kiambu and Bomet had low level of carbonyl group. Samples obtained from Murang'a region had relatively high level of carbonyl group implying that the samples can oxidize or fermented since it contains low level of catechins.

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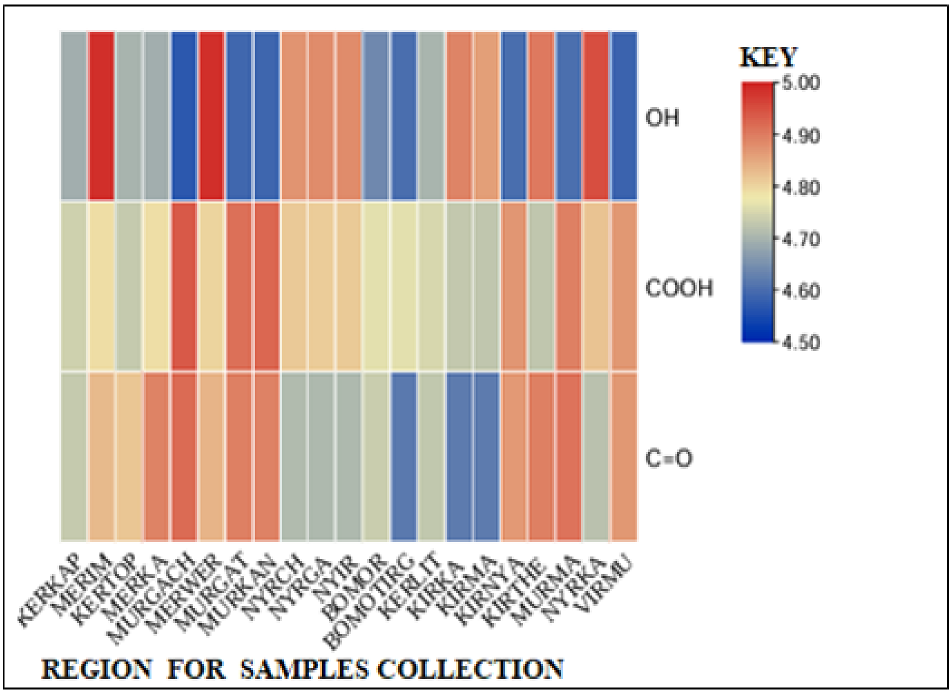

Figure 8. Heatmap of UV-vis spectral data of aqueous extracts

CONCLUSION

This study demonstrates the effectiveness of chemical fingerprinting techniques, specifically FTIR and UV-Vis spectroscopy combined with PCA and heat map analyses, in distinguishing the geographical origin of Kenyan teas. The FTIR-PCA model successfully differentiated tea samples from various regions, with Murang'a (MUR) exhibiting the most distinct chemical profile. Notably, samples from Vihiga (VIHMU) and Kisii (KINYA) clustered closely, likely due to their proximity to the Lake Victoria basin and shared climatic conditions. FTIR-based heat maps further enabled the discrimination of premium teas from specific factories rather than broad regions, with samples from MERKA displaying the highest concentrations of phytochemicals and macro elements, followed by KERTOP and NYRGA.

UV-Vis spectral data, analyzed through PCA, also revealed clear clustering of teas from similar geographical regions, reinforcing the consistency of regional chemical signatures. The UV-Vis heat maps provided additional resolution, distinguishing Murang'a teas by their conspicuously high levels of carbonyl (C=O) and carboxylic acid (COOH) groups and low hydroxyl (OH) content. Similarly, teas from Meru were identified by their elevated levels of multiple functional groups, including phenolic compounds, carboxylic acids, alkanes, and macromolecules, collaborating findings from the FTIR analysis.

It is important to acknowledge, however, that environmental factors such as altitude, rainfall, temperature, soil characteristics, and sunlight exposure are known to significantly influence the chemical composition of tealeaves. While these variables likely contribute to the observed regional differences, their detailed assessment was beyond the scope of this study. On the other hand, tea processing methods at the sampling points have minimal influence on the variance of the data since they are standard methods regulated by Kenya Tea Development Authority Kenya (KTDA) and are strictly adhered to by all KTDA factories in the regions of study. The study has not validated the model using an independent lab test set and the findings have not been compared with findings from other methods of origin tracing, however this is planned for the near future as this is an ongoing project. Future research shall incorporate comprehensive agro-environmental data to improve the robustness and accuracy of the model as applied to Kenyan tea. In addition more advanced methods of data analysis will be adopted in future studies as the results represented herein are preliminary.

Overall, the findings provide a promising foundation for the development of a tea traceability model grounded in region-specific chemical profiles.

ACKNOWLEDGMENT

The corresponding author acknowledges the contributions of the supervisors at Murang'a University of Technology Dr. Warren Andrew Andayi, Dr. Jane Mburu, Dr Sharon Kiprotich and Dr Kimani Shadrack for conceptualization of the study, their guidance and support. The Murang'a University is thanked for allowing James Waweru access to analytical instrumentation and equipment.

Conflict of Interest

The authors declare no conflict of interest

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