YEAR-TO-YEAR DIFFERENTIATION OF BLACK TEA THROUGH SPECTROSCOPIC AND CHEMOMETRIC ANALYSIS

A Thesis Submitted to
the Graduate School of Engineering and Sciences of
İzmir Institute of Technology
in Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE

in Food Engineering

by
Hilal YORULMAZ

December 2024
İZMİR

We approve the thesis of **Hilal YORULMAZ**

Examining Committee Members:

Prof. Dr. Banu ÖZEN
Department of Food Engineering, İzmir Institute of Technology

Prof. Dr. Figen TOKATLI
Department of Food Engineering, İzmir Institute of Technology

Prof. Dr. Kamile Nazan TURHAN
Department of Gastronomy and Culinary Arts, İzmir University of Economics

6 December 2024

Prof. Dr. Banu ÖZEN
Supervisor, Department of Food Engineering, İzmir Institute of Technology

Prof. Dr. Efe SEZGİN
Head of the Department of Food Engineering

Prof. Dr. Mehtap EANES
Dean of the Graduate
School of Engineering
and Sciences

ACKNOWLEDGEMENTS

Firstly, I would like to express my deepest gratitude to my advisor Prof. Dr. Banu ÖZEN for her endless patience, support and knowledge sharing. She was there for me at every moment of this journey and working with her made me realize how precious and highly qualified a mentor she is.

I would like to thank Çağrı ÇAVDAROĞLU for his boundless enthusiasm for teaching—not only for assisting me through the experiments but also for being a constant source of mental support and boosting my belief in myself. I am also grateful to Elif ÇAVDAROĞLU for her warm encouragement and making me feel like part of the family.

I would like to express my heartfelt thanks to Ege ŞİMŞEK. His belief in my goals gave me strength and became my source of motivation. Even though we are miles apart, his loving support always felt so close.

I am thankful to İpek ARKCA, my travel buddy, who has been with me since high school, for sharing these years with me. Additionally, I would like to express my deep appreciation to my lab mates, as well as Başak ÇOBAN and Nazlı EDEN, who made this journey enjoyable.

I am grateful to my family members Arda, Ayten and Bilgi for their unwavering support and love.

Lastly, I also would like to thank the Döhler Gmbh. for providing the tea samples used in this study

ABSTRACT

YEAR-TO-YEAR DIFFERENTIATION OF BLACK TEA THROUGH SPECTROSCOPIC AND CHEMOMETRIC ANALYSIS

In this study, the impact of the harvest year on various spectral profiles of black tea samples was examined, aiming to differentiate black tea samples from two harvest years. Utilizing the advantages of each methodology, the capabilities of mid-infrared, UV-visible and fluorescence spectroscopy have been extensively studied in combination with multivariate statistical methods.

Due to changing climate conditions and seasonal cycles, the composition of food products like tea has become more variable from one harvest year to the next, influencing the overall product quality. Therefore, mid-infrared, UV-visible, and fluorescence spectroscopy were used to examine 205 tea samples that were harvested in 2021 and 2022. Mid-infrared spectra were gathered for both infused and powdered sample forms, whereas only the infused samples were analyzed using the other spectroscopic methods. Partial least-squares discriminant analysis (PLS-DA), orthogonal partial least-squares discriminant analysis (OPLS-DA) and soft independent modeling of class analogy (SIMCA) models were developed to classify the samples by harvest year. Models based on mid-infrared data achieved correct classification rates of 93.33% for powdered samples and 90.33% for infused samples. Additionally, fluorescence and UV-visible spectral data yielded highly accurate results, with success rates of 98.3% and 100%, respectively. Although SIMCA showed lower performance compared to other multivariate methods, these findings suggest that integrating spectroscopic techniques with chemometric approaches can effectively monitor black tea across different years. This could contribute to improved quality control and classification processes in tea production.

Keywords: *Black tea, Infrared spectroscopy, Uv-visible spectroscopy, Fluorescence spectroscopy, Chemometrics*

ÖZET

SİYAH ÇAYIN SPEKTROSKOPİK VE KEMOMETRİK ANALİZLER YOLUYLA YILDAN YILA AYRIMININ YAPILMASI

Bu çalışmada, hasat yılının siyah çayın çeşitli spektral profilleri üzerindeki etkisi incelenerek, iki hasat yılından siyah çay örneklerinin ayırt edilmesi amaçlanmıştır. Her metodolojinin avantajlarından yararlanılarak, orta kızılötesi, UV-görünür ve floresan spektroskopisinin yetenekleri, çok değişkenli istatistiksel yöntemlerle birlikte kapsamlı bir şekilde incelenmiştir.

Değişen iklim koşulları ve mevsimsel döngüler nedeniyle, çay gibi gıda ürünlerinin bileşimi bir hasat yılından diğerine daha değişken hale gelmiş ve genel ürün kalitesini etkilemiştir. Bu nedenle, 2021 ve 2022'de hasat edilen 205 çay örneğini incelemek için orta kızılötesi, UV-görünür ve floresan spektroskopisi kullanılmıştır. Orta kızılötesi spektrumlar hem demlenmiş hem de toz örnek formları için toplanırken, yalnızca demlenmiş örnekler diğer spektroskopik yöntemler kullanılarak analiz edilmiştir. Örnekleri hasat yılına göre sınıflandırmak için PLS-DA, OPLS-DA ve SIMCA modelleri geliştirilmiştir. Orta kızılötesi verilere dayanan modeller toz örnekler için %93,33 ve aşılanmış örnekler için %90,33 doğru sınıflandırma oranlarına ulaşmıştır. Ek olarak, floresan ve UV-görünür spektral veriler sırasıyla %98,3 ve %100 başarı oranlarıyla oldukça doğru sonuçlar vermiştir. SIMCA diğer çok değişkenli yöntemlerle karşılaştırıldığında daha düşük performans gösterse de bulgular spektroskopik tekniklerin kemometrik yaklaşımlarla entegre edilmesinin siyah çayı farklı yıllarda etkili bir şekilde izleyebileceğini böylece, çay üretiminde kalite kontrol ve sınıflandırma süreçlerinin iyileştirilmesine katkıda bulunabilir.

Anahtar kelimeler: *Siyah çay, İnfrared spektroskopisi, Uv-görünür spektroskopisi Floresans spektroskopisi, kemometri*

TABLE OF CONTENTS

LIST OF TABLES ix
LIST OF FIGURES ix
CHAPTER 1. INTRODUCTION 1

CHAPTER 2. LITERATURE REVIEW 3
2.1. Historical Overview and Global Market……….…………..…...…..3
2.2. Production Process of Black Tea 5
2.2.1. Withering 6
2.2.2. Rolling 7
2.2.3. Fermentation (Oxidation) 7
2.2.4. Drying 8
2.3. Chemical Compounds in Black Tea 10
2.3.1. Catechins 9
2.3.2. Theaflavins and Thearubigins 11
2.3.3. L-Theanine 14
2.3.4. Caffeine 15
2.3.5. Gallic Acid 15
2.3.6. Other Components 16
2.4. Features Affecting Black Tea Quality 16
2.5. Spectroscopic Methods 17
2.5.1. FTIR Spectroscopy 19
2.5.2. UV-visible Spectroscopy 21
2.5.3. Fluorescence Spectroscopy 26

CHAPTER 3. MATERIALS AND METHODS 25

3.1. Materials 25

3.2. Infusion of Tea Samples 25

3.3. Methods 25

3.3.1. Fourier Transform Infrared Spectroscopy 26

3.3.3. Fluorescence Spectroscopy 27

3.3.4. Total Phenolic Content 27

3.3.5. Soluble Solids Content 29

3.3.6. Statistical Methods 30

CHAPTER 4 RESULTS AND DISCUSSION 31

4.1. Soluble Solids Content and Total Phenolic Content 31

4.2. Spectral Assignments of Tea Samples 32

4.3. Classification Using Soft Independent Modeling of Class Analogies (SIMCA) 35

4.3.1. Application of SIMCA on FTIR Spectroscopic Data for Tea in Powder Form 37

4.3.2. Application of SIMCA on FTIR Spectroscopic Data for Infused Tea Samples 40

4.3.3. Application of SIMCA on UV-visible Spectroscopic Data for Infused Tea Samples 43

4.3.4. Application of SIMCA on Fluorescence Spectroscopic Data for Infused Tea Samples 46

4.4. Classification Using Partial Least-Square Discriminant (PLS-DA) and Orthogonal Partial Least-Square Discriminant Analyses (OPLS-DA) 49

4.4.1. Application of OPLS-DA and PLS-DA on FTIR Spectroscopic Data for Tea Samples in Powder Form 49

4.4.2. Application of OPLS-DA and PLS-DA on FTIR Spectroscopic Data for Infused Tea Samples 50

4.4.3. Application of OPLS-DA and PLS-DA on UV-visible Spectroscopic Data for Infused Tea Samples 55

4.4.4. Application of OPLS-DA and PLS-DA on Fluorescence Spectroscopic Data for Infused Tea Samples 55

CHAPTER 5. CONCLUSION 62

REFERENCES 63

LIST OF TABLES

Table **Page**

Table 2.1. Four main catechins in black tea ………………..……………………..…11

Table 2.2. Important theaflavins found in black tea (Koch,2020)……………….….…….13

Table 4.1. Soluble solids content of infused tea samples……………….………….…..31

Table 4.2. Total phenolic content of infused tea samples…………….………..……….…..32

Table 4.3. SIMCA results of FTIR-Transmission data for the differentiation of black tea according to harvest year…..…….……………….……………….………………...38

Table 4.4. SIMCA results of FTIR-ATR data for the differentiation of black tea according to harvest year…...……………….……………….....…...……..…………..…..41

Table 4.5. SIMCA results of UV-visible data for the differentiation of black tea according to harvest year.……………...……….……………….…………………………..44

Table 4.6. SIMCA results of Fluorescence data for the differentiation of black tea according to harvest year…...………………......................…………….………………..47

Table 4.7. Statistical parameters of year-to-year discrimination chemometric models of black tea in powder form for FTIR-Transmission data……...……..………..51

Table 4.8. Statistical parameters of year-to-year discrimination chemometric models of infused black tea for ATR-FTIR data…………………………....………..53

Table 4.9. Statistical parameters of year-to-year discrimination chemometric models of infused black tea for UV-visible spectroscopic data………...……..………..56

Table 4.10. Statistical parameters of year-to-year discrimination chemometric models of infused black tea for Fluorescence spectroscopic data……………………………………………...…………...….……………..….58

LIST OF FIGURES

Figure **Page**

Figure 2. 1. Global black tea production in 2022 and predicted production for 2032 (Source: FAO, 2024)................... …………………………………………..……………4

Figure 2. 2. Tea consumption in 2022 (Source: FAO, 2024)………..……..………..…5

Figure 2. 3. Main catechin structure found in black tea (Source: Kuhnert, 2013)....... 11

Figure 2. 4. Main theaflavin structures found in black tea (Source: Pereira-Caro et al., 2017) 13

Figure 2. 5. Caffeine structure (Sahoo, 2015) 15

Figure 2. 6. Basic set-up of FTIR spectroscopy (Source: Nielsen, 1994)........................ 20

Figure 2. 7. Basic set-up of UV-visible spectroscopy (Source: Nielsen, 1994)................ 22

Figure 2. 8. Basic set-up of Fluorescence spectroscopy (Source: Nielsen, 1994).……...24

Figure 4. 1. Spectral data of infused and powdered tea samples (a. FTIR spectra of powdered black tea, b. FTIR spectra of black tea infusions, c.UV-visible spectra of black tea infusions, d.Fluorescence spectra of black tea infusions) ……………………………………………………...………...…….36

Figure 4. 2. Cooman's plot obtained from SIMCA analysis of FTIR-Transmission for the differentiation of black tea according to harvest years (samples harvested in 2021 represented by orange cycles, samples harvested in 2022 represented by maroon circles)………….....…..…39

Figure 4. 3. Cooman's plot obtained from SIMCA analysis of FTIR-ATR for the differentiation of black tea according to harvest years (samples harvested in 2021 represented by orange cycles, samples harvested in 2022 represented by maroon circles)…………………..……..……..……43

Figure 4. 4. Cooman's plot obtained from SIMCA analysis of UV-visible for the differentiation of black tea according to harvest years (samples harvested in 2021 represented by orange cycles, samples harvested in 2022 represented by maroon circles)………………......…..……45

Figure 4.5. Cooman's plot obtained from SIMCA analysis of Fluorescence for the differentiation of black tea according to harvest years (samples harvested in 2021 represented by orange cycles, samples harvested in 2022 represented by maroon circles)………………………………………..…..…...48

Figure **Page**

Figure 4.6. a) Second derivative FTIR-Transmittance spectra of black tea leaves, b) Score plot of OPLS-DA model obtained from second derivative FTIR-Transmittance black tea spectra for the differentiation of two harvest years(samples harvested in 2021 represented by red cycles, samples harvested in 2022 represented by black circles)…………………………………………………………………...52

Figure 4.7. a) Square transformed FTIR spectra of black tea infusions, b) Score plot of OPLS-DA model obtained from square transformed FTIR black tea infusion spectra for the differentiation of two harvest years (samples harvested in 2021 represented by red cycles, samples harvested in 2022 represented by black circles)………….. ……………..……………54

Figure 4.8. a) First derivative+SNV transformed UV-visible spectra of black tea infusions, b) Score plot of OPLS-DA model obtained from first derivative+SNV transformed UV-visible black tea infusion spectra for the differentiation of two harvest years (samples harvested in 2021 represented by red cycles, samples harvested in 2022 represented by black circles)……...............…57

Figure 4.9. a) First derivative transformed Fluorescence spectra of black tea infusions, b) Score plot of OPLS-DA model obtained from first derivative transformed fluorescence black tea infusion spectra for the differentiation of two harvest years (samples harvested in 2021 represented by red cycles, samples harvested in 2022 represented by black circles)……....….….…..59

CHAPTER 1

INTRODUCTION

Tea is the most consumed non-alcoholic beverage in the world after water. With a history spanning thousands of years, tea is consumed by more than three billion people in 160 countries worldwide (Pan et al., 2022). The popularity of tea dates back centuries. The first documented tea records date back to the 3rd century AD; however, it is known to have been consumed even before Christ (Sanlier et al., 2018). Since ancient times, tea has been mostly prepared by brewing the leaves of the *Camelia sinensis* plant with water or milk. In addition to its unique flavor, tea has also been used for medicinal purposes due to its therapeutic and antioxidant properties (Aaqil et al., 2023). Beneficial effects of tea on health such as its antioxidant qualities, heart health benefits, cerebral and cognitive function enhancements have been mostly associated with phenolic compounds (Naveed et al., 2018; Ruxton, 2009). These compounds in tea can vary depending on the type of tea, as different processing methods affect the levels of bioactive compounds found in tea.

Tea can be classified according to its production processes. After harvesting, tea leaves usually go through withering, rolling, fermentation, and drying processes. Tea types are separated from each other, especially during fermentation, and gain their characteristic features during this process (Sharma and Rao, 2009). Black tea accounts for approximately 75% of tea consumption worldwide and is particularly popular in North America and Europe, followed by green tea at 22%, which is more popular in Asia (Zhang et al., 2019). Black tea is completely fermented, while green tea does not undergo fermentation. Throughout the fermenting phase, catechins are converted into theaflavins and thearubigins by oxidation and polymerization catalyzed by polyphenol oxidase (PPO) and peroxidase (POD). During these transformations, the color of the tea leaves changes from green to reddish brown (Chen et al., 2022).

Conventional methods for assessing the quality of black tea such as sensory analysis, have the disadvantage that the evaluation results are greatly affected by human factors and are inevitably subjective even if the participants are professional and well-trained before the assessment (Li et al., 2019). Since the differences in sensory properties are indicative of changes in their chemical structures, it is also possible to notice structures in spectroscopic methods and can provide more accurate and reproducible results. Spectroscopic methods, also known for being non-invasive, fast, and generating minimal waste, are widely used in food analysis. Bioactive chemical compounds such as polyphenols, caffeine and pigments in tea can be determined quickly and objectively by spectroscopic methods and these methods are also quite suitable for monitoring quality changes.

Additionally, as plant product quality may vary in harvest years due to the factors described earlier it is important to monitor annual changes in products. Raw materials may exhibit desirable or undesirable quality characteristics in certain harvest years and the initial determination of quality aspects allows the implementation of necessary measures throughout the processing stages. When acquiring raw materials, processors need to rapidly evaluate quality parameters and make swift decisions by utilizing the rapid analysis capabilities of spectroscopic methods. These methods, can serve as useful tools in studying how the harvest year influences tea characteristic, offering deeper insights into the quality parameters of product.

Spectroscopic methods provide rapid results, and a single measurement can facilitate tea analysis process by replacing multiple chemical analyses. When the full range of spectroscopic measurements is evaluated, the resulting data provide a holistic perspective that incorporates multiple components into statistical analysis. This approach enables classification to be conducted by considering all these compounds collectively, thereby enhancing the success rate of the classification model. Chemometric methods have been used to construct statistical models to effectively interpret spectroscopic methods. This combination provides a more comprehensive understanding of the underlying chemical changes, leading to improved classification models and more accurate prediction of product quality.

With this study, the capabilities of mid-infrared, Ultraviolet-Visible (UV-visible) and fluorescence spectroscopy were comprehensively investigated in combination with multivariate statistical methods by exploiting the strengths of each technique. The main objective of this research is to evaluate the effect of harvest year on black tea and to distinguish black samples from two harvest year by spectroscopic and chemometric methods. This may eventually lead to improved classification models and better quality control methods for tea production.

CHAPTER 2

LITERATURE REVIEW

2.1. Historical Overview and Global Market

Apart from water, tea is the most extensively consumed and affordable beverage worldwide. According to anthropologists, the discovery of native tea trees growing wild in the forests of southwestern China was made by prehistoric people around 2700 BC, making tea one of the oldest beverages in history (Heiss and Heiss, 2011; Pan et al., 2022). Tea leaves were likely discovered during the search for edible plants and found to provide an energizing effect when chewed. After learning how to make fire, people began to boil tea leaves with the other forest plants and obtained various stimulating drinks. Around 1766-1050 BC, tea was used in this region for its medicinal purposes due to its therapeutic and antioxidant properties and was often mixed with the other herbs to treat ailments. Later, around 1122-256 BC, tea was first consumed by boiling it in water alone in the neighboring region, and it was transformed from a medicinal remedy into a bitter but stimulating drink (Heiss and Heiss, 2011). In the following centuries Turkish traders began transporting it westward. In the seventeenth century, it was first introduced in Russia. Later, it spread to the rest of Europe (Ahmed Klasra et al., 2007; Palacios-Morillo et al., 2013). As tea has traveled around the world via trade routes, its production has spread around the world and became a global market. The global tea market is expected to increase at a compound annual growth rate (CAGR) of 5.8% over the forecast period, reaching an estimated \$70.19 billion by 2028 from \$56.12 billion in 2024 (Ltd, 2024). Due to its growing global appeal, approximately 3 billion people are tea drinkers and tea plant grows over 60 nations across six continents (Pan et al., 2022).

The Food and Agriculture Organization (FAO) lists China, India, Kenya, Sri Lanka, and Türkiye as some of the few countries that control the majority of the global market for tea production. China, the birthplace of tea, leads as the world’s largest producer, accounting for nearly half of global tea production. India, the second-largest producer of tea globally, leads the world in black tea production (FAO, 2024). In terms of output of black tea, Türkiye ranked fifth globally in 2022.

Figure 2.1 shows the distribution by country of black tea production worldwide in 2022, with the estimated production amounts in 2032. In terms of tea consumption, Türkiye ranked as the third largest consumer globally, held the top spot in per capita tea consumption, and placed 27th in exports in 2022 (Figure 2.2).

Given these statistics, the tea production of Türkiye meets a significant portion of domestic consumption and keeps the supply-demand balance stable and has an important place in daily life in Türkiye. For a tea plant to grow well, the ambient temperature range needs to be between 18 and 25°C (Aaqil et al., 2023). Due to seasonal suitability and abundant rainfall, the Black Sea region dominates the production of black tea in Türkiye.

.

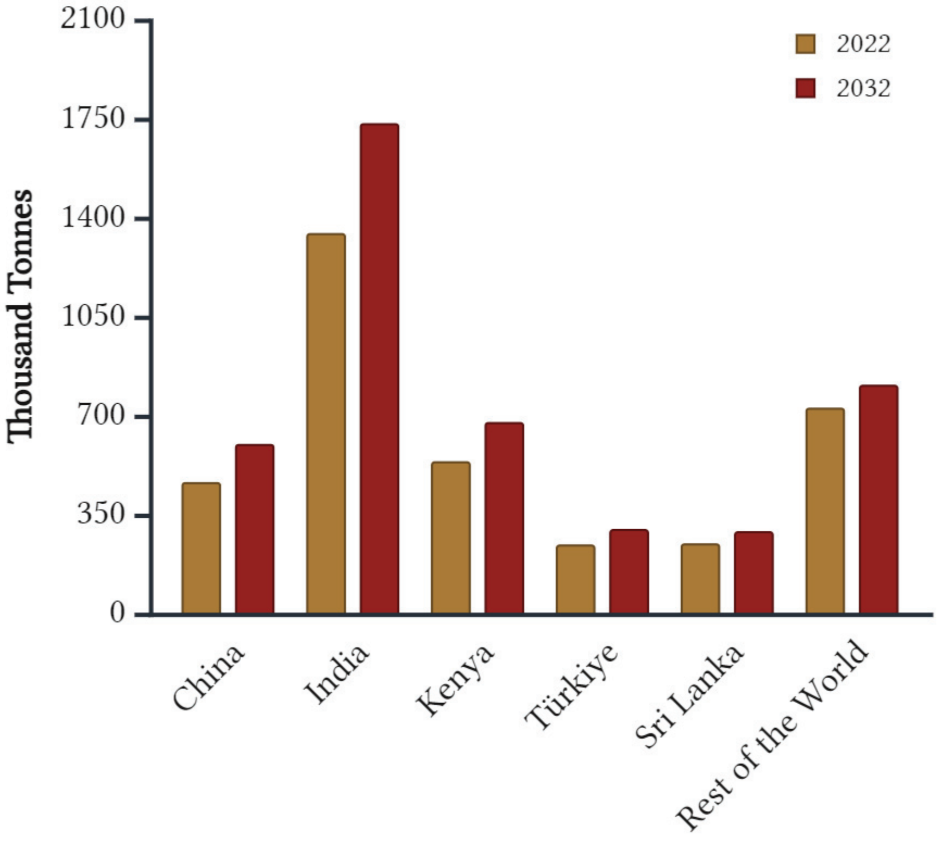


Figure 2. 1. Global black tea production in 2022 and predicted production for 2032 (Source: FAO, 2024).

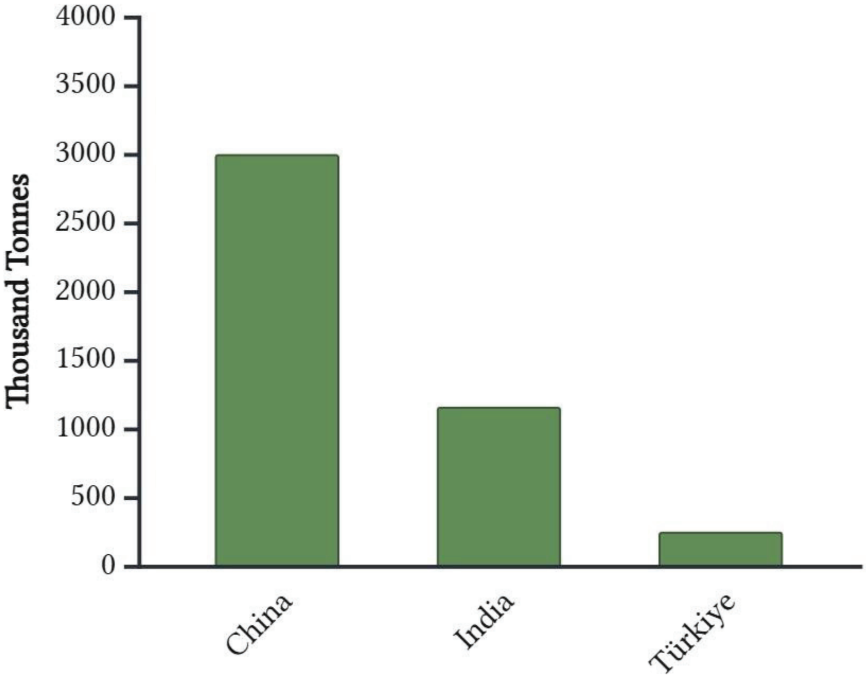


Figure 2. 2. Tea consumption in 2022 (Source: FAO, 2024)

Black tea is the most popular type of tea worldwide and in Türkiye, accounting for 75-78% of global tea production. Green tea follows with approximately 20%, while other varieties, such as yellow and oolong teas, make up the remaining 2-5% (Zhang et al., 2019). Western and some Asian countries consume more black tea, whereas eastern countries prefer other types of tea such as green and oolong (Naveed et al. 2018; Ren et al. 2023; Truong and Jeong 2021). Tea types are shaped based on the manufacturing methods applied after harvesting the fresh leaves of the *Camellia sinensis* plant. The chemical contents of tea types differ from each other due to the different processes that fresh leaves go through during production.

2.2. Production Process of Black Tea

Black tea undergoes full fermentation, unlike green tea. This fermentation process is the main factor behind the chemical and sensory characteristics of black tea, though other processing steps also contribute to its composition. Fermentation in tea manufacturing refers to the organic browning process catalyzed by enzymes in tea leaves and is an oxidation reaction as opposed to any other type of fermentation or an exogenous process (Harbowy et al., 1997). The degree of fermentation is determined by the color changes of the tea leaves caused by oxidation (Kim et al., 2011). Following harvest, black tea undergoes a series of processes including withering, rolling, fermenting, and drying. Each step from plucking fresh tea leaves to making the product ready for consumption has a significant impact on characteristics and quality attributes of the product.

2.2.1. Withering

Withering is the process after the leaves are collected. In this step, the tea leaves are usually blown with air to reduce the moisture content of the leaves and to give them flexibility. At this stage, variables such as withering temperature and withering time affect the quality of the tea (Deb and Jolvis Pou, 2016). In the withering process, air is usually blown at 25-30 °C for about 12-16 hours (Macheka et al., 2022). In this method, the moisture content in the leaves drops from 80-70% relative humidity to 70-60% (Deb and Jolvis Pou, 2016).

The reactions that form the basic characteristics of the finished product begin at this stage. When tea leaves wither, they undergo both chemical and physical changes. Proper withering is vital to enhance flavor, aroma and other characteristics. The physical change in tea consists of changes in its structure due to the loss of water in the leaf with controlled air flow in the environment. When the leaves lose some of their water content, the leaf becomes looser with the decrease in pressure. The tea leaves are more flexible after withering and following the breaking process, rolling, takes place. This process also leads to an increase in the sap concentration in the cells of the tea leaf (Aaqil et al., 2023).

The chemical changes in tea begin immediately after the tea is picked; therefore, they are independent of moisture and dependent on temperature and time. During withering, the concentration of enzymes in the cell increases due to the decrease in cell sap. One of the most important enzymes whose concentration increases during this process is polyphenol oxidase (PPO). Activity of PPO fluctuates during the manufacturing period (Bortolini et al., 2021). As moisture decreases during withering, PPO activity increases, facilitating the oxidation of catechins into flavonoids and thearubigins. At the end of withering, PPO activity reaches a level, which is 2.9 times that of fresh leaf PPO activity (Zou et al., 2024). Another enzyme that becomes active is the peptidase. The peptidase enzyme catalyzes the breakdown of some proteins into amino acids. Carbohydrates are also broken down like proteins and are converted into simple sugars that react with amino acids. Each of these reactions serves as a precursor for flavor formation (Deb and Jolvis Pou, 2016).

2.2.2. Rolling

During the withering process, tea leaves lose water, causing their cell walls to become flexible. The leaves are then shaped through the rolling process. After tea leaves undergo rolling process, the deformation of cell structure of tea initiates oxidation. There is no reaction prior to this step since the oxidase enzymes and substrates are in distinct media (Aaqil et al., 2023). When the external physical force applied during rolling damages the tea leaves, the enzymes whose activity begins to increase during withering begin to come into contact with the substrates (Chiang et al., 2022; Zhang et al., 2023). In addition, the reduction of leaf size causes an increase in the surface area in contact with oxygen. As the tea leaves are bent and crushed while rolling, the cell sap is squeezed out and covers the leaf surface. Phenolic compounds, PPO and oxygen in the tea leaf damaged by external physical force come into contact in the same environment (Zhang et al., 2019). Polyphenol and oxygen contact is achieved at this stage. This action ensures that substrates and enzymes are thoroughly mixed, supporting the biochemical reactions necessary for tea oxidation. The PPO enzyme, which plays a critical role in oxidation, continues to increase its activity during the rolling process and reaches the highest level of activity in the entire production line (Chiang et al., 2022).

2.2.3. Fermentation (Oxidation)

During oxidation, which is one of the important processes in tea production, the components found in fresh tea leaves are oxidized and/or polymerized or undergo other changes during the process. At the end of this process, 80–85% of the leaves turn black. The oxidation process is one of the most vital processing procedures for tea because it is at this stage that the aroma and value of the tea are determined (Sharma and Rao, 2009).

The oxidation stage also separates different categories of tea. During fermentation, catechins oxidized into theaflavins (TF) followed by thearubigins (TR) through enzyme-catalyzed reactions (Koch, 2020).

In black tea production, two main enzymes play an important role during fermentation. One of these enzymes, PPO, oxidizes flavonoids (catechins) to compounds such as theaflavins and thearubigins (Samanta et al., 2015).

The other enzyme, peroxidase (POD) is an enzyme that plays a role in oxidative processes by reacting with peroxides such as hydrogen peroxide (H_2O_2) formed by the action of PPO on some flavonoids. POD activity in fresh tea leaves is five times higher than the activity of PPO and increases during black tea processing (Abudureheman et al., 2022).

Catechins and caffeine in tea leaves play an important role in determining the quality of black tea. TFs determine the brightness, vibrancy and quality of the tea liquor, while TRs are responsible for the color, body and flavor of the tea. Proper fermentation ensures a balanced ratio between TF and TR compounds, resulting in ideal quality (Rahman et al., 2020). In addition, an optimum fermentation ensures a proper balance between TFs and TRs. On the other hand, the presence of catechins (flavonoids) and caffeine in tea leaves also play an important role in determining the quality of black tea (Ruxton, 2009).

2.2.4. Drying

After fermentation, the leaves are dried to stop the enzymatic oxidation processes. Drying is usually the final stage of tea processing. Leaves are exposed to a minimum of 80°C to ensure aroma and flavor stability; however, temperatures above 110°C can negatively affect the quality (Temple et al., 2001). During drying, chemical changes are driven by heat rather than enzymes, making temperature a crucial factor. This process influences the tea's appearance, flavor, and taste by altering and breaking down its original compounds (Guo et al., 2018; Zhang et al. 2019). First, depending on the type of tea, the leaves are dried at a high temperature of around 100°C and then dried at a lower temperature around 75°C (Sharma and Rao, 2009). The final moisture content in black tea drops to around 3%, which also ensures that the teas are preserved throughout their shelf life. However, drying processes lead not only to water loss, but also to the reduction of some volatile substances, the combination of polyphenols with other compounds, the increase of carboxylic acids and the color change by the Maillard reaction (Sharma and Rao, 2009; Zhang et al., 2019). While many of these changes contribute positively to the quality of the tea, some can have undesirable effects. When PPO and POD enzymes are deactivated, biochemical reactions largely cease. In the early stages of drying, enzymes become active, and reactions accelerate as the temperature increases, but these processes cease when the temperature reaches a level where the enzymes become inactive (Aaqil et al., 2023).

2.3. Chemical Compounds in Black Tea

Tea is a widely preferred beverage worldwide for its stimulating and unique taste. Previous studies have confirmed that the majority of tea's nutritional benefits come from its bioactive compounds, such as flavonoids and phenolic acids. These compounds exhibit a range of biological functions, including antioxidant, antimicrobial, and anti-inflammatory activities, immune function enhancement, cancer risk reduction, and protective effects against diabetes, hyperlipidemia, and obesity. (Bortolini et al., 2021; Li et al., 2013; Sharma and Rao, 2009; Suryoprabowo, 2024). In addition, these compounds are associated with tea’s astringency, bitterness, sweetness, and saltiness, and determine its sensory properties (Ren et al. 2023). Specifically, black tea contains flavonoids, phenolic acids, caffeine, carbohydrates, vitamins A, C, K and theanine (Li et al., 2013). Moreover, flavonoids have important potential in antioxidant regulation of apoptosis (Naveed et al., 2018). Apart from its numerous health advantages, tea is widely utilized in the food sector for food coloring, preparation of ready to drink products and edible coatings (Suryoprabowo, 2024).

2.3.1. Catechins

Catechins are colorless, water-soluble compounds that add astringency to infused samples. Catechins are a type of flavonoid and belong to the class of polyphenols, specifically the flavan-3-ols. The basic structure of catechins is characterized by three rings: two benzene rings (A and B) and a dihydropyran ring (C) that carries a hydroxyl group. This core structure is also known as the C6-C3-C6 structure.

There are four main catechins in fresh tea leaves (-)-epicatechin (EC), (-)-epicatechin gallate (ECG, (-)-epigallocatechin (EGC) and (-)-epigallocatechin gallate (EGCG) and their chemical structures are presented in Figure 2.3 and Table 2.1. (Balentine et al., 1997; Sang, 2015).

Tea catechins are prone to oxidation when exposed to enzymes, acids, and heat. During black tea production, the presence of PPO leads to the rapid oxidation of catechins, resulting in the formation of more complex theaflavins and thearubigins (Lee et al. 2016; Łuczaj and Skrzydlewska, 2005). Since catechins exhibit significant level of oxidation, concentration of catechins decreases with more processing, resulting in less catechin content for black tea compared to green tea (Bortolini et al., 2021; Vuong et al., 2010). In a study conducted by Liang et al. (2003), supporting this information, the average EGCG concentration in black tea corresponds to 8.4% of the concentration in green tea. However, the overall polyphenol content of black tea is similar between the two (Tanaka and Matsuo, 2020). Four main catechins compose almost 30% of the dry weight of fresh tea leaves while in black tea extract, this ratio is around 10%. Along with this, theaflavins and thearubigins constitute between 10 and 30% of black tea extract (Abudureheman et al., 2022; Kuhnert, 2013; Yang and Liu, 2013).

There are two types of catechins according to their chemical structure namely free and esterified forms. Free catechins (EC, EGC) have considerably less astringent and sweeter taste than esterified catechins (ECG, EGCG) (Vuong et al., 2010). During the complete breakdown and transformation of most catechins in black tea, especially during oxidation, gallic acid is released from the galloyl parts of these compounds (Lee et al., 2016). As a result of this transformations, trans-catechins, which are more commonly found in green tea, are rarely found in black tea. Instead, in black tea main constituents are gallic acid, caffeine, and theaflavins which provide its distinctive flavor, color, and bioactivity (Zhang et al., 2019). According to the literature, epicatechin quinone further oxidizes the theaflavins formed during black tea fermentation, contributing to the synthesis of thearubigins (Lee et al., 2016).

Table 2.1. Four main catechins in black tea (Source: Sang, 2015)

Catechin		**R_1**	**R_2**
Epicatechin	EC	H	H
Epicatechin-3-O-gallate	ECG	H	Galloyl Group
Epigallocatechin	EGC	OH	H
Epigallocatechin-3-O-gallate	EGCG	OH	Galloyl Group

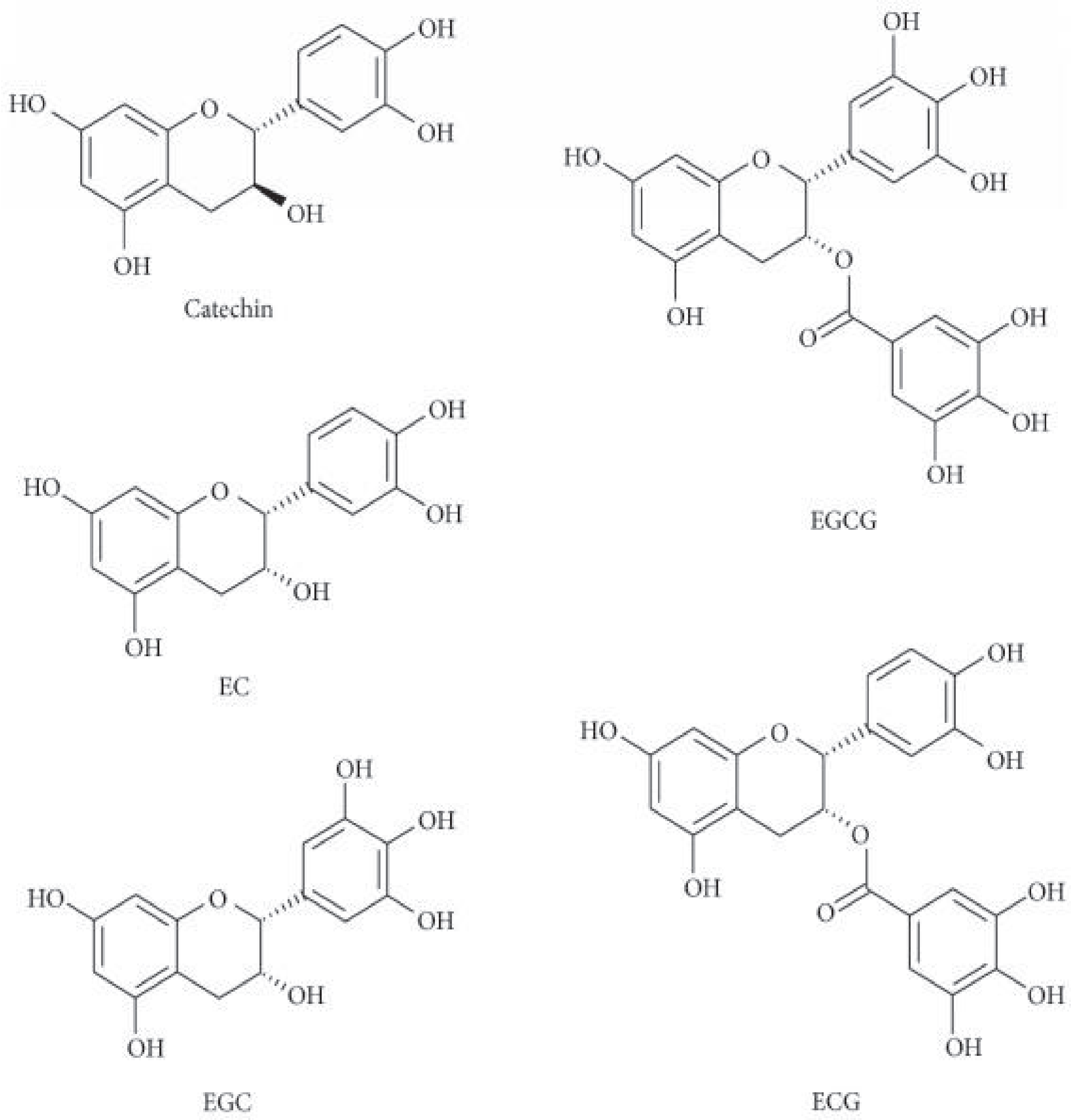


Figure 2. 3. Main catechin structures found in black tea (Source: Kuhnert, 2013)

2.3.2. Theaflavins and Thearubigins

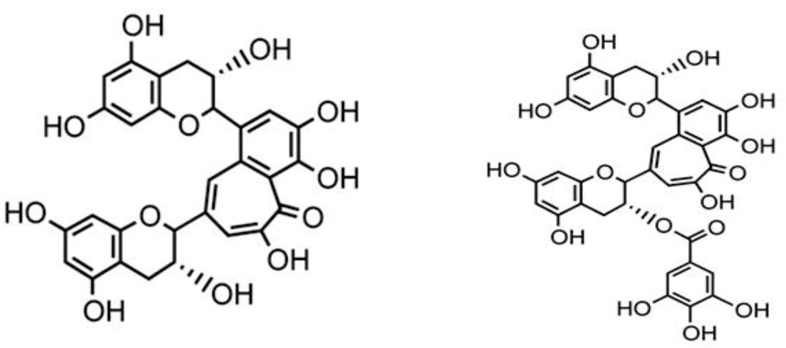
The two main polyphenols, theaflavins and thearubigins, formed during oxidation of tea leaves are important regulators of apoptosis, cell proliferation, and aging in human cancer cell lines. They also have the potential to function as antioxidants (Naveed et al., 2018). Moreover, it has been known for nearly half a century that theaflavin content is an important chemical compound in determining the quality of black tea (Liang et al., 2003). Thearubigins constitute the majority of the total flavonoids in black tea, accounting for 50–60%, while theaflavins account for 10% (Zhang et al., 2019). These two compounds also play an important role in the sensory properties of black tea. Theaflavins give bright orange-red color while thearubigins give dark-brown color to tea (Abudureheman et al., 2022; Lee et al., 2016). Furthermore, an optimal fermentation duration is crucial for preserving the quality and sensory properties of black tea. The theaflavins-to-thearubigins ratio should ideally range between 1:10 and 1:12 (Rahman et al., 2020).

Theaflavins are dimerized catechins and conversion of catechins to theaflavins is a two-step process (Table 2.2 and Figure 2.3). Catechins found in fresh leaves are oxidized to quinones, which are already present in fresh leaves and whose activity increases during the process and they serve as substrates for subsequent transformations by the enzymes PPO and POD. In the second step, the catechin quinones formed can interact with one another through various pathways.

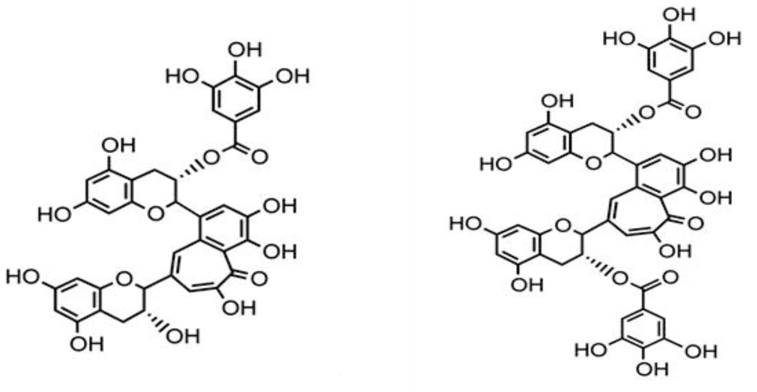
Theaflavins are formed by the polymerization reaction of EC or ECG quinones and EGC or EGCG quinones. The PPO enzyme is responsible for converting flavanols into theaflavins and thearubigins, whereas the POD enzyme oxidizes phenols to quinones using hydrogen peroxide. POD activity is typically higher in fresh tea leaves and increases further during the production process. However, the role of peroxidase in the synthesis of complex polyphenols in black tea has not yet been fully understood (Koch, 2020). As a result, the four major epicatechins are oxidized to four major theaflavins namely, theaflavin, theaflavin-3-gallate, theaflavin-3'-gallate and theaflavin-3,3' gallate (Lee et al., 2016; Łuczaj and Skrzydlewska, 2005). It has been reported that in black tea infusions, theaflavins primarily enhance brightness, while theaflavin gallates contribute more to tea cream formation. Additionally, theaflavin content is associated with variations in tea flavor (Liang et al., 2003).

Table 2.2. Important theaflavins found in black tea (Source: Koch, 2020)

Theaflavin	**R_1**	**R_2**	**Monomers**	**Percent (%)**
Theaflavin (TF_1)	OH	OH	EC+EGC	18
Theaflavin-3-gallate (TF_2A)	Galloyl Group	OH	EC+EGCG	18
Theaflavin-3'-gallate (TF_2B)	OH	Galloyl Group	EGC+EGC	20
Theaflavin-3,3'-digallate (TF_3)	Galloyl Group	Galloyl Group	EGC+EGCG	40



Theaflavin Theaflavin-3-gallate



Theaflavin-3’-gallate Theaflavin-3,3’-gallate

Figure 2.4. Main theaflavin structures found in black tea (Source: Pereira-Caro et al., 2017)

Thearubigins, the other important phenols in black tea, constitute the largest active ingredient portion of black tea. Therefore, it is known that most of the catechins are converted to thearubigins due to oxidation, however information about thearubigins is limited and their chemistry is not sufficiently understood because of their high molecular weights (Zhang et al., 2019). In fact, the general opinion is that theaflavins are involved in the formation of thearubigins (Rahman et al., 2020; Truong and Jeong, 2021; Zhu et al., 2021).

Various studies have reported that theaflavin reaches its highest level during fermentation and then begins to decrease. It is suggested that this decrease may be due to the degradation of theaflavins or their transformation into thearubigins (Aaqil et al., 2023; Lee et al., 2016). However, it is not possible to explain all thearubigin content solely through this transformation, indicating the involvement of other complex oxidation reactions during tea fermentation (Long et al., 2023). Thearubigins have been reported to exhibit several health benefits, including anti-cancer, cardiovascular disease reduction, antioxidant, and anti-inflammatory effects (Kuhnert, 2013). These polyphenolic compounds also contribute to the characteristic color, astringency, and body of black tea, enhancing its sensory attributes (Aaqil et al., 2023; Long et al., 2023).

2.3.3. L-theanine

Theanine, the major amino acid present both in green and black tea, is a non-proteinogic amino acid specific to tea and comprises half of the amino acids in tea. It plays a significant role in contributing to the umami taste of black tea, enhancing its sensory profile (Guo et al., 2018; Li et al., 2013; Vuong, 2014). Previous studies have shown that theanine increases cognitive function, reduce anxiety and assist in managing obesity in humans (Sari and Velioglu, 2013).

Consuming L-theanine, a key component in black tea, orally may have anti-stress benefits by inhibiting cortical neuron excitability, which reduces stress on the body and mind (Suryoprabowo, 2024). However, it has been determined that theanine content decreases throughout the tea processing due to oxidation and enzymatic reactions. Therefore, less theanine is detected in black tea compared to other tea types. Despite this decrease, theanine remains a bioactive compound that contributes to the health benefits of black tea consumption. (Bortolini et al. 2021; Sari and Velioglu, 2013).

2.3.4. Caffeine

Caffeine is an alkaloid compound with the chemical formula $C_8H_{10}N_4O_2$. Its structure consists of a fused ring system with two rings: a purine skeleton known as xanthine (Figure 2.5). Caffeine is another important compound found in black tea that triggers fat burning and reduces mental fatigue, in addition to having a fat-soluble stimulant effect (Bortolini et al., 2021). As a member of the methylxanthine group, caffeine is the primary chemical that gives tea its stimulatory action (Sari and Velioglu, 2013). Caffeine is well known for its stimulant properties and its ability to improve cognitive abilities. Caffeine has been suggested to change dopaminergic transmission and boost serotonin release and it is also in charge of giving the black tea taste with theaflavin (Vuong, 2014). The temperature, brewing time, and leaf size all affect how much caffeine is in tea drinks. Additionally, the caffeine contents of black and green tea is the same, suggesting that caffeine remains highly stable throughout the fermentation process (Sang, 2015).

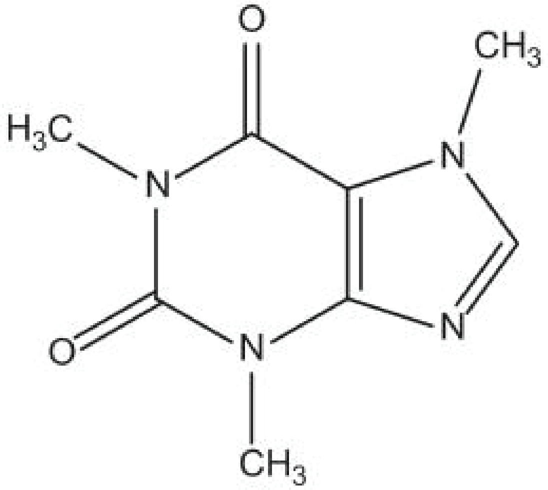


Figure 2.5. Caffeine structure (Source: Sahoo, 2015)

2.3.5. Gallic Acid

Gallic acid, a naturally occurring polyphenolic compound, has the chemical formula $C_7H_6O_5$ and contains a benzene ring. It is a type of phenolic acid found in a variety of plants, teas, and fruits, known for its antioxidant and antimicrobial properties. During black tea production, nearly all catechins decrease, whereas gallic acid levels increase, as catechins undergo an oxidation reaction during processing, their galloyl moieties are broken down, resulting in an increase in gallic acid content (Lee et al., 2016). The ester hydrolysis of the 3-galloyl catechins, during fermentation, greatly raises the amount of gallic acid in black tea (Li et al., 2013). Gallic acid can interact with oxidized catechins to generate the quinone form (Łuczaj and Skrzydlewska, 2005). In addition, gallic acid undergoes oxidation to create epitheaflavic acids, which then condense with theaflavins to generate thearubigins (Rahman et al., 2020).

2.3.6. Other Components

Fresh tea leaves also contain large molecules that do not dissolve in water, such as carbohydrates and proteins. Tea contains a significant amount of carbohydrates, with about one-third being cellulosic fiber. While tea leaves also have small amounts of free sugars, the majority of the carbohydrates are insoluble and do not dissolve in the brewed tea (Harbowy et al., 1997; Vuong, 2014). Tannins are a group of water-soluble polyphenols present in many plant-based foods. They represent a diverse class of compounds and offer a range of health benefits (Khasnabis et al., 2015).

2.4. Features Affecting Black Tea Quality

The elements that provide the rich aroma and taste of black tea are not limited to the processing alone. Also, the composition of the tea varies depending on the soil, seasonality of the plant, growth location, and agricultural practices, and potential taste and odor differences occur depending on these factors (Vuong, 2014). Especially in the last decades, changes in average precipitation and climate conditions due to global warming, and changes such as increasing surface temperature of the Earth have also reduced agricultural productivity. In a study conducted by Jayasinghe and Kumar (2021), it was reported that climate change has increased significantly in recent years and this situation will have unexpected effects on tea cultivation.

The changes in chemical properties of plant products from year to year have affected the quality of tea products. For instance, variations in climate-related factors such as seasonality, water availability, geographical conditions, light exposure, altitude, herbivore activity, microbial presence, temperature, and soil characteristics have been recognized as contributing to changes in secondary metabolites. These changes can cause the levels of these compounds to fluctuate, potentially increasing or decreasing by as much as 50% (Ahmed et al., 2019). Changing climate can threaten regional economies by causing changes in tea types (Jayasinghe and Kumar, 2021). Additionally, as plant product quality may vary in harvest years due to the factors described earlier it is important to monitor annual changes in products. Raw materials may exhibit desirable or undesirable quality characteristics in certain harvest years.

Climate change can cause some changes in the quality of tea by affecting seasonality. It has been stated in previous studies that contents of compounds such as polyphenols, volatiles and caffeine vary according to the season (Ahmed et al., 2019; Jayasinghe and Kumar, 2021; Li et al., 2013). Previous studies have found that increasing carbon dioxide gas due to climate change reduces caffeine and free amino acids in tea leaves (Jayasinghe and Kumar, 2021; Wang et al., 2011; Wei et al., 2012).

Changes in chemical composition are evident in the sensory properties. Sensory and physical and chemical component tests have long been preferred to determine the quality and content of tea. Even though the participants in sensory analysis are professional and well-educated, human factors are inevitably subjective. Moreover, in physical and chemical component analyses, there is a possibility of using toxic and hazardous chemicals and they can be time-consuming (Li et al., 2019; Ren et al., 2023). Rapid evaluation of tea quality is an essential element of supply chain management for this high volume of production and consumption (Panigrahi et al., 2016). Therefore, instead of these traditional methods, spectroscopic methods, known for being fast, non-invasive, and producing minimum waste, have begun to be widely used in food analysis.

2.5. Spectroscopic Methods

Spectroscopy involves generating, measuring, and interpreting spectra that arise from the interaction between electromagnetic radiation and matter (Nielsen, 1994). A wide range of spectroscopic techniques exists, each designed to address diverse analytical challenges. These techniques are applied in both qualitative and quantitative analysis of various tea products and are often combined with chemometric methods in order to classify, distinguish and interpret the results. For this reason, the combination of spectroscopic profiles with chemometrics has been used in the determination of quality parameters and variety separation in teas as well as in various foods. The concentrations of quality parameters, including phenolic and flavonoid compounds, pigments, caffeine, and pesticides, were assessed from coupling various and chemometric methods (Panigrahi et al., 2016; Wang et al., 2019). Near infrared (NIR) hyperspectral imaging technique was effectively used to distinguish black tea samples based on their storage years, while the effectiveness of both traditional machine learning methods and deep learning techniques to classify these tea samples was evaluated (Hong et al., 2021). In another study, NIR spectroscopic data was evaluated using multivariate statistical analysis techniques, leading to a classification based on tea varieties (Chen et al., 2020). NIR spectroscopy used to verify the authenticity of tea is another application field. This spectroscopic technique was used to examine the mixing of Darjeeling tea with other varieties of tea by analyzing the data using chemometric techniques (Firmani et al., 2019) In determining some properties of teas, more than one spectroscopic method has been used together. In a study conducted by Dankowska and Kowalewski (2019), it is shown that black, green, white, yellow, dark and oolong teas produced by different methods were characterized by using NIR, UV-visible and synchronous fluorescence spectroscopy together. In another study, the combination of Raman spectroscopy and Fourier transform infrared (FTIR) spectroscopy brought the feasibility of rapid tea catechin detection and verification for high-quality tea products (Xia et al., 2020). Using more than one spectroscopic method together can reduce the error rate and increase the reliability, and methods in different wavenumber ranges can complement each other (Arifah et al., 2022; Dankowska and Kowalewski 2019; Xia et al., 2020).

In the current study, three different approaches were utilized which focused on different molecular properties and offered complimentary insights into the samples. Functional groups and bonds can be found via FTIR spectroscopy, which also provides precise molecular structures. Tea's primary quality indicators, pigments and phenolic chemicals, are especially sensitive to UV-visible spectroscopy. When it comes to identifying specific polyphenols and other fluorescent chemicals, fluorescence spectroscopy is highly effective and provides increased sensitivity for these substances.

Depending on the type of radiation-matter interaction observed, spectroscopy using UV-visible radiation can be broadly classified into two types: absorbance spectroscopy and fluorescence spectroscopy and the infrared region of spectrum is great assistance for food product analysis, both quantitatively and qualitatively. (Nielsen, 1994). Spectroscopic techniques can also be helpful in examining how the harvest year affects tea, and the data obtained may help to clarify the product's quality standards. The findings obtained offer a comprehensive view that integrates several elements into statistical analysis when the full spectrum of spectroscopic techniques is assessed. This method increases the success rate of the classification model by enabling classification to be carried out by taking into account each of these compounds collectively

2.5.1. FTIR Spectroscopy

FTIR is one of the spectroscopic methods frequently used in the food industry for purposes such as adulteration detection and variety differentiation since samples can be analyzed quickly with high reliability. Infrared radiation, which is frequently used in food applications, is electromagnetic energy with wavelengths longer than visible light but shorter than microwaves and is divided into three groups: near-IR (12000-4000 cm^{-1}), mid-IR (4000-670 cm^{-1}), and far-IR (670-100 cm^{-1}) (Nielsen, 1994). Infrared spectroscopy explores the vibrations of molecules by passing infrared radiation through a sample. Some of the radiation is absorbed, while the rest is transmitted. The fundamental vibrations of functional groups are correlated with distinctive infrared absorption bands that can be linked to them. Specific functional groups are linked to distinctive infrared absorption bands that correspond to their fundamental vibrations. Since no two molecular structures produce identical IR spectra, this technique is valuable for many analytical purposes (Berthomieu and Hienerwadel 2009; Dutta 2017).

In FTIR spectroscopy, radiation is not dispersed; instead, all wavelengths reach the detector simultaneously. FTIR employs an interferometer, which splits and recombines a light beam (Figure 2.6). By adjusting the path length of one beam, the two beams can interfere constructively or destructively depending on their phase difference. This interferogram is then directed to the sample chamber. Here, the sample absorbs energy at specific frequencies in the interferogram. Depending on the chemical structure of the sample, some frequencies are absorbed while others pass through. The detector records changes in light over time, revealing the frequencies of energy absorbed by the sample. Fourier transform software then processes the interferogram to obtain the sample's spectrum, which indicates the specific frequencies at which energy is absorbed and identifies the chemical bonds present (Berthomieu & Hienerwadel, 2009; Nielsen, 1994).

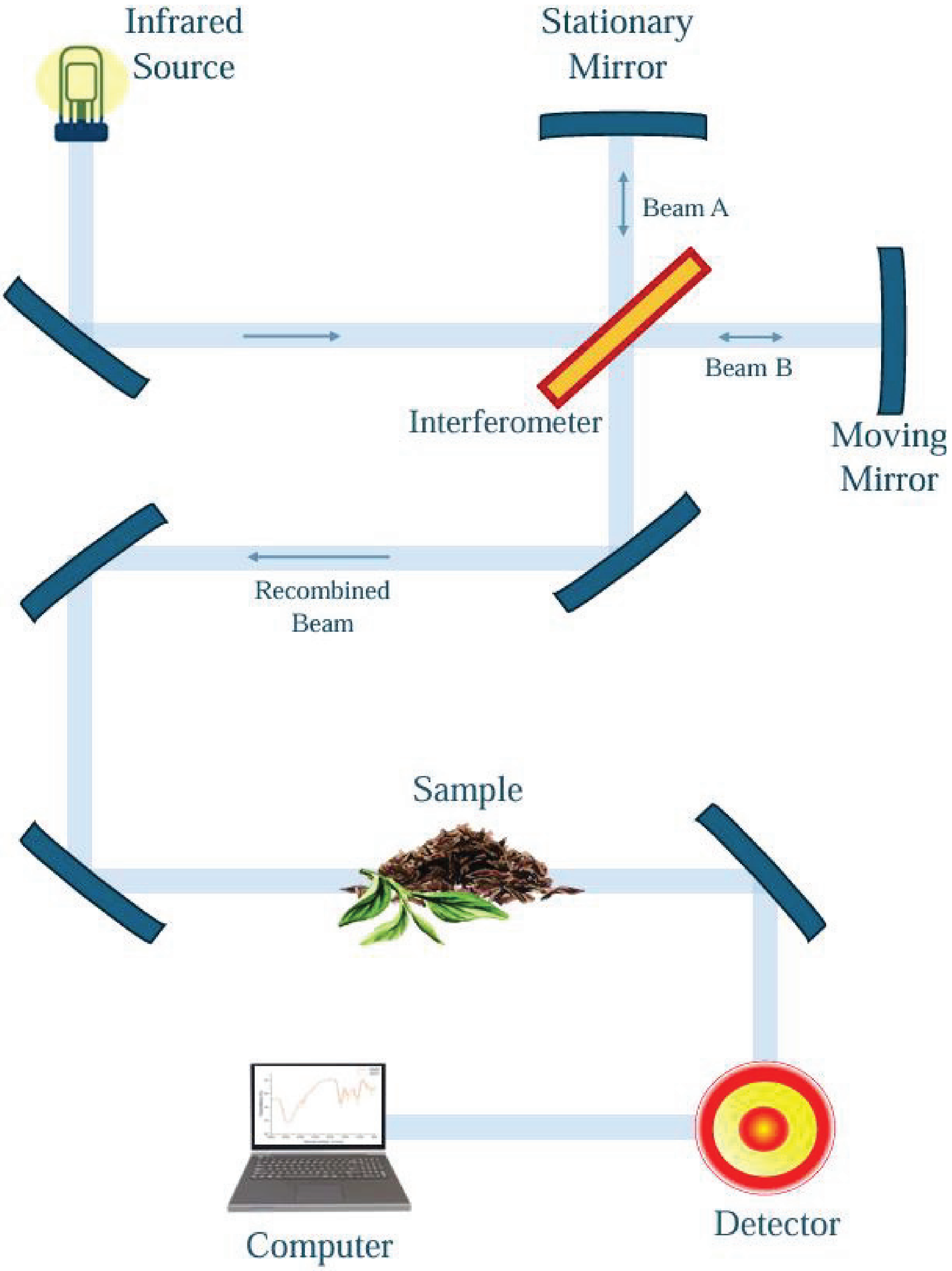


Figure 2.6. Basic set-up of FTIR spectroscopy (source: Nielsen, 1994)

Fourier-transform infrared (FTIR) spectroscopy was used to differentiate teas with respect to their types and geographical growth locations. In one study, FTIR spectroscopy combined with adaptive improved possibilistic c-means (AIPCM) clustering effectively classified tea varieties, achieving a prediction accuracy of 98.5% (Zhou et al., 2020). Another study employed FTIR spectroscopy with the partial least square (PLS) method and a self-organizing map neural network to analyze tea polysaccharides. Using the neural network approach, the study successfully classified common Chinese tea types with a 100% accuracy rate (Cai et al., 2015).

2.5.2. UV-Visible Spectroscopy

UV-visible spectroscopy is based on the absorption of light in the ultraviolet (180-380 nm) and visible light (380-780 nm) wavelengths (Pratiwi and Nandiyanto, 2022). The measurement of the amount of light absorbed from a reference beam as it passes through the sample solution provides the basis for the analysis. As the beam passes through the solution, the absorbing species absorbs photons, which causes the radiant power to drop. Therefore, the radiant power of the radiation entering the absorption cell will be substantially higher than that of the radiation exiting the cell from the opposite side (Nielsen, 1994). Absorbance generally shows a linear relationship between concentration and path length. Therefore, in most applications, absorbance is usually measured to determine the UV-visible spectrum of samples (Picollo et al., 2019).

The wavelength of the absorbed light is the energy required to move an electron from a lower energy level to a higher energy level. In UV-Visible spectroscopy, light from a UV-Visible source is directed through a monochromator to select a specific wavelength range (Figure 2.7). This light then passes through the sample, where a particular wavelength is absorbed, before reaching the detector. The main components of a spectrophotometer include an energy source, a monochromator (which isolates a narrow range of wavelengths), a sample holder (cuvette), a detector, and a signal output that displays the results for the analyst.

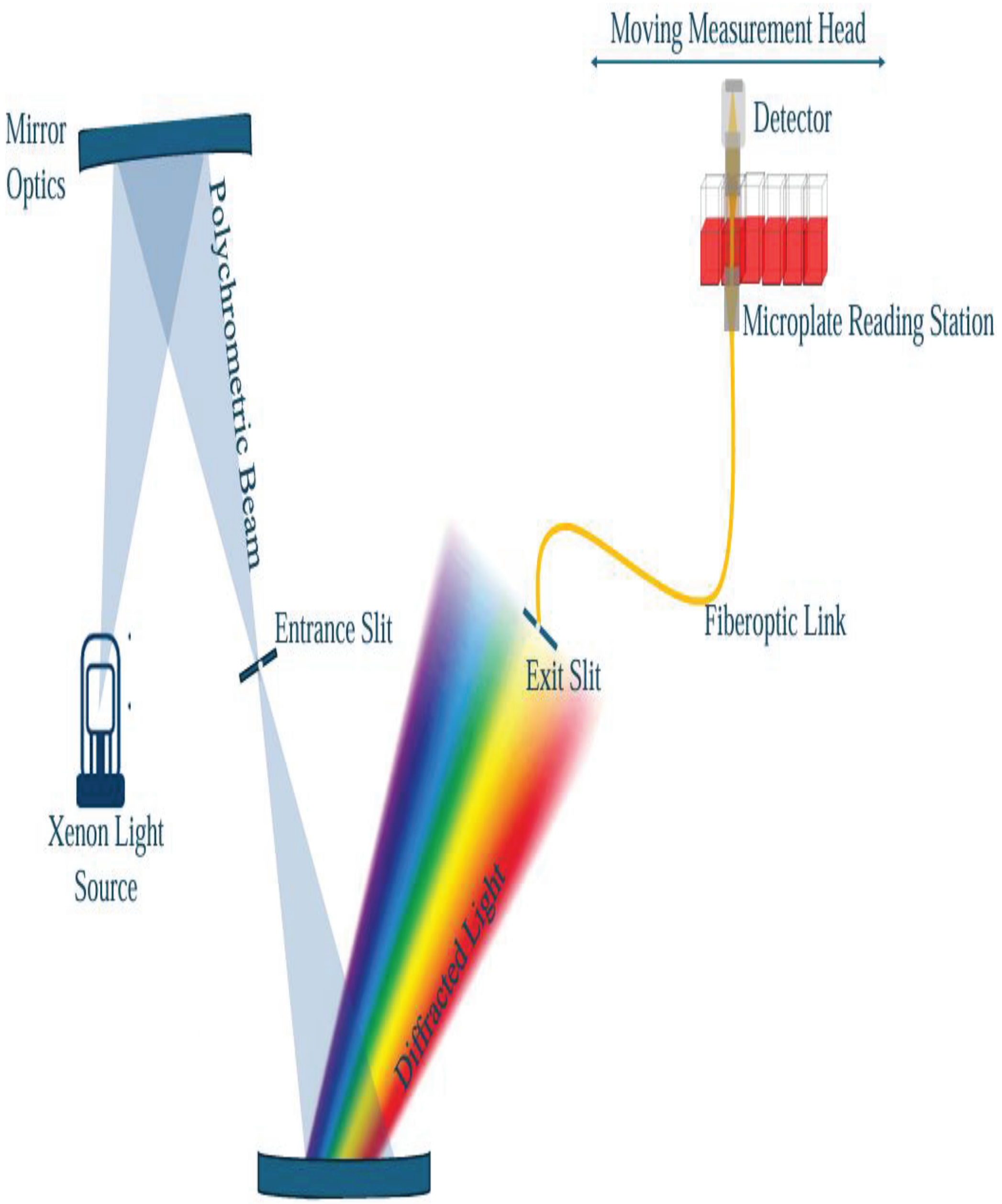


Figure 2.7. Basic set-up of UV-visible spectroscopy (Source: Nielsen, 1994)

UV-visible spectroscopy has been applied to differentiate infused tea samples based on their variety and geographical origin, leading to the conclusion that this technique may serve as an alternative method for assessing tea quality (Diniz et al. 2016). Another study also explored tea variety discrimination by combining UV-visible and FTIR spectroscopy, using various chemometric approaches with differing levels of success (Arifah et al., 2022).

2.5.3. Fluorescence Spectroscopy

Fluorescence spectroscopy is a technique used to quantify trace amounts of inorganic, organic, and biomolecules. A small amount of compound can exhibit fluorescence, besides, there is an ideal wavelength of radiation for excitation and emission for every distinct molecular composition. In other words, no two substances have fluorescence spectra that are precisely the same. Therefore, this method is an extremely sensitive and selective technique, and it can offer relatively large number of physical parameters (Dankowska and Kowalewski, 2019; Du et al., 2020; Nielsen, 1994). The concentration of the molecule under study is directly correlated with the fluorescence intensity of the emitted light at specific excitation and emission wavelengths (Sennaroglu, 2006).

In fluorescence spectroscopy, the measured signal comes from the electromagnetic radiation emitted by the analyte as it returns to its ground state from an excited electronic energy level. Initially the analyte gains energy and is excited to this higher energy level by absorbing radiation in the UV or visible range (Nielsen, 1994). During a fluorescence measurement, the activation and deactivation processes take place concurrently (Wehry, 1997). A molecule is excited by absorbing light of a certain wavelength and rises to a higher energy level. This energy level then drops again and emits light at a longer wavelength.

The equipment utilized for UV-visible absorption spectroscopy and fluorescence spectroscopy are nearly identical. Fluorometers essentially require two wavelength selectors: one for the emission beam and one for the excitation beam. Figure 2.8 provides a visual representation of the basic set-up of fluorescence spectroscopy.

Fluorescence spectroscopy is also used in the authentication of green tea, and differentiation of tea categories with respect to factors such as variety, geographic location, and fermentation degree (Hu et al., 2023; Seetohul et al., 2013). In a study, three-dimensional fluorescence spectroscopy with distance discrimination was used to identify matcha tea’s producer and grade, highlighting fluorescence spectroscopy’s potential for precise origin and quality verification (Xu et al., 2023).

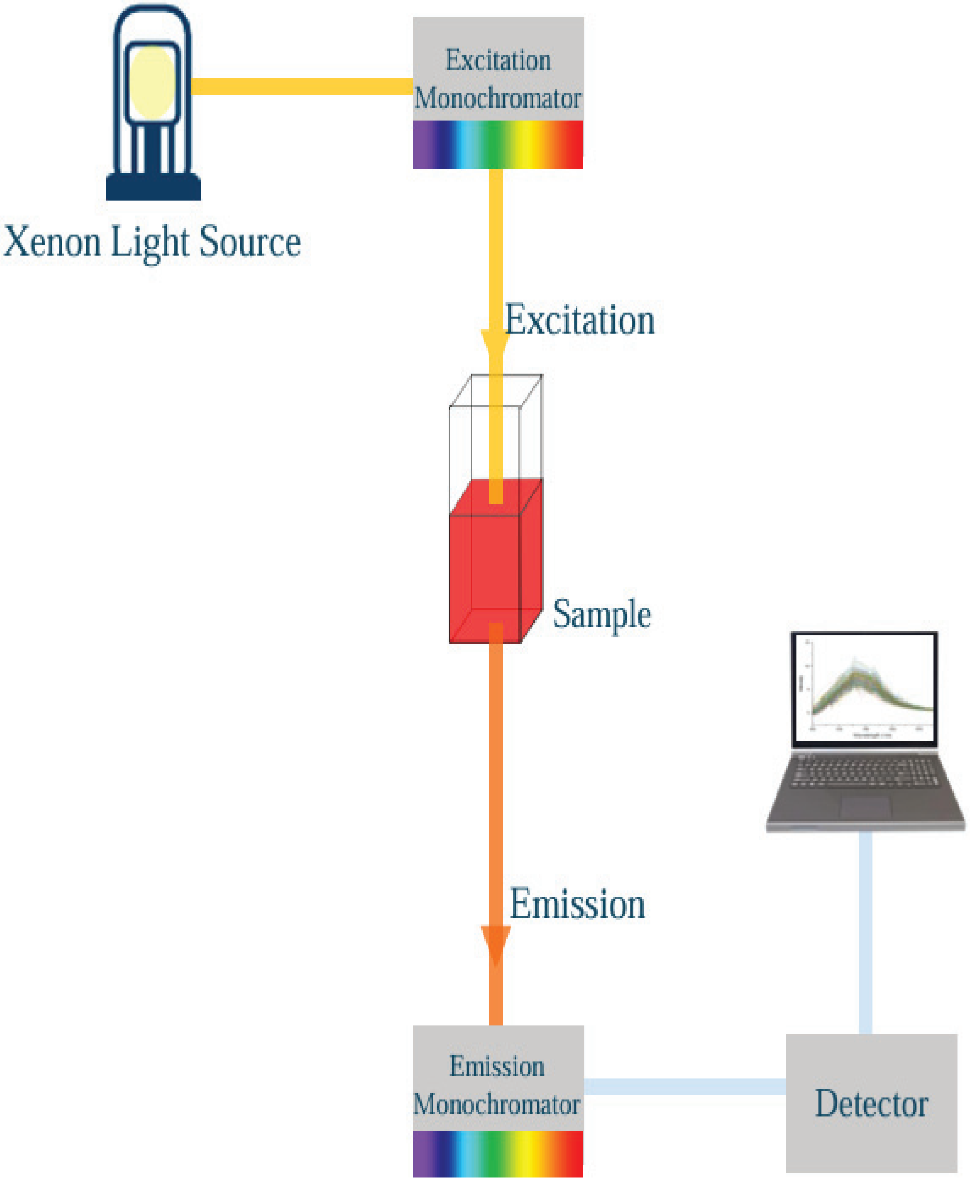


Figure 2.8. Basic set-up of fluorescence spectroscopy (Source: Nielsen, 1994)

When sourcing raw materials, processors need to quickly evaluate quality parameters and make timely decisions. Spectroscopic methods provide rapid results, allowing a single measurement to substitute for multiple chemical analyses, thus simplifying the tea analysis process.

Since variations in the quality of plant products can occur between harvest years due to previously discussed factors, it's essential to monitor year-to-year changes. Raw materials may possess either favorable or unfavorable quality traits in particular harvest years and recognizing these quality aspects early on allows for the implementation of necessary measures during the processing stages.

CHAPTER 3

MATERIALS AND METHODS

3.1. Materials

The black tea samples used within the scope of the thesis were harvested in the cities of Rize and Trabzon in the East Black Sea region of Turkey. These two cities alone supply approximately 87% of the tea production in Türkiye (TEPGE, 2024). 100 tea samples were harvested in 2021, and 105 tea samples were harvested in 2022 were transferred to bags after their processing and shipped to the Food Engineering Department of the İzmir Institute of Technology.

The tea samples were ground using a grinder (Renas Makina, Istanbul, Türkiye) then sieved (mesh 25) to become fine powder for analysis.

3.2. Infusion of Tea Samples

To prepare infused samples, 5 grams of powdered tea samples were transferred to tea filter bags (Prateaco, Turkiye). Tea filter bags were gently placed in 75 mL of distilled water (tea samples:water, 1:15, w/w) at 85°C. Infused tea samples were obtained by keeping them in a water bath for 15 minutes. These samples were analyzed after they were left to cool to room temperature.

3.3. Methods

In this section, analysis methods of infused and powdered form of tea samples were explained.

3.3.1. Fourier Transform Infrared Spectroscopy

Mid-infrared spectroscopic profiles were obtained for both infused and powdered tea samples. Spectra of infused tea samples were collected using a Fourier Transform Infrared Spectrometer (FTIR) (Spectrum 100, Perkin-Elmer, Waltham, Massachusetts, USA). Horizontal zinc-selenium attenuated total reflectance (ATR) accessory and deuterated triglycine sulfate (DTGS) detector were utilized to evaluate the infused samples. After taking the spectra of air as background, 2 mL of undiluted infused tea samples were spread homogeneously on the ATR cell surface, leaving no gaps. The spectra were measured in the range of 4000–800 cm^{-1}, with 96 scans at a resolution of 4 cm^{-1} and 1 cm/s per spectrum. The average of two spectra represents each sample.

Spectra of powdered tea samples were obtained with the same spectrometer in transmission mode. 1.5 mg of powdered tea was mixed with 148.5 mg of KBr powder (1:99, w/w) after grinding with a mortar (50-mm, P/N 161-5050, Pike Technologies, Wisconsin, USA) at room temperature to prepare pellets. Tea and KBr were placed into the die set (13 mm pellets, Pike Technologies, Wisconsin, USA) and 20,000 kPa pressure was applied by the hydraulic press (Wir Sas, Camilla'95, Germany) for three minutes. The resulting pellet was removed from the die set and placed in the magnetic pellet holder (Perkin-Elmer, Waltham, Massachusetts, USA), and transmittance spectra were obtained with 64 scans at 4 cm^{-1} resolution. Average of four readings were taken for each. As background, spectra of air were collected at the same conditions before every sample measurement

3.3.2. UV-Visible Spectroscopy

Infused tea samples were transferred to a 96-well flat-bottom polystyrene plate (Isolab, Wertheim, Germany) by taking 75 µL of each sample at room temperature without any pretreatment and a cuvette was filled with 75 µL distilled water to serve as a blank. The absorbance spectra were obtained using a UV-visible spectrophotometer (Thermo Scientific Multiskan GO Microplate Spectrophotometer, Fisher Scientific, Vantaa, Finland) within the wavelength range of 200 to 1000 nm. The data analysis involved using the average of two spectra for each sample.

3.3.3. Fluorescence Spectroscopy

Infused tea samples (200 µL) were placed in a black 96-well flat bottom polystyrene plate (Isolab, Wertheim, Germany). Fluorescence spectra of the samples were obtained between 350 and 600 nm with a fluorescence spectrophotometer (Thermo Scientific Varioskan, Fisher Scientific, Vantaa, Finland) and subjected to excitation at 320 nm. Slit width was set to 12 nm. The average of two spectra for each sample was used in the data analysis.

3.3.4. Total Phenolic Content

Total phenolic contents (TPC) were determined for infused tea samples as described by Fu et al. (2011) with minor modifications. 0.1 mL of infused tea samples were mixed with 0.9 mL distilled water in Eppendorf tubes (1.5 mL) to dilute the tea samples ten-fold. 50 µL of the diluted tea samples were transferred to other Eppendorf tubes and 250 µL of the Folin-Ciocalteu reagent (10%, v/v) were added then mixtures kept in the dark for 5 minutes.

After adding 200 µL of sodium carbonate (7.5%, w/v) into tubes, they were stored for one hour in the dark. As a last step, 200 µL of the samples was transferred to the 96-well flat-bottom polystyrene plate (Isolab, Wertheim, Germany) and absorbance was measured at 760 nm with a spectrophotometer (Thermo Scientific Multiskan GO Microplate Spectrophotometer, Fisher Scientific, Vantaa, Finland). As a reference, the absorbance of distilled water was measured as well. Two replicates were taken from each sample and the average of two readings was used.

A gallic acid standard curve was constructed as described by Shirazi et al. (2014), with slight modifications. 1 g of gallic acid was dissolved in 100 mL methanol to get 1%

solution of gallic acid (10 mg/ml). A standard gallic acid curve was constructed by preparing the dilutions of (0.1, 0.5, 1.0, 2.5 and 5 mg/ml) in methanol from standard solution of gallic acid. The equation of the gallic acid curve was found to be $y = 0.0011x$.

100 µL of each of these dilutions were mixed with 500 µL of water and then with 100 µL of Folin-Ciocalteu reagent and allowed to stand for 5 minutes. Then, 1 mL of sodium carbonate (7.5 % w/v) and 500 µL of distilled water was added to the reaction mixture. The absorbance was recorded after one hour at 760 nm with a spectrophotometer (Thermo Scientific Multiskan GO Microplate Spectrophotometer, Fisher Scientific, Vantaa, Finland). All stages of the experiment were carried out at room temperature.

The formula below was used to determine the TPC of the samples in terms of Gallic Acid Equivalent (mg GAE/L)

$$\text{Total Phenolic Content}\left(\frac{\text{mg GAE}}{\text{L}}\right)=\frac{\text{Sample Absorbance}-\text{Blank Absorbance}}{0.0011}\times 10\times\frac{170.12}{1000} \qquad (3.1)$$

where molecular weight of gallic acid: 170.12 g/mole, dilution factor: 10 and the slope of gallic standard curve: 0.0011.

3.3.5. Soluble Solids Content

As the first step of determining dry matter content of infused tea samples, petri plates were dried in a laboratory oven (Binder, Tuttlingen, Germany) at 105°C for 2 hours and then cooled in a desiccator until their weights do not change. Then, 25 mL of infused tea samples were spread onto petri plates and the samples dried at 70°C in a laboratory oven overnight. The following formula was used to calculate the dry matter content.

$$\text{Soluble-Solids Content}\left(\frac{\text{g}}{\text{g}}\right)=\frac{\text{Total Soluble Solids}}{\text{Tea Sample}} \qquad (3.2)$$

3.3.6. Statistical Methods

To assess whether there is a significant difference on the dry matter content and total phenolic content of infused tea samples between the two harvest years, Tukey’s Test was applied at 95% confidence interval (Minitab, v.19.1, Minitab Inc., Pennsylvania, USA).

Spectral profiles were evaluated with chemometric methods to investigate the separation of tea samples from different harvest years. As the first step of multivariate statistical analysis, the data were treated with principal component analysis (PCA). A noticeable trend of differentiation was observed between harvest years in the score plot of the first and second principal components. PCA is used as an unsupervised recognition method and gives only the clustering tendency of the spectroscopic profiles of tea sample; therefore, it was decided to perform supervised multivariate statistical methods; partial least square-discriminant analysis (PLS-DA), orthogonal partial least square-discriminant analysis (OPLS-DA) and soft independent modeling of class (SIMCA) to separate black tea samples according to the harvest year.

Preceding model fitting, data transformation methods were applied to the spectral data to eliminate the uninformative variables. These methods are First (FD), second (SD), and third derivatives (TD), standard normal variate (SNV), multiplicative scatter correction (MSC), Savitzky-Golay filtering (SGF), square transformation (SQ), FD+SNV, FD+MSC, SD+SNV, SD+MSC, TD+SNV and TD+MSC.

In the next step, all 205 samples were divided into two as stated in the literature (Quansheng et al., 2009), 137 of which was used to build a model (calibration set) and 68 of which were used to test the reliability of the generated models (validation set) using a stratified random sampling technique. Raw and transformed data obtained from all spectroscopic techniques were used to create chemometric models.

OPLS-DA and PLS-DA analyses were conducted using the 'ropls package' (Version 3.12) within the R programming language (Thévenot et al., 2015). R^2, root mean square of error (RMSE), latent variable (LV), sensitivity, specificity, and correct classification rate for validation were used to test the performance of the models. Score plots were also created to check the success of the models.

Soft Independent Modeling of Class Analogy (SIMCA) is a chemometric method used for classification in multivariate data analysis and it is one of the most widely applied class modeling approaches used in the literature, SIMCA builds individual models for each class based on PCA. Each class is modeled independently, and each sample is assigned to the class where it best fits. SIMCA was applied to all spectral data by using the SIMCA statistical software program (version 14.1, Sartorius, Göttingen, Germany). Cooman's plots were constructed to visually compare how well a sample fits into two different models (or classes).

The results of the chemometrics analyses are reported by providing sensitivity, specificity and correct classification rate of the established models. Sensitivity indicates the proportion of samples from a category that are correctly accepted by the class, while specificity is the proportion of objects from other categories that are correctly rejected by the corresponding model (Firmani et al., 2019). The correct classification rate is expressed as the percentage of tea samples correctly predicted in the harvest year to all tea samples. Sensitivity, specificity and correct classification rate were calculated according to the definitions given in the literature (Cavdaroglu and Ozen 2023).

Sensitivity was calculated as the ratio of the number of correctly identified samples harvested in 2022 to the total number of samples identified as harvested in 2022 and its calculation is given below:

$$S\text{ensitivity} = \frac{\text{TP}}{\text{TP+FN}} \tag{3.3}$$

where samples classified as true positive (TP) and false negative (FN),

Specificity was measured as the ratio of correctly identified number of samples harvested in 2021 to number of all samples identified as harvested in 2021 and its calculation is given below:

$$\text{Specificity} = \frac{\text{TN}}{\text{FP+TN}} \tag{3.4}$$

where samples classified as true negative (TN) and false positive (FP),

Finally, the correct classification rate is defined by the percentage of all correctly classified tea samples to all tea samples.

$$\text{The Correct Classification Rate} = \frac{\text{TP+TN}}{\text{Total number of tea samples}}\ x\ 100 \tag{3.5}$$

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Soluble Solids Content and Total Phenolic Content

The dry matter of black tea, composed of components like polyphenols, caffeine, nitrogen, amino acids, carbohydrates, and vitamins, is a key factor in evaluating tea quality. These compounds are responsible for the tea's color, flavor, and brightness (Liang et al., 2003; Turkmen et al., 2009). Amounts of these compounds in tea depend on the growing conditions besides the other factors such as processing and storage conditions (Kowalska et al., 2021).

The ranges and means of the total soluble solids content of black tea samples used in this study from two harvest years are listed in Table 4.1. Soluble solids of black tea samples varied between 0.032 and 0.175 g/g sample in 2021 while 0.003 and 0.146 g/g sample in 2022. Pairwise comparison for dry matters between two different harvest years was done using Tukey's Comparison test. A statistically significant difference was observed in the 95% confidence interval.

Table 4.1. Soluble solids content of infused tea samples

Harvest year	**Number of samples**	**Soluble solids (g/g sample)**	
		Range	**Mean***
2021	100	0.032-0.175	0.108^a
2022	105	0.003-0.146	0.071^b

*Different letters indicate a statistically significant difference at $p<0.05$.

In addition to soluble solids content, total phenolic contents (TPC) of infused tea samples were also determined. Phenols, which play an important role in sensory properties, are quality indicators, as higher phenolic content is generally associated with stronger antioxidant activity. Therefore, the presence of polyphenols in tea is also associated with a reduction in cardiovascular risk (Samadi and Fard, 2020). To determine the effect of harvest year, pairwise comparison test applied to TPC data.

For tea samples harvested in 2021, the TPC values varied between 640.5-786.5 mg GAE/L, and for 2022 harvesting year, the TPC values varied between 647.7-790.1 mg GAE/L. TPC values of tea samples originated from two different harvest years did not significantly differ from each other. ($p>0.05$).

Considering the solid/water ratio and infusing method in this study, TPC measurements were consistent with the values given in literature. In the study conducted by Erturk et al. (2010), TPC values of black tea infusions prepared using boiling water are reported as 555.53 and 999.927 mg GAE/L for 50 and 100 mg/mL concentration, respectively.

Table 4.2. Total phenolic content of infused tea samples

Harvest year	**Number of samples**	**Total Phenolic Content (mg GAE/L)**	
		Range	**Mean**
2021	100	640.5-786.5	713.5^a
2022	105	647.7-790.1	718.9^a

*Different letters indicate a statistically significant difference at $p<0.05$.

The fact that the change seen in soluble solids content is not seen in TPC suggests that this change might be due to components other than phenolics. Non-phenolic substances exist in black tea, such as carbohydrates, amino acids or other organic components, might be the cause of this change.

4.2. Spectral Assignments of Tea Samples

The components in black tea are distinctly reflected in their spectral patterns. These patterns contain unique spectral features that characterize the presence and composition of the chemical components in the tea (Arifah et al., 2022). Mid-IR spectra of powdered and infused tea and UV-visible and fluorescence spectra of infused tea samples are shown in Figure 4.1.

The functional group characteristics of tea samples could be identified by analyzing the spectra within the 4000-400 cm^{-1} range. Moreover, the vibration frequencies of the FTIR spectra are divided into the fundamental frequency range of approximately 4000-1800 cm^{-1} and the fingerprint frequency range of 1800-400 cm^{-1}. The fingerprint region is a crucial region for the recognition of inorganic and organic compounds since it encompasses the vibration frequencies of chemical bonds belonging to these compounds. Different functional groups have absorption bands that appear within this specific region, and these bands generally have consistent positions (Zhou et al., 2020).

Mid-IR spectral data of powdered tea samples could be seen in Figure 4.1a. Various peaks are observed in all samples between the wavenumber ranges of 3700-3300 cm^{-1}, 2950-2700 cm^{-1}, 1750-1500 cm^{-1} and 1500-500 cm^{-1}, indicating the presence of certain functional groups.

The broad peak around 3300 cm^{-1} is attributed to O–H stretching vibrations originating from hydroxyl groups of phenols, alcohols, carboxylic acid and water (Muheddin et al., 2023). Absorption due to stretching vibrations of methyl group occurs around 2930 cm^{-1} and 2850 cm^{-1} (Li et al., 2013). Peaks at 650 cm^{-1} and 1515 cm^{-1} are specifically attributed to the C=O stretching of catechins and flavonoids and the C=C stretching of alkenes in caffeine. In addition, peaks around 2930 cm^{-1}, 1700–1460 cm^{-1}, 1400–1200 cm^{-1} and 800–500 cm^{-1} can be associated with tea polysaccharides.

FT-IR spectra obtained for infused tea samples could be seen in Figure 4.1b. The peaks in the infused tea spectra generally parallel with the powdered tea spectra. The most prominent peaks in spectra are observed around 3300 cm^{-1}, 2200 cm^{-1}, 1650-1630 cm^{-1}, and within the 1550-800 cm^{-1} range.

As it is true for powder FTIR spectra, the broad peak of O–H stretching modes around 3600-3100 cm^{-1} is representative of the presence of many phenolic compounds in infused tea along with water (Arifah et al., 2022; Zhou et al., 2020;). Besides, in this range C–H vibrations of saturated and unsaturated fatty acids may also be present but masked by the broadness of the other peaks (Arifah et al., 2022).

Triple and cumulative bonds exhibit stretching vibrations between 2500-2000 cm^{-1}; therefore, the peak around 2200 cm^{-1} might indicate a C≡C bond or a C≡N bond (Arifah et al., 2022; Zhou et al. 2020). The wavenumber ranges of the stretching vibrations of double bonds are around 2000–1500 cm^{-1} (Giorgini et al., 2024; Zhou et al., 2020). Specifically, C=O stretching associated with catechin, and flavonoids is seen around

1650-1630 cm^{-1}. However, C=O peaks from other carbonyl groups might overlap in this region (Arifah et al., 2022). In addition, vibration of single bonds is around 1550-600 cm^{-1}. For example, C-C and C-O bonds from caffeine and phenolic compounds in the spectra are located in 1550-830 cm^{-1} region.

Water related peaks are predominant features of the infused tea spectra. The main reason for this is that these samples contain a significant amount of water as a result of infusion.

By observing the spectra, peak around 3300 cm^{-1} is more evident compared to powdered tea spectra due to the -OH stretching vibrations from water. Moreover, infused tea samples exhibit weaker absorption peaks in 1500-800 cm^{-1} region.

UV-visible spectroscopy, a method that measures the absorption of ultraviolet and visible light that interacts with the sample. The peaks encountered in UV-visible spectra are generally non-specific and broad (Arifah et al., 2022). In Figure 4.1c, spectra of infused black tea recorded in the 200-800 nm range is shown and broad peak in the 200-400 nm range and a shoulder in the 250-300 nm range are observed.

Peak observed in 200-400 nm region is assigned with the $n \rightarrow \pi$ electronic transition of phenolic compounds (Arifah et al., 2022). Shoulder that can be seen in 350-380 nm range is associated specifically with theaflavins and thearubigins which are present in black tea but not in green tea as a result of the fermentation process of polyphenols. These compounds also have influence on the tea's sensory properties like color (Arifah et al., 2022; Palacios-Morillo et al., 2013).

Fluorescence spectroscopy is the last spectroscopic technique used in the scope of this thesis, and spectra can be seen in Figure 4.1c. Fluorescence is a sensitive and selective method frequently used for the detection of chemical compounds even in small amounts. Therefore, fluorescence can be used complementary to other spectroscopic methods (Dankowska and Kowalewski, 2019; Du et al. 2020;).

One major and a smaller peak were identified around 425 nm and 475 nm in fluorescence spectra of infused black tea samples as a result of excitation at 320 nm. Peaks in 300-500 nm region of fluorescence spectra are mainly attributed to tea polyphenols and phenolic compounds (Hu et al., 2023).

The effective combination of spectroscopic methods and chemometric approaches has been frequently reported in literature. In a study, the ability of FTIR spectroscopy to categorize Chinese tea types based on tea polysaccharides were evaluated and 100% classification was obtained by PLS method (Cai et al., 2015).

In other study, FTIR spectroscopy and adaptive improved probability c-means (AIPCM) clustering were used, and 98.5% accuracy rate was achieved in the prediction of tea varieties (Zhou et al., 2020).

UV-visible spectroscopy also has an important application area in distinguishing tea varieties and their geographical location. Studies conducted show that this method can be an alternative in the evaluation of tea quality (Diniz et al., 2016), or could be used for differentiating black, green and Pu-erh tea types (Palacios-Morillo et al., 2013). In a study by Seetohul et al. (2013), a technique that used total luminescence spectroscopy and PCA data classification to distinguish teas from 11 different plantations in Sri Lanka was able to do so with 100% accuracy.

Furthermore, literature includes studies using more than one spectroscopic method and complementary use of spectroscopic techniques with each other. In research using combination of UV-visible, synchronous fluorescence and NIR spectroscopy with data fusion techniques showed a good ability to classify tea samples according to their production processes (Dankowska and Kowalewski, 2019).

It has been observed that spectroscopic methods provide very effective results for the classification and quality assessment of tea varieties. In addition, the enhancement of these methods with chemometric methods is used to characterize and control the quality of teas in both scientific research and industrial applications. Since chemometric methods are sufficient to recognize even very small deviations, entire ranges in all spectral data were used in statistical analyses to distinguish two harvest years

4.3. Classification Using Soft Independent Modeling of Class Analogies (SIMCA)

Spectral data obtained from infused and powdered tea samples were evaluated with SIMCA to differentiate two harvest years. SIMCA is a class modeling technique that emphasizes similarities among the samples by creating an individual model for each class. New samples are then assigned to the class whose model best represents their characteristics (Firmani et al., 2019). Several transformations were applied to the data prior to SIMCA analysis. Correct classification rates for calibration and validation sets and specificity and sensitivity for validation set were calculated.

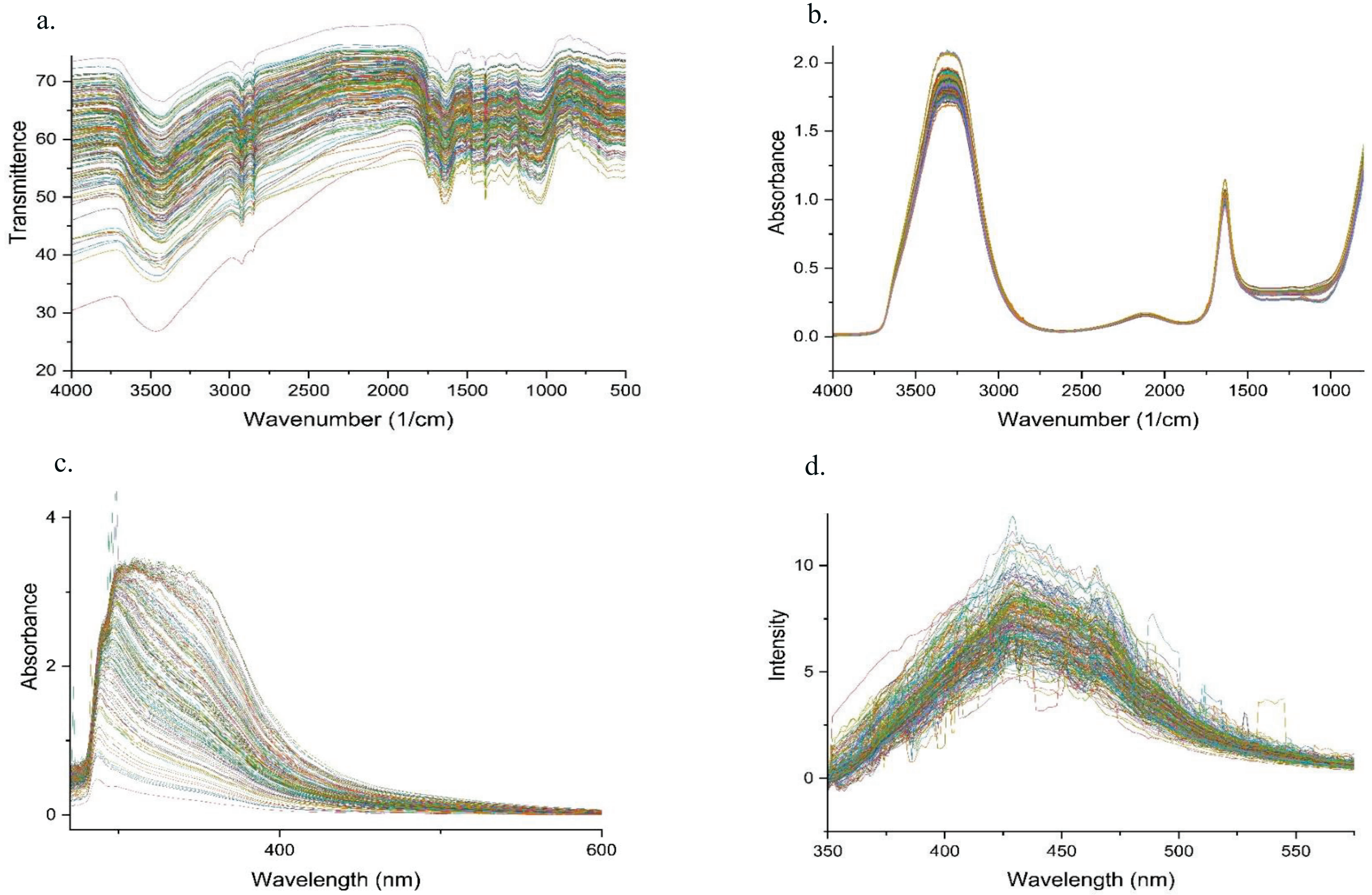


Figure 4.1. Spectral data of infused and powdered tea samples (a. FTIR spectra of powdered black tea, b. FTIR spectra of black tea infusions, c. UV-visible spectra of black tea infusions, d. Fluorescence spectra of black tea infusion

4.3.1. Application of SIMCA on FTIR Spectroscopic Data for Tea in Powder Form

Considering transmission FTIR spectral data of tea in powder form, the best three pre-treatment methods are shown in Table 4.3. These methods include the first derivative, as well as the first derivative (FD) combined with SNV and MSC separately. The correct classification rates for these models range from 85.4% to 92.7% for the calibration set, and from 72.06% to 80.88% for the validation set. All three models show similar performance in terms of classification accuracy, sensitivity, selectivity, and efficiency.

For the validation set, the specificity is calculated as the highest value (1) for both the FD and the FD combined with SNV, indicating that all tea samples from 2021 were correctly classified. Specificity is calculated as 0.88 in combination with the FD and MSC. However, this value does not extend to the 2022 harvest year, where the sensitivity ranges from 0.65 to 0.74. The highest sensitivity values, 0.73 and 0.74, are observed in the combinations of the FD with SNV and MSC, respectively. Efficiency, defined as the geometric mean of sensitivity and specificity, falls between 80.6% and 85.4% across the three methods, with the highest efficiency in the FD and SNV combination. Besides, model belonging to the combination of the FD and MSC explains 92.1% of the 2021 harvest model, while the FD and SNV combination explains 88.72%. For the 2022 harvest year, these values are 88.9% and 81%, respectively. Based on these metrics, the combination of the FD and SNV was chosen as the suitable data preprocessing method in the analysis performed with SIMCA.

Cooman's plot for the SIMCA model of the validation set after applying this pre-treatment is shown in Figure 4.2. The horizontal and vertical dashed green lines indicate the threshold for acceptance by the individual class models. This threshold acceptance is 1.2 for tea samples harvested in both 2021 and 2022. Since some samples from both years do not clearly belong to any one group, and most of the samples from both years are clustered closely together, this suggests that the data from both years share similar characteristics.

Table 4.3. SIMCA results of FTIR-Transmission data for the differentiation of black tea according to harvest year

Data Transform Method	**Correct Classification Rate (%)**		**Sensitivity***	**Specificity***	**Efficiency (%) ***	**Number of Principal Components***		**R^{2*}**	
	Calibration	**Validation**				**2021**	**2022**	**2021**	**2022**
1st Derivative	85.4	72.06	0.65	1	80.6	12	8	0.944	0.888
1 st Der + SNV	90.51	80.88	0.73	1	85.4	10	11	0.887	0.81
1 st Der + MSC	92.7	77.94	0.74	0.88	80.7	11	11	0.921	0.889

*These values correspond to validation set.

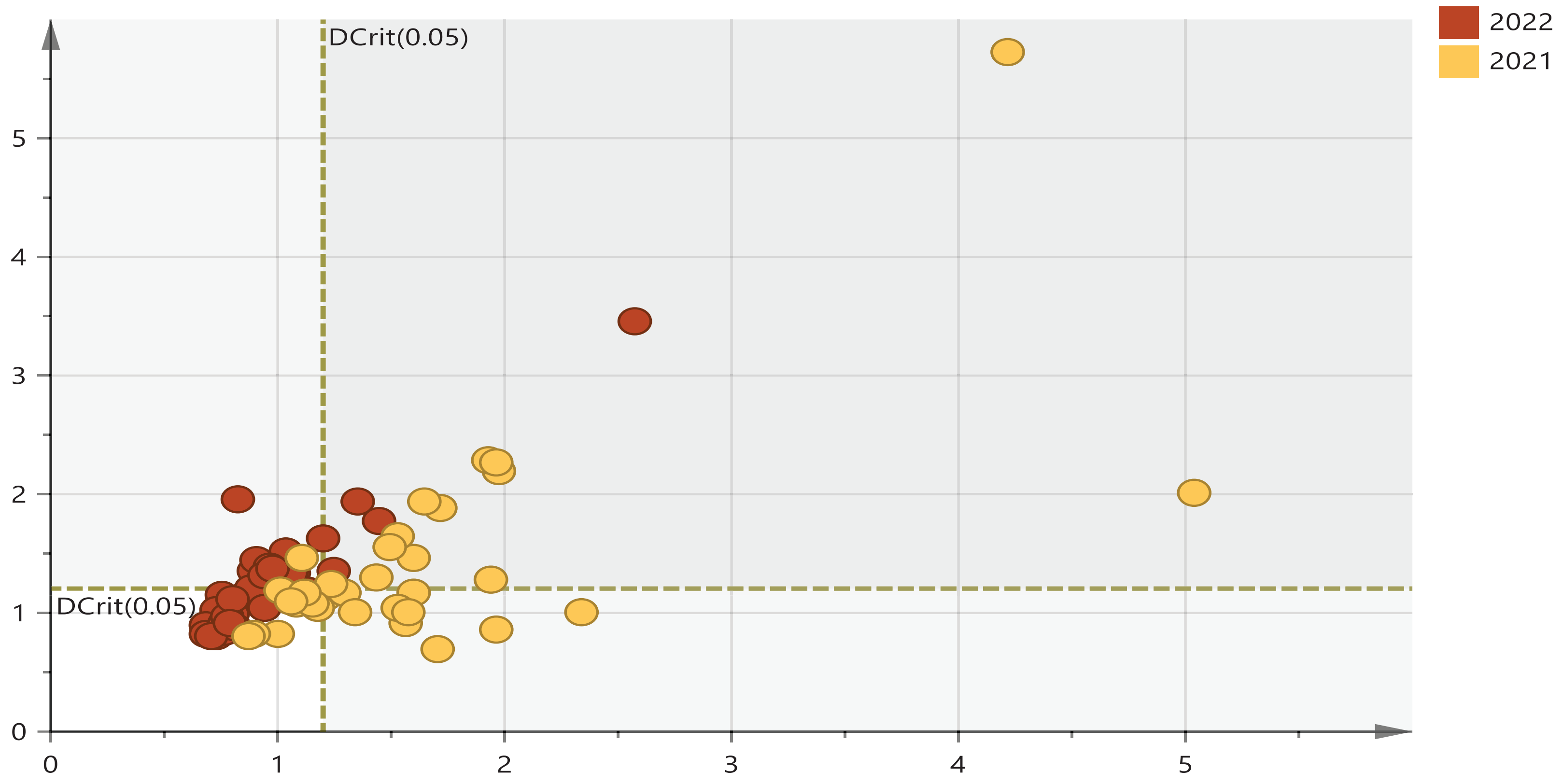


Figure 4.2. Cooman's plot obtained from SIMCA analysis of FTIR-Transmission data for the differentiation of black tea according to harvest year (samples harvested in 2021 represented by orange cycles, samples harvested in 2022 represented by maroon circles)

4.3.2. Application of SIMCA on FTIR Spectroscopic Data for Infused Tea Samples

Data processing techniques were applied to the ATR-FTIR spectral data of infused tea samples as previously mentioned and the best three preprocessing methods for this data modeled with SIMCA are listed in Table 4.4. Given models have similar values for correct classification rate, sensitivity, selectivity, and efficiency. Efficiency was calculated by taking the geometric mean of sensitivity and specificity. Although sensitivity results of these models had the highest value specificity values vary from 0.73 to 0.75 and correct classification rates for validation set change between 82.35 to 83.82%.

The number of the principal component in the models established with the best three preprocessing methods are also close to each other and are between 4 and 6 for 2021 and 5 and 7 for 2022. R^2, which gives the model's fit to the calibration set, varies between 0.743-0.954 and 0.709-0.984 for harvest years 2021 and 2022, respectively. The most suitable data processing method, MSC, was selected as the approach leading to the highest model statistics. In this model, sensitivity and specificity were calculated as 1 and 0.75, respectively. The high sensitivity of this model indicates that all teas harvested in 2022 were correctly identified. However, the relatively low specificity indicates that 11 out of 33 samples harvested in 2021 were incorrectly classified. This misclassification may be due to water-dominant peaks in the ATR-FTIR spectral profile, which may obscure critical differences.

The Cooman's plot belonging to MSC transformed SIMCA model of the validation set is shown in Figure 4.3. Threshold acceptance is 1.119 for tea samples harvested in 2021 and 1.196 for samples harvested in 2022. The proximity of the threshold acceptances may mean that both models have similar performance. However, it is seen that some samples from 2022 harvesting year do not fit well into the defined classes of the SIMCA model. Although there is a tendency for the two harvest years to differ from each other it can be said that the two harvest years are very close to the origin and there is significant overlap between the samples.

Table 4.4. SIMCA results of FTIR-ATR data for the differentiation of black tea according to harvest year

Data Transform Method	**Correct Classification Rate (%)**		**Sensitivity***	**Specificity***	**Efficiency (%) ***	**Number of Principal Components***		**R^{2*}**	
	Calibration	**Validation**				**2021**	**2022**	**2021**	**2022**
1st Derivative	86.86	82.35	1	0.73	85	4	6	0.743	0.703
SNV	87.59	83.82	1	0.75	87	6	5	0.954	0.979
MSC	86.86	83.82	1	0.75	87	6	7	0.954	0.984

*These values correspond to validation set.

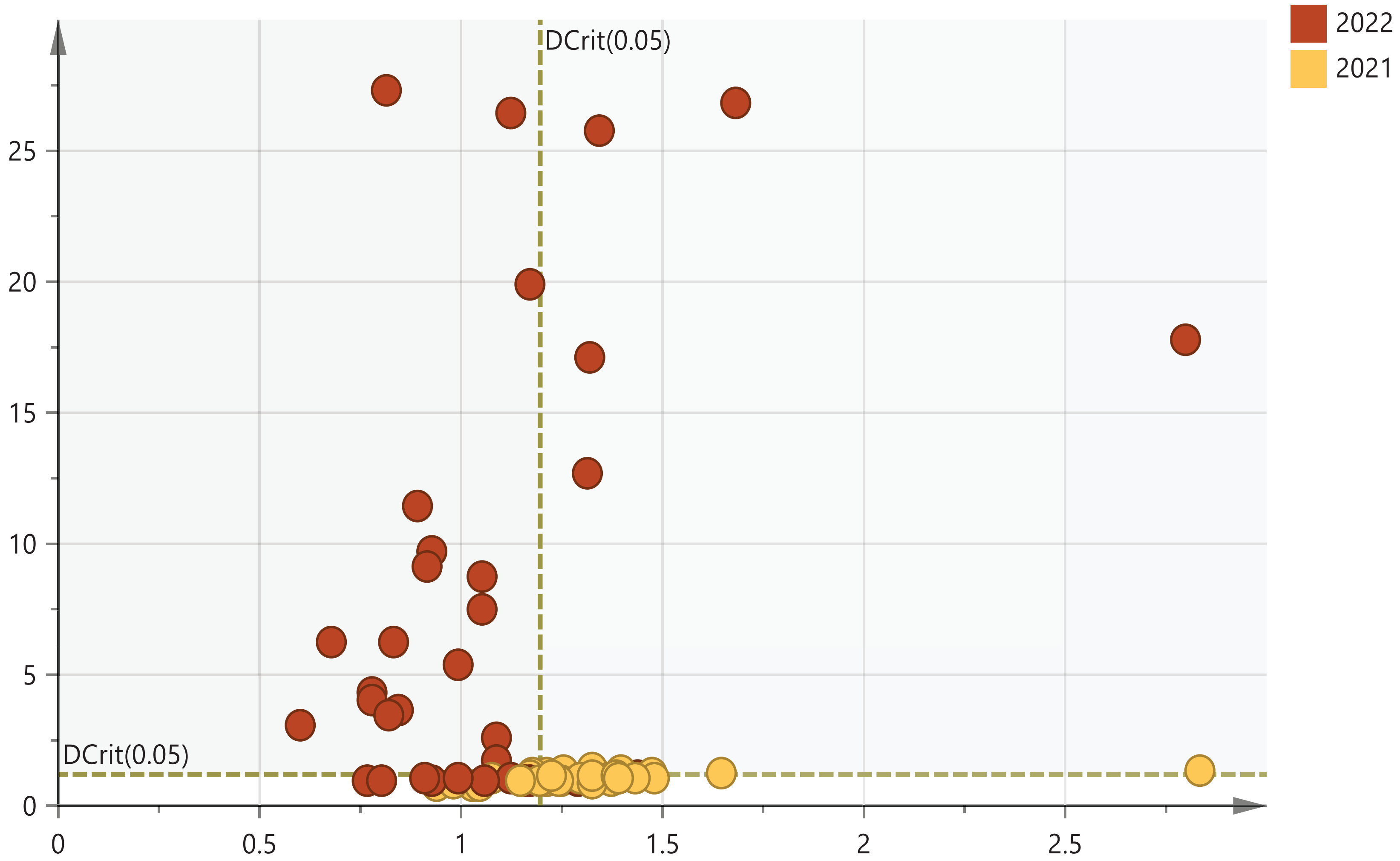


Figure 4.3. Cooman's plot obtained from SIMCA analysis of FTIR-ATR data for the differentiation of black tea according to harvest year (samples harvested in 2021 represented by orange cycles, samples harvested in 2022 represented by maroon circles)

4.3.3. Application of SIMCA on UV-Visible Spectroscopic Data for Infused Tea Samples

As in other spectroscopic data evaluations, UV-visible spectroscopic data were also analyzed with SIMCA after various transformations. The data obtained from SIMCA modeling are given in Table 4.5.

It was found that the correct classification rate was over 90% for all data processing methods in the calibration set and varied between 80.88% and 91.18% for the validation set. Sensitivity values were calculated between 0.84 and 0.94, while specificity results were between 0.78 and 0.94. The second derivative pre-processing has the highest value for both parameters. It can be said that for this data transformation, the model performed quite well in correctly identifying the teas harvested in both years with a sensitivity and specificity of 0.94. Considering the correct classification rates and efficiency, the results obtained in this model are higher than the results obtained from the other spectral data. This model is effective in correctly classifying the samples and distinguishing different groups with a good level of reliability. Additionally, the R^2 value of 0.573 for the second derivative indicates that approximately 57.3% of the variance in the data is explained by the model, which is a fair fit. Although not a perfect fit, it does indicate that the model is able to explain a large portion of it.

Figure 4.4 presents the Cooman's plot for validation set's second derivative transformed SIMCA model. Both models share the same threshold value of 1.212. A certain number of samples that were taken in both years are beyond model thresholds since they are not well explained by these models. However, most samples are highly grouped within the critical boundaries.

Table 4.5. SIMCA results of UV-visible spectroscopic data for the differentiation of black tea according to harvest year

Data Transform Method	**Correct Classification Rate (%)**		**Sensitivity***	**Specificity***	**Efficiency (%) ***	**Number of Principal Components***		**R^{2*}**	
	Calibration	**Validation**				**2021**	**2022**	**2021**	**2022**
1st Derivative	93.43	80.88	0.84	0.78	80.94	6	8	0.846	0.886
2nd Derivative	98.54	91.18	0.94	0.94	94	8	9	0.573	0.593
3rd Derivative	95.62	85.29	0.91	0.85	87.95	10	9	0.559	0.495

*These values correspond to validation set.

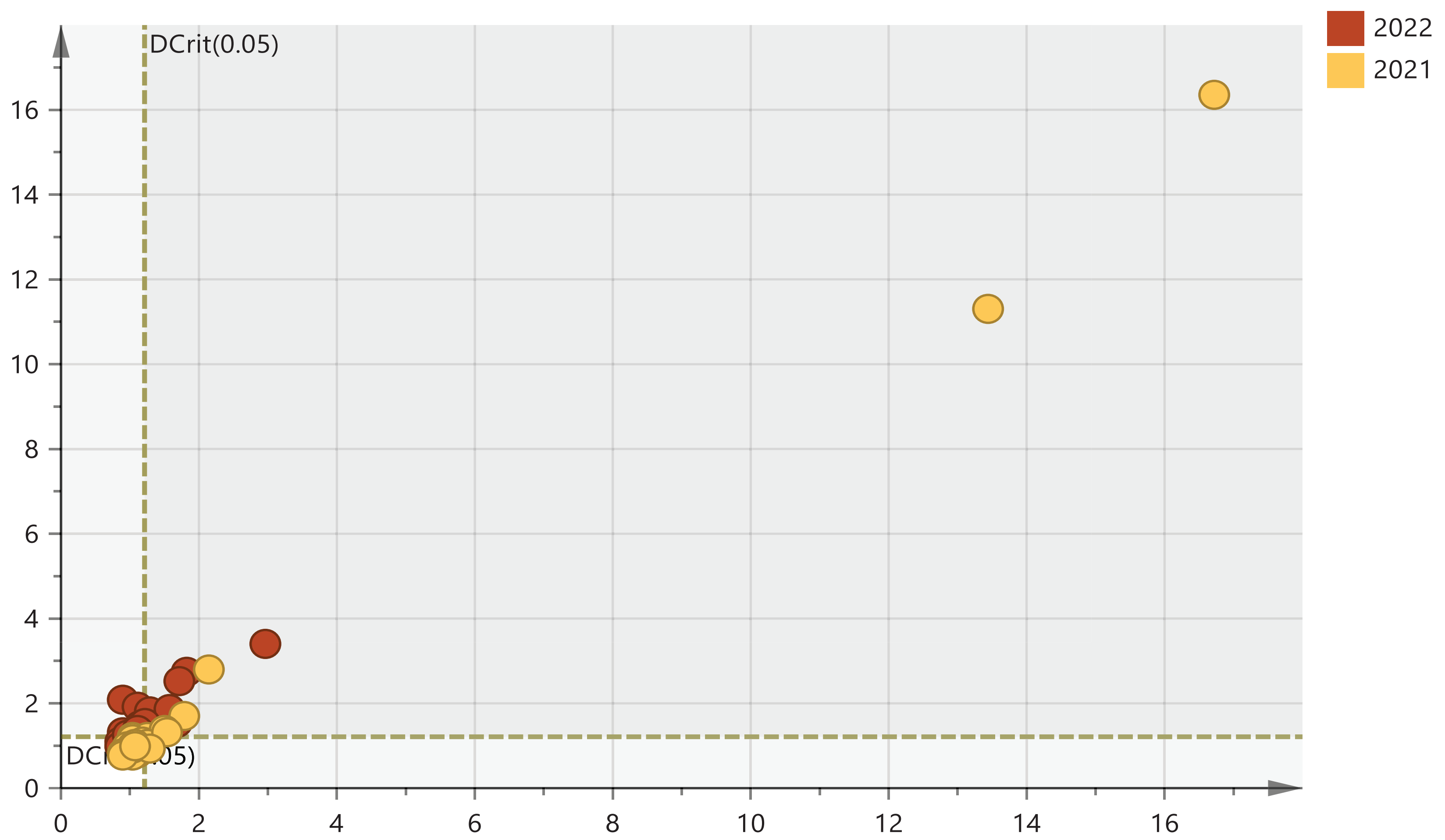


Figure 4.4. Cooman's plot obtained from SIMCA analysis of UV-visible (samples harvested in 2021 represented by orange cycles, samples harvested in 2022 represented by maroon circles)

4.3.4. Application of SIMCA on Fluorescence Spectroscopic Data for Infused Tea Samples

Similar to the evaluation of other spectroscopic data, fluorescence spectroscopic data were analyzed using SIMCA after various data transformations. Data transformation methods, SNV and SNV combined with the FD and SD resulted the highest performance indicators listed in Table 4.2.

The correct classification rate of the calibration set varied between 67.88 and 88.32%. This value was found between 73.53 and 88.24% for the validation set and the highest value for both sets was found in the combination of the SD with SNV. In the model created with this data transformation method, efficiency was found to be 88.5 and was selected as the best data pretreatment method. In the model obtained with the SD transformation of SNV, the number of principal components is 15 for both 2021 and 2022 harvest years and this model can explain approximately 80% of the harvested tea.

Figure 4.5 shows the Cooman's plot for SIMCA model after transformation of validation set data with a combination of SD and SNV. Threshold acceptance is 1.245 for tea samples harvested in both 2021 and 1.237 for those from 2022.

The samples for this model have a wider distribution, with some falling outside the thresholds for both years indicating that these models do not fully explain the data. Moreover, some samples positioned between the two thresholds may reflect characteristics common to both models. This suggests that while the models capture the key aspects of the data, they may not be comprehensive enough to distinguish all the nuances between the tea samples from the two harvest years.

.

Table 4.6. SIMCA results of fluorescence spectroscopic data for the differentiation of black tea according to harvest year

Data Transform Method	**Correct Classification Rate (%)**		**Sensitivity***	**Specificity***	**Efficiency (%) ***	**Number of Principal Components***		**R^{2*}**	
	Calibration	**Validation**				**2021**	**2022**	**2021**	**2022**
SNV	67.88%	73.53%	0.67	0.9	77.7	8	9	0.861	0.892
1st Der + SNV	76.64%	73.53%	0.7	0.81	75.3	12	13	0.853	0.871
2nd Der+SNV	88.32%	88.24%	0.86	0.91	88.5	15	15	0.798	0.771

*These values correspond to validation set.

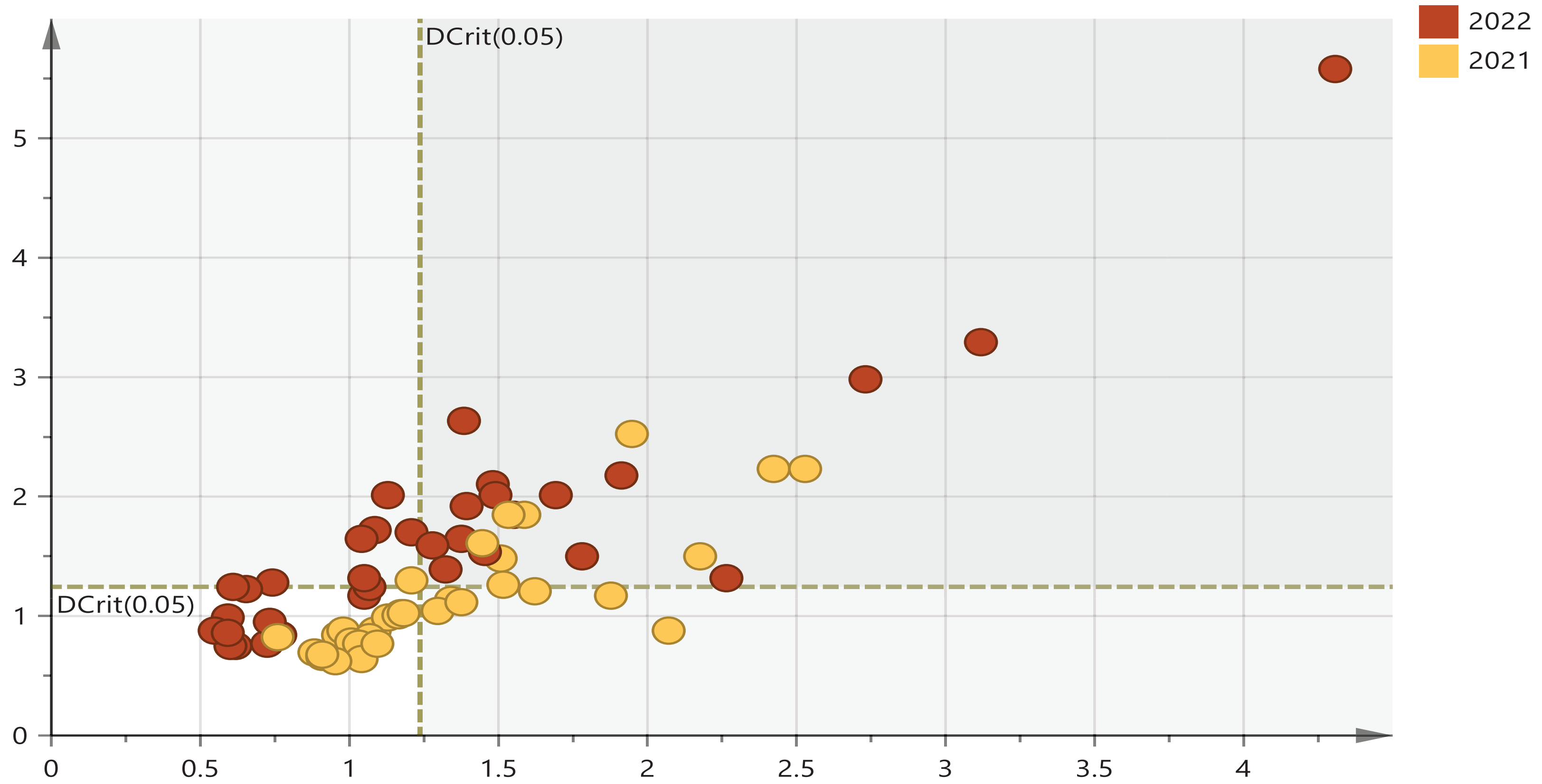


Figure 4.5. Cooman’s plot for SIMCA analysis of fluorescence data for the differentiation of black tea according to harvest year (samples harvested in 2021 represented by orange cycles, samples harvested in 2022 represented by maroon circles)

4.4. Classification Using Partial Least-Square Discriminant (PLS-DA) and Orthogonal Partial Least-Square Discriminant Analyses (OPLS-DA)

Spectral data obtained from powdered tea and infused tea samples were also analyzed using PLS-DA and OPLS-DA to investigate whether classification by harvest year was possible. Discriminant classification refers to methods used to classify samples based on their significant differences. PLS-DA and OPLS-DA are effective when dealing with highly correlated variables such as in spectroscopic data (Firmani et al., 2019).

Thirteen different data transformations, along with the raw data, were applied before model fitting. The classification models were built in three iterations, each time using a newly randomized dataset for calibration and validation. In each iteration, LV, R^2, RMSE, sensitivity, specificity and efficiency were assessed for both calibration and validation models. The final models were selected based on their consistent and strong performances across all three iterations. This method aimed to reduce the impact of specific samples on model development (Cavdaroglu and Ozen, 2023). This approach was applied to data from all spectroscopic techniques examined in the study

4.4.1. Application of OPLS-DA and PLS-DA on FTIR Spectroscopic Data for Tea Samples in Powder Form

The top three models for FTIR powder spectra, along with results from the three iterations, are shown in Table 4.7. All three models consist of various combinations of SD preprocessing. The resulting models showed consistently high correct classification rate, sensitivity, specificity, and R^2 values, low RMSE values for both calibration and validation regardless of the sample used. As can be seen from Table 4.7, the correct classification rate for the validation set of these models was found to be higher than 90% for all of them and an average of 93.3%, the sensitivity was 0.9 on average, and the specificity was the highest for all models. All these values are the same and high in all models; therefore, the models show strong sample identification according to the harvest year of the samples.

Figure 4.6a displays the SD of black tea samples spectra, which is one of the transformations that generated a successful discriminating model. The spectra of the samples from different years differ visibly in the 1550–800 cm^{-1} region. The main components of this spectral region are pigments, phenolic compounds, caffeine and carbohydrates. Furthermore, chemometric analysis can uncover differences that might not be readily apparent. Figure 4.6b displays the score plot for the second derivative OPLS-DA model. This plot shows that the data from 2021 and 2022 are clearly differentiated from one another in terms of the first latent variable (LV1). According to the established model, chemometric analysis and FTIR transmission data evaluation may successfully separate black tea samples based on the year of harvest.

4.4.2. Application of OPLS-DA and PLS-DA on FTIR Spectroscopic Data for Infused Tea Samples

The best models developed with the FTIR spectroscopic data obtained from infused tea samples along with the outcomes of three iterations are listed in Table 4.8. These models offer acceptable classification abilities with a high correct classification rate, sensitivity, and specificity, which range from 90.33 to 95%, 0.87 to 0.92, and 0.97 to 1, respectively. Figure 4.7a shows the square transformed data, and Figure 4.7b displays the score plot for this model.

The transformed spectra reveal noticeable differences in the peak corresponding to the 3600-3000 cm^{-1} region. The score plot for the samples from the two harvest years shows some overlap. In comparison to the FTIR transmission spectra, water extracts from tea samples exhibit comparatively modest absorption peaks in the 1500–800 cm^{-1} area.

In certain spectral regions, water-related peaks are the most noticeable features. Nonetheless, useful models have been developed to accurately differentiate tea samples based on their harvest year.

Table 4.7. Statistical parameters of year-to-year discrimination chemometric models of black tea in powder form for FTIR-Transmission data

Data Transform and Analysis Method	**LV***	**R^{2*}**	**RMSE***	**Correct Classification Rate (%) ***	**Sensitivity***	**Specificity***
2^{nd} derivative + SNV/ PLS-DA	7	0.990	0.050	95	0.92	1.00
	6	0.984	0.064	90	0.85	1.00
	7	0.993	0.042	95	0.92	1.00
2^{nd} derivative + MSC/ PLS-DA	7	0.992	0.044	95	0.92	1.00
	6	0.985	0.063	90	0.85	1.00
	7	0.992	0.046	95	0.92	1.00
2^{nd} derivative/ OPLS-DA	1+3	0.923	0.140	95	0.92	1.00
	1+4	0.990	0.050	90	0.85	1.00
	1+4	0.990	0.050	95	0.92	1.00

*These values correspond to validation set.

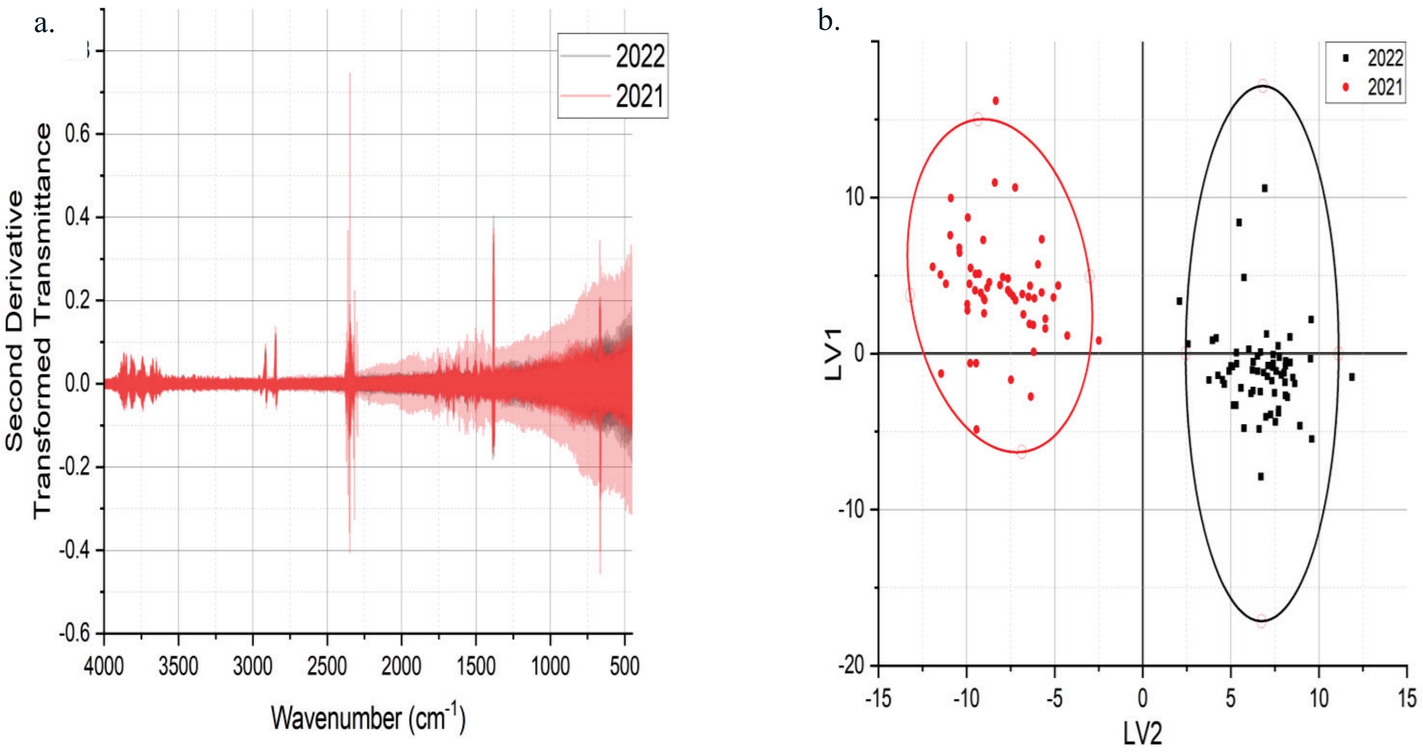


Figure 4.6. a. Second derivative FTIR-Transmittance spectra of black tea leaves, b. Score plot of OPLS-DA model obtained from second derivative FTIR- Transmittance black tea spectra for the differentiation of two harvest years (samples harvested in 2021 represented by red cycles, samples harvested in 2022 represented by black circles

Table 4.8. Statistical parameters of year-to-year discrimination chemometric models of infused black tea for ATR-FTIR data

Data Transform and Analysis Method	**LV***	**R^{2*}**	**RMSE***	**Correct Classification Rate (%) ***	**Sensitivity***	**Specificity***
Square transformed/ OPLS-DA	1+11	0.807	0.230	95	0.92	1.00
	1+11	0.845	0.207	95	0.92	1.00
	1+11	0.822	0.219	95	0.92	1.00
Savitzky-Golay filtering/ PLS-DA	11	0.706	0.282	90	0.91	0.90
	15	0.904	0.165	90	0.85	1.00
	14	0.889	0.174	95	0.92	1.00
3rd derivative/ PLS-DA	6	0.989	0.054	86	0.83	0.89
	6	0.988	0.057	95	0.92	1.00
	6	0.988	0.055	90	0.85	1.00

*These values correspond to validation set

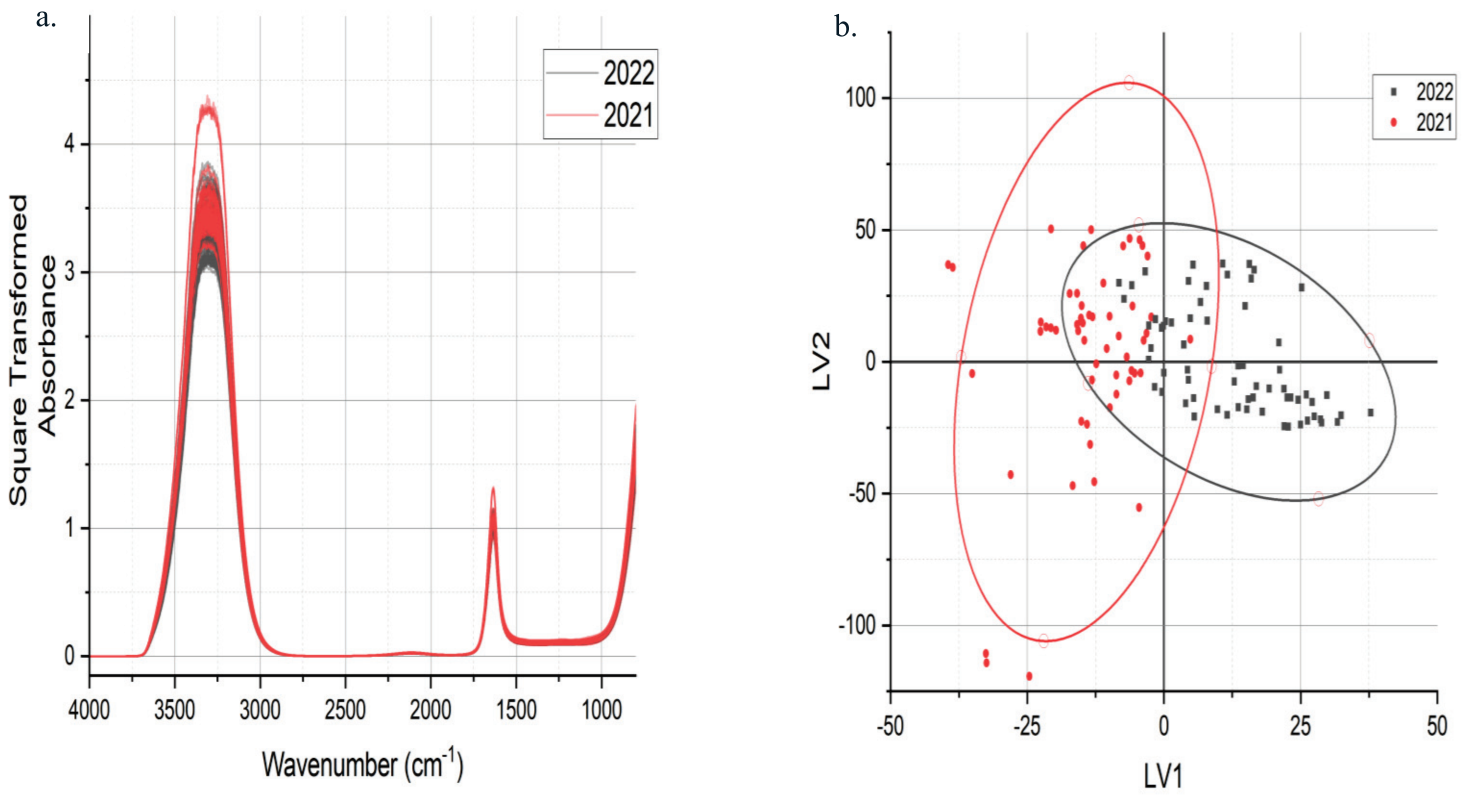


Figure 4.7. a. Square transformed FTIR spectra of black tea infusions, b. Score plot of OPLS-DA model obtained from square transformed FTIR black tea infusion spectra for the differentiation of two harvest years (samples harvested in 2021 represented by red cycles, samples harvested in 2022 represented by black circles

4.4.3. Application of OPLS-DA and PLS-DA on UV-Visible Spectroscopic Data for Infused Tea Samples

The UV-visible spectroscopic data were analyzed using PLS-DA and OPLS-DA after applying various transformations. Overall, the statistical models developed were quite successful; however, the models highlighted in Table 4.9. stand out as the best due to their high R^2 values and low RMSE, along with strong classification rates, sensitivity, and specificity. One of the transformed spectra, specifically the first derivative-SNV transformation, is displayed in Figure 4.8a, indicates clear differences in the 200-300 nm range. Additionally, the score plot from the OPLS-DA model using the FD-SNV transformation demonstrates a clear separation of the infused tea samples along the first latent variable (LV) in Figure 4.8b.

This separation suggests that there are significant differences in the pigment composition of the black tea samples from the two harvest years, as UV-visible spectroscopy primarily detects color compounds.

4.4.4. Application of OPLS-DA and PLS-DA on Fluorescence Spectroscopic Data for Infused Tea Samples

Among the best models for using fluorescence spectra to differentiate black tea depending on harvest year are the PLS-DA and OPLS-DA analyses of the raw data, as well as the OPLS-DA of the FD transformed data which are listed in Table 4.10. The FD transformed OPLS-DA model achieved a correct classification rate of 98.33%, with a sensitivity of 1 and specificity of 0.97. The spectra from this transformation are illustrated in Figure 4.9a, while the score plot is shown in Figure 4.9b.

Notable differences can be observed throughout the spectral range of the samples from different harvest years. Additionally, the score plot demonstrates a strong separation of the samples corresponding to the different harvest years along the first latent variable (LV).

Table 4.9. Statistical parameters of year-to-year discrimination chemometric models of infused black tea for UV-visible spectroscopic data

Data Transform and Analysis Method	**LV***	**R^{2*}**	**RMSE***	**Correct Classification Rate (%) ***	**Sensitivity***	**Specificity***
1st derivative + SNV/ OPLS-DA	1+3	0.855	0.192	100	1.00	1.00
	1+3	0.862	0.188	100	1.00	1.00
	1+4	0.925	0.138	100	1.00	1.00
2nd derivative/ PLS-DA	6	0.982	0.067	100	1.00	1.00
	6	0.987	0.058	100	1.00	1.00
	5	0.981	0.070	100	1.00	1.00
3rd derivative + SNV/ OPLS-DA	1+2	0.775	0.237	100	1.00	1.00
	1+1	0.724	0.263	100	1.00	1.00
	1+2	0.771	0.240	100	1.00	1.00

*These values correspond to validation set.

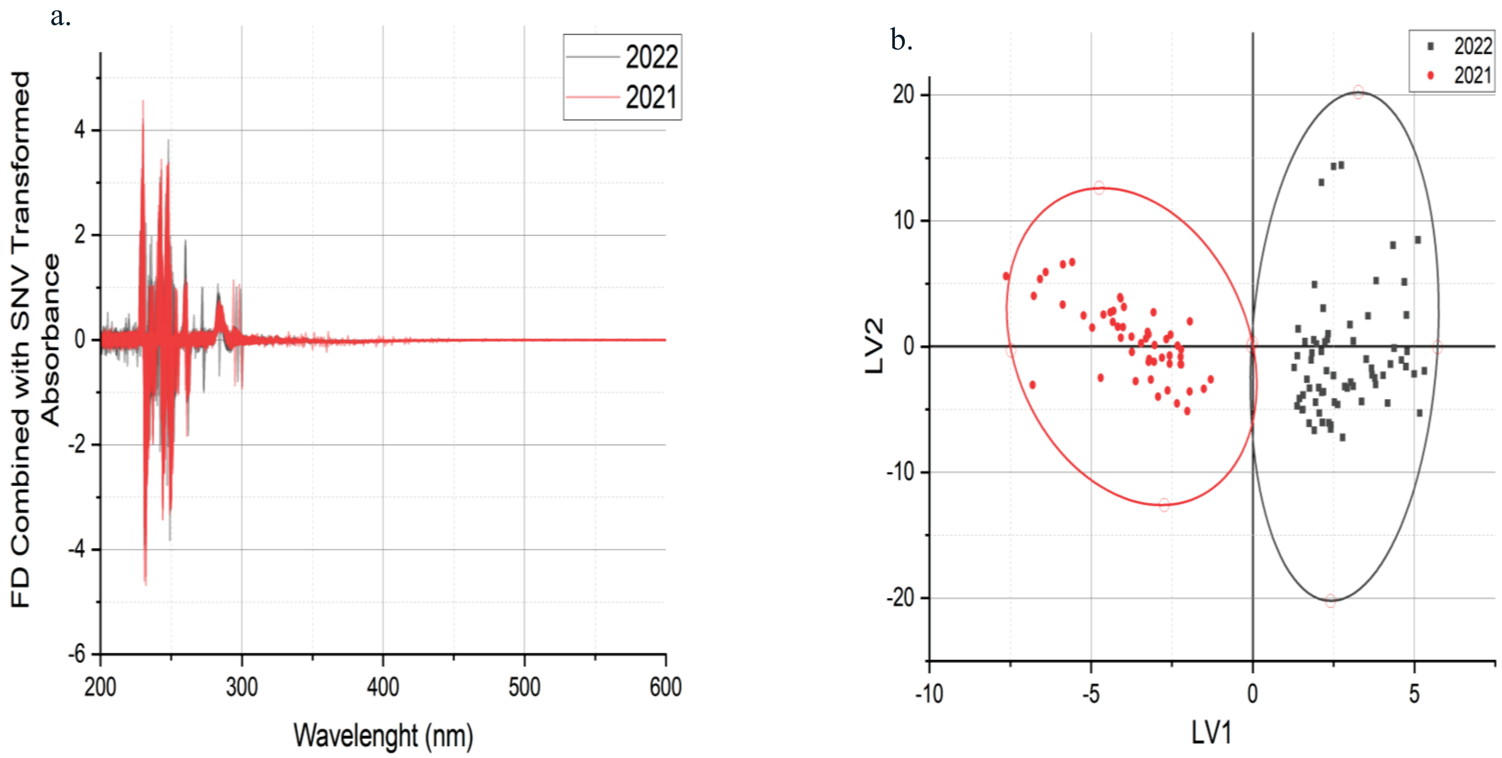


Figure 4.8. a. First derivative+SNV transformed UV-visible spectra of black tea infusions, b. Score plot of OPLS-DA model obtained from first derivative+SNV transformed UV-visible black tea infusion spectra for the differentiation of two harvest years (samples harvested in 2021 represented by red cycles, samples harvested in 2022 represented by black circles)

Table 4.10. Statistical parameters of year-to-year discrimination chemometric models of infused black tea for fluorescence spectroscopic data

Data Transform and Analysis Method	**LV***	**R^{2*}**	**RMSE***	**Correct Classification Rate (%)***	**Sensitivity***	**Specificity***
Raw/ PLS-DA	10	0.849	0.202	100	1.00	1.00
	8	0.760	0.254	100	1.00	1.00
	14	0.923	0.145	95	1.00	0.91
Raw/ OPLS-DA	1+13	0.926	0.145	100	1.00	1.00
	1+5	0.655	0.302	100	1.00	1.00
	1+11	0.889	0.173	95	1.00	0.91
1st derivative/ OPLS-DA	1+2	0.812	0.219	100	1.00	1.00
	1+3	0.907	0.156	95	1.00	0.91
	1+5	0.944	0.119	100	1.00	1.00

*These values correspond to validation set.

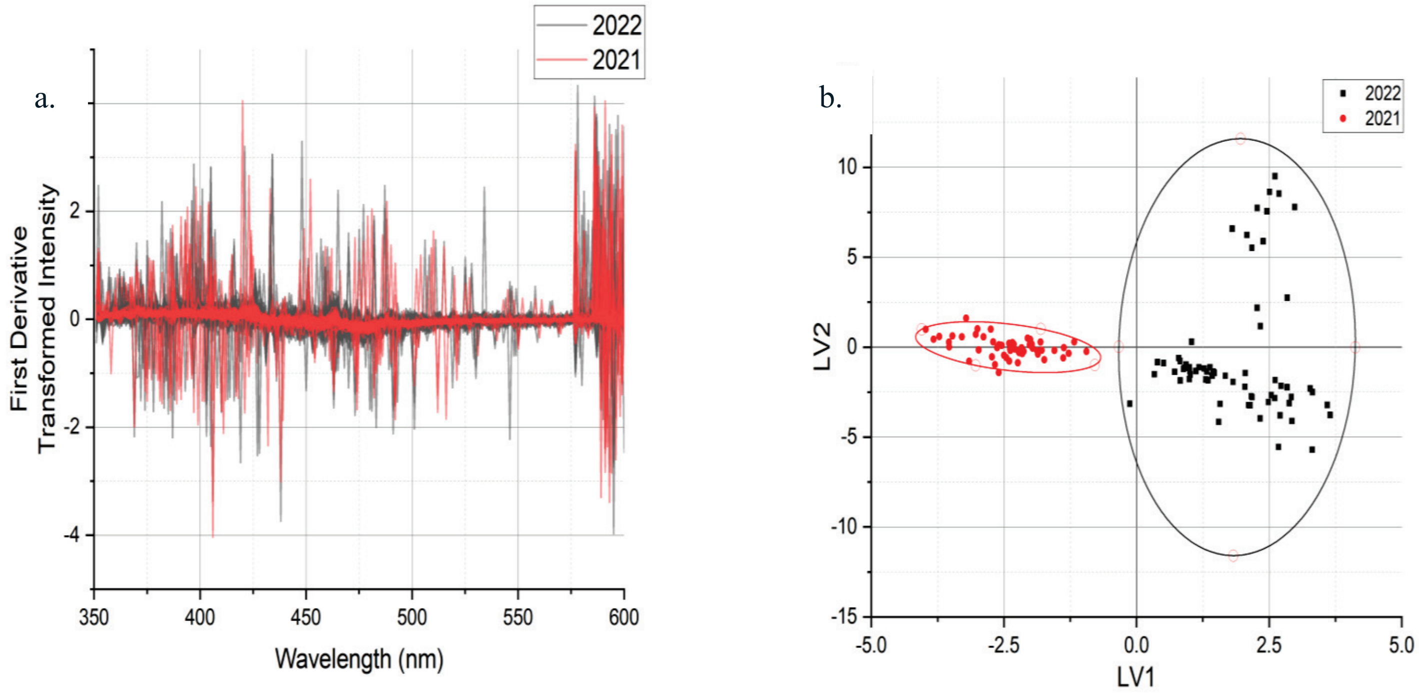


Figure 4.9. a. First derivative transformed Fluorescence spectra of black tea infusions, b. Score plot of OPLS-DA model obtained from first derivative transformed fluorescence black tea infusion spectra for the differentiation of two harvest years (samples harvested in 2021 represented by red cycles, samples harvested in 2022 represented by black circles)

There are previous studies combining various spectroscopic methods with chemometrics to determine different properties of tea. In the study conducted by (Diniz et al., 2016), various multivariate statistical analyses including PLS-DA and SIMCA were performed on UV-visible spectra of tea infusions in order to distinguish geographical origin and variety. In another study, UV-visible and FTIR data were investigated to distinguish between black and green tea types and it was concluded that these two spectroscopic methods can be used for this purpose (Arifah et al., 2022). Furthermore, combination of UV-Vis, NIR and fluorescence spectroscopy were used with the help of different chemometric methods for the classification of tea samples (Dankowska and Kowalewski, 2019). Combination of near and mid infrared spectroscopy has also been used to determine the dry-matter content in tea with chemometric methods, including PLS (Li et al., 2013). Fluorescence spectroscopy is aimed to determine the catechin content in tea rapidly (Du et al., 2020).

Previous studies combining spectroscopic methods with SIMCA and PLS analysis-based techniques also exist. In the study by Firmani et al. (2019), NIR spectroscopy with PLS-DA and SIMCA methods were used to detect the adulteration of black tea. Although both methods were successful in distinguishing these authentic samples from adulterated samples, the PLS-DA method proved to be a highly effective method for this type of studies by correctly classifying 138 out of 140 test samples. In another study, SIMCA method was used to rapidly identify tea varieties using spectral data obtained from NIR spectroscopy, and only one of the four tea varieties was predicted with 80% accuracy, while the remaining three varieties were predicted with 100% accuracy. In the same study, PLS was used to estimate the caffeine and total polyphenol content in tea (Chen et al., 2006).

The criteria used to construct models is the most important difference between PLS analysis-based techniques and SIMCA. While PLS finds directions in the data space that discriminate against classes directly, PCA sub-models in SIMCA are computed with the objective of capturing variations within each class. As a result, in these situations, SIMCA classification consistently provides lower results compared to PLS analysis-based techniques (Firmani et al., 2019; Galtier et al., 2011). Although studies in literature have proven that spectroscopic and chemometric methods are effective in rapid and effective food analysis and predicting content in food substances, as far as our knowledge goes, no investigation has performed for tea harvested in different years until this study.

This study utilized FTIR, UV-visible, and fluorescence spectroscopy combined with chemometric models to classify black tea samples by harvest year. Each technique offers shared benefits, including non-destructiveness, rapid analysis, and minimal sample preparation, while also demonstrating unique strengths and limitations.

FTIR spectroscopy effectively captured chemical details, especially for phenolic compounds, and delivered high classification accuracy for both powdered and infused samples. Its sensitivity to specific molecular structures (e.g., -OH, C=O, C-H) enables detailed chemical profiling, aiding in year-based differentiation. The slightly reduced accuracy for infused samples may result from water interference.

UV-visible spectroscopy provided insights into pigment composition, particularly theaflavins and thearubigins, yielding the highest classification accuracy for infused samples. While effective for pigments and UV-visible absorbing compounds, its scope is narrower than FTIR.

Fluorescence spectroscopy excelled in identifying phenolic compounds, achieving the highest classification rate. It distinguished samples by detecting minor compositional shifts in fluorescent compounds, such as tea polyphenols, with peak intensities around 425 nm and 475 nm highlighting harvest-year differences.

Preprocessing methods, like SNV, MSC, and derivatives, enhanced model performance by reducing noise. Among chemometric models, PLS-DA and OPLS-DA performed well, with OPLS-DA showing slightly better differentiation by excluding uncorrelated variation.

Despite promising results, the study's limitations include the complexity of chemometric models for practical applications and limited geographic scope. Future research could broaden geographic sampling and span more years to capture year-to-year variability, strengthening the generalizability and robustness of findings.

These spectroscopic techniques hold promise for routine quality control in the tea industry. Their ability to classify tea by harvest year enables rapid assessment of batch consistency and identification of compositional deviations due to environmental factors. UV-visible and fluorescence spectroscopy are especially valuable for monitoring phenolic compounds and pigments, key to flavor and quality. Implementing these methods at critical points can help producers ensure consistent quality, aligning with consumer expectations.

CHAPTER 5

CONCLUSION

Spectroscopic analyses offer the advantages of speed, accuracy, and environmental friendliness. Consequently, numerous spectroscopic methods and studies on tea— the world’s most consumed beverage after water, valued for its unique flavor, therapeutic benefits, and antioxidant properties— are readily available in literature. However, most of the literature on the application of various spectroscopic techniques in tea primarily focuses on classifying the product by its variety, geographical origin, or identifying specific compounds. Whereas the extensive capabilities of spectroscopic and chemometric methods can also be effectively and easily applied to monitor changes in tea quality across different harvest years. Monitoring changes in tea quality is important since plants are critically affected by factors such as seasonality, water availability, geographical conditions, light exposure, altitude, microbial presence, temperature and soil properties related to climate. In addition to these critical factors, changes in average precipitation and climate conditions due to global warming, especially in recent years, and changes such as increasing Earth's surface temperature have also reduced agricultural productivity. Therefore, it is vital for both producers and future research to be able to quickly and easily determine annual and seasonal changes with spectroscopic methods. Results of spectroscopic and chemometric techniques provide a comprehensive perspective, and the information collected can help clarify quality criteria of the product. In this study, the FTIR, UV-visible and fluorescence spectral profiles, together with the help of multivariate statistical analyses PLS-DA, OPLS-DA, and SIMCA, proved that a total of 205 tea samples harvested in two different years, correctly distinguished black tea according to the harvest year. Combination of spectroscopic methods with multivariate statistical analysis, can support the evaluation process of new harvests, enable the development of efficient products in the following years, and enable healthier decisions to be made during quality control. Finally, it can allow for a better understanding of the effects of the above-mentioned factors on plants. Future research should aim to broaden sample range by incorporating diverse geographical regions and processing conditions, enhancing the validation and generalizability of these models for wider applications.

REFERENCES

Aaqil, Muhammad, Chunxiu Peng, Ayesha Kamal, Taufiq Nawaz, Fei Zhang, and Jiashun Gong. 2023. "Tea Harvesting and Processing Techniques and Its Effect on Phytochemical Profile and Final Quality of Black Tea: A Review" *Foods* 12, no. 24: 4467. https://doi.org/10.3390/foods12244467.

Abudureheman, Buhailiqiemu, Xiaochun Yu, Dandan Fang, and Henghui Zhang. 2022. "Enzymatic Oxidation of Tea Catechins and Its Mechanism" *Molecules* 27, no. 3: 942. https://doi.org/10.3390/molecules27030942

Klasra, Mushtaq Ahmed, Khalid Mahmood Khawar, and Muhammed Aasim. 2007. "History of Tea Production and Marketing in Turkey." *International Journal of Agriculture & Biology* 9, no. 3: 523-529.

Ahmed, Selena, Timothy S. Griffin, Debra Kraner, M. Katherine Schaffner, Deepak Sharma, Matthew Hazel, Alicia R. Leitch, et al. 2019. “Environmental Factors Variably Impact Tea Secondary Metabolites in the Context of Climate Change.” *Frontiers in Plant Science* 10 (August). https://doi.org/10.3389/fpls.2019.00939.

Arifah, Mitsalina Fildzah, Agustina Ari Murti Budi Hastuti, and Abdul Rohman. 2022. “Utilization of UV-Visible and FTIR Spectroscopy Coupled with Chemometrics for Differentiation of Indonesian Tea: An Exploratory Study”. *Indonesian Journal of Pharmacy* 33 (2): 200–207. https://doi.org/10.22146/ijp.3795.

Balentine, Douglas A., Sheila A. Wiseman, and Liesbeth C. M. Bouwens. 1997. “The Chemistry of Tea Flavonoids.” *Critical Reviews in Food Science and Nutrition* 37 (8): 693–704. https://doi/10.1080/10408399709527797.

Berthomieu, Catherine, and Rainer Hienerwadel. 2009. “Fourier Transform Infrared (FTIR) Spectroscopy.” *Photosynthesis Research* 101 (2–3): 157-170. https://doi.org/10.1007/s11120-009-9439-x.

Bortolini, Débora Gonçalves, Charles Windson Isidoro Haminiuk, Alessandra Cristina Pedro, Isabela de Andrade Arruda Fernandes, and Giselle Maria Maciel. 2021. “Processing, Chemical Signature and Food Industry Applications of Camellia Sinensis Teas: An Overview.” *Food Chemistry: X* 12 (December). https://doi.org/10.1016/j.fochx.2021.100160.

Cai, Jian xiong, Yuan feng Wang, Xiong gang Xi, Hui Li, and Xin lin Wei. 2015. “Using FTIR Spectra and Pattern Recognition for Discrimination of Tea Varieties.” *International Journal of Biological Macromolecules* 78 (July). Elsevier B.V.: 439–46. https://doi.org/10.1016/j.ijbiomac.2015.03.025.

Cavdaroglu, Cagri, and Banu Ozen. 2023. “Applications of UV–Visible, Fluorescence and Mid-Infrared Spectroscopic Methods Combined with Chemometrics for the

Authentication of Apple Vinegar" *Foods* 12, no. 6: 1139. https://doi.org/10.3390/foods12061139

Chen, Qincao, Yin Zhu, Yafang Liu, Yang Liu, Chunwang Dong, Zhi Lin, and Jie Teng. 2022. "Black Tea Aroma Formation during the Fermentation Period." *Food Chemistry* 374 (April). Elsevier Ltd. https://doi.org/10.1016/j.foodchem.2021.131640.

Chen, Quansheng, Jiewen Zhao, and Hao Lin. 2009. "Study on Discrimination of Roast Green Tea (Camellia Sinensis L.) According to Geographical Origin by FT-NIR Spectroscopy and Supervised Pattern Recognition." *Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy* 72 (4): 845–50. https://doi.org/10.1016/j.saa.2008.12.002.

Chen, Quansheng, Jiewen Zhao, Haidong Zhang, and Xinyu Wang. 2006. "Feasibility Study on Qualitative and Quantitative Analysis in Tea by near Infrared Spectroscopy with Multivariate Calibration." *Analytica Chimica Acta* 572 (1): 77–84. https://doi.org/10.1016/j.aca.2006.05.007.

Chen, Xi, Danhong Wang, Jie Li, Taotao Xu, Keqiang Lai, Qi Ding, Hetong Lin, Lin Sun, and Mengshi Lin. 2020. "A Spectroscopic Approach to Detect and Quantify Phosmet Residues in Oolong Tea by Surface-Enhanced Raman Scattering and Silver Nanoparticle Substrate." *Food Chemistry* 312 (May): Elsevier Ltd. https://doi.org/10.1016/j.foodchem.2019.126016.

Chiang, Shu Hua, Kai Min Yang, Shiu Yu Wang, and Chih Wei Chen. 2022. "Enzymatic Treatment in Black Tea Manufacturing Processing: Impact on Bioactive Compounds, Quality, and Bioactivities of Black Tea." *LWT* 163 (June): https://doi.org/10.1016/j.lwt.2022.113560.

Dankowska, A., and W. Kowalewski. 2019. "Tea Types Classification with Data Fusion of UV–Vis, Synchronous Fluorescence and NIR Spectroscopies and Chemometric Analysis." *Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy* 211 (March). Elsevier B.V.: 195–202. https://doi.org/10.1016/j.saa.2018.11.063.

Deb, Saptashish, and K.R. Jolvis Pou. 2016. "A Review of Withering in the Processing of Black Tea." *Journal of Biosystems Engineering* 41 (4). Korean Society for Agricultural Machinery: 365–72. https://doi.org/10.5307/jbe.2016.41.4.365.

Diniz, Paulo Henrique Gonçalves Dias, Mayara Ferreira Barbosa, Karla Danielle Tavares De Melo Milanez, Marcelo Fabián Pistonesi, and Mário César Ugulino De Araújo. 2016. "Using UV-Vis Spectroscopy for Simultaneous Geographical and Varietal Classification of Tea Infusions Simulating a Home-Made Tea Cup." *Food Chemistry* 192 (July): 374–79. https://doi.org/10.1016/j.foodchem.2015.07.022.

Du, Chenxu, Chaoqun Ma, Jiao Gu, Lei Li, Chun Zhu, Lvming Chen, Tingyu Wang, and Guoqing Chen. 2020. "Rapid Determination of Catechin Content in Black Tea by Fluorescence Spectroscopy." *Journal of Spectroscopy* 2020. https://doi.org/10.1155/2020/2479612.

Dutta, Aastha. 2017. “Fourier Transform Infrared Spectroscopy.” In *Spectroscopic Methods for Nanomaterials Characterization*, 2:73–93. Elsevier. https://doi.org/10.1016/B978-0-323-46140-5.00004-2

Erturk, Yasar, Sezai Ercisli, Memnune Sengul, Zeynep Eser, Ayhan Haznedar, and Metin Turan. 2010. “Seasonal Variation of Total Phenolic, Antioxidant Activity and Minerals in Fresh Tea Shoots (Camellia Sinensis Var. Sinensis).” *Pakistan journal of pharmaceutical sciences*, 23(1): 69-74.

FAO. 2024. “Current Global Market Situation and Medium Term Outlook.” https://openknowledge.fao.org/handle/20.500.14283/cd0688en.

Firmani, Patrizia, Silvia De Luca, Remo Bucci, Federico Marini, and Alessandra Biancolillo. 2019. “Near Infrared (NIR) Spectroscopy-Based Classification for the Authentication of Darjeeling Black Tea.” *Food Control* 100 (June). Elsevier Ltd: 292–99. https://doi.org/10.1016/j.foodcont.2019.02.006.

Fu, Li, Bo-Tao Xu, Ren-You Gan, Yuan Zhang, Xiang-Rong Xu, En-Qin Xia, and Hua-Bin Li. 2011. "Total Phenolic Contents and Antioxidant Capacities of Herbal and Tea Infusions" *International Journal of Molecular Sciences* 12, (4): 2112-2124. https://doi.org/10.3390/ijms12042112

Galtier, O., O. Abbas, Y. Le Dréau, C. Rebufa, J. Kister, J. Artaud, and N. Dupuy. 2011. “Comparison of PLS1-DA, PLS2-DA and SIMCA for Classification by Origin of Crude Petroleum Oils by MIR and Virgin Olive Oils by NIR for Different Spectral Regions.” *Vibrational Spectroscopy* 55 (1): 132–140. https://doi.org/10.1016/j.vibspec.2010.09.012.

Giorgini, Elisabetta, Valentina Notarstefano, Roberta Foligni, Patricia Carloni, and Elisabetta Damiani. 2024. “First ATR-FTIR Characterization of Black, Green and White Teas (Camellia Sinensis) from European Tea Gardens: A PCA Analysis to Differentiate Leaves from the In-Cup Infusion.” *Foods* 13 (1): Multidisciplinary Digital Publishing Institute (MDPI). https://doi.org/10.3390/foods13010109.

Guo, Xiangyang, Chuankui Song, Chi Tang Ho, and Xiaochun Wan. 2018. “Contribution of L-Theanine to the Formation of 2,5-Dimethylpyrazine, a Key Roasted Peanutty Flavor in Oolong Tea during Manufacturing Processes.” *Food Chemistry* 263 (October): 18–28. https://doi.org/10.1016/j.foodchem.2018.04.117.

Harbowy, Matthew E., Douglas A. Balentine, Alan P. Davies, and Ya Cai. 1997. “Tea Chemistry.” In *Critical Reviews in Plant Sciences*, 16:415–80. Informa UK Limited. https://doi.org/10.1080/07352689709701956

Hong, Zhiqi, Chu Zhang, Dedong Kong, Zhenyu Qi, and Yong He. 2021. “Identification of Storage Years of Black Tea Using Near-Infrared Hyperspectral Imaging with Deep Learning Methods.” *Infrared Physics and Technology* 114 (May): Elsevier B.V. https://doi.org/10.1016/j.infrared.2021.103666.

Hu, Xian-Chun, Huicheng Yu, Ying Deng, Ying Chen, Xiao Hua Zhang, Hui Wen Gu, and Xiao Li Yin. 2023. “Rapid Authentication of Green Tea Grade by Excitation-

Emission Matrix Fluorescence Spectroscopy Coupled with Multi-Way Chemometric Methods.” *European Food Research and Technology* 249 (3): Springer Science and Business Media Deutschland GmbH: 767–75. https://doi.org/10.1007/s00217-022-04174-w.

Jayasinghe, Sadeeka Layomi, and Lalit Kumar. 2021. "Potential Impact of the Current and Future Climate on the Yield, Quality, and Climate Suitability for Tea [*Camellia sinensis* (L.) O. Kuntze]: A Systematic Review" *Agronomy* 11, no. 4: 619. https://doi.org/10.3390/agronomy11040619

Khasnabis, Jyotismita, Chandan Rai, and Arindam Roy. 2015. "Determination of Tannin Content by Titrimetric Method from Different Types of Tea." *Journal of Chemical and Pharmaceutical Research* 7 (6): 238-241. Accessed September 3, 2024. https://www.jocpr.com/articles/determination-of-tannin-content-by-titrimetric-method-from-different-types-of-tea.pdf.

Kim, Youngmok, Kevin L. Goodner, Jong Dae Park, Jeong Choi, and Stephen T. Talcott. 2011. “Changes in Antioxidant Phytochemicals and Volatile Composition of Camellia Sinensis by Oxidation during Tea Fermentation.” *Food Chemistry* 129 (4): 1331–1342. https://doi.org/10.1016/j.foodchem.2011.05.012.

Koch, Wojciech. 2020. “Theaflavins, Thearubigins, and Theasinensins.” In *Handbook of Dietary Phytochemicals*, 1–29. Springer Singapore. https://doi.org/10.1007/978-981-13-1745-3_20-1.

Kuhnert, Nikolai. 2013. “Chemistry and Biology of the Black Tea Thearubigins and of Tea Fermentation.” In *Tea in Health and Disease Prevention*, 343–60. Elsevier. https://doi.org/10.1016/B978-0-12-384937-3.00029-X.

Lee, Lan Sook, Young Chan Kim, Jong Dae Park, Young Boong Kim, and Sang Hee Kim. 2016. “Changes in Major Polyphenolic Compounds of Tea (Camellia Sinensis) Leaves during the Production of Black Tea.” *Food Science and Biotechnology* 25 (6): Kluwer Academic Publishers: 1523–1527. https://doi.org/10.1007/s10068-016-0236-y.

Li, Shiming, Chih-Yu Lo, Min-Hsiung Pan, Ching-Shu Lai, and Chi-Tang Ho. 2013. “Black Tea: Chemical Analysis and Stability.” *Food and Function*. https://doi.org/10.1039/c2fo30093a.

Li, Xiaoli, Juanjuan Jin, Chanjun Sun, Dapeng Ye, and Yufei Liu. 2019. “Simultaneous Determination of Six Main Types of Lipid-Soluble Pigments in Green Tea by Visible and near-Infrared Spectroscopy.” *Food Chemistry* 270 (January): Elsevier Ltd: 236–42. https://doi.org/10.1016/j.foodchem.2018.07.039.

Li, Xiaoli, Liubin Luo, Yong He, and Ning Xu. 2013. “Determination of Dry Matter Content of Tea by near and Middle Infrared Spectroscopy Coupled with Wavelet-Based Data Mining Algorithms.” *Computers and Electronics in Agriculture* 98. Elsevier B.V.: 46–53. https://doi.org/10.1016/j.compag.2013.07.014

Liang, Yuerong, Jianliang Lu, Lingyun Zhang, Shan Wu, and Ying Wu. 2003. “Estimation of Black Tea Quality by Analysis of Chemical Composition and

Colour Difference of Tea Infusions." *Food Chemistry* 80 (2): https://doi.org/10.1016/S0308-8146(02)00415-6.

Long, Piaopiao, Kanyasiri Rakariyatham, Chi Tang Ho, and Liang Zhang. 2023. "Thearubigins: Formation, Structure, Health Benefit and Sensory Property." *Trends in Food Science and Technology* 133. 37-48 Elsevier Ltd. https://doi.org/10.1016/j.tifs.2023.01.013.

Ltd, R. Tea Global Market Report. 2024 - Research and Markets. Research and Markets Ltd 2024. https://www.researchandmarkets.com/reports/5806952/tea-global market-report#tag-pos-23.

Łuczaj, W., and E. Skrzydlewska. 2005. "Antioxidative Properties of Black Tea." *Preventive Medicine* 40 (6): Academic Press Inc.: 910–918. https://doi.org/10.1016/j.ypmed.2004.10.014.

Macheka, Lesley, Veronica Chifamba, Juliet Mubaiwa, Godwil M Madamombe, Ruth T Ngadze, and Faith Manditsera. 2022. "Effect Of Withering Temperature and Time on Biochemical Properties of Vegetatively Propagated and Seedling Tea." *Nutrition and Food Science International Journal* 11 (3): Juniper Publishers. https://doi.org/10.19080/nfsij.2022.11.555814.

Muheddin, Daron Q., Shujahadeen B. Aziz, and Pshko A. Mohammed. 2023. "Variation in the Optical Properties of PEO-Based Composites via a Green Metal Complex: Macroscopic Measurements to Explain Microscopic Quantum Transport from the Valence Band to the Conduction Band" *Polymers* 15 (3): 771. https://doi.org/10.3390/polym15030771

Naveed, Muhammad, Jannat BiBi, Asghar Ali Kamboh, Imran Suheryani, Ihsanullah Kakar, Sarfaraz Ali Fazlani, Xia FangFang, et al. 2018. "Pharmacological Values and Therapeutic Properties of Black Tea (Camellia Sinensis): A Comprehensive Overview." *Biomedicine and Pharmacotherapy*. Elsevier Masson SAS.: 521-531 https://doi.org/10.1016/j.biopha.2018.02.048.

Palacios-Morillo, Ana, Ángela Alcázar, Fernando De Pablos, and José Marcos Jurado. 2013a. "Differentiation of Tea Varieties Using UV-Vis Spectra and Pattern Recognition Techniques." *Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy* 103 (February): 79–83. https://doi.org/10.1016/j.saa.2012.10.052.

Pan, Si-Yuan, Qu Nie, Hai Chuan Tai, Xue Lan Song, Yu Fan Tong, Long Jian Feng Zhang, Xue Wei Wu, et al. 2022. "Tea and Tea Drinking: China's Outstanding Contributions to the Mankind." *Chinese Medicine (United Kingdom)*. BioMed Central Ltd. https://doi.org/10.1186/s13020-022-00571-1.

Panigrahi, Niranjan, Chandra Sekhar Bhol, and Bhabani Sankar Das. 2016. "Rapid Assessment of Black Tea Quality Using Diffuse Reflectance Spectroscopy." *Journal of Food Engineering* 190 (December): Elsevier Ltd: 101–8. https://doi.org/10.1016/j.jfoodeng.2016.06.020.

Picollo, Marcello, Maurizio Aceto, and Tatiana Vitorino. 2019. “UV-Vis Spectroscopy.” *Physical Sciences Reviews* 4 (4). De Gruyter. https://doi.org/10.1515/psr-2018-0008.

Pratiwi, Restiani Alia, and Asep Bayu Dani Nandiyanto. 2022. “How to Read and Interpret UV-VIS Spectrophotometric Results in Determining the Structure of Chemical Compounds.” *Indonesian Journal of Educational Research and Technology* 2 (1). Universitas Pendidikan Indonesia (UPI): 1–20. https://doi.org/10.17509/ijert.v2i1.35171.

Rahman, M. M., M. M. Hossain, R. Das, and I. Ahmad. 2020. “Changes in Phytochemicals and Determination of Optimum Fermentation Time during Black Tea Manufacturing.” *Journal of Scientific Research* 12 (4). Bangladesh Journals Online (JOL): 657–664. https://doi.org/10.3329/jsr.v12i4.45452.

Ren, Guangxin, Xusheng Zhang, Rui Wu, Lingling Yin, Wenyan Hu, and Zhengzhu Zhang. 2023. “Rapid Characterization of Black Tea Taste Quality Using Miniature NIR Spectroscopy and Electronic Tongue Sensors.” *Biosensors* 13 (1). https://doi.org/10.3390/bios13010092.

Ruxton, Carrie H.S. 2009. “The Health Effects of Black Tea and Flavonoids.” *Nutrition and Food Science* 39(13): https://doi.org/10.1108/00346650910957546.

Samadi, Saba, and Fatemeh Raouf Fard. 2020. “Phytochemical Properties, Antioxidant Activity and Mineral Content (Fe, Zn and Cu) in Iranian Produced Black Tea, Green Tea and Roselle Calyces.” *Biocatalysis and Agricultural Biotechnology* 23 (January): https://doi.org/10.1016/j.bcab.2019.101472.

Sang, S. 2015. “Tea: Chemistry and Processing.” In *Encyclopedia of Food and Health*, 268–72. Elsevier Inc. https:10.1016/B978-0-12-384947-2.00685-1.

Sanlier, Nevin, Buşra Basar Gokcen, and Mehmet Altuğ. 2018. “Tea Consumption and Disease Correlations.” *Trends in Food Science and Technology*. Elsevier Ltd. https://doi.org/10.1016/j.tifs.2018.05.026.

Sari, Ferda, and Y. Sedat Velioglu. 2013. “Changes in Theanine and Caffeine Contents of Black Tea with Different Rolling Methods and Processing Stages.” *European Food Research and Technology* 237 (2). Springer Verlag: 229–236. https://doi.org/10.1007/s00217-013-1984-z.

Seetohul, L. Nitin, Simon M. Scott, William T. O’Hare, Zulfiqur Ali, and Meez Islam. 2013. “Discrimination of Sri Lankan Black Teas Using Fluorescence Spectroscopy and Linear Discriminant Analysis.” *Journal of the Science of Food and Agriculture* 93 (9): 2308–2314. https://doi.org/10.1002/jsfa.6044.

Sharma, Vasundhara, and L. Jagan Mohan Rao. 2009. “A Thought on the Biological Activities of Black Tea.” *Critical Reviews in Food Science and Nutrition* 49 (5): 379–404. https://doi.org/10.1080/10408390802068066.

Suryoprabowo, Steven. Bryan Laywith, Wang Zhongxing, Albert Hendriko and Wang Wenbin “Black Tea Manufacturing Process, Health Benefit, and Application in

Food Industry: A Review.” *Food Science and Nutrition* 10 (2): 1–6. https://doi.org/10.24966/FSN-1076/100180.

Tanaka, Takashi, and Yosuke Matsuo. 2020. “Production Mechanisms of Black Tea Polyphenols.” *Chem. Pharm. Bull* (68). https://doi.org/10.1248/cpb.c20-00295

Temple, S. J., C. M. Temple, A. J.B. Van Boxtel, and M. N. Clifford. 2001. “The Effect of Drying on Black Tea Quality.” *Journal of the Science of Food and Agriculture* 81 (8): 764–72. https://doi.org/10.1002/jsfa.881.

TEPGE. 2024. “TARIM ÜRÜNLERİ PİYASALARI Agricultural Products Markets.” Accessed October16,2024. https://arastirma.tarimorman.gov.tr/tepge/Belgeler/PDF%20Tar%C4%B1m%20%C3%9Cr%C3%BCnleri%20Piyasalar%C4%B1/2024-Temmuz%20Tar%C4%B1m%20%C3%9Cr%C3%BCnleri%20Raporu/%C3%87ay%20Tar%C4%B1m%20%C3%9Cr%C3%BCnleri%20Piyasalar%C4%B1%20Raporu%20Temmuz-2024-v4.pdf.

Truong, Van-Long, and Woo-Sik Jeong. 2021. "Cellular Defensive Mechanisms of Tea Polyphenols: Structure-Activity Relationship" *International Journal of Molecular Sciences* 22, no. 17: 9109. https://doi.org/10.3390/ijms22179109

Turkmen, Nihal, Ferda Sarı, and Y. Sedat Velioglu. 2009. “Factors Affecting Polyphenol Content and Composition of Fresh and Processed Tea Leaves”. *Akademik Gıda* 7(6): 29-40.

Ullah Shirazi, Ovais, Muhammad Muzaffar Ali Khan Khattak, Nor Azwani Mohd Shukri, Jalan Sultan Ahmad Shah. 2014. “Determination of Total Phenolic, Flavonoid Content and Free Radical Scavenging Activities of Common Herbs and Spices.” *Journal of Pharmacognosy and Phytochemistry* 3 (3).

Vuong, Quan V. 2014. “Epidemiological Evidence Linking Tea Consumption to Human Health: A Review.” *Critical Reviews in Food Science and Nutrition* 54 (4): 523–536. https://doi.org/10.1080/10408398.2011.594184.

Vuong, Quan V., John B. Golding, Minh Nguyen, and Paul D. Roach. 2010. “Extraction and Isolation of Catechins from Tea.” *Journal of Separation Science*. https://doi.org/10.1002/jssc.201000438

Wang, Jingjing, Muhammad Zareef, Peihuan He, Hao Sun, Quansheng Chen, Huanhuan Li, Qin Ouyang, et al. 2019. “Evaluation of Matcha Tea Quality Index Using Portable NIR Spectroscopy Coupled with Chemometric Algorithms.” *Journal of the Science of Food and Agriculture* 99 (11). John Wiley and Sons Ltd: 5019–5027. https://doi.org/10.1002/jsfa.9743.

Wang, L. Y., K. Wei, Y. W. Jiang, H. Cheng, J. Zhou, W. He, and C. C. Zhang. 2011. “Seasonal Climate Effects on Flavanols and Purine Alkaloids of Tea (Camellia Sinensis L.).” *European Food Research and Technology* 233 (6): 1049–55. https://doi.org/10.1007/s00217-011-1588-4.

Wehry, Earl L. 1997. "Molecular Fluorescence and Phosphorescence Spectrometry." In Handbook of Instrumental Techniques for Analytical Chemistry, edited by Frank A. Settle, 507-531. Upper Saddle River, NJ: Prentice Hall.

Wei, Kang, Li Yuan Wang, Jian Zhou, Wei He, Jian Ming Zeng, Yong Wen Jiang, and Hao Cheng. 2012. "Comparison of Catechins and Purine Alkaloids in Albino and Normal Green Tea Cultivars (Camellia Sinensis L.) by HPLC." *Food Chemistry* 130 (3): 720–724. https://doi.org/10.1016/j.foodchem.2011.07.092.

Xia, Jing, Dan Wang, Pei Liang, De Zhang, Xiaoqing Du, Dejiang Ni, and Zhi Yu. 2020. "Vibrational (FT-IR, Raman) Analysis of Tea Catechins Based on Both Theoretical Calculations and Experiments." *Biophysical Chemistry* 256 (January). Elsevier B.V. https://doi.org/10.1016/j.bpc.2019.106282.

Xu, Yue, Xiangyang Zhou, and Wenjuan Lei. 2023. "Identifying the Producer and Grade of Matcha Tea through Three-Dimensional Fluorescence Spectroscopy Analysis and Distance Discrimination" *Foods* 12(19): 3614. https://doi.org/10.3390/foods12193614

Yang, Jun, and Rui H. Liu. 2013. "The Phenolic Profiles and Antioxidant Activity in Different Types of Tea." *International Journal of Food Science and Technology* 48 (1): 163–171. https://doi.org/10.1111/j.1365-2621.2012.03173.x.

Zhang, Hua, Ruili Qi, and Yoshinori Mine. 2019. "The Impact of Oolong and Black Tea Polyphenols on Human Health." *Food Bioscience* 29 (June). Elsevier Ltd: 55–61. https://doi.org/10.1016/j.fbio.2019.03.009.

Zhang, Liang, Chi Tang Ho, Jie Zhou, Jânio Sousa Santos, Lorene Armstrong, and Daniel Granato. 2019. "Chemistry and Biological Activities of Processed Camellia Sinensis Teas: A Comprehensive Review." *Comprehensive Reviews in Food Science and Food Safety* 18 (5). Blackwell Publishing Inc.: 1474–1495. https://doi.org/10.1111/1541-4337.12479.

Zhou, Haoxiang, Haijun Fu, Xiaohong Wu, Bin Wu, and Chunxia Dai. 2020. "Discrimination of Tea Varieties Based on FTIR Spectroscopy and an Adaptive Improved Possibilistic C-Means Clustering." *Journal of Food Processing and Preservation* 44 (10). Blackwell Publishing Ltd. https://doi.org/10.1111/jfpp.14795.

Zhu, Kun, Jie Ouyang, Jianan Huang, and Zhonghua Liu. 2021. "Research Progress of Black Tea Thearubigins: A Review." *Critical Reviews in Food Science and Nutrition* 61 (9). Bellwether Publishing, Ltd.: 1556–66. https://doi.org/10.1080/10408398.2020.1762161.

Zou, Chun, Xin Zhang, Yongquan Xu, and Junfeng Yin. 2024. "Recent Advances Regarding Polyphenol Oxidase in *Camellia sinensis*: Extraction, Purification, Characterization, and Application" *Foods* 13(4): 545. https://doi.org/10.3390/foods13040545