# APPLICATION OF NON-TARGETED ANALYSIS METHODS IN ADULTERATION DETECTION AND PREDICTION OF PROCESS PARAMETERS OF VINEGARS

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**Çağrı ÇAVDAROĞLU**

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We approved the thesis of **Çağrı ÇAVDAROĞLU**

**Examining Committee Members:**

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

**Assoc. Prof. Dr. Efe SEZGİN**
Department of Food Engineering, İzmir Institute of Technology

**Prof. Dr. Durmuş ÖZDEMİR**
Department of Chemistry, İzmir Institute of Technology

**Prof. Dr. Kamile Nazan TURHAN**
Department of Food Engineering, İzmir University of Economics

**Prof. Dr. Neriman BAĞDATLIOĞLU**
Department of Food Engineering, Manisa Celal Bayar University

**24 November 2023**

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

**Assoc. Prof. Dr. Ayşe Handan BAYSAL**
Head of the Department of
Food Engineering

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

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# ABSTRACT

## APPLICATION OF NON-TARGETED ANALYSIS METHODS IN ADULTERATION DETECTION AND PREDICTION OF PROCESS PARAMETERS OF VINEGARS

Vinegar plays a multifaceted role in human diet, encompassing nutritive, functional, and taste-enhancing aspects. Quality of vinegar is influenced by quality of raw materials and production methods employed. Spectroscopic techniques offer distinct advantages in terms of speed and environmental friendliness, making them valuable tools for monitoring and controlling food production processes. This study has two major parts. In the first part, traditional and commercial grape vinegar production were monitored using both chemical parameters (total acidity, pH, brix, ethanol etc.) and mid-infrared (mid-IR) and UV-visible (UV-vis) profiles. These measured chemical parameters were predicted from spectral profiles in combination with multivariate statistical analysis techniques. In the second part, mid-IR, UV-vis and fluorescence spectroscopic techniques were used in determination of adulteration of both grape and apple vinegar with acetic acid and spirit vinegar at various ratios. Capability of spectroscopic methods combined with chemometrics were tested for prediction of various chemical parameters of vinegar as well as detection of adulteration of vinegar with different adulterants. Those techniques have proven to be effective in estimating the overall quantities of sugars, phenolics, flavonoids, and organic acids. Utilizing chemometric models with UV–vis and mid-IR data yielded high rates of correct classification, sensitivity, and specificity, particularly for adulteration levels exceeding 5% in vinegar. The performance of mid-IR spectroscopy demonstrated success in detecting the presence of spirit vinegar and acetic acid in apple vinegar. Overall, with this thesis, the usefulness of spectroscopic methods was highlighted by emphasizing the importance of chemometric tools for the parameter prediction and detection of vinegar adulteration.

# ÖZET

## SİRKEDE TAĞŞİŞİN TESPİT EDİLMESİNDE HEDEFSİZ ANALİZ YÖNTEMLERİNİN KULLANILMASI VE PROSES PARAMETRELERİNİN TAHMİN EDİLMESİ

Sirke, insan beslenmesinde besleyici, işlevsel ve tat arttırıcı bir rol oynar. Sirke kalitesi, kullanılan hammaddelerin kalitesinden ve üretim yöntemlerinden etkilenir. Spektroskopik teknikler hız ve çevre dostu olma açısından belirgin avantajlar sunarak onları gıda üretim süreçlerinin izlenmesi ve kontrolünde değerli araçlar haline getirmektedir. Bu çalışma iki ana bölümden oluşmaktadır. İlk bölümde, geleneksel ve ticari üzüm sirkesi üretimi hem kimyasal parametreler (toplam asitlik, pH, briks, etanol vb.) orta kızılötesi (mid-IR) ve UV-görünür (UV-vis) profilleri kullanılarak izlenmiştir. Ölçülen kimyasal parametreler, çok değişkenli istatistiksel analiz teknikleriyle spektral profillerden tahmin edilmiştir. İkinci bölümde üzüm ve elma sirkesinin asetik asit ve beyaz sirke ile çeşitli oranlarda tağşişinin belirlenmesinde mid-IR, UV-vis ve floresans spektroskopik teknikleri kullanılmıştır. Kemometri ile birleştirilmiş spektroskopik yöntemlerin başarısı, sirkenin çeşitli kimyasal parametrelerinin belirlenmesinin yanı sıra sirkenin farklı tağşiş maddeleri ile hilesinin tespiti için test edilmiştir. Bu tekniklerin şeker, fenolik madde, flavonoid ve organik asitlerin genel konsantrasyonunu tahmin etmede etkili olduğu kanıtlanmıştır. Ancak toplam şeker, fenolik madde veya organik asitlere ait ayrı ayrı bileşenlerin konsantrasyonu yüksek hassasiyetle tahmin edilememiştir. UV-vis ve mid-IR verileriyle kemometrik modellerin kullanılması, özellikle üzüm sirkesi için %5'in üzerindeki tağşiş seviyelerini değerlendirirken yüksek oranda doğru sınıflandırma, hassasiyet ve özgüllük elde edilmesini sağlamıştır. Mid-IR'nin performansı, elma sirkesinde beyaz sirke ve ilave asetik asit varlığının tespitinde başarı göstermiştir. Genel olarak, bu tezle, konsantrasyon tahmini ve sirke tağşişinin tespiti için kemometrik araçların önemi gösterilerek spektroskopik yöntemlerin kullanışlılığı vurgulanmıştır.

*Dedicated to my beloved family*

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| | |
|---|---|
| ANN | Artificial neural networks |
| CE | Catechin equivalent |
| DTGS | Deuterated triglycine sulfate |
| EHEC | Enterohemorrhagic *Escherichia coli* |
| EPEC | Enteropathogenic *Escherichia coli* |
| FD | First derivative transformation |
| FTIR | Fourier transform infrared |
| GAE | Gallic acid equivalent |
| GC-MS | Gas chromatography/mass spectrometry |
| HS-SPME | Headspace solid-phase microextraction |
| IRMS | Isotope ratio mass spectrometry |
| LV | Latent variables |
| mid-IR | Mid-infrared |
| MSC | Multiplicative signal correction |
| NIR | Near-infrared |
| NMR | Nuclear magnetic resonance |
| OPLS | Orthogonal partial least square |
| OSC | Orthogonal signal correction |
| PARAFAC | Parallel factor analysis |
| PDO | Protected Designation of Origin |
| PGI | Protected Geographical Indication |
| PLS | Partial least square |
| RMSEC | Root mean square of error for calibration |
| RMSEP | Root mean square of error for prediction |
| RPD | Residual predictive deviation |
| SD | Second derivative transformation |
| SGF | Savitzky-Golay filtering |
| SNV | Standard normal variate |
| TBV | Traditional Balsamic Vinegar |
| TBVM | Traditional Balsamic Vinegar of Modena |
| TD | Third derivative transformation |
| TFC | Total flavonoid content |
| TPC | Total phenolic content |
| UV-Vis | UV-Visible |
| VIP | Variable importance in projection |
| WCTS | Wavelet condensed time series |
| WDTS | Wavelet denoising decimation |
| ZnSe | Zinc-Selenide |

# CHAPTER 1

# INTRODUCTION

Vinegar is a beneficial product derived from a variety of raw materials using different fermentation methods. Commonly employed raw materials encompass a wide range of grains and fruits, mostly grapes and apples. The major components of vinegar consist of acetic acid and water, but there are also low concentration of other organic acids, alcohol, phenolic compounds, and amino acids. These minor constituents play a significant role in shaping the sensory attributes of the final product.

Spectroscopic techniques have been invaluable tools for characterizing and verifying various types of vinegars. Spectroscopy has also been used to predict the chemical composition of diverse food products (Cavdaroglu and Ozen 2021a). Monitoring major chemical parameters like total acidity, sugar content, acetic acid levels, and ethanol concentrations at different stages of vinegar production as well as minor components, such as volatiles, phenolic profiles, and total phenol content throughout the fermentation process is crucial for the process and the quality control (Cavdaroglu and Ozen 2021b). The use of chemometric techniques for analyzing spectral data allows for the simultaneous estimation of chemical constituents in different types of fermented food products. As a result, some studies have collected various spectroscopic profiles during vinegar production or solely of the final product to predict its quality and chemical attributes.

Given the complexity of vinegar, which comes in many diverse varieties, detecting adulteration has become a challenging task. Adulteration detection methods can be categorized as targeted or non-targeted techniques. Targeted techniques focus on the presence or absence of specific compounds as indicators of adulteration. Conversely, non-targeted methods aim to create general profiles of the analyzed products.

Targeted adulteration testing focuses on specific compounds that can serve as markers of adulteration (Hattori et al. 2010). The presence or absence of certain compounds may signal potential adulteration. However, many of these targeted compounds exist in low concentrations within food products, which can be a limitation

of targeted analysis, as sophisticated adulteration techniques may make small changes undetectable.

On the contrary, non-targeted analysis takes a comprehensive approach, aiming to provide a holistic assessment of the analyzed food product. Various non-targeted techniques are currently in use, with spectroscopic methods, such as Fourier transform infrared (FTIR), near-infrared (NIR), hyperspectral imaging, Raman, and nuclear magnetic resonance (NMR) spectroscopy. Spectroscopic measurements often involve complex differences between authentic and adulterated spectra that may not be readily discriminable through visual inspection. As a result, chemometrics arises as a valuable tool for extraction of meaningful information from the data. Chemometrics proves highly effective in identifying food samples based on geographical origin and species variety, while also aiding in the detection of contamination and adulteration. It is commonly integrated with spectroscopic techniques to analyze and interpret the data effectively.

Among the various types of fraudulent activities related to vinegar, the most common one involves blending a less economically valuable product with one of higher value. For instance, an often-cited example is the mixing of spirit vinegar with authentic vinegar. In economically motivated fraud cases involving vinegar, spirit vinegar and acetic acid are frequently used adulterants.

Misrepresenting the geographical origin of a product is another fraudulent practice (Rios-Reina et al. 2020). Both targeted and non-targeted methods employed in authentication studies have been put to use in detecting vinegar adulteration. As spectroscopic methods generate a multitude of variables even in a single measurement, the preferred approach is often to employ chemometric methods to evaluate this type of data rather than univariate statistical analysis techniques.

In the light of these, this thesis has three main aims which will be covered under Chapters 3 to 5 as listed below.

- In Chapter 3, the study involved monitoring the production of vinegar through both traditional and submerged fermentation methods using two different grape varieties. The aim was to predict 22 quality and chemical parameters, including brix, total phenolic content, total flavonoid content, titratable acidity, pH, and the concentrations of individual phenolic compounds using different spectroscopic data and chemometric techniques.
- In chapter 4, objective was to detect adulteration with spirit vinegar and diluted glacial acetic acid in grape vinegars. UV-Vis and Fourier transform infrared

(FTIR) spectroscopic data using partial least square (PLS), orthogonal PLS (OPLS) methods and artificial neural networks (ANN).

- In chapter 5, the purpose was to evaluate and compare the capabilities of different spectroscopic methods, including UV-visible, fluorescence, and mid-infrared, in combination with PLS and OPLS techniques, for the detection of adulteration of apple vinegars with spirit vinegar and synthetic acetic acid.

Each chapter refers to an article. Therefore, at the beginning of each chapter, bibliographic information of the publications is given. In order to keep the integrity of the thesis structure, some necessary elements which were not placed in publications were supplied in the appendices.

# CHAPTER 2

# AUTHENTICATION OF VINEGARS WITH TARGETED AND NON-TARGETED METHODS

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## 2.1. Abstract

There has been a growing interest in vinegar, especially after the increasing reports about its beneficial health effects. Bioactive compounds of vinegar are associated with its antimicrobial, antioxidant, antidiabetic, antitumor, and anti-obesity types of activities. Quality of vinegar is related with the authenticity of the product besides the amounts of bioactive compounds in its composition. Addition of cheaper substitutes to higher quality vinegars and false labeling are some common authentication problems for this product. There are various examples of the use of targeted and untargeted methods in authentication studies for vinegars. Specific constituents and properties of vinegars such as molecular isotope ratios and individual volatile compounds were used to detect adulteration with targeted methods. On the other hand, untargeted methods, mostly in the form of the application of spectroscopic techniques, such as infrared and fluorescence spectroscopy in combination with chemometrics, provide an overall measurement. This review mainly focuses on adulteration types and elaborates on different targeted and non-targeted methods used to authenticate vinegars.

## 2.2. Introduction

Vinegar is defined as “a liquid fit for human consumption, produced from a suitable raw material of agricultural origin, containing starch, sugars or starch and sugars such as fruit, berries, cereal grains, malted barley, whey, honey; by the process of double fermentation, alcoholic and acetous, and contains a specified amount of acetic acid” by Joint FAO/WHO Food Standards Programme.[1] There are some variations in regulations regarding vinegar depending on the legal entity. Codex[1] specifies that vinegar shall not contain more than 0.5% alcohol and less than 50 g/L acetic acid. According to Food and Drug Administration (FDA), the final vinegar at the end of processing should contain “4 g acetic acid per 100 mL”. European Union (EU) recognizes that “acetic acid when diluted with water (4–30% by volume) could be used as a food or food ingredient in the same manner as vinegars from agricultural origin (Commission Regulation 2016/23)”. However, “in some Member States only vinegars obtained from the fermentation of agricultural products are allowed to be named vinegars”, according to the same regulation.

Vinegar is consumed as a seasoning, preservation agent, and one of the main ingredients in salad dressings, ketchup and other sauces.[1, 2] Vinegars can be classified into five groups: cereal, wine and grape, traditional balsamic (TBV), Jerez, and cider vinegars.[3] However, this classification does not include types of vinegars such as spirit vinegars produced by acetic oxidation of ethanol derived from the distillation of fermented mashes or petrochemical ethanol. Codex also provides definitions for the following vinegar groups: 1. wine, fruit vinegar, berry vinegar, cider vinegar, 2. Spirit vinegar, 3. Grain vinegar, 4. Malt vinegar, 5. Distilled malt vinegar, 6. Whey vinegar, 7. Honey vinegar. Vinegar may contain some optional ingredients such as plants, particularly herbs, spices and fruits, whey, concentrated or fresh fruit juices, sugars, honey and food-grade salts, according to Codex again.

History of vinegar has been evolved from its production as a by-product of wine processing to the production of a wide spectrum of vinegars, including cheap to quite expensive products. With increasing number of research on its beneficial effects on health, this product is getting even more consumer attention. However, this increased attention makes product, especially certain economically valuable traditional ones, more

prone to counterfeiting. There are various reports about mixing different types of vinegars with different adulterants to obtain extra profit. Since vinegar could be a very complex liquid depending on its type, it can be quite challenging to determine its adulteration. There are examples of the use of both targeted and non-targeted approaches in authentication studies that could be found in the literature. Targeted methods identify specific constituents of vinegars such as certain phenolic compounds or volatiles, while non-targeted methods are based on overall measurement of the sample as in spectroscopic analyses. It is aimed to provide a literature review about the use of targeted and non-targeted techniques for vinegar authentication. However, it is important to understand the characteristics of the product itself to better evaluate these frauds. Therefore, a brief information about the types, production, composition and functional properties of the vinegar will be provided first.

## 2.3. Vinegar Types

Diversity of the vinegars is due to not only by raw materials but also processes used in the production. Various raw materials including grape, apple, cereals, and other starch and sugar-containing foods, such as pomegranate, lemon, artichoke, tomato, onion, bamboo, ginseng, are used in the production of vinegar. Production and aging steps could also specify the characteristics of vinegar. As an example, balsamic and sherry vinegars are differentiated from the others by their production through traditional processes. In this part, some significant vinegar types will be described.

### 2.3.1. Wine Vinegar

Wine vinegar is made from red or white wine and is the most commonly used vinegar in the households of the Mediterranean countries and Central Europe. Wine vinegars are mostly produced using the semi-continuous submerged process.[2] The acetic acid content of wine vinegar is set as at least 6% (w/v), and the maximum allowed ethanol

concentration is specified as 1.5% (v/v) by European Union Regulation (EC) 1493/1999. Phenolic acids and aldehydes are indicated as useful quality parameters of wine vinegars besides their major components.[4]

### 2.3.2. Balsamic Vinegar

Production of balsamic vinegar was first originated from Italy and there are two types of balsamic vinegar: “balsamic vinegar of Modena” (BVM) and “traditional balsamic vinegar of Modena” (TBVM). The first one is a flavored wine vinegar obtained by blending cooked must and wine vinegar and, in some cases, by adding a small amount of caramel. TBVM is produced in Modena and Reggio Emilia with cooked grape must, through a three-step process: conversion of sugars to ethanol by yeasts; oxidation of ethanol to acetic acid by acetic acid bacteria; and, finally, at least 12 years of aging. The final product is a highly dense, dark-brown aged vinegar, having a sweet and sour taste, fruity and complex in flavor.[3] Grapes, from the northern region of Italy near Modena, which are used in vinegar production are left on the vine for as long as possible to increase the sugar level, as ripened grapes contain higher sugar levels. TBV may age up to 25 years.[5] The commercial version of balsamic vinegar is designated as Aceto Balsamico di Modena (BVM) and must be aged for a minimum of two months and up to three years to meet the minimum requirements to claim protected geographical indication.[6]

### 2.3.3. Sherry Vinegar

Sherry vinegar, considered as a traditional food product, has been commonly used as a seasoning and a condiment.[7] As for balsamic type, this vinegar is also a high-quality product with fame all over the world.[8] It can be produced by both traditional methods and submerged culture acetification followed by aging in wood (dynamic or static system). Special type of traditional methods, the “solera” system and the static method, are used in its production. According to aging time in barrels, Sherry vinegar is defined

as “Vinagre de Jerez”, “Reserva”, and “Gran Reserva”.[9] These vinegars from Spain have also Protected Designated of Origin (PDO) status, which shows that the product quality is attributed to the region of production.

### 2.3.4. Cider Vinegar

Processed apple products, apple juice, or fermented apple cider can be used as raw materials in the production of cider vinegar through a double fermentation: alcoholic and acetic.[3] Most natural raw materials do not require the addition of extra nutrients, but apple cider is usually low in nitrogenous materials; for this reason, addition of extra nitrogen in the form of ammonium phosphate and thiamin is a common practice.[10]

### 2.3.5. Cereal Vinegar

Malt and rice vinegars are the most widely produced cereal vinegars. Malt vinegar is an aged and filtered product made by alcoholic and subsequent acetous fermentation, without distillation, of an infusion of barley malt with or without the addition of other cereals.[10] Malt has a distinctive flavor that contributes to the flavor of the deriving vinegar. Malt vinegar is popular for pickling, especially walnut pickles. It is the most famous one as a condiment for fish and chips.[11] Rice vinegar is a traditional seasoning that has long been used in China, Japan, and Korea.[12] Rice vinegar is produced from fermented polished and unpolished rice and there are amber, red and black colored rice vinegars having different acidity values and usages.[3]

## 2.4. Production

Production of vinegar is a two-stage fermentation process: conversion of fermentable sugars to ethanol by yeasts, usually *Saccharomyces* species, at acidic pH and the oxidation of ethanol by bacteria, usually *Acetobacter* species.[13] Acetic acid fermentation occurs in two steps, first ethanol is oxidized to acetaldehyde, then further oxidation yields acetic acid. These reactions are catalyzed by cytoplasmic enzymes, alcohol dehydrogenase, and aldehyde dehydrogenase. During alcohol fermentation, anaerobic conditions prevail and after the consumption of sugars by yeasts aerobic conditions develop at the surface with further progress of the process. Factors including starter culture, ethanol concentration at the start of fermentation, fermentation temperature, oxygen flow rate, method of maturation, storage conditions, bottling, and pasteurization influence the quality of the product.[13] After acetification of mash, vinegar can be maturated or aged. Currently, oak is the most commonly used wood in enology for aging wines, spirits, and vinegars.[14]

The traditional process is one of the main methods of vinegar production and it is based on surface culture fermentation, where the acetic acid bacteria is placed on the air–liquid interface in direct contact with atmospheric air. The presence of the bacteria is limited to the surface of the acidifying liquid and hence, it is also considered as a static method.[2] This method includes gradual filling of the barrel with slime or "mother of vinegar" and the rate of reaction is slow with low efficiency.[15] Traditional vinegar production taking place in wood barrels is known as Orleans process and is especially used in the production of high-quality table vinegars.

Submerged culture system is the other common method of vinegar production. In this type of system, must is spread through a large area with a slow flow rate and acetic acid fermentation takes place with the inoculation of acetic acid bacteria. *Acetobacter xylinum*, *Acetobacter pasteurianus*, *Acetobacter aceti*, *Acetobacter hansenii*, *Acetobacter lovaniensis*, *Acetobacter liquefaciens* are commonly used cultures for this purpose. Fermentation occurs with the activity of bacteria which are homogeneously spread in the must. In this type of production, fermentation takes place on the whole media by the airing of reactor. High production capacity is obtained with fast conversion to acetic acid. Because of the faster processing and the higher productivity, commercial vinegar

production is mostly done with this method.[13] The Frings Acetator is the most widely used equipment for the production of all kinds of vinegar.[10] The rotor is installed on the shaft of a motor mounted under the fermenter, connected to an air suction pipe, and surrounded by a stator. It sucks air and pumps liquid, creating an air-liquid emulsion which is ejected through the stator, radially outward at a given speed, chosen so that the turbulence of the stream causes a uniform distribution of the air over the whole cross-section of the fermenter in commercial scale.[15]

The vinegar production process is generally carried out in a semi-continuous manner, and the final product reaches 12–15% acetic acid concentration at the end of this process. The process continues in cycles that start with the addition of fresh mash to the fermenter and 1/3 of this fermenter is filled with the previous fermentation product to obtain 7–10% acetic acid and ca. 5% ethanol concentration. When an alcohol concentration is in the range of 0.05–0.3% in the fermenting liquid, a quantity of vinegar is discharged from the fermenter, and it is refilled with fresh mash.

In the literature, there are limited number of studies that compare the properties of traditional and commercial vinegars. In several comparison studies, physicochemical properties, phenol profiles, antioxidant and antimicrobial properties, volatile components, and sensory properties of vinegars produced with different techniques were assessed. As a result, differences in almost all tested characteristics were observed between commercial and traditional techniques.[11, 16–18]

## 2.5. Composition

Composition of vinegar is directly related with its raw materials' composition, as a result, it depends on factors, such as variety and growing conditions of raw material and also production techniques of the product. Major raw materials used in the production of vinegar are grape, apple, and wine. Total acidity of vinegars produced from different raw materials varies between 3.9% and 12.2% (as acetic acid equivalent) and the rest of the medium is organic acids, alcohols, polyphenols, amino acids, etc.[13] Acetic acid is the most dominant component of vinegar; however, citric, formic, lactic, malic and succinic acids are also present.[19, 20] Concentrations of organic acids and reducing sugars are quite

high in TBV. Although tartaric acid is one of the main components of grape, it is not present in high concentrations in TBV.[20] The total amount of glucose and fructose ranges between 43 and 63 g/100 g, while the sum of organic acids and sugars is more than 50% of the composition. Acetic acid, other organic acids, esters, ketones, and aldehydes are the sources of the distinctive aroma of vinegar and these aromatic compounds form especially during acetic acid fermentation.[21] In a study conducted with different classes of Sherry vinegars, 58 aroma, and 80 odor compounds were identified using gas chromatography/mass spectrometry (GC-MS) and gas chromatography/olfactometry (GC-O), respectively.[7] While the presence of some of the aroma compounds, such as ethyl heptanoate, ethyl furoate, ethyl benzoate, and sotolon were known; ethyl 2-methylbutyrate, ethyl heptanoate, ethyl furoate, ethyl benzoate, acetophenone, and nonanoic acid were recorded in the samples for the first time. Besides, research team was able to discriminate Sherry vinegars from other types of vinegars according to their aroma compounds using multivariate statistical analysis techniques.[7]

Vinegar is a good source of various phenolic compounds, such as gallic acid, catechin, vanillic acid, syringic acid, and caffeic acid. The total phenolic contents of different vinegars were determined in several studies. According to Bakir et al.,[22] balsamic vinegar had the highest total flavonoid (960 mg catechin equivalent (CE)/L) and total phenolic contents (2550 mg gallic acid equivalent (GAE)/L). This study was supported by another research conducted in which the amount of total phenolic contents of commercial apple, rice, balsamic, red wine, rose, white wine, grape and pomegranate vinegars were investigated, and the highest total phenolic content was measured in balsamic vinegar with 2141.64 ± 25.07 mg GAE/L while rice vinegar contained the lowest with 14.36 ± 0.16 mg GAE/L.[23] Several chemical and functional properties of BVM and TBVM were determined.[24] The mean of total phenolics, total flavonoids, and total tannins for TBVM, extra old TBVM, and BVM were determined as 7515 ± 3768, 1771 ± 963, and 1291 ± 724 mg CE/L, respectively. The results of this study also showed that extra old TBVM had the highest phenolic content. This was associated with evaporation of water and diffusion of phenolics from barrel to vinegar. Phenolic contents and antioxidant capacities of eight commercial vinegars and 10 homemade vinegars were also examined in another study[25] and it was concluded that polyphenol content of the examined vinegar samples showed significant variations due to their raw materials and the production techniques. Total phenolic and total flavonoid contents of homemade red wine and red balsamic vinegars were considerably higher than other samples.

Anthocyanin content of red wine vinegar was investigated in another study[4] and 20 anthocyanin compounds such as catechyl, pyranocyanidin-3-glucoside, acetyl vitisin B, and coumaroyl vitisin B were determined in this type of vinegar. Twenty traditional home-made and five industrial vinegars, produced from grape, grape wine, apple, artichoke, pomegranate, lemon, and sour cherry, were inspected by Ozturk et al.[16] Vinegars had extremely variable total phenolic content values, ranging between 42.04 and 2228.79 mg GAE/L. The total phenolic content of traditional home-made vinegars was higher than commercial vinegars. The highest total phenolic content was obtained in grape vinegars among traditional vinegar samples and in sour cherry vinegar among industrial vinegars.

Several studies monitored the changes in compositional parameters, particularly bioactive compounds, during vinegar production. Effect of acetification process on phenolic profile and total phenolic content of cider, red and white vinegar production was studied and up to 50% decrease in phenolic content was observed.[26] The effects of production techniques on the composition of the vinegar were also investigated. It was shown that vinegars produced from the same raw material (Uluğbey Karası grapes) using different techniques (traditional surface and industrial submerge methods) had different phenolic contents.[17] Vinegar, produced by the traditional surface method, contained 2690 mg GAE/L, while industrial vinegar had 2461 mg GAE/L total phenolic content. Two vinegars also differed by the amounts of catechin and chlorogenic acid.

Aging is a part of vinegar production and this section of production also has an effect on the chemical composition of vinegar. Through NMR spectroscopic investigation, it was found out that vinegars, aged in acacia (*Robinia pseudoacacia*) wood barrels, contained (+)-dihydrorobinetin[8] Amount of (+)-dihydrorobinetin in vinegar was proportionally increased with aging duration; however, limited migration was observed in toasted barrels.[8]

## 2.6. Functional Properties

Vinegar has not only antioxidant and antibacterial properties but also has a role in the acceleration of glycogen repletion and calcium absorption in the human body. Studies

have shown that vinegar consumption provides protection from hypertension and decreases the serum cholesterol levels. A brief summary of these functional properties of vinegars is provided in this section.

It was shown that chronically alcoholic rats having vinegar supplemented diet had reduced serum triglycerides, total cholesterol, and liver total cholesterol concentration.[27] In another study, the effect of dietary vinegar consumption on calcium absorption was investigated.[28] Experimental results of the study on ovariectomized rats fed on a low-calcium diet suggested that dietary vinegar improved intestinal calcium absorption by increasing calcium solubility and by the trophic effect of the acetic acid. The effect of the vinegar uptake on aiding the recovery from fatigue in rats was also investigated.[29] Studies showed that rats with a diet containing acetic acid had enhanced glycogen repletion in muscles and liver. Tests done on spontaneously hypertensive rats indicated that acetic acid lowered blood pressure and renin activity; however, any change in concentration of angiotensin I-converting enzyme activity was not observed. Kondo et al.[30] concluded that anti-hypertensive benefits of vinegar are due to acetic acid content and its mechanism caused lowering of renin activity in blood plasma.

Antioxidant properties of vinegar are shown in several studies. One of the famous traditional Chinese vinegar, Shanxi vinegar, was investigated for its antioxidant effect on hydrogen peroxide-induced oxidative stress, superoxide dismutase, catalase and glutathione levels. Vinegar treatment in cells treated with $H_2O_2$ reduced reactive oxygen species significantly.[31] Similarly, antioxidant effects of soy vinegar on Swiss albino male mice was also studied.[32] These mice were treated with allopurinol (10 mg/kg) and soy vinegar (100, 200, and 400 mg/kg) once a day for seven days. The control group and experimental group which were fed with 400 mg/kg vinegar daily had the same xanthine oxidase activity. Moreover, this study showed that vinegar might be an alternative treatment to allopurinol for potassium oxonate-induced hyperuricemic mice.

The effect of apple vinegar uptake in 70 patients with type 2 diabetes and dyslipidemia was observed by Gheflati et al.[33] Any significant differences in the blood pressure and homocysteine concentration were not noted. However, daily consumption of apple vinegar showed a reducing effect on glycemic indices and an increasing effect in the total antioxidant capacity. Clinical nutrition studies conducted on three men and seven women, aged between 22 and 51, with normal body mass showed that vinegar supplemented diet significantly lowered the postprandial glucose and insulin levels.[34] In another study, Ostman et al.[35] inspected the effect of vinegar supplementation to lower

the glycemic index of a starchy meal, and the dose-response relationship of postprandial glucose and insulin levels on 12 healthy participants. As a result, vinegar containing diet reduced postprandial responses of blood glucose and insulin.

Additionally, 24 obese mice were monitored during 10 weeks to observe the effect of vinegar consumption on body weight.[36] In this period, mice were divided into three groups. The control group was fed with a high-fat diet while two other experimental groups' diets were supplemented with 0.08 mL and 2 mL coconut vinegar per kg body weight. At the end of 10 weeks, approximately 8.7–17.9% reductions in body weights were detected.

There are also studies that indicate the immune system support of vinegar. Active group, control group, and placebo group, consisting of people aged between 30 and 60 years, were observed during 8 weeks and change in the rate of release of secretory immunoglobulin A was recorded in the study.[37] Uptake of active food (vinegar with mashed garlic) was closely correlated with an increase in the release of secretory immunoglobulin A in saliva. Responses of the immune system to persimmon vinegar uptake were investigated in the intestinal system of mice at different doses for 20 days.[38] Concentration of Immunoglobulin A in intestinal fluids and feces was recorded four times higher than in the control group. In both studies, consumption of vinegar did not show any adverse or cytotoxic effect.

Antimicrobial effect of vinegar was also demonstrated and it was shown that 18 vinegar types (apple, grape, pomegranate, balsamic, blueberry, rosehip, gilaburu, lemon, blackberry, artichoke, mulberry, rice, apricot, date, and hawthorn vinegars) were effective on the inhibition of *Salmonella typhimurium*, *Staphylococcus aureus*, and *Escherichia coli*.[16] In another study, inhibitory effects of acetic and lactic acids on of *Salmonella enteritidis* and *E. coli* were examined and the results showed that the undissociated organic acids have antimicrobial activity.[39] Besides, synergism was observed between acetic and lactic acids. Food poisoning is one of the main reasons for outbreaks; therefore, bacteriostatic and bactericidal effects of vinegar on 17 strains of food-borne pathogenic bacteria including *E. coli* (EHEC, EPEC), *S. enteritidis*, *Vibrio parahamolyticus*, *Aeromonas hydrophila*, *S. aureus*, *Bacillus cereus* were studied.[40] The growth inhibition of all strains was observed at 0.1% (w/w) acetic acid concentration. Moreover, sodium chloride and treatment temperature had synergistic effect with acetic acid concentration on bacterial growth. Besides to its un-dissolved organic acid content, phenolic and volatile compounds of vinegars also provide antimicrobial activity. A study in the

literature indicated that grape vinegar samples had higher antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* than apple vinegar samples and this was associated with the higher antioxidant capacity of grape vinegar compared with apple vinegar.[41] Due to its antimicrobial effect, vinegar can be used as a cleaning and disinfection agent in home environmental surfaces. Cleaning and disinfection effects of various agents including vinegar, bleach, club soda, and tea tree oil on common home surfaces, and against two common bacteria, *S. aureus* and *E. coli*, were compared in an investigation.[42] The mixture of vinegar, club soda, and tea tree oil was found to be an adequate alternative to bleach for cleaning, in the cases of which complete elimination of microorganisms was not required. Vinegars produced from physalis (*Physalis Pubescens* L.) and red pitahaya (*Hylocereus Monacanthus*) were also reported to have antimicrobial effects due to both acetic acid and phenolic contents. *E. coli*, *Listeria monocytogenes*, *S. aureus*, and *S. enteritidis* were subjected to vinegar produced from these raw materials. The minimum inhibitory concentrations and minimal bactericidal concentration of vinegars were determined as 0.5% and 1%, respectively.[43]

## 2.7. Authentication

Different types of adulteration practices exist for vinegar, and the main type of economic adulteration is the use of an ingredient of lower value or cost than the authentic product. Adding edible alcohol made from molasses or glacial acetic acid to vinegar and declaring the product as traditional vinegar is a common practice.[44] Although grape must caramel (E-150d) is legal to add even into more expensive special type of vinegars it could be also used in vinegars with the purpose of imitating a longer storage time or covering undesirable attributes.[45]

Differences in the production processes between and within Protected Designation of Origin (PDO) and Protected Geographical Indication (PGI) categories are reflected in their commercial price.[46] The products such as foodstuffs, agricultural products, and wines registered as PDO are produced, processed and prepared in a specific region. PGI label shows, on the other hand, at least one of the stages of production, processing or preparation takes place in a specific geographic region where quality, reputation or

characteristic is linked to.[47] Labeling non-PDO or non-PGI products as PDO or PGI is a type of fraud. In addition, false labeling of the age of vinegar, in which the quality is associated with, is another problem. Labeling vinegar obtained from dried grapes with the addition of water as wine vinegar is reported as another type of authentication case.[48]

Vinegar with many different varieties is a complex liquid; therefore, detection of adulteration has become even more of a daunting task with the increasing number of adulterants that are mixed with the pure product. Adulteration detection methods can be grouped as targeted and non-targeted techniques. Targeted adulteration testing is based on the detection of specific compounds that can be used to trace abnormality. As an example, the presence or absence of certain pigment or phenolic compounds could be an indication of adulteration. However, most of the targeted compounds have low concentrations in food products and this could be regarded as a weakness for targeted analysis because adulteration techniques are becoming more sophisticated and can be undetectable by small changes. Amounts of targeted compounds in food products could be directly measured with any suitable analytical method such as chromatographic techniques.

Non-targeted analysis, on the other hand, has a holistic approach and aims to obtain an overall measurement of the analyzed food product. There is a variety of non-targeted techniques currently available: especially spectroscopic methods, such as Fourier transform infrared (FTIR), near-infrared (NIR), hyperspectral imaging, Raman and nuclear magnetic resonance (NMR), are the important non-targeted analysis tools, although some of these techniques could also be used in targeted measurements. In spectroscopic measurements, differences between spectra could be too complicated to detect visually. Chemometrics is a useful multivariate statistical analysis tool to extract the information from the data to differentiate classes and to eliminate unnecessary elements of the data. Chemometrics can be used to identify food samples based on geographical origin, species variety as well as highlighting the contamination and adulteration of a sample and it is very commonly used in combination with spectroscopic techniques to evaluate the data.

### 2.7.1. Non-targeted Analyses

Non-targeted analyses are attracting attention due to their rapid, low cost and small amounts of sample and minimum amounts of chemicals requiring nature. Especially in the past 15 years, various scientific studies aiming to detect the origin of the vinegar, to classify according to raw material, to characterize, and to authenticate the quality of vinegar have been published. In this part of the review, researches that were performed using non-targeted techniques will be discussed first. These techniques have been mostly used in detection of mixtures and identifying false labeling frauds (Table 2.1).

Cocchi et al.[49] aimed to discriminate TBVM "affinato", aged at least 12 years, and "extravecchio", aged at least 25 years, using whole volatile profiles obtained by head-space mass spectrometry and evaluating the data with multivariate analysis techniques. Score plots showed that reasonable classification with respect to aging was obtained. The potential of non-targeted methods combined with multivariate statistical techniques was also shown in another study.[53] Wine vinegar, balsamic, sherry and cider vinegar samples were analyzed with headspace solid-phase microextraction/gas chromatography to classify four types of vinegars. Again based on their distinctive overall volatile profiles, samples were differentiated successfully. Nuclear magnetic resonance (NMR) spectroscopy combined with chemometrics is one of the other spectroscopic methods used to classify vinegars and predict their properties. Seventy-two balsamic vinegar samples having different ages were successfully classified and predicted with high precision with this technique.[50] Hierarchical projection to latent structure discriminant analysis of NMR data provided differentiation of samples as young (<12 years), old (between 12 and 25 years) and extra old (>25 years). Fluorescence spectroscopy is the other technique to produce data used in the classification of vinegars. Determination of synthetic vinegars in Shanxi aged vinegars, a traditional Chinese vinegar type, was performed with excitation-emission matrix fluorescence spectroscopy and evaluation of the data with parallel factor analysis (PARAFAC) and multi-way partial least square discriminant analysis resulted in 100% correct classification of adulterated vinegars.[52] Rios-Reina et al.[57] used multidimensional fluorescence spectroscopy with parallel factor analysis and partial least squares-discriminant analysis to characterize and authenticate Spanish PDO wine vinegars. Results showed that the combination of these techniques

**Table 2.1.** Studies performed with various non-targeted approaches for authentication of vinegars.

| Aim | Type of Vinegar | Method | Result | Ref |
|---|---|---|---|---|
| Classification of products according to aging process | Traditional balsamic vinegar of Modena | Head-space mass spectrometry (HS-MS) | Reasonable classification with respect to ageing was accomplished | [49] |
| Prediction of the ageing | Balsamic and traditional balsamic vinegar of Modena | 1H Nuclear magnetic resonance spectroscopy (1H NMR) | 1H NMR spectra combined with PLS-DA and Naive Bayes approaches showed their strong classification and prediction capability | [50] |
| Classification of production methods and prediction of vinegar properties such as total acidity | Vinagres de Montilla-Moriles; produced by submerged culture and Orleans methods | Near infrared reflectance spectroscopy | Submerged culture and Orleans methods were differentiated and vinegar properties were predicted with high accuracy | [51] |
| Developing and comparing robust classification models for the identification of adulterated aged vinegars | Authentic Shanxi aged vinegars and synthetic vinegars samples adulterated with glacial acetic ranging from 10 to 100% | Excitation-emission matrix (EEM) fluorescence spectroscopy | Adulterated samples were recognized with a rate of 100% in both training and prediction sets | [52] |

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**Table 2.1.** (Cont.)

| Aim | Type of Vinegar | Method | Result | Ref |
|---|---|---|---|---|
| Classification of vinegars with respect to their types | White and red, balsamic, sherry and cider vinegars | Headspace solid-phase microextraction (HS-SPME) coupled with gas chromatography (GC) | Use of HS-SPME/GC in combination with chemometrics provided a simple, fast and reliable discrimination between different vinegar types on the basis of their total volatile profiles | [53] |
| Discrimination of origins of vinegars | Vinagre de Jerez, Vinagre de Montilla-Moriles, and Vinagre de Condado de Huelva, Industrial Balsamic Vinegar | UV-visible and fluorescence spectroscopy | Well discrimination of vinegar origins was obtained | [54] |
| Characterization and classification of PDO wine vinegars | Vinagre de Jerez and Vinagre Condado de Huelva | Fourier transform mid infrared spectroscopy (FTIR) with attenuated total reflectance | FTIR analysis was useful for a simple characterization of the established aging categories of high quality wine vinegars protected under PDO | [55] |

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**Table 2.1.** (Cont.)

| Aim | Type of Vinegar | Method | Result | Ref |
|---|---|---|---|---|
| Characterization and classification of PDO wine vinegars | Vinagre de Jerez, Vinagre de Condado de Huelva and Vinagre de Montilla-Moriles | Mid-infrared spectroscopy (MIR), near infrared spectroscopy (NIR), excitation-emission multidimensional fluorescence (EFM), 1H-Nuclear Magnetic Resonance (1H-NMR) spectroscopy | Application of data fusion methods improved the characterization and authentication of PDO wine vinegars | [56] |
| Characterization and authentication of Spanish PDO wine vinegars | Vinagre de Jerez, Vinagre de Montilla-Moriles and Vinagre de Condado de Huelva | Fluorescence analysis | Fluorescence spectroscopy and chemometrics combination was proposed as a potential routine analysis for PDO regulatory councils | [57] |
| Classification of PDO wine vinegars and their discrimination from commercial wine vinegars | Vinagre de Jerez, Vinagre de Condado de Huelva and Vinagre de Montilla-Moriles of different categories, and wine vinegars without PDO | Near infrared spectroscopy (NIR) | Combination of NIR with chemometrics was useful for a rapid characterization and classification of the Spanish PDO wine vinegars with >90% correct classification | [58] |

(cont. on next page)

**Table 2.1.** (Cont.)

| Aim | Type of Vinegar | Method | Result | Ref |
|---|---|---|---|---|
| Differentiation of aged and PDO wine vinegars from commercial (rapid) vinegars | Vinagre de Condado de Huelva, Vinagre de Jerez, Vinagre de Montilla-Moriles and 20 wine vinegars without PDO | UV-visible spectroscopy | Hierarchical classification model developed using UV-visible spectra showed promising classification of aged and PDO vinegars | [59] |
| Determination and quantification of adulterants in Sherry vinegar | Sherry vinegar adulterated with molasses, rice, cider and wine vinegars | Laser diode fluorescence spectroscopy | Adulteration ratios of Sherry vinegar were determined with small mean absolute percentage errors | [60] |

can be the standard for PDO investigations. Following this study, same group completed other classification and authentication studies using Fourier transform mid-infrared, NIR, UV–visible, excitation-emission multidimensional fluorescence, and $^{1}$H-NMR spectroscopy as analytical tools combined with various chemometric methods and obtained successful results for the characterization and authentication of PDO wine vinegars.[55, 56, 58, 59] In one of these studies, data from various spectroscopic techniques including NIR, Mid-IR, $^{1}$H-NMR, and multidimensional fluorescence spectroscopy were fused to improve the classification performance of these techniques for Spanish PDO wines.[56] Mid-level data fusion and common component and specific weights analysis multi-block method were the two data fusion approaches used in this study.

In addition to the identification of raw material or detection of aging duration, non-targeted methods can be used to determine high-quality products. la Haba et al.[51] aimed to characterize Vinagres de Montilla-Moriles wine vinegars, which were protected with PDO certification, using NIR reflectance spectroscopy. Submerged culture and Orleans methods were differentiated and also prediction of vinegar properties was performed with high accuracy in the same study. In a study that aimed to detect and quantify cheaper and low-quality vinegars from molasses, rice, cider and white wine in high-quality sherry vinegars, laser diode fluorescence spectroscopy was used and the data were evaluated with varying success using several intelligent chaotic algorithms.[60] Argentinean, Italian and Spanish vinegars were examined using a combination of UV–visible and fluorescence spectroscopies, aiming discrimination of their origins. Data were analyzed using principal component analysis and parallel factor analysis. Well discrimination of vinegar origins was reported.[54] As part of our still ongoing study, evaluation of the second derivative of combined FTIR and UV–visible spectral data with orthogonal partial least square discriminant analysis provided a good separation between pure apple vinegars and apple vinegars adulterated with spirit vinegar and synthetic vinegar (diluted acetic acid) separately. Figure 2.1 and Figure 2.2 show mid-IR and UV–visible spectral differences between vinegar and adulterated vinegars and differentiation of apple vinegar and adulterated samples regardless of adulterant, respectively.

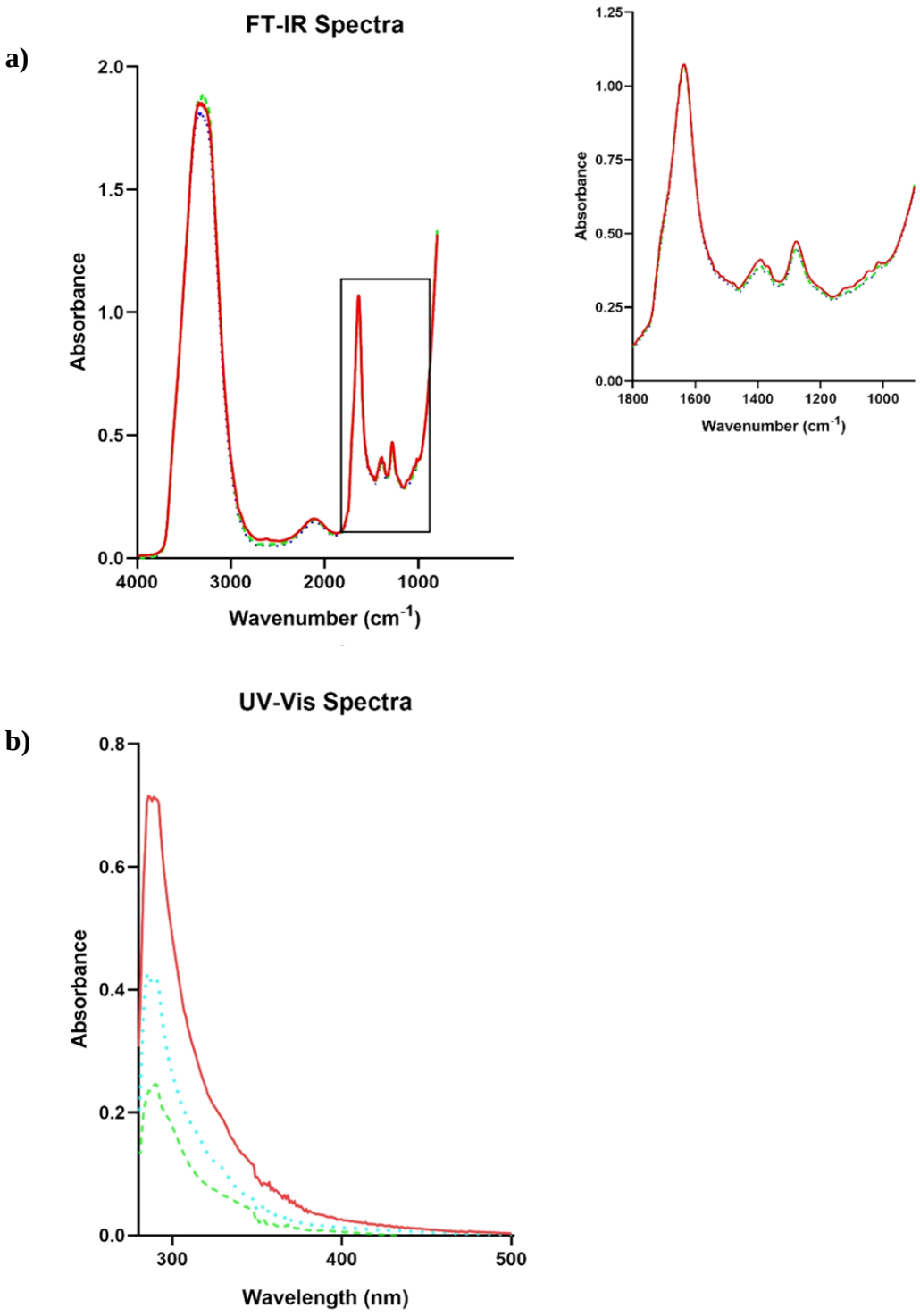


**Figure 2.1.** (a) Mid-infrared spectra of pure apple vinegar (–), apple vinegar adulterated with spirit vinegar (—) and apple vinegar adulterated with synthetic vinegar (. . . . . .); (b) UV–visible spectra of pure apple vinegar (–), apple vinegar adulterated with spirit vinegar (—) and apple vinegar adulterated with synthetic vinegar (. . . . . .).

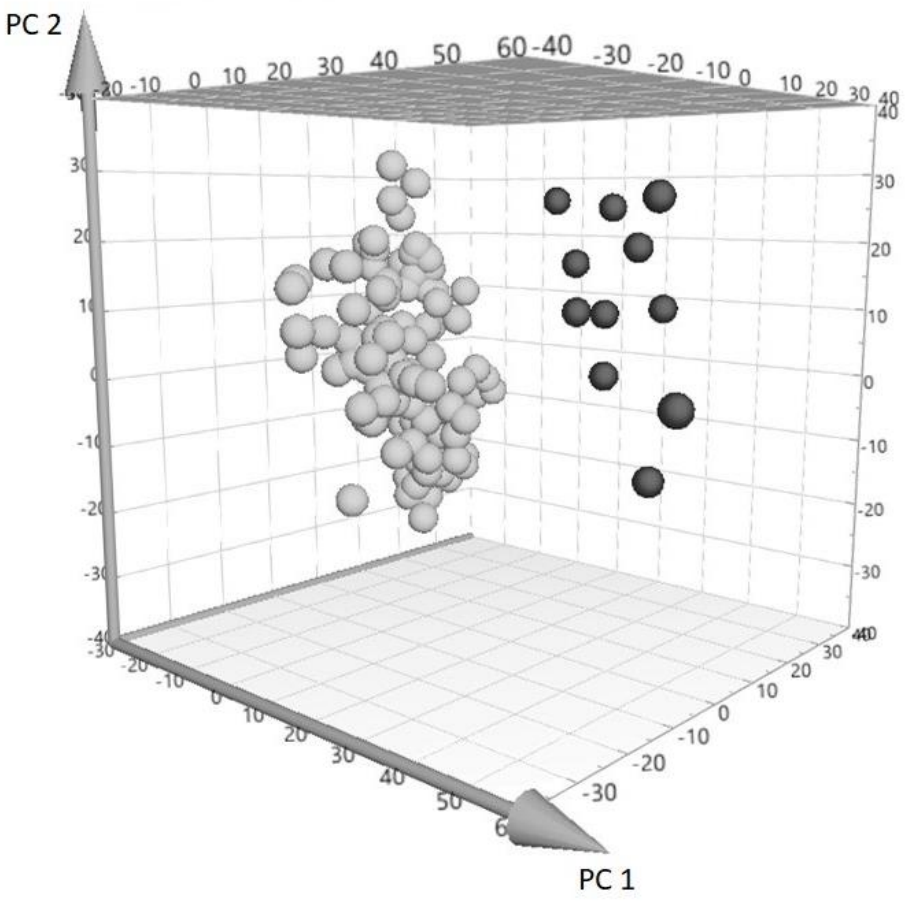


**Figure 2.2.** Orthogonal partial least square discriminant analysis model built with using combined mid-infrared and UV–visible spectral data and showing discrimination between pure apple vinegars (dark color) and apple vinegar adulterated with spirit vinegar and synthetic vinegars (light color).

## 2.7.2. Targeted Analyses

Targeted testing, on the other hand, aims to differentiate products with respect to their specific properties and constituents and has been used in various authentication studies of vinegar (Table 2.2). Since molecular isotope ratios provide information regarding the precursor molecules, measurement of stable isotope ratio was introduced as a useful tool to differentiate the botanical and geographical origin of food products. Therefore, it could be possible to classify the fermentation of raw materials with respect to their sources in the case of vinegar production with this technique. For this purpose, 14

vinegars, fermented from 7 different raw materials, were examined using headspace solid-phase microextraction combined with gas chromatography-high temperature conversion or combustion–isotope ratio mass spectrometry to provide differentiation according to raw materials.[64] Hydrogen and carbon isotope ratios were determined as effective parameters to discriminate the botanical origins of the acetic acid. The difference between C3 and C4 plants was clearly observed. Following this study, same sample composition was also used to determine $\delta^{13}C$ values of methyl and carboxyl carbons of acetic acid with gas chromatography–pyrolysis–gas chromatography–combustion–isotope ratio mass spectrometry (GC-Py-GC-C-IRMS) combined with headspace solid-phase microextraction (HS-SPME) since each carbon isotope ratios of methyl and carboxyl groups in acetic molecules could be indicators of the origin.[63] Therefore, findings of this study were expected to assist in the determination of indigenously and exogenously produced sources of acetic acid. Stable isotope methods using hydrogen, carbon and oxygen isotope analyses by isotope ratio mass and H-2-NMR spectrometry were also proposed to check the authenticity of balsamic vinegar.[63, 68] Scatter plot of $\delta^{13}C$ versus $\delta^{2}H$ values of acetic acid (calcium acetate) from balsamic vinegar demonstrated successful visual discrimination of pure wine acetic acid, C4 plant acetic acid added samples, and C3 plant acetic acid added samples. $\delta^{18}O$ analysis of water with isotope ratio mass spectrometer was used to determine the production of wine vinegar through fermentation of dried gapes and dilution with tap water which is against the EU regulation (EU Regulation 555/2008).[69] Limit values for $\delta^{18}O$ which are the indications of this type of fraud were established for this purpose. Another study assessed $\delta^{18}O$ and $\delta^{13}C$ as the fingerprints for the discrimination of Spanish wine vinegars according to their origin and $\delta^{18}O$ was found useful for this purpose.[70] Site-specific natural isotopic fractionation by NMR spectroscopy was another technique used to determine the deuterium to hydrogen ratio at the methyl group of acetic acid. Hsieh et al.[65] showed that the deuterium to hydrogen ratio at the methyl group is different for rice, molasses spirit, and synthetic vinegars. Moreover, as rice vinegar was adulterated with synthetic vinegar or molasses spirit vinegar, the ratio increased proportionally and the ratio versus adulteration level had a high correlation ($R^2 > 0.97$). Another property to classify vinegars is isotropic $^{13}C/^{12}C$ ratio of glycerol in balsamic vinegar. Sighinolfi et al.[67] studied 112 TBVM and BVM using this technique and it was concluded that this approach could be used as an additional tool for balsamic vinegar authentication. Glucose and fructose

isoforms in TBVM were measured through $^{13}C$ NMR spectroscopy and especially fructose isoforms were found useful in authentication of this type of vinegar.[72]

PDO vinegars of Spain were differentiated with respect to their individual volatile components determined with head space stir bar sorptive extraction GC-MS and it was shown that certain volatile components were inherent to each of three different vinegar types.[61] As a result, 100% correct classification for these vinegars was obtained with the evaluation of the data with a chemometric technique. Aroma profiles of Spanish PDO vinegars were also shown to have a discriminatory power.[74]

In a recent study, an acid-sensitive sensor array was used in identification of the types and ages of 32 traditional Chinese cereal vinegars and discrimination was based on organic acids and melanoidins present in vinegars.[71] Analysis of vinegar components with multivariate statistical techniques can also be used to authenticate high-quality vinegars. A total of 76 samples containing TBV and BVM samples aged for different durations were determined. Compositional properties such as brix value, concentration of acetic acid, ethanol, formic acid, 5-hydroxymethylfurfural, lactic acid, malic acid, succinic acid, and tartaric acid were analyzed using principal component analysis, factor analysis, and general discriminant analysis. Scatter plot of the first two discriminant functions of the general discriminant analysis showed very distinct groups visually.[66]

In some studies, both targeted and non-targeted methods were used together to validate each other and/or provide comparison between methods. The study performed by Rios-Reina et al.[45] is a good example for the use of targeted and non-targeted methods together. Although vinegar adulteration with grape-must caramel can be detected using multidimensional fluorescence, validation of this technique with the conventional chromatographic (HPLC) method was required.

**Table 2.2.** Studies performed with various targeted and combination of targeted and non-targeted approaches for authentication of vinegars.

| Aim | Type of Vinegar | Targeted | Method | Result | Ref |
|---|---|---|---|---|---|
| Authentication of high quality Spanish wine vinegars | Wine vinegars from Vinagre de Jerez, Vinagre de Condado de Huelva, and Vinagre de Montilla-Moriles | Targeted | Headspace sorptive extraction gas chromatography-mass spectrometry analysis (HSSE-GC-MS) | Successful classification was obtained using individual volatile compounds | [61] |
| Authentication of high quality Italian and Spanish wine vinegars | Vinagre de Jerez, balsamic vinegars of Modena and traditional balsamic vinegars of Modena | Targeted | High-performance liquid chromatography (HPLC) analysis | The amino acid and biogenic amine composition can be used to authenticate high quality Italian and Spanish vinegars | [62] |
| Determination of carbon isotope distribution in vinegar acetic acid | Commercial Japanese vinegars | Targeted | Improved gas chromatography-pyrolysis-gas chromatography-combustion-isotope ratio mass spectrometry (GC-Py-GC-C-IRMS) combined with headspace solid-phase microextraction (HS-SPME) | Low concentrations of acetic acid in complex media such as food products were measurable by the SPME technique | [63] |

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**Table 2.2.** (Cont.)

| Aim | Type of Vinegar | Targeted | Method | Result | Ref |
|---|---|---|---|---|---|
| Discrimination of raw materials used in fermentation | Rice vinegar, tomato vinegar, apple vinegar, pineapple vinegar, lychee vinegar, grain vinegar, wheat vinegar | Targeted | Head space solid-phase microextraction (HS-SPME) combined with gas chromatography-high temperature conversion or combustion-isotope ratio mass spectrometry (GC-TC/C-IRMS) | Hydrogen and carbon isotope ratios were good parameters to discriminate the botanical origins of the acetic acid. The difference between C3 and C4 plants was clearly shown. | [64] |
| Identification of the adulteration by addition of molasses spirit vinegar and synthetic acetic acid into rice vinegar | Rice vinegar | Targeted | Site-specific natural isotopic fractionation by nuclear magnetic resonance (SNIF-NMR) | Deuterium to hydrogen ratio of acetic acid varies with the source of vinegar | [65] |
| Classification of balsamic vinegars | Balsamic vinegar of Modena (BVM) and traditional balsamic vinegar of Modena (TBVM) | Targeted | 1H Nuclear magnetic resonance (1H NMR) spectroscopy | Constituents such as acetic acid, ethanol, formic acid, 5-hydroxylmethylfurfural, lactic a., malic a., succinic a. and tartaric a. were effective in BVM and TBVM characterization and quality control | [66] |

(cont. on next page)

**Table 2.2.** (Cont.)

| Aim | Type of Vinegar | Targeted | Method | Result | Ref |
|---|---|---|---|---|---|
| Characterization and classification of PDO wine vinegars | Traditional balsamic vinegar of Modena and the industrial balsamic vinegar of Modena products | Targeted | High-performance liquid chromatography (HPLC) analysis, gas chromatographic-combustion-Isotopic ratio mass spectrometry (GC-C-IRMS) | Carbon isotopic ratio of glycerol polyalcohol varied with origin, varietal or provenance; therefore, the discriminating potential of these species could be useful to elucidate balsamic vinegar production process | [67] |
| Detection of adulterations of balsamic vinegars through addition of water, acetic acid not only from wine, and/or other sugars to grape must | Balsamic vinegar | Targeted | Gas chromatography isotope ratio mass spectrometry (GC-IRMS) validated by 1H NMR | A stepwise procedure based on different isotopes was validated | [68] |
| Detection of addition of rehydrated grapes to fermentation medium | 14 groups of authentic samples including wine vinegars, raw vinegars, diluted vinegars | Targeted | 18O/16O ratio analyses by isotope ratio mass spectrometers | 18O/16O ratio values were determined for authenticated vinegars and vinegars produced from rehydrated grapes | [69] |

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**Table 2.2.** (Cont.)

| Aim | Type of Vinegar | Targeted | Method | Result | Ref |
|---|---|---|---|---|---|
| Determination of origin of vinegars from different parts of Spain | Vinagre de Condado de Huelva, Vinagre de Jerez, Vinagre de Montilla-Moriles and commercial vinegars from Northern Spain | Targeted | Stable isotope analysis ($^{13}C$ and $^{18}O$) | Î´18O ratio analysis provided better results than the Î´13C for determination of origin of vinegar | [70] |
| Determination of types and aging times of Chinese vinegars | Chinese traditional cereal vinegars | Targeted | CdTe-TGA sensor array and fluorescence spectroscopy | Types and aging time of Chinese vinegars were successfully predicted using organic acids and melanoidins | [71] |
| Authentication of Italian PDO vinegars | Traditional balsamic vinegar of Modena (TBVM), balsamic vinegar of Modena (BVM) | Targeted | $^{13}C$ NMR spectroscopy | Glucose and fructose isoforms were measured and fructose isoforms were found effective in determination of fraud | [72] |
| Detection of addition of synthetic acetic acid into spirit vinegar | Spirit vinegar | Targeted | Isotopic ratios, $^{2}H/^{1}H$ and $^{13}C/^{12}C$, individual volatile compounds, sensory analysis | Isotopic ratios provided the most successful identification of the presence of acetic acid in spirit vinegar | [73] |

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**Table 2.2.** (Cont.)

| Aim | Type of Vinegar | Targeted | Method | Result | Ref |
|---|---|---|---|---|---|
| Classification of vinegars | Wine vinegars and alcohol vinegars | Both | Near-infrared (NIR) spectroscopy, high-performance liquid chromatography | Vinegar NIR spectra is closely related to sample origin | [44] |
| Detection and quantification of grape-must caramel in vinegars | Vinagre de Jerez PDO and Vinagre de Montilla-Moriles | Both | Fluorescence spectroscopy, high-performance liquid chromatography (HPLC) analysis, sensory analysis | Multidimensional fluorescence coupled with a suitable chemometric method identified as a valuable tool for detecting and quantifying the addition of grape-must caramel to wine vinegars without sample treatment | [45] |

## 2.8. Conclusion

Vinegar has nutritive, functional, and taste and flavor enhancing roles in the human diet. The use of vinegar provides protection of foods against microorganisms while addition to sauces enhances aroma and taste. Moreover, positive effects of vinegar consumption on human health are proven with in vivo, in vitro and clinical experiments. Raw material diversity and the presence of different production methods define classes of vinegars by their quality. With increasing demand to high-quality vinegars, adulteration practices are also in rise and fast and low-cost authentication methods are in high demand for detection of low-quality ingredients, estimation of the age of the product and identification of false labeling. Both targeted and non-targeted methods have been used for determination of different types of adulteration in vinegar. However, more studies especially using combination of different techniques and various data analysis methods particularly data fusion approaches are needed to improve the detection of adulteration of this product.

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# CHAPTER 3

# PREDICTION OF VINEGAR PROCESSING PARAMETERS WITH CHEMOMETRIC MODELLING OF SPECTROSCOPIC DATA

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## 3.1. Abstract

Spectroscopic methods have the advantages of being rapid and environmentally friendly and can be used in measurement and control of processing parameters during food production. It was aimed to predict several quality and chemical parameters of vinegar processing from UV-visible and mid-infrared spectroscopic profiles. Two processing lines of both traditional and submerged vinegar production from 2 separate grape varieties (green and red grapes) were monitored. Some of the important markers of the fermentation processes; pH, brix, total acidity, total flavonoid content, total and individual phenolic contents, organic acid, sugar, ethanol concentrations as well as UV-visible and mid-infrared spectra were obtained during both types of vinegar processing and quality and chemical parameters were predicted from spectroscopic data using chemometric methods. Individual UV-visible and mid-infrared spectral profiles along with low level of data fusion were used in building of chemometric prediction models. Accurate, reliable and robust prediction models ($R^2_{cal}$ and $R^2_{val}$ >0.9) were obtained for quality parameters mostly with combination of two spectroscopic datasets. Predictive models used for phenolic components were below average except for p-coumaric and syringic acids. Citric and acetic acids were the most accurately estimated ones among

organic acids along with ethanol. Close agreements between reference and predicted values were obtained during the monitoring of changes of some quality parameters for vinegar fermentation process through rapid and simultaneous spectroscopic measurements.

## 3.2. Introduction

Vinegar production is a two-stage process: alcoholic and acetic acid fermentations. Sugar source is converted into ethanol and $CO_2$ in the first stage and fermentation takes place with the activity of Saccharomyces cerevisiae strains in anaerobic conditions. During the second stage of processing, acetic acid and water are produced from ethanol by acetic acid bacteria in aerobic conditions.

Vinegar is commonly produced with traditional and submerged fermentation techniques. Traditional vinegar processing involves fermentation by the microbial culture which forms a film on the surface. A relatively longer time of around 6–14 weeks is required for acetification of the must using this method [1]. Submerged fermentation, on the other hand, is a faster production technique. Fermentation takes place with the activity of acetic acid bacteria which is homogenously distributed in must [1]. Bioreactor is aerated from the bottom so that fermentation occurs not only on the surface but also throughout all fermentation media. Therefore, this type of production allows fast conversion to acetic acid and high yield and is preferred as a commercial processing technique. Acetic acid at 8–9% levels can be obtained within 24–48 h after ethanol fermentation.

Vinegar composition mainly depends on raw material and production technique. Acetic acid and water constitute most of the vinegar; however, small amounts of organic acids, alcohol, phenolic compounds and amino acids are also present. Minor compounds are especially important for sensorial characteristics of this product.

Various spectroscopic methods have been used especially in the characterization and authentication of different types of vinegars [26], [27], [28], [29] and use of these techniques for vinegar was reviewed in literature [5], [25]. Spectroscopic methods have been also applied to predict the chemical compositional parameters of different types of

food products. There are studies in literature which monitored the critical compositional parameters such as total acidity, sugar, acetic acid and ethanol contents at different stages of vinegar production and minor components such as volatiles, phenolic profile and total phenol content were also determined throughout fermentation processes [2], [7], [15], [37]. Spectral data evaluated with chemometric techniques allow the simultaneous estimation of the concentrations of chemical constituents of different types of fermented food products [4], [10], [9], [11], [19]. Therefore, in some studies, various spectroscopic profiles during vinegar production or only of final product were also collected to predict the quality and chemical parameters. Fourteen parameters including total acidity, volatile and non-volatile acids, organic acids, L-proline, dry matter, ash and chlorine contents of wine vinegar were successfully predicted from partial least square (PLS) regression models of near infrared (NIR) spectroscopic data [30]. Acetification process of vinegar produced from onion waste was followed with ethanol, acetic acid, biomass and NIR spectral measurements and these parameters were determined from the spectral data with PLS regression modelling [12]. NIR spectroscopy was also used in estimating the ethanol and acetic acid concentrations in culture broth samples obtained from rice vinegar fermentation [41]. In another study, Raman spectroscopy was used in monitoring grape vinegar production and, changes in glucose, fructose, ethanol and acetic acid concentrations were predicted with high coefficient of determination values through the evaluation of spectral data with PLS regression [39]. In this study, traditional and submerged fermentation types of vinegar production from two grape varieties were monitored with the determination of 22 quality and chemical parameters (brix, total phenolic content, total flavonoid content, titratable acidity, pH, and concentrations of citric acid, lactic acid, malic acid, succinic acid, tartaric acid, acetic acid, caffeic acid, catechin, epicatechin, coumaric acid, gallic acid, syringic acid, vanillic acid, ethanol, sucrose, glucose, fructose) along with the collection of UV-visible (UV-Vis) and Fourier transform infrared (FTIR) spectra. It was aimed to predict these quality and chemical parameters from spectral data using various chemometric techniques in order to determine several parameters simultaneously and rapidly during vinegar production.

## 3.3. Materials and Methods

Dried Sultaniye (white grape) and Alicante Bouchet (red grape) types of grapes were used separately in the production of vinegars. Samples from submerged culture fermentation were obtained from a commercial vinegar production line for these two grape varieties separately. Sampling was done at various times of alcoholic and acetic acid fermentations twice. 29 and 71 samples were collected during alcoholic and acetic acid fermentations, respectively.

Traditional type (surface fermentation) of vinegar processing was done with the same type of grapes separately and 2 batches were prepared for each grape variety. Grape musts obtained from a commercial vinegar processing plant were used as raw materials for this type of production. Mother of vinegar obtained during pre-trials were added to grape musts (18 Brix) and musts, in glass bottles covered with cotton cloths, were kept in a dark place. Sampling was done at $0^{th}$, $2^{nd}$, $4^{th}$, $6^{th}$, $10^{th}$, $15^{th}$, $20^{th}$, $25^{th}$, $30^{th}$ and $40^{th}$ days and a total of 40 samples were obtained during traditional production. In addition, 26 commercial vinegars were obtained from markets to widen the range of measured variables and to increase the number of the samples which are critical in building prediction models.

### 3.3.1. Brix, pH and Total Acidity Measurements

pH of the samples was measured with a pH meter (WTW, Germany). Brix was determined with a digital refractometer (Isolab, Germany). Total acidity was measured with titration using NaOH [14] and expressed as volumetric percentage (% v/v).

### 3.3.2. Total Phenolic and Flavonoid Contents

Total phenolic content (TPC) of the samples were determined with a spectrophotometric Folin-Ciocalteu assay adapted to microscale [24]. Results were reported as mg gallic acid/L. Total flavonoid content (TFC) was measured at 510 nm with a spectrophotometer [43] and expressed as mg catechin/L.

### 3.3.3. Phenolic Profiles

Concentrations of individual phenolic compounds were determined according to a method described in the literature [38]. Samples were filtered through a syringe filter (0.45 μm, cellulose acetate) before chromatographic analysis. Then, they are injected into an HPLC-DAD system (Perkin Elmer 200, Waltham, MA, USA) according to the conditions given in the same reference. C18 column (250×4.6 mm, 5 μm, ACE, Aberdeen, Scotland) was used in the analyses. Phenolic contents were calculated from at least 5 points standard curves of catechin, epicatechin, gallic acid, caffeic acid, syringic acid, p-coumaric acid and vanillic acid. All phenolic standards were purchased from Sigma-Aldrich (Germany).

### 3.3.4. Organic Acid, Sugar and Ethanol contents

Organic acid, sugar and ethanol concentrations of vinegars were determined simultaneously with an HPLC having refractive index detector (Agilent 1200, Santa Clara, CA, USA) according to a method in literature [6]. Aminex 87H column (300×7.8 mm, 9 μm, Bio-Rad Laboratories, Hercules, CA, USA) was used for the analysis. Acetic, citric, malic, tartaric and succinic acids, glucose, fructose, sucrose and ethanol concentrations were determined from standard curves. All standards were obtained from Sigma-Aldrich (Germany).

### 3.3.5. UV-Visible Spectroscopy

UV-visible (UV-Vis) spectra of the samples were collected in 200–550 nm range with a Thermo Multiscan UV-Vis spectrophotometer (Thermofisher Scientific, Waltham, MA, USA).

### 3.3.6. Fourier Transform Infrared Spectroscopy

Mid-infrared spectra of the samples were obtained with an FTIR spectrophotometer having a horizontal ZnSe-ATR accessory and a DTGS detector (Spectrum 100, Perkin Elmer, Waltham, MA, ABD) in 4000–800 $cm^{-1}$ range. Measurements were performed with 128 scans and 4 $cm^{-1}$ resolution. Spectra of air were taken as background before each sample reading.

### 3.3.7. Chemometric Modelling

All chemometric analyses were performed with 'ropls package' (Version 3.12) in R [33]. 2/3 of the data were used for calibration and 1/3 was separated to validate the models, and 107 and 62 samples were used in the development of calibration and validation models, respectively. Stratified random sampling was applied prior to multivariate statistical analyses to choose calibration and validation samples [31]. For measured properties, every sample was split into subgroups based on percentiles and random sampling was done within these subgroups.

Chemometric models were constructed to predict the chemical parameters of the samples that were obtained during two types of vinegar production along with commercial vinegars from individual UV-Vis and FTIR spectra. FTIR and UV-Vis measurements contain absorbance values between 4000 and 800 $cm^{-1}$ wavenumbers and 200–550 nm wavelengths of the samples, respectively. Low level data fusion with the

combinations of two spectroscopic data were also used in model building. Complementary integration of homogeneous FTIR data with UV-Vis data was applied to increase descriptive power and to reduce information gaps [32].

Partial least square (PLS) and orthogonal partial least square (OPLS) regression methods were used to generate the prediction models. All spectroscopic data including two individual spectroscopic profiles along with their combinations were transformed with square, first, second and third derivative transformations, Savitzky-Golay filtering (SGF), standard normal variate (SNV) and multiplicative signal correction (MSC) methods before construction of prediction models for each variable. More information regarding the pre-processing techniques can be found in literature [20], [23]. Fourteen models were generated for each parameter and the performance of these models were tested with the number of latent variables (LV), coefficient of determination for calibration ($R^2_{cal}$), coefficient of determination for validation ($R^2_{val}$), root mean square of error for calibration (RMSEC), root mean square of error for prediction (RMSEP) and residual predictive deviation (RPD) [36]. $R^2$ values close to 1 and small RMSE values relative to measurement ranges show the reliability of the models. RPD can be used as an indicator for the evaluation of a model's predictive ability. RPD value which is less than 1.5 indicates that the model's predictive capability is poor. Model is classified as average when the RPD value is between 1.5 and 2.0. RPD values between 2.0 and 2.5 shows that the model effect is relatively good and it is suitable for quantitative analysis. RPD values between 2.5 and 3.0 shows that the model is very effective and higher values than 3 indicates that the model has a very good prediction ability [42].

## 3.4. Results and Discussion

Quality parameters and concentrations of several important components during grape vinegar production with two different techniques (traditional and submerged culture fermentation) were determined using reference methods. Two different grape types and two production techniques along with commercial vinegar samples provided a wide range of parameters. Range and spread of measured values and number of analyzed samples are critical to obtain good prediction models. Reference results were compared

with predicted results obtained from PLS and OPLS models developed by using FTIR and UV-Vis spectroscopic data along with their combinations. Several transformations were applied to all data before model building for each parameter as explained in Section 2.4 and only the results of the best models are presented here.

Sample UV-Vis and FTIR spectra obtained during vinegar production are shown in Figure 3.1. As expected, both spectra have variations in the absorbance values of the peaks with respect to process stage due to reactions taking place throughout the processing. The peaks in 280–500 nm region of UV-Vis spectra are associated with phenolic compounds and organic acids [35], [40]. As far as the FTIR spectra is concerned, major differences were observed in 1500–900 $cm^{-1}$ region although all peaks varied somewhat with processing stage. Peak in 3800–2790 $cm^{-1}$ region is attributed to –OH group of water and C – H stretching of acetic acid. In addition, 1300–1000 $cm^{-1}$ is related with absorption due to organic acids while the peak in 1100–1000 $cm^{-1}$ belongs to C – O stretching. Then, peaks at 1065–1030 $cm^{-1}$ are associated with O – H and $–CH_2$ groups of sugars. Absorptions due to C = O stretching of aldehydes, – C – O and – OH groups of phenolic compounds take place in 1700–1600 $cm^{-1}$ and 1800–900 $cm^{-1}$ regions of FTIR spectra, respectively [10], [26].

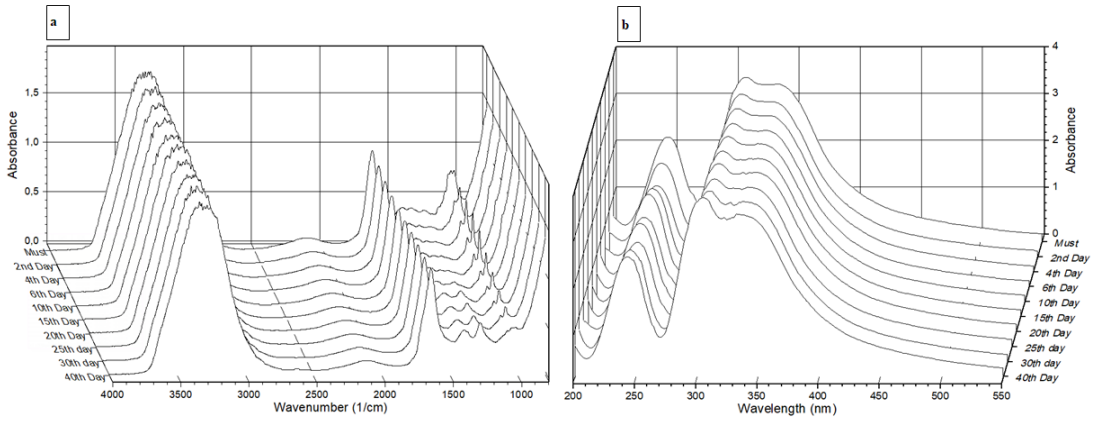


**Figure 3.1.** FTIR (A) and UV-Vis (B) spectra of the samples collected during traditional vinegar processing of Sultaniye grape must.

### 3.4.1. Prediction of Quality Parameters

Prediction results of five quality parameters (brix, TPC, TFC, pH and titratable acidity) are shown in Table 3.1. Brix values of the samples range between 0.5 and 31.2. PLS and OPLS regression analyses indicated that the use of only FTIR and UV-Vis data resulted in average models according to the performance criteria explained in Section 3.3.7; however, low level data fusion improved prediction vigor. Combination of FTIR and UV-Vis spectral data analyzed with OPLS after transformation with SNV generated the best results ($R^2_{cal}$ = 0.984, $R^2_{val}$ = 0.983, RMSEC = 0.468, RMSEP = 0.478, RPD = 2.652). TPC of the samples were determined as in the range of 120 – 3020 ppm. Similarly, combined dataset without any transformation provided the best predictive model ($R^2_{cal}$ = 0.992, $R^2_{val}$ = 0.969, RMSEC = 53.07, RMSEP = 107.5, RPD = 2.704), while the model predictions created with FTIR and UV-Vis datasets had average precisions. Although RMSEC values of TPC predictive models are a little bit high, measured TPC values are also high and comparison should be done considering measured values. A reason for high RMSEC values can be the relatively higher standard deviations of TPC measurements. However, these standard deviations are taken into account in RPD calculations and RPD and $R^2$ values of TPC model indicate very effective predictive ability of the data fusion model for this variable [42]. Maximum and minimum TFC values of the samples were 1.62 and 1500 ppm, respectively. TPC and TFC had wide ranges since both white and red grape types were used in the production. Although combined dataset estimated the closest values to the reference measurements ($R^2_{cal}$ = 0.986, $R^2_{val}$ = 0.973, RMSEC = 18.03, RMSEP = 26.11, RPD = 2.993) similar to the previous parameters, UV-Vis dataset seemed to be dominant over FTIR dataset in the combination model. Since UV-Vis spectroscopy is based on absorption of colored components its dataset provided good results for TPC and TFC predictions. Since grape juice has already acidic properties, pH values of the samples varied between 2.78 and 4.44. In the prediction of this parameter, UV-Vis dataset provided less contribution compared with the previous parameters. As a result, square transformed FTIR dataset with PLS regression had the most accurate results with $R^2_{cal}$ = 1, $R^2_{val}$ = 0.999, RMSEC = 0.055, RMSEP = 0.099, RPD = 2.481. Titratable acidity of vinegars results from the presence of different organic acids at different stages of fermentation. Late fermentation stages were dominated by acetic acid produced by the

activity of acetic acid bacteria, while acidity is originated from fruit itself at the beginning of the fermentation. Maximum and minimum titratable acidity values of the samples were recorded as 0.25 and 7.94, respectively. UV-Vis dataset was unsuccessful to create robust prediction model; however, FTIR dataset and FTIR dominated combined dataset analyzed with PLS and OPLS resulted in excellent predictive models ($R^2_{cal}$ = 0.986, $R^2_{val}$ = 0.991, RMSEC = 0.355, RMSEP = 0.273, RPD = 5.351). Low level data fusion was more successful in construction of predictive models for quality parameters except pH compared with individual spectroscopic data. FTIR spectroscopic data alone provided a better result for estimation of pH values of the samples. Although number of LV's are between 9 and 11 for these models, models were built using 3450 variables (3200 for FTIR and 250 for UV-Vis) with combination of two spectroscopic data sets. Graphs of measured vs. predicted values plotted using the best prediction models are shown in Figure 3.2. As can be seen from these graphs and Table 3.1, very good agreements between measured and predicted values were obtained.

Several studies that used individual or combination of spectroscopic techniques with chemometric methods on vinegar samples are present in the literature. Some of these articles focused on commercial final products while the others aimed to monitor fermentation process. Soluble solids content and pH of white vinegars were determined using Vis/NIR data that was analyzed with least square support vector machine (LS-SVM) and PLS regression methods [3]. In another study, total acidity of traditional Chinese vinegars was predicted correctly using NIR data analyzed with non-linear regression technique [8]. Mid-IR spectroscopy connected with flow lines was used to determine acidity on a group of samples containing wine, cherry, apple and balsamic vinegars [21]. Analysis of data with parallel factor analysis (PARAFAC) and PLS regression provided excellent predictive models. TPC of apple, rice, grape, pomegranate, balsamic, white, rose and red wine vinegars was determined using FTIR spectroscopy data and PLS regression technique [16]. As in the examples of these studies in literature, successful models also were obtained for the estimation of the quality parameters during vinegar processing in this study.

**Table 3.1.** Statistical parameters of predictive models for quality measurements.

| Component | Dataset | Transformation* | Method | LV | $R^2_{Cal}$ | $R^2_{Val}$ | RMSEC | RMSEP | RPD |
|---|---|---|---|---|---|---|---|---|---|
| Brix | Combined | SNV | OPLS | 9 | 0.98 | 0.98 | 0.47 | 0.48 | 2.65 |
| | FTIR | SNV | PLS | 7 | 0.98 | 0.97 | 0.47 | 0.62 | 1.92 |
| | UV-Vis | Raw Data | PLS | 6 | 0.97 | 0.97 | 0.61 | 0.65 | 1.72 |
| TPC | Combined | Raw Data | OPLS | 9 | 0.99 | 0.97 | 53.07 | 107.54 | 2.70 |
| | FTIR | MSC | PLS | 6 | 0.97 | 0.96 | 96.64 | 119.21 | 2.39 |
| | UV-Vis | MSC | PLS | 5 | 0.97 | 0.96 | 96.24 | 113.78 | 2.30 |
| TFC | Combined | SNV | PLS | 11 | 0.99 | 0.97 | 18.03 | 26.11 | 2.99 |
| | FTIR | MSC | PLS | 6 | 0.96 | 0.89 | 31.79 | 52.64 | 1.38 |
| | UV-Vis | SGF | PLS | 6 | 0.94 | 0.93 | 37.90 | 41.38 | 1.99 |
| pH | Combined | Square | PLS | 12 | 1.00 | 1.00 | 0.04 | 0.10 | 2.29 |
| | FTIR | Square | PLS | 10 | 1.00 | 1.00 | 0.06 | 0.10 | 2.48 |
| | UV-Vis | SGF | OPLS | 19 | 1.00 | 0.99 | 0.11 | 0.33 | 0.92 |
| Titratable Acidity | Combined | Raw Data | PLS | 9 | 0.99 | 0.99 | 0.36 | 0.27 | 5.35 |
| | FTIR | Raw Data | OPLS | 5 | 0.97 | 0.99 | 0.50 | 0.30 | 5.12 |
| | UV-Vis | SGF | OPLS | 20 | 0.94 | 0.54 | 0.73 | 2.09 | 0.91 |

* SNV: standard normal variate, MSC: multiplicative signal correction, SGF: Savitzky-Golay Filtering.

### 3.4.2. Prediction of Phenolic Compounds

In addition to TPC, estimation of phenolic compounds, that are known to be in vinegar, were studied. Reference values for those phenolic compounds were measured with HPLC. Prediction results of individual phenolic compounds are shown in Table 3.2. Concentration value ranges were measured as 0 – 30 ppm for gallic acid, 0– 60 ppm for catechin, 0 – 15 ppm for epicatechin, 0 – 10 ppm for coumaric acid, 0 – 40 ppm for caffeic acid, 0 – 15 ppm for vanillic acid and 0 – 3.5 ppm for syringic acid. Similar phenolic compounds were determined in studies performed with grape vinegars in the literature [18], [22] and the concentrations of these compounds are function of the grape type and processing type and processing stage. Although usage of combined dataset was more successful in the prediction of quality parameters, a generalization for prediction of phenolic compounds is not possible. Combined dataset with SGF resulted in the most successful prediction model for syringic acid, while first derivative transformation of UV-Vis dataset with PLS regression was preferable for p-coumaric acid. FTIR dataset alone did not produce any model better than UV-Vis data for the phenolic compounds. Combination models for gallic acid and epicatechin have high $R^2_{cal}$ (0.97 and 0.99) and average $R^2_{val}$ (0.82 and 0.78) values; however, their RPD values are not satisfactory. Expanded uncertainty [34] for gallic acid was calculated as 2.23 while RMSEP value for this parameter was 1.23. None of the datasets produced any reliable model for catechin, caffeic acid and vanillic acid. Since these components are minor compounds of food matrix, statistical analyses resulted in average and below average success rates for predictive models.

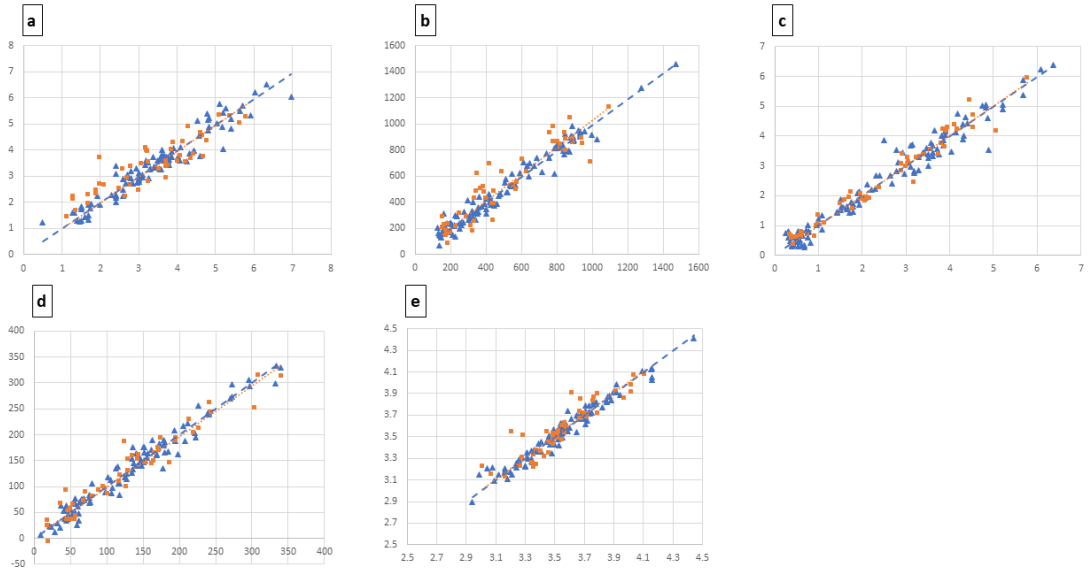


**Figure 3.2.** Measured vs predicted plots constructed with the best chemometric regression models for a) brix, b) total phenol content (TPC), c) titratable acidity, d) total flavonoid content (TFC), and e) pH.

### 3.4.3. Prediction of Organic Acids and Sugars

Sugars and organic acids are the major components of fruits. Concentrations of these compounds and ethanol, produced during fermentation, are important parameters to monitor the process. Although total titratable acidity includes sum of individual organic acid concentration, source of acidity may have importance for several processes such as wine fermentation. Obtained spectral data was also analyzed in order to test predictive capabilities in terms of sugars, acids and ethanol. Reference values for organic acids, sugars and ethanol were measured with HPLC. Prediction results of these compounds are shown in Table 3.3. Measured values ranged between 1 and 150 ppm for citric acid, 100–10,000 ppm for lactic acid, 4–2500 ppm for malic acid, 100–4500 ppm for succinic acid, 0–4730 ppm for tartaric acid, 0–7.14 % (v/v) for acetic acid, 0–300 ppm for sucrose, 0–3500 ppm for glucose, 0–10,000 ppm for fructose and 0–8 % (v/v) for ethanol. Although amounts of organic acids varied with grape type and processing, same type of organic acids were also determined in studies performed with grape vinegars [13], [18], [22].

**Table 3.2.** Predictive models for phenolic compounds content.

| Component | Dataset | Transformation | Method | LV | $R^2_{Cal}$ | $R^2_{Val}$ | RMSEC | RMSEP | RPD |
|---|---|---|---|---|---|---|---|---|---|
| Gallic Acid | Combined | Raw Data | OPLS | 11 | 0.97 | 0.82 | 0.46 | 1.23 | 1.24 |
| | FTIR | SNV | OPLS | 6 | 0.89 | 0.81 | 0.9 | 1.27 | 1.07 |
| | UV-Vis | Square | PLS | 5 | 0.84 | 0.79 | 1.1 | 1.34 | 0.97 |
| Catechin | Combined | SGF | PLS | 11 | 0.92 | 0.61 | 0.53 | 1.07 | 0.8 |
| | FTIR | SGF | PLS | 7 | 0.84 | 0.64 | 0.74 | 1.02 | 0.65 |
| | UV-Vis | SGF | OPLS | 8 | 0.74 | 0.69 | 0.95 | 0.98 | 0.68 |
| Epicatechin | Combined | SNV | PLS | 16 | 0.99 | 0.78 | 0.61 | 2.5 | 1.32 |
| | FTIR | Square | PLS | 9 | 0.91 | 0.72 | 1.44 | 2.83 | 0.85 |
| | UV-Vis | Square | PLS | 7 | 0.83 | 0.68 | 2 | 3.01 | 1.02 |
| p-coumaric Acid | Combined | SNV | PLS | 14 | 0.98 | 0.87 | 0.1 | 0.32 | 1.84 |
| | FTIR | Savitzky-Golay Filtering | OPLS | 11 | 0.97 | 0.6 | 0.15 | 0.57 | 1.06 |
| | UV-Vis | First Derivative | PLS | 5 | 0.93 | 0.87 | 0.21 | 0.32 | 1.92 |
| Caffeic Acid | Combined | Square | OPLS | 7 | 0.85 | 0.77 | 1.83 | 2.17 | 1.36 |
| | FTIR | SGF | OPLS | 15 | 0.99 | 0.53 | 0.52 | 3.67 | 1.12 |
| | UV-Vis | SGF | PLS | 7 | 0.81 | 0.8 | 2.04 | 2.08 | 1.45 |
| Vanillic Acid | Combined | MSC | OPLS | 12 | 0.98 | 0.31 | 0.1 | 0.76 | 0.79 |
| | FTIR | SGF | PLS | 8 | 0.93 | 0.48 | 0.22 | 0.64 | 0.67 |
| | UV-Vis | Third Derivative | PLS | 5 | 0.96 | 0.37 | 0.16 | 0.72 | 0.67 |
| Syringic Acid | Combined | SGF | PLS | 10 | 0.97 | 0.86 | 0.06 | 0.15 | 1.81 |
| | FTIR | Raw Data | PLS | 6 | 0.91 | 0.83 | 0.11 | 0.16 | 1.38 |
| | UV-Vis | SGF | OPLS | 7 | 0.85 | 0.54 | 0.14 | 0.27 | 0.89 |

Combined dataset produced better results for citric acid ($R^2_{cal}$ = 0.97, $R^2_{val}$ = 0.85) and tartaric acid ($R^2_{cal}$ = 0.99, $R^2_{val}$ = 0.87) prediction; nevertheless, FTIR data was favorable for estimation of ethanol ($R^2_{cal}$ = 0.88, $R^2_{val}$ = 0.82) and acetic acid ($R^2_{cal}$ = 0.98, $R^2_{val}$ = 0.91) concentrations. RMSEP value for citric acid was determined as 311, while expanded uncertainty was 568.

UV-Vis dataset did not create any preferable predictive models. In the prediction of lactic acid, malic acid, succinic acid sucrose, fructose and glucose concentrations; none of the datasets were successful enough. Since these components can exist in very small amounts depending on the fermentation stage, concentrations in the data range are not well distributed and this causes generation of poor prediction models for some compounds. In literature, Vis/NIR data and various multivariate statistical analysis techniques were used in combination to predict organic acid content of plum vinegars and LS-SVM was determined as the most precise technique [17] and better prediction models using variable selection were developed for acetic, tartaric and lactic acids compared to current study.

Spectroscopic methods combined with chemometric techniques can provide opportunities to determine several quality parameters of food products simultaneously, rapidly and easily. In the current study, successful results were obtained for the estimation of brix, pH, titratable acidity, TPC and TFC along with average predictions of ethanol, acetic acid, citric acid, p-coumaric acid and syringic acid and, mostly combination of FTIR and UV-vis data provided better predictions.

Some of the most successful models (pH, titratable acidity, TPC and TFC) were used in predicting the changes during vinegar production. For this purpose, quality parameters of vinegar samples of both grape varieties which were collected during the production with surface fermentation technique are compared with the predicted values (Figure 3.3). As can be seen from the figure, quite close agreements between predicted and measured values especially for pH and titratable acidity are observed. There are some deviations in TPC and TFC estimations. As can be seen from the plots (Figure 3.3), particularly TPC measurements have relatively higher standard deviations. Therefore, deviations in predictions of these variables can be related with higher variability in measurements. Despite this, prediction models for quality variables can be considered as quite effective in monitoring the vinegar processing and can be used in monitoring of vinegar process.

**Table 3.3.** Predictive models for sugars, ethanol and organic acids.

| Component | Dataset | Transformation | Method | LV | $R^2_{Cal}$ | $R^2_{Val}$ | RMSEC | RMSEP | RPD |
|---|---|---|---|---|---|---|---|---|---|
| Citric Acid | Combined | SNV | OPLS | 11 | 0.97 | 0.85 | 131.32 | 311.27 | 1.99 |
| | FTIR | MSC | OPLS | 4 | 0.77 | 0.79 | 343.83 | 363.42 | 1.54 |
| | UV-Vis | SGF | PLS | 7 | 0.81 | 0.8 | 313.45 | 353.52 | 1.46 |
| Lactic Acid | Combined | Square | PLS | 12 | 0.99 | 0.77 | 443.84 | 1947.51 | 1.05 |
| | FTIR | Square | OPLS | 13 | 0.99 | 0.7 | 362.79 | 2237.19 | 1.07 |
| | UV-Vis | SGF | OPLS | 16 | 0.93 | 0.49 | 987.23 | 3311.03 | 0.9 |
| Malic Acid | Combined | SNV | OPLS | 17 | 0.99 | 0.67 | 88.85 | 555.39 | 0.94 |
| | FTIR | Square | PLS | 11 | 0.97 | 0.51 | 155.75 | 694.85 | 0.82 |
| | UV-Vis | SNV | PLS | 6 | 0.86 | 0.64 | 353.05 | 564.47 | 0.77 |
| Succinic Acid | Combined | MSC | PLS | 9 | 0.91 | 0.79 | 524.75 | 874.39 | 1.05 |
| | FTIR | Square | PLS | 13 | 0.99 | 0.66 | 201.98 | 1110.15 | 0.89 |
| | UV-Vis | SGF | OPLS | 9 | 0.89 | 0.84 | 578.77 | 765.58 | 1.1 |
| Tartaric Acid | Combined | SNV | PLS | 14 | 0.99 | 0.87 | 149.5 | 667.07 | 1.46 |
| | FTIR | Raw Data | PLS | 8 | 0.97 | 0.74 | 285.33 | 1013.74 | 1.23 |
| | UV-Vis | SGF | OPLS | 10 | 0.93 | 0.64 | 461.66 | 1300.2 | 1 |
| Acetic Acid | Combined | Raw Data | PLS | 8 | 0.98 | 0.9 | 0.37 | 0.95 | 1.92 |
| | FTIR | MSC | PLS | 6 | 0.98 | 0.91 | 0.4 | 0.91 | 1.94 |
| | UV-Vis | SGF | OPLS | 15 | 0.9 | 0.53 | 0.88 | 2.33 | 0.96 |

(cont. on next page)

**Table 3.3.** (Continued).

| Component | Dataset | Transformation | Method | LV | $R^2_{Cal}$ | $R^2_{Val}$ | RMSEC | RMSEP | RPD |
|---|---|---|---|---|---|---|---|---|---|
| Sucrose | Combined | Square | PLS | 13 | 0.99 | 0.49 | 11.69 | 84.27 | 0.77 |
| | FTIR | Square | PLS | 12 | 0.99 | 0.45 | 12.43 | 96.17 | 0.86 |
| | UV-Vis | Square | OPLS | 6 | 0.87 | 0.31 | 39.95 | 97.76 | 0.76 |
| Glucose | Combined | SNV | PLS | 11 | 0.93 | 0.28 | 276.76 | 978.03 | 0.88 |
| | FTIR | MSC | OPLS | 8 | 0.92 | 0.36 | 301.81 | 879.08 | 0.79 |
| | UV-Vis | Square | OPLS | 12 | 0.86 | 0.1 | 396.36 | 956.77 | 0.74 |
| Fructose | Combined | SNV | OPLS | 10 | 0.92 | 0.26 | 610.52 | 2827.76 | 0.83 |
| | FTIR | Raw Data | OPLS | 10 | 0.99 | 0.16 | 248.66 | 3019.96 | 0.71 |
| | UV-Vis | Raw Data | OPLS | 13 | 0.92 | 0.34 | 604.57 | 2622.5 | 0.85 |
| Ethanol | Combined | MSC | OPLS | 9 | 0.89 | 0.84 | 0.31 | 0.45 | 1.84 |
| | FTIR | SNV | PLS | 5 | 0.88 | 0.82 | 0.4 | 0.39 | 2.08 |
| | UV-Vis | First Derivative | PLS | 6 | 0.92 | 0.37 | 0.26 | 1.47 | 1.06 |

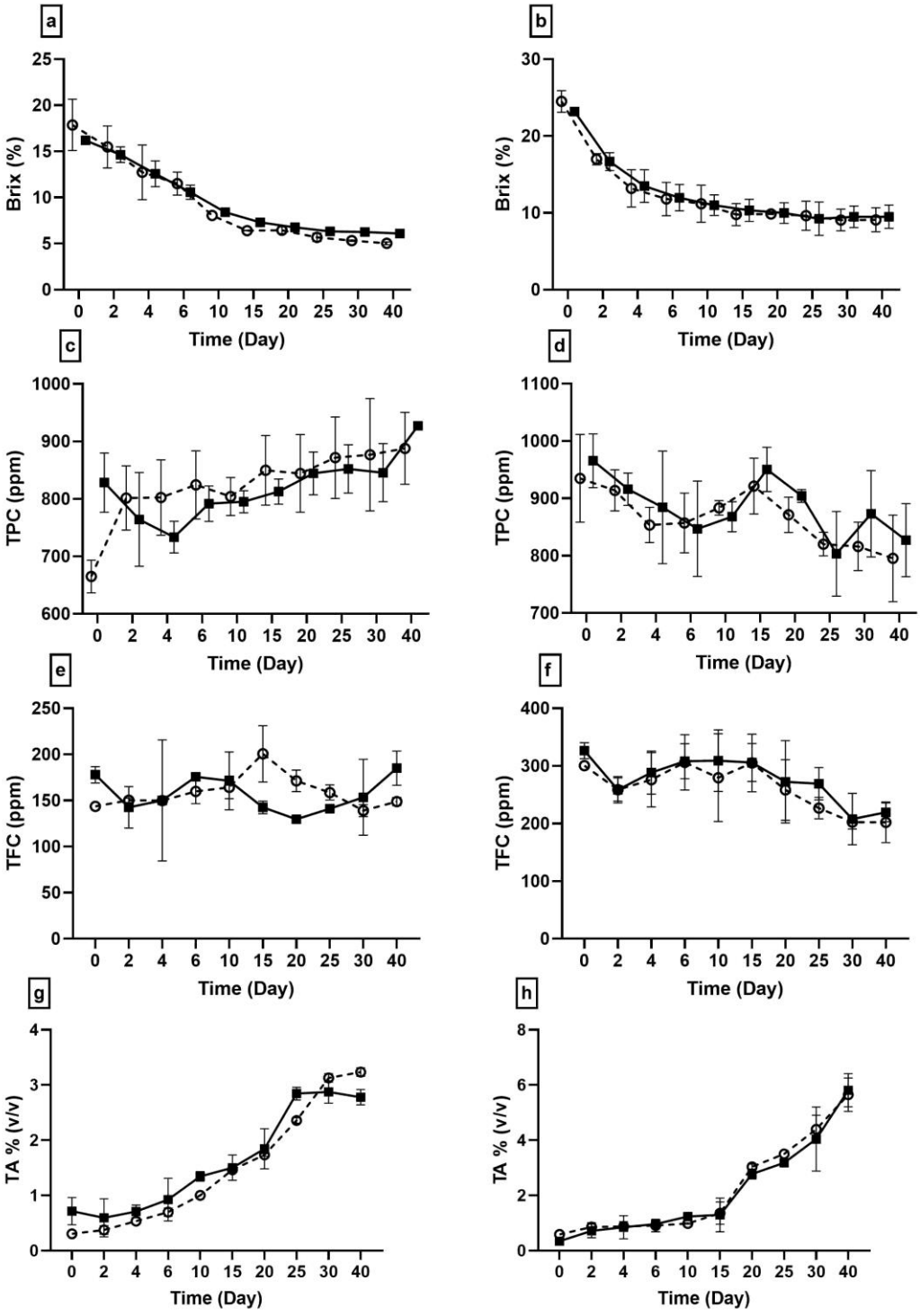


**Figure 3.3.** Changes in quality parameters during surface fermentation process of vinegar with respect to time. a, c, e and g vinegar production with Sultaniye grapes and b, d, f and h are vinegar production with Alicante Bouchet grapes. Solid and dashed lines represent measured and predicted values, respectively.

## 3.5. Conclusion

In this study, various quality parameters, phenolic compounds, organic acid and sugar profiles of vinegars produced from Sultaniye and Alicante grape varieties by submerged and surface fermentation techniques are estimated from FTIR and UV-Vis spectral data in combination with PLS and OPLS regression analyses.

Spectral data and chemometric methods are successful in prediction of total amount of sugars, phenolics, flavonoids and organic acids. However, concentration of individual components which are portion of total sugar, phenolics or organic acids cannot be predicted with high precision. Successful results showed that FTIR and UV-Vis spectral data analyzed with chemometrics have potential to be cheap, non-hazardous, and fast methods in order to monitor vinegar fermentation processes. Simultaneous analyses of these parameters would provide better control of quality during fermentation and also can be helpful in determining the authenticity of the product.

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# CHAPTER 4

# DETECTION OF VINEGAR ADULTERATION WITH SPIRIT VINEGAR AND ACETIC ACID USING UV–VISIBLE AND FOURIER TRANSFORM INFRARED SPECTROSCOPY

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Cavdaroglu, C., & Ozen, B. (2022). Detection of vinegar adulteration with spirit vinegar and acetic acid using UV–visible and Fourier transform infrared spectroscopy. Food Chemistry, 379, 132150.

## 4.1. Abstract

Vinegar is one of the commonly adulterated food products, and variations in product and adulterant spectrum make the detection of adulteration a challenging task. This study aims to determine adulteration of grape vinegars with spirit vinegar and synthetic acetic acid using different spectroscopic methods. For this purpose, grape vinegars were mixed separately with spirit vinegar and diluted synthetic acetic acid (4%) at 1–50% (v/v) ratios. Spectra of vinegars and mixtures were obtained with UV–visible and Fourier-transform infrared (FTIR) spectrometers. Data were evaluated with various chemometric methods and artificial neural networks (ANN). Correct classification rates of at least 94.3% and higher values were obtained by the evaluation of both spectroscopic data along with their combination with chemometric methods and ANN for discrimination of non-adulterated and adulterated vinegars. UV–vis and FTIR spectroscopy can be rapid and accurate ways of detecting adulteration in vinegars regardless of adulterant type.

## 4.2. Introduction

Vinegar is a product which can be produced from different raw materials through different processing techniques. Common raw materials are various types of grains and fruits, particularly grape and apple. Processing takes place in two stages as ethanol and acetic acid fermentations and traditional (surface) and submerged type of fermentations are the techniques commonly used in production.

Vinegar is a product which has a rich composition of various bioactive compounds including mainly organic acids, phenolic compounds, vitamins and minerals (Xia et al., 2020). In recent years, several researches indicated many beneficial effects of vinegar on human health such as cholesterol reduction, anti-infection property, blood glucose control, lipid metabolism regulation and blood pressure reduction along with its antimicrobial and antioxidant effects (Chen et al., 2016a). Vinegar is one of the food products which has been exposed to different types of frauds (Callejon et al., 2018). The most common type of these frauds is to add a less economically valuable product to another one with a higher price. One example for this is mixing spirit vinegar with a regular vinegar. Spirit vinegar and acetic acid are two of the most commonly used adulterants in economically motivated frauds of vinegar (Callejon et al., 2018). Another practice is to provide misinformation regarding the origin of the product which has a geographical indication status. Various targeted and untargeted methods used in authentication studies have been also applied to vinegar adulteration detection (Cavdaroglu and Ozen, 2021, Ríos-Reina et al., 2020, Ríos-Reina et al., 2020). In targeted techniques, presence or absence of specific compounds indicates the adulteration. With non-targeted methods, on the other hand, it is aimed to obtain general profiles of the analyzed products and spectroscopic techniques are the most common methods used as non-targeted analysis. Since spectroscopic methods produce many variables, even with a single measurement, chemometric methods are generally used to evaluate this type of data instead of univariate statistical analysis methods. Various studies are available in the literature which investigated the application of different spectroscopic methods including nuclear magnetic resonance (NMR), fluorescence, near infrared (NIR) and mid-infrared (mid-IR), in combination with chemometric techniques for the authentication of various types of vinegars. Evaluation of NMR spectroscopic data with discriminant analysis

resulted in successful separation of vinegars with respect to their ages (Consonni et al., 2008). Adulteration of a traditional Chinese vinegar with acetic acid was determined accurately with fluorescence spectroscopy (Peng et al., 2019). Non-targeted spectroscopic methods including NMR, NIR, mid-IR, fluorescence and UV–visible (UV–vis) have been also used in classification of vinegars regarding their origin (Rios-Reina et al., 2017a, Rios-Reina et al., 2018, Rios-Reina et al., 2019a, Rios-Reina et al., 2019b, Rios-Reina et al., 2019c). To the best of our knowledge, there is no study in literature which investigated the detection of vinegar/acetic acid and vinegar/spirit vinegar mixtures using mid-IR and UV–vis spectroscopy alone or in low data fusion form.

This study aims to determine the adulteration of grape vinegars with spirit vinegar and diluted glacial acetic acid by using UV–vis and Fourier transform infrared (FTIR) spectroscopic data with chemometric methods and artificial neural networks (ANN) regardless of the type of these adulterants. Hypothesis of this research is that evaluation of UV–vis and FTIR spectroscopic data with chemometric techniques and ANN could be effective in determining the adulteration of vinegar with spirit vinegar and acetic acid.

## 4.3. Materials and Methods

Twenty grape vinegars were obtained from 11 reliable commercial producers. To prepare the adulterated sample set, eleven randomly chosen vinegars were mixed with two spirit vinegars and diluted glacial acetic acid (4% v/v) separately at 1, 5, 10, 20, 30, 40 and 50% concentrations. Total number of samples was 251 (231 adulterated and 20 authentic samples).

### 4.3.1. pH, Brix, and Total Acidity Measurements

pH values were measured with a pH meter. A digital refractometer (Isolab, Germany) was used in determination of Brix values. Total acidity was measured with a titration using NaOH (OIV, 2000) and expressed as volumetric percentage (% v/v).

### 4.3.2. Determination of Compositional Parameters

Acetic acid, tartaric acid and ethanol concentrations of authentic samples were determined according to a method in the literature (Castellari et al., 2000). HPLC with a refractive index detector (Agilent 1200, Santa Clara, CA, USA) were used in the analyses of the samples. Aminex 87H column (300 × 7.8 mm, 9 μm, Bio-Rad Laboratories, Hercules, CA, USA) was the column for HPLC analyses. Concentrations of acetic acid, tartaric acid and ethanol in the samples were determined from standard curves.

### 4.3.3. UV–Visible Spectroscopy

UV–vis spectra of all samples were obtained in 200–550 nm range with a UV–Vis spectrophotometer (Thermo Scientific Multiskan GO Microplate Spectrophotometer, Thermo Fisher Scientific, Vantaa, Finland). UV–Vis spectra scans were recorded by loading 200 μL of 5 times diluted samples into a 96-well flat bottom polystyrene plate (Isolab, Wertheim, Germany). Spectrum of each sample was collected twice and they were averaged.

### 4.3.4. Fourier Transform Infrared Spectroscopy

An FTIR spectrophotometer having a DTGS detector (Spectrum 100, Perkin Elmer, Waltham, MA, ABD) was used in collection of mid-infrared spectra of the samples against air as background. Measurements were performed with a horizontal ZnSe ATR accessory in 4000–800 $cm^{-1}$ range. Measurement parameters were 128 scans and 4 $cm^{-1}$ resolution. Spectrum of each sample was collected twice and they were averaged.

### 4.3.5. Statistical Analysis

Partial least square discriminant analysis (PLS-DA) and orthogonal partial least square discriminant analysis (OPLS-DA) were used in classification of pure and adulterated vinegars from spectroscopic data. UV–vis and FTIR spectroscopic data separately and low level data fusion with the combination of two data sets were used in model building. Prior to modeling, data were transformed through first, second and third derivative, standard normal variate (SNV), multiplicative scatter correction (MSC), orthogonal signal correction (OSC), wavelet condensed time series (WCTS) and wavelet denoising time series (WDTS). PLS-DA and OPLS-DA were performed by SIMCA-P v.14.1 (Umetrics, Umea, Sweden). ANN model was created with the 'neuralnet package' (Version 1.44.2) in R programming language (Fritsch et al., 2019). Preliminary trials showed that ANN models trained using raw data with 125, 25 and 3 neurons in three hidden layers resulted in higher correct classification rate. ANN model yielded possible classifications of the samples and possibility ratios. All PLS-DA, OPLS-DA and ANN models were calibrated using 2/3 of the collected spectral data and validated by cross validation and remaining 1/3 of the data as external validation. $R^2$ values for calibration ($R^2_{cal}$) and validation ($R^2_{val}$) were calculated for developed models. In addition, score plots were generated along with correct classification matrix for PLS-DA and OPLS-DA. Definitions given in literature were used to calculate correct classification rate, sensitivity and specificity (Bajoub et al., 2017). To predict adulteration ratio, ANN, PLS and OPLS regression models were constructed following the same transformations mentioned above. Performance of these models were checked with the number of latent variables (LV), $R^2_{cal}$, $R^2_{val}$, root mean square of error of calibration (RMSEC) and prediction (RMSEP). Variable importance in projection (VIP) values were determined to see the importance of variables and VIP values above 1 are the indication of the significance of each variable.

## 4.4. Result and Discussion

pH, brix and total acidity of 20 non-adulterated vinegars used in this study varied between 2.78-3.48, 2.4–23.5 and 2.5–4.9% (v/v), respectively. These authentic samples had acetic acid concentrations in the range of 2.07 – 5.69% (v/v) and their ethanol concentrations were in not detectable levels. Besides, amount of tartaric acid which originates from grape as the raw material were determined as in the range of 444.36 – 3252.86 ppm. A set of randomly chosen vinegars were adulterated with spirit vinegar and diluted acetic acid (4%) separately. Spirit vinegars had total acidity of 3.95–4.05% (v/v), pH values of 3.05–3.11 and Brix of 1.1–1.5 Adulterated sample set contains vinegars adulterated with 2 different types of adulterants. Chemometric models were constructed using both adulterant together with the assumption that type of adulterant would be unknown during analysis in control laboratories.

Both UV–vis and FTIR spectra of adulterated and non-adulterated samples were collected and these spectra were evaluated with various chemometric methods after pre-treatment. UV–vis spectra of vinegars adulterated with spirit vinegar and acetic acid are provided in Figure 4.1a and b, respectively. Spectra for both cases resemble to each other and non-adulterated vinegar spectra have similar features with the ones in the literature (Torrecilla et al., 2016, Yalçın et al., 2021). The highest absorbance values were observed in 280–300 nm region and 275–350 nm region is associated with phenolic compounds and organic acids (Torrecilla et al., 2016, Yalçın et al., 2021). The absorbance values of the peaks in this region vary with respect to adulterant concentration.

Effects of adding spirit vinegar and acetic acid on FTIR spectra are shown in Figure 4.2. Adulteration of vinegar with spirit vinegar and acetic acid caused changes in the same regions of FTIR spectra. According to literature, band at 3800–2790 $cm^{-1}$ of vinegar FTIR spectra is attributed to – OH group of water and C – H stretching of acetic acid. C – O stretching of organic acid is at 1300–1000 $cm^{-1}$ region while C – O stretching of ethanol takes place at 1100–1000 cm−1. Peaks at 1065–1030 $cm^{-1}$ are associated with O – H and $–CH_2$ groups of sugars. Absorption band at 1700–1600 $cm^{-1}$ belongs to C – O stretching of aldehydes while 1800–900 cm−1 region is for – C – O and – OH groups of phenolic compounds (Rios-Reina et al., 2017b).

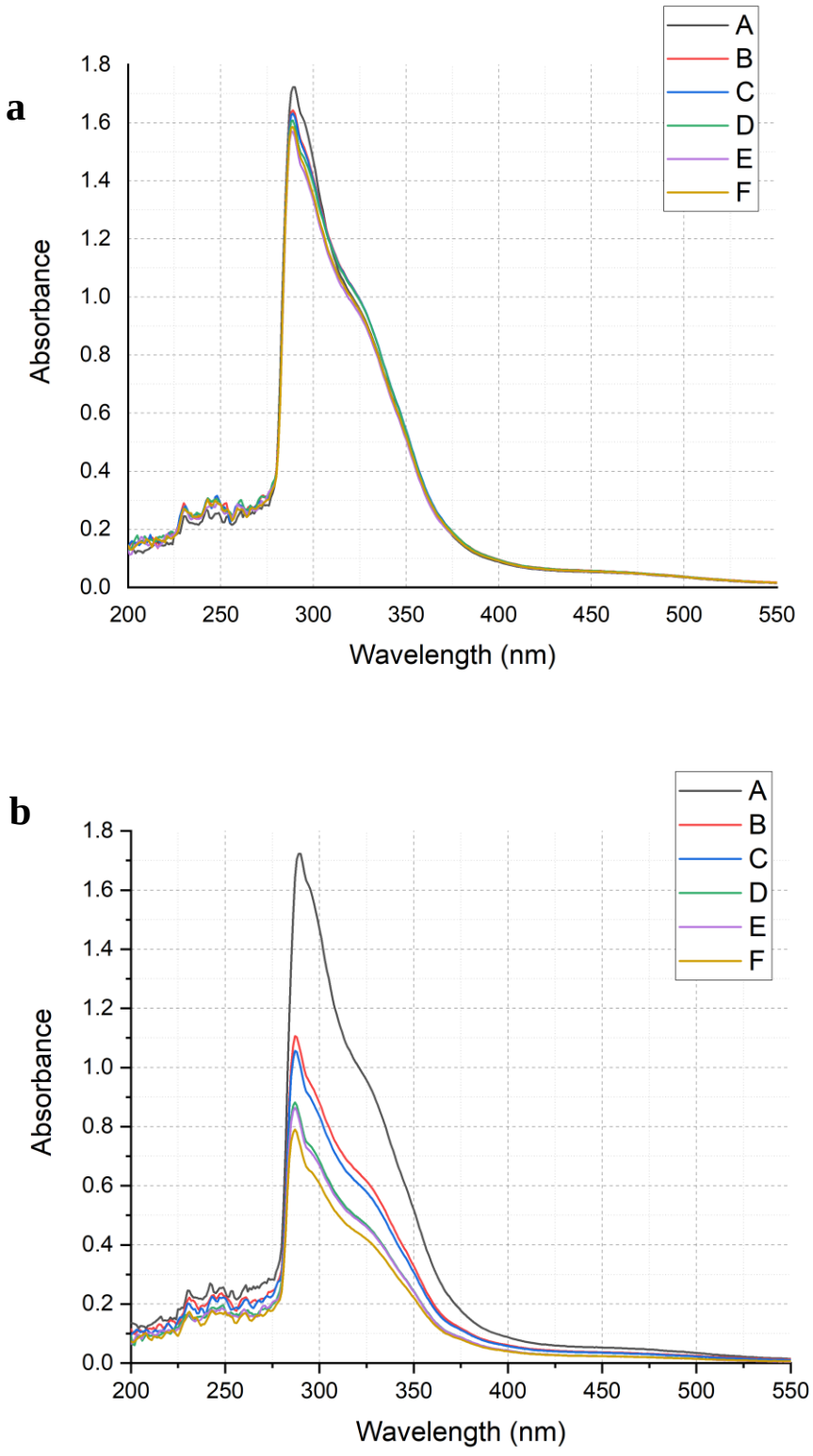


**Figure 4.1.** UV–vis spectra of non-adulterated vinegar and vinegar adulterated with a) spirit vinegar, b) acetic acid. (A: non-adulterated, B: 10%, C: 20%, D: 30%, E: 40%, F: 50%).

All spectra obtained with two spectroscopic methods were pre-treated with first, second and third derivative, SNV, MSC, OSC, WCTS and WDTS transformations. Chemometric models were first constructed with all the data obtained. However, later on, adulterated samples having 1% and 5% adulterant levels were excluded from the models to improve the model performances. Since it is indicated that generally adulteration levels around 30% and higher are used in mixing vinegars with adulterants (oral communication with vinegar producers) it would be acceptable to remove low levels of adulterated sample set for better estimation of adulteration. As a result, models were built again with 123 samples and validated with 62 samples. At least 16 models were generated for each individual spectroscopic data and also for their fused data with the combination of different transformations and chemometric methods (PLS-DA and OPLS-DA). Best classification models among these were chosen by considering number of LVs, $R^2_{cal}$ and $R^2_{val}$ values. Same data were also evaluated with ANN using 125, 25, 3 neurons in three hidden layers.

First, only UV–vis spectra were used in model building for adulteration detection. OPLS-DA model (1 + 9 + 0 LV, $R^2_{cal} = 0.97$, $R^2_{val} = 0.5$) of third derivative transformed UV–vis data produced the best classification model for non-adulterated and spirit and acetic acid adulterated vinegars. Score plot of OPLS-DA model shows a very good separation of adulterated samples from pure vinegars (Figure 4.3a). Correct classification rate for the constructed model is 100% for calibration set and 95.16% for validation set (1 out of 7 non-adulterated and 2 out of 55 adulterated samples are misclassified) (Table 4.1). Sensitivity and specificity of this model are determined as 85.71% and 96.36%, respectively.

Evaluation of UV–vis spectroscopic data with ANN resulted in 97.6% and 95.2% correct classification for calibration and validation models, respectively (Table 4.1). OPLS-DA calibration model has a higher success rate in calibration set while correct classification rate of ANN and OPLS-DA models of UV–vis spectroscopic data are almost the same. High specificity value of ANN model is comparable with OPLS-DA models but ANN model has a lower sensitivity value for validation model (71%).

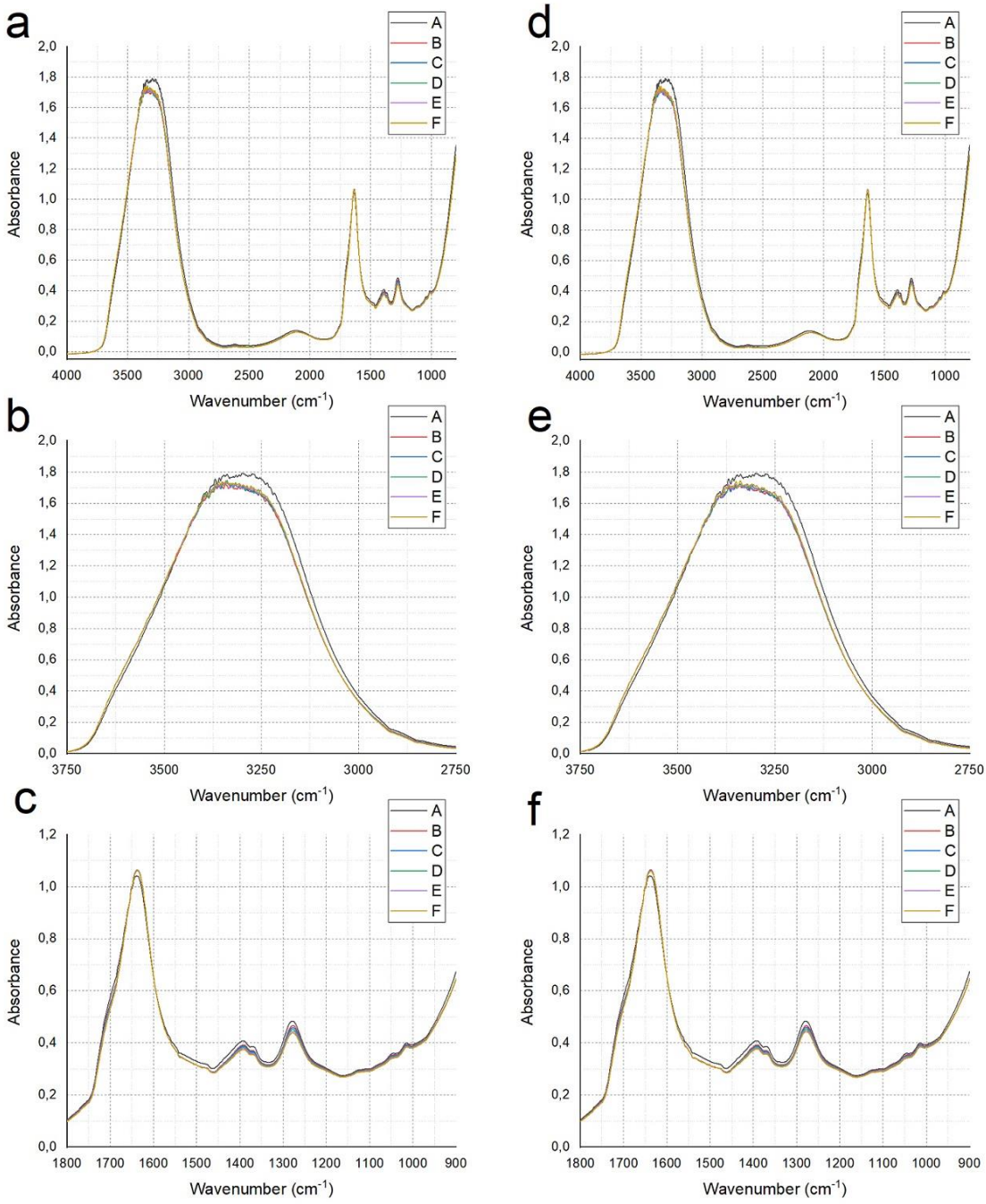


**Figure 4.2.** FTIR spectra of non-adulterated vinegar and vinegar adulterated with a, b, c) spirit vinegar, d, e, f) acetic acid vinegars. (A: non-adulterated, B: 10%, C: 20%, D: 30%, E: 40%, F: 50%).

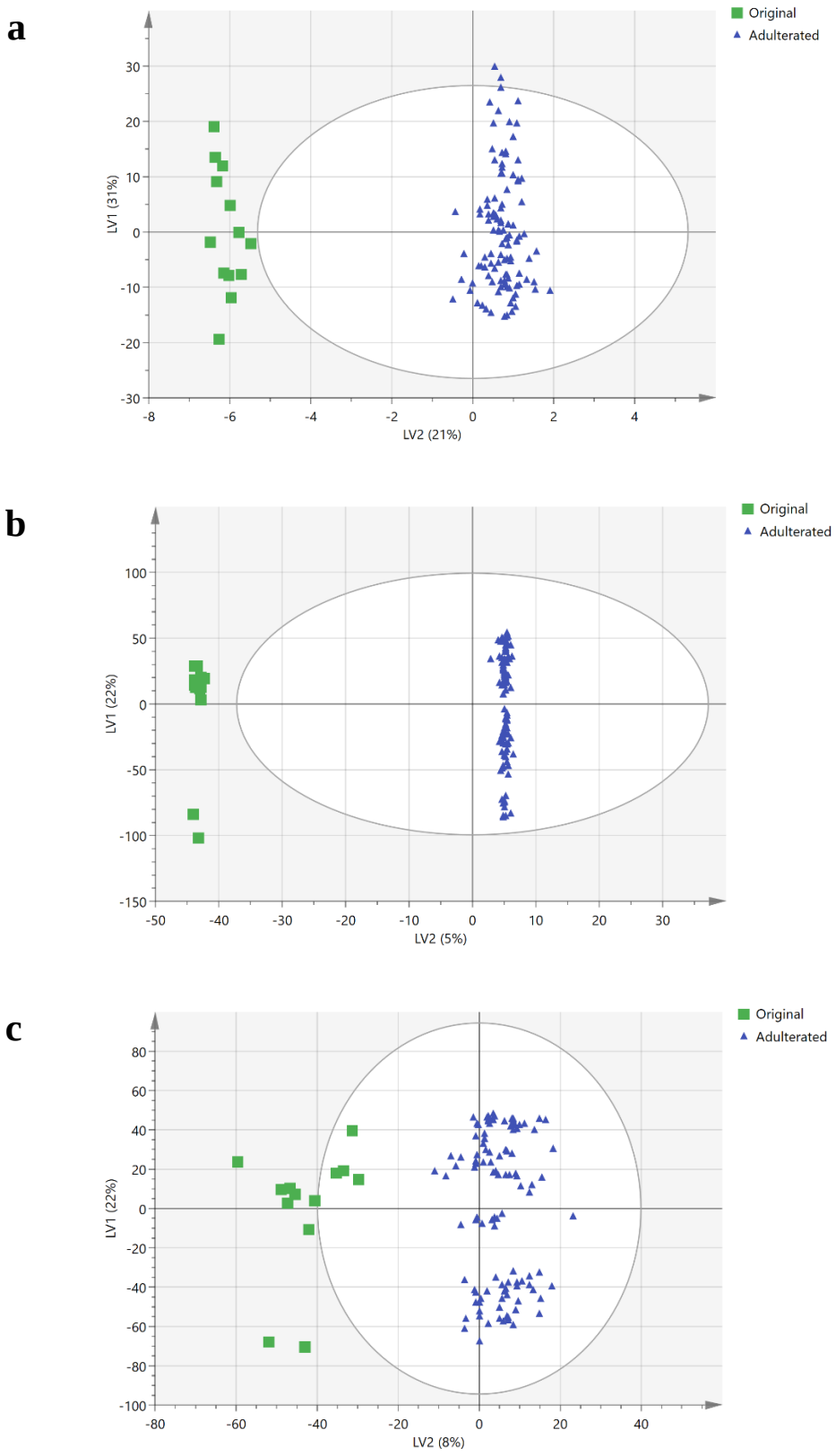


**Figure 4.3.** OPLS-DA score plots showing the discrimination between non-adulterated and adulterated vinegars using a) UV–Vis spectroscopic data, b) FTIR spectroscopic data, c) FTIR + UV–vis spectroscopic data (squares and triangles represent authentic and adulterated samples, respectively).

**Table 4.1.** Statistical parameters of chemometric and ANN models for discrimination of non-adulterated and adulterated vinegars using UV–Vis, FTIR and UV–vis + FTIR spectroscopic data.

| Spectroscopic Technique | Model | Type | Correct Classification % | Sensitivity % | Specificity % |
|---|---|---|---|---|---|
| UV-vis | Third derivative transformation and OPLS-DA | Calibration | 100 | 100 | 100 |
| | | Validation | 95.2 | 85.7 | 96.4 |
| | ANN | Calibration | 97.6 | 100 | 97 |
| | | Validation | 95.2 | 71 | 98 |
| FTIR | WDTS transformation and OPLS-DA | Calibration | 100 | 100 | 100 |
| | | Validation | 96.8 | 85.7 | 98.2 |
| | ANN | Calibration | 95.2 | 100 | 95 |
| | | Validation | 94.3 | 100 | 94 |
| UV-vis + FTIR | WDTS transformation and OPLS-DA | Calibration | 100 | 100 | 100 |
| | | Validation | 96.8 | 85.7 | 98.2 |
| | ANN | Calibration | 97.6 | 100 | 97 |
| | | Validation | 95.2 | 100 | 97 |

FTIR spectroscopic data were also used in creating chemometric models for the determination of adulteration after various transformations. As far as chemometric analysis is concerned, the best classification between non-adulterated and adulterated vinegars was obtained with an OPLS-DA model (LV = 1 + 13 + 0, $R^2_{cal}$ = 0.99, $R^2_{val}$ = 0.67) after WDTS transformation.

Score graph for this model is shown in Figure 4.3b and it can be observed from this graph that there is a very well separation between adulterated and non-adulterated vinegars with respect to the first LV. Correct classification rates of this model are provided for both calibration and validation sets in Table 4.1. According to this table, correct classification rate for calibration model is 100% while success rate of classification in validation set is determined as 96.8% (1 out of 54 of adulterated and 1 out of 7 non-adulterated samples were misclassified). This model has 85.7% sensitivity and 98.2% specificity.

Correct classification rates obtained with ANN treatment of FTIR spectroscopic data are 94.3% and 95.2% for calibration and validation sets, respectively (Table 4.1). These values are a little bit lower than OPLS-DA models of FTIR data. Sensitivity and specificity values of ANN analysis provided high values and these values are mostly similar with chemometric models. One exception is higher sensitivity (100%) of ANN validation model compared to OPLS-DA model (85.7%).

Low data fusion was also applied to the data and UV–vis and FTIR data sets were combined together for chemometric model building to improve adulteration detection. OPLS-DA model (LV: 1 + 7 + 0; $R^2_{cal}$ = 0.99; $R^2_{val}$ = 0.85) after WDTS transformation resulted in the best model for differentiation of non-adulterated and adulterated vinegars and score graph of this model can be seen in Figure 4.3c. Score graph shows a good classification of the samples. However, 2 classes are closer to each other compared to the score plots generated using individual spectroscopic data (Figure 4.3). Correct classification table for the combined data is shown in Table 4.1. Model built using both spectroscopic data resulted in 100% and 96.77% correct classification rates for calibration and validation data sets, respectively. This model has the same sensitivity (85.71%) and specificity (98.18%) values as the model developed using FTIR data. Combining the data did not provide any significant improvement with respect to the use of individual UV–vis and FTIR spectroscopic data.

Classification success rates (97.6% and 95.2%) for UV–vis + FTIR data evaluated with ANN is slightly lower compared to OPLS-DA models (Table 1). Other than higher

sensitivity (100%) of validation model, sensitivity and specificity values of ANN and OPLS-DA models are comparable. Use of combined UV–vis and FTIR data in ANN analysis did not improve classification rates as it happened in other multivariate techniques.

In all cases, PLS-DA, OPLS-DA and ANN treatments of the data which have adulteration levels higher than 5% provided mostly similar success rates. UV–vis and FTIR spectroscopic data are equally useful in detecting the adulteration of vinegars mixed with either spirit vinegar or diluted acetic acid. Combined data did not allow better classification rates. VIP values of the successful models indicated that all peaks in the UV–vis and FTIR spectra are responsible for the differentiation of authentic and adulterated samples and this means that differentiation is not only due to one component but combinations of different components. Spectroscopic methods provide a holistic approach and this is one of the advantages of these methods as also observed in this study.

There are several studies regarding the detection of acetic acid and spirit vinegar added to vinegars with various techniques in the literature. δ13C values originating from methyl and carboxyl groups in acetic acid were determined with head space-solid phase micro extraction (HS-SPME) combined with gas chromatography pyrolysis gas chromatography combustion isotope ratio mass spectrometry (GC-Py-GC-C-IRMS) technique and used successfully in detecting the addition of low levels of acetic acid to commercial Japanese vinegars (Hattori et al., 2010). In another study, determination of acetic acid added to balsamic vinegar was aimed and it was investigated to build a multi-step method including GC-IRMS measurement which was validated with $^{1}$H NMR spectroscopic technique (Werner and Roßmann, 2015). Isotopic ratios obtained with GC-Py-GC-C-IRMS technique were also used in detection of acetic acid in spirit vinegar with successful results (Hattori et al., 2011). SNIF-NMR method was applied to determine the addition of acetic acid and spirit vinegar into rice vinegar and it was reached to a conclusion that deuterium/H ratio can be used to find out the acetic acid adulteration (Hsieh et al., 2013). It was reported that another spectroscopic method, fluorescence spectroscopy, provided 100% accurate results in determination of acetic acid addition into Chinese aged Shanxi vinegars (Peng et al., 2019). Since Shanxi vinegar is an aged product, accumulation of some substances such as phenolic compounds during aging as indicated in literature (Chen et al., 2016b) can cause better differentiation of authentic and adulterated samples. Therefore, the success of differentiation is also related with the product. If the vinegar is quite rich in terms of its organic compounds coming from the

raw material it is more likely that spectroscopic methods could provide more accurate detection of adulteration. As demonstrated in this study, UV–vis and FTIR spectroscopy can be also alternatives to these existing methods in the literature and it has the advantages of being easy to measure, rapid and minimum waste generating techniques as other spectroscopic techniques.

## 4.5. Conclusions

Potential of UV–vis and FTIR spectroscopy in combination with chemometric methods and ANN analysis was investigated for the detection of adulteration of grape vinegars with acetic acid and spirit vinegar. ANN and OPLS-DA models of UV–vis and FTIR spectroscopic data provided high correct classification rates, sensitivity and specificity values at adulteration levels higher than 5%. Both UV–vis and FTIR spectroscopic data as well as ANN and chemometric models have, in general, similar success rates in determination of vinegar adulteration with specified adulterants.

This study was based on the hypothesis that investigated spectroscopic techniques would be effective in determining the vinegar adulteration with spirit vinegar and acetic acid and this hypothesis is confirmed with the results obtained. UV–vis and FTIR spectroscopy in combination with chemometric methods can provide easy detection of grape vinegar adulteration with minimum sample preparation.

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# CHAPTER 5

# APPLICATIONS OF UV–VISIBLE, FLUORESCENCE AND MID-INFRARED SPECTROSCOPIC METHODS COMBINED WITH CHEMOMETRICS FOR THE AUTHENTICATION OF APPLE VINEGAR

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## 5.1. Abstract

Spectroscopic techniques as untargeted methods have great potential in food authentication studies, and the evaluation of spectroscopic data with chemometric methods can provide accurate predictions of adulteration even for hard-to-identify cases such as the mixing of vinegar with adulterants having a very similar chemical nature. In this study, we aimed to compare the performances of three spectroscopic methods (fluorescence, UV–visible, mid-infrared) in the detection of acetic-acid/apple-vinegar and spirit-vinegar/apple-vinegar mixtures (1–50%). Data obtained with the three spectroscopic techniques were used in the generation of classification models with partial least square discriminant analysis (PLS-DA) and orthogonal partial least square discriminant analysis (OPLS-DA) to differentiate authentic and mixed samples. An improved classification approach was used in choosing the best models through a number of calibration and validation sets. Only the mid-infrared data provided robust and accurate classification models with a high classification rate (up to 96%), sensitivity (1) and

specificity (up to 0.96) for the differentiation of the adulterated samples from authentic apple vinegars. Therefore, it was concluded that mid-infrared spectroscopy is a useful tool for the rapid authentication of apple vinegars and it is essential to test classification models with different datasets to obtain a robust model.

## 5.2. Introduction

Vinegar is a product which can be produced from various raw materials, mostly belonging to plant origins, with sugar as the substrate using a double fermentation process (ethanol fermentation and acetification). Vinegar can be classified with respect to its raw materials or production systems. Common types, considering the raw materials used, include wine, fruit, spirit/white (produced from diluted ethanol), cereal, malt, honey and whey vinegars, and they are most commonly produced through either surface culture (traditional) or submerged culture methods. Compositions of vinegars vary with respect to the raw materials from which they are produced. The major constituent is acetic acid; however, they also have various organic acids including citric, formic, lactic, malic and succinic acids, alcohols, sugars (glucose and fructose), amino acids, volatile compounds and phenolic compounds. The presence of phenolic compounds such as gallic acid, catechin, vanillic acid, syringic acid, caffeic acid and volatiles including ethyl heptanoate, ethyl furoate, ethyl benzoate and sotolon have been determined in vinegars [1]. Regulations about vinegars generally involve the amounts of acetic acid and ethanol in the product, which can vary slightly from country to country. The Food and Drug Administration (FDA) of the USA specifies the level of acetic acid as 4 g/100 mL, while the levels of acetic acid and ethanol are 50 g/L and less than 0.5% in the Codex, respectively. The European Union set a minimum of 5% (w/v) acidity and a maximum of 0.5% (v/v) ethanol levels for vinegars. While some countries allow the mixing of vinegar with acetic acid, others do not [1].

A projected compound annual growth rate of approximately 1.6% is expected between 2021–2026 for the vinegar market [2]. The increase in the global demand for vinegar is a result not only of its increased use in the food industry but also of its expanding applications in the cleaning, healthcare and agricultural industries. Besides its

antimicrobial and antioxidant properties, another factor causing the consumer interest in vinegars is studies that have uncovered the positive health effects of this product [3,4]. Different claims such as weight loss, laxative effects and blood glucose lowering effects for type-2 diabetes patients, some of which require further confirmation studies, have also been made, particularly for apple vinegar [5]. However, this increased interest has also resulted in a rise in different types of fraud practices surrounding this product. Food fraud is described as 'any deliberate action of businesses or individuals to deceive others in regards to the integrity of food to gain undue advantage', and it is stated that this definition includes 'adulteration, substitution, dilution, tampering, simulation, counterfeiting, and misrepresentation' in addition to others [6]. The rising demand of consumers for good-quality, safe and healthy foods goes in parallel with the increase in the sophisticated ways that fraudsters misrepresent/adulterate these food products. Chemically similar and cheaper replacements of products can be very challenging to detect; therefore, there is always a need for alternative methods to determine different types of food fraud.

Variations in raw materials, production methods and regulations regarding the definition of the product along with the levels of acetic acid and ethanol add up to difficulties in adulteration detection for vinegars. Various adulterants are mixed with authentic vinegars to obtain economic profit. Adulteration can be achieved by adding chemical acetic acid, spirit vinegar, coloring compounds such as caramel and by mixing different types of vinegars. The false labeling of regular vinegars as high-priced vinegars with a protected-designation-of-origin status (PDO) or mixing PDO vinegars with adulterants is also an authenticity problem. Besides the economic effects of mixing, the addition of acetic acid can have particularly negative consequences, since it contains more heavy metals [1].

The targeted and untargeted methods available for the detection of vinegar adulteration have been summarized in several reviews in the literature [7,8]. Targeted methods such as chromatographic measurements focus on specific compounds such as a particular organic acid, a pigment or a phenolic compound [9,10]. Although valuable information can be obtained from the analysis of products using this type of approach, it also has disadvantages, as the used methods require time-consuming steps of sample pre-treatments that mostly involve the use of chemicals. On the other hand, untargeted methods, depending on their working principle, provide data originating from the many compounds in the analyzed product. Spectroscopic techniques, used mostly as untargeted methods, have the advantages of being rapid and generating relatively less waste, and

they produce fingerprints of the analyzed samples. They are also very suitable for use as sensors [11,12,13]. Since spectroscopic techniques produce a large number of variables, multivariate statistical analysis tools are commonly used to evaluate these data. These chemometric methods can be used in classifying samples or for the prediction of chemical properties. Various spectroscopic methods have been investigated for the authentication of vinegars in the literature. Near-infrared (NIR) spectroscopy has been used in the classification of vinegars with regard to their production methods, and vinegars produced with the submerged and Orleans methods have been successfully differentiated [14]. The separation of balsamic and traditional balsamic vinegars of Modena with respect to their ages was achieved through the evaluation of nuclear magnetic resonance (NMR) spectroscopic data with chemometric methods, partial least square discriminant analysis (PLS-DA) and naive Bayes approaches [15]. Various studies about vinegar authentication have also been focused on the discrimination of vinegars according to their origin, and spectroscopic methods including NIR, mid-infrared (mid-IR), fluorescence, UV–visible and NMR spectroscopies have been applied for this purpose [16,17,18,19,20,21]. UV–visible and fluorescence spectral data were evaluated with principal component analysis (PCA) and parallel factor analysis (PARAFAC) in the discrimination of vinegars with respect to the country of production [21]. Spanish PDO vinegars, “Vinagre de Jerez” and “Vinagre Condado de Huelva”, were characterized with mid-IR spectroscopy, and the data were analyzed with PCA [16]. The performances of several spectroscopic methods, namely mid-IR spectroscopy, NIR spectroscopy, excitation–emission multidimensional fluorescence spectroscopy and 1H nuclear magnetic resonance (1H-NMR) spectroscopy, were compared in the classification of Spanish PDO vinegars, namely Vinagre de Jerez, Vinagre de Condado de Huelva and Vinagre de Montilla-Moriles, and the data were treated with data fusion techniques [20].

Spectroscopic methods were also used in differentiating mixtures of vinegars and, as an example, detection of the adulteration of sherry vinegars with molasses, rice, cider and wine vinegars was investigated with laser diode fluorescence spectroscopy in conjunction with chaotic algorithms [22]. Excitation–emission fluorescence spectroscopy, on the other hand, was used in differentiating authentic Shanxi aged vinegars from this vinegar mixed with acetic acid in combination with chemometric methods, and a 100% discrimination was achieved [23]. Although there have been many studies focusing on the different aspects of vinegar authentication, the number of studies on the detection of spirit vinegar and synthetic acetic acid is limited. In an earlier study,

mid-IR and UV–visible spectroscopies were used to detect the adulteration of grape vinegars with spirit vinegar and acetic acid, and both techniques in combination with PLS-DA and orthogonal PLS-DA (OPLS-DA) were found to be successful in identifying adulterated grape vinegars [24]. The current study compared three spectroscopic techniques (UV–visible, fluorescence and mid-infrared) for their potential in the authentication of apple vinegars considering two adulterants. There are a limited number of studies regarding the mixing of vinegars with spirit vinegar and acetic acid, and the detection of these adulterants poses a challenge due to their similar chemical nature to vinegar. More studies are required to investigate the effect of the type of vinegar on the performances of various spectroscopic techniques in combination with chemometric methods so that suitable analytical and chemometric methods can be chosen for adulteration detection.

This study was designed to test and compare the potentials of various spectroscopic methods, namely UV–visible, fluorescence and mid-infrared, in conjunction with chemometric methods for detecting mixtures of apple vinegars with spirit vinegar and synthetic acetic acid.

## 5.3. Materials and Methods

Seventeen authentic apple vinegars were supplied by eleven trusted producers. Two batches were obtained from each of two producers and five batches were obtained from one producer while the other producers supplied one batch. Two adulterated sample sets were prepared: apple-vinegar/spirit-vinegar and apple-vinegar/acetic-acid mixtures. Each set had adulterant levels of 1, 5, 10, 20, 30, 40 and 50% (v/v). Glacial acetic acid used as an adulterant was diluted to a typical vinegar acetic acid level of 4% (v/v) before mixing with the vinegars. Eight apple vinegars were randomly chosen among seventeen vinegars to mix with two spirit vinegars and acetic acid separately, and one hundred and eighty-five adulterated samples were prepared.

### 5.3.1. Measurement of Quality Parameters

pH and Brix values of the authentic vinegars were determined with a pH meter (WTW, Weilheim, Germany) and a digital refractometer (Isolab, Wertheim, Germany), respectively. Total acidity expressed as a volumetric percentage was measured via titration analysis using sodium hydroxide [25]. A microscale Folin–Ciocalteu spectrophotometric assay was used in the measurement of the total phenolic content in terms of mg gallic acid/L of the authentic vinegars [26]. The total phenolic contents of the authentic apple vinegars were determined using a 5-point gallic acid standard curve.

### 5.3.2. Fluorescence Spectroscopy

Spectra of authentic and adulterated samples were collected with a fluorescence spectrophotometer (Thermo Scientific Varioskan, Fisher Scientific, Vantaa, Finland) at 320–800 nm with 1 nm intervals. Excitation wavelengths were 320, 330, 340 and 350 nm [27]. The best results were obtained at 320 nm. The slit width was 5 nm. Samples were diluted 5 times, and the spectra of 200 μL samples in a black 96-well flat bottom polystyrene plate (Isolab, Wertheim, Germany) were collected. Two spectra from each sample were averaged.

### 5.3.3. UV–Visible Spectroscopy

A total of 200 μL from all the samples diluted 5× with distilled water in 96-well flat bottom polystyrene plates (Isolab, Wertheim, Germany) was scanned in 200–550 nm range with a UV–vis spectrophotometer (Thermo Scientific Multiskan GO Microplate Spectrophotometer, Fisher Scientific, Vantaa, Finland). The average of two spectra for each sample was used in the statistical analyses.

### 5.3.4. Fourier Transform Infrared Spectroscopy

Mid-IR spectra of the samples were obtained with a Fourier transform infrared (FTIR) spectrophotometer with a horizontal ZnSe ATR accessory and a deuterated triglycine sulfate (DTGS) detector (Spectrum 100, Perkin Elmer, Waltham, MA, USA). The spectra were collected in 4000–800 $cm^{-1}$ range with 128 scans and a 4 $cm^{-1}$ resolution against an air spectrum. Two measurements were taken for each sample, and they were averaged.

### 5.3.5. Statistical Analysis

One of the unsupervised techniques, principal component analysis (PCA), was performed as a preliminary analysis. A discrimination trend between the authentic and adulterated samples in the scatter plot of the first and second principal components was observed; therefore, it was decided to continue with a higher-level multivariate analysis. Differentiation of the authentic and adulterated apple vinegars was conducted with two supervised chemometric methods, namely partial least square discriminant analysis (PLS-DA) and orthogonal partial least square discriminant analysis (OPLS-DA). PLS-DA and OPLS-DA are supervised multivariate classification techniques, and they convert data to a lower dimension through linear transformation. The authentic samples were defined as one class, and all the adulterated samples were assigned to another class. The raw and transformed data from the 3 spectroscopic techniques were used in the chemometric model building. Along with intensity values at different emission wavelengths for fluorescence spectroscopy, the absorption values of the samples at different wavenumbers and wavelengths for the mid-IR and UV–vis spectroscopy, respectively, were individually collected in column-wise vectors. After the collection of the data, individual observations were combined in a row-wise matrix prior to the multivariate analysis. The following data transformations were applied: first (FD), second (SD) and third (TD) derivatives, square, standard normal variate (SNV), multiplicative scatter correction (MSC) and Savitzky–Golay (SG). In addition, the following combinations of these

transformations were also used: FD + SNV, FD + MSC, SD + SNV, SD + MSC, TD + MSC and TD + MSC. Every feature in the collected and transposed dataset was normalized using the scaling of 0–1, which is called a min-max normalization. Models were created using the 'ropls package' (Version 3.12) in the R programming language [28]. Two-thirds of the data were used for building the calibration models, while the external validation was conducted with the rest of the data. The samples were assigned to the calibration and validation sets using stratified random sampling [29]. The goodness of the classification models was evaluated using the number of latent variables (LV), $R^2$ values for calibration ($R^2_{cal}$) and validation ($R^2_{val}$), root mean square of error (RMSE), sensitivity, specificity, correct classification rates for calibration and validation. Definitions of correct classification rates, sensitivity and specificity are provided in the literature [30]. Sensitivity was measured as the ratio of the true number of correctly identified apple vinegars to all the samples identified as apple vinegar and was calculated using:

$$Sensitivity = \frac{True\ Positive}{True\ Positive + False\ Negative}$$

where TP and FN are samples identified as true positive and false negative, respectively. On the other hand, dividing the number of correctly identified adulterated samples to all the samples identified as adulterated provided the specificity:

$$Specificity = \frac{True\ Negative}{True\ Negative + False\ Positive}$$

where TN and FP are samples identified as true negative and false positive, respectively. The correct classification rate was calculated by dividing the number of correctly determined samples to all the samples, and it was determined for both the calibration and validation sets as follows:

$$Correct\ classification\ rate = \frac{True\ Positive + True\ Negative}{Total\ number\ of\ samples}\ x\ 100$$

## 5.4. Results and Discussion

Various properties of the authentic apple vinegars are shown in Table 5.1. The authentic vinegar samples had pH and Brix ranges of 2.74–2.99 and 0.6–5.3, respectively. The total acidity of these samples varied between 4.08 and 5.49%. The vinegars had total phenolic contents of 163.15–547.40 mg gallic acid/L. These measurements were in agreement with the values given in the literature [31].

**Table 5.1.** Various properties of authentic apple vinegar samples.

| Number of Samples | pH Range | Brix Range | Total Phenolic Content Range (mg Gallic Acid/L) |
|---|---|---|---|
| 17 | 2.74–2.99 | 0.6–5.3 | 163.15–547.40 |

### 5.4.1. Spectroscopic Profiles

The authentic apple vinegars had strong double absorption peaks in 280–300 nm range of the UV–visible spectra (Figure 5.1a), and these peaks were associated with phenolic compounds, as reported in the literature [32,33]. The authentic vinegars had a wide absorption range in the UV–visible range, which was most probably due to their varying phenolic compositions, and this was confirmed by the measured total phenolic contents of the authentic apple vinegars, which were in the range of 163.15–547.4 mg gallic acid/L. The UV–visible, fluorescence and mid-IR spectra of an example set of adulterated spirit vinegar and acetic acid vs. authentic vinegars are shown in Figure 5.2.

In general, the absorbance of the adulterated samples in 280–400 nm range decreased with increasing adulteration ratio due to the dilution of phenolic compounds with an adulterant (Figure 5.2a,b). This decrease was more obvious in the spirit-vinegar-adulterated samples, while the dilution effect was visible at around 20% for the acetic-acid-mixed samples for this particular sample set. Spirit vinegar is produced from bio-resources through fermentation, while acetic acid is a synthetic product without any ingredients from biological sources. Therefore, these differences in the adulterated sample spectra can be related with the sources of the adulterants.

For the authentic apple vinegars, a wide variation in intensity was also observed in their fluorescence spectra (Figure 5.1b). The spectra could be characterized by strong intensity peaks in 300–600 nm region. Phenolic compounds are designated as having fluorescent properties, and this range corresponds to the intensity due to these compounds [23,34]. The peak at around 470–500 nm was attributed to brown pigments, which can be produced by acetic acid bacteria [27]. As was observed in the UV–visible spectra, the fluorescence spectra of the authentic vinegar vs. the spirit vinegar adulterated apple vinegar and the authentic vinegar vs. acetic acid adulterated apple vinegar sample sets indicated a dilution effect but at higher concentrations compared to the UV–visible spectra (Figure 5.2c,d).

The mid-IR spectra were collected in 4000–800 $cm^{-1}$ region; however, it is generally hard to see major differences if the full spectra are shown. Therefore, part of the spectra corresponding to 1500–800 $cm^{-1}$ region are presented for all the authentic apple vinegars in Figure 5.1c. The mid-IR spectra of the adulterated and authentic samples had significant differences, especially in 1500–1000 $cm^{-1}$ region (Figure 5.2e,f). The peaks in the 1400–1350 $cm^{-1}$ region were attributed to –OH stretching of alcohol and organic acids [24], and the adulterated samples had a higher absorption in this region, as was expected. However, the absorption intensity decreased with respect to the ratio for the adulterated samples in 1150–1000 $cm^{-1}$ region where absorption took place due to compounds such as sugars and phenolic compounds, and this decrease in the absorption intensity was also attributed to the addition of adulterants. Differences in the spectra obtained by these three spectroscopic methods were also evaluated by chemometric methods, which can reveal even small changes in the spectra that are not very visible, and this is especially useful for spectroscopic methods with large number of variables, as is the case in mid-IR spectroscopy.

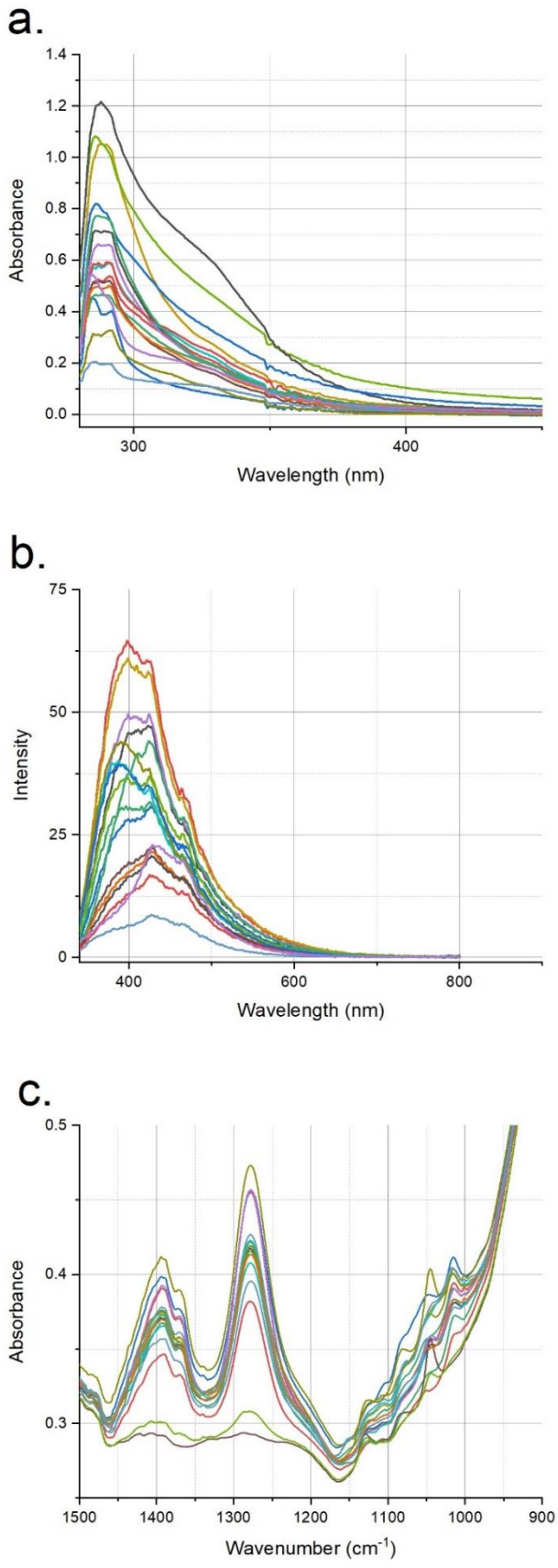


**Figure 5.1.** (a) UV–visible, (b) fluorescence and (c) mid-IR spectra of all authentic apple vinegars used in this study.

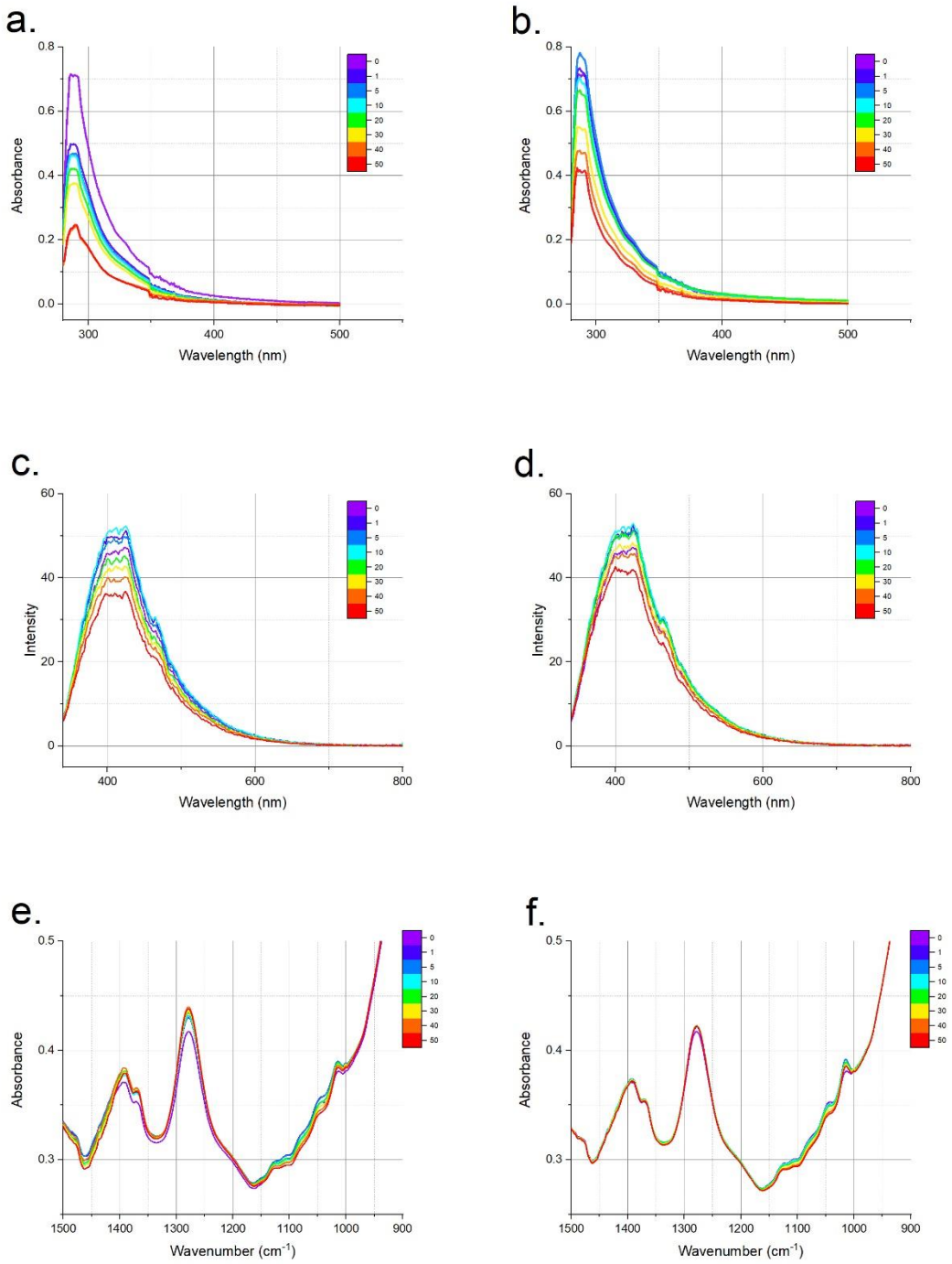


**Figure 5.2.** UV–visible spectra of (a) spirit-vinegar-added and (b) acetic-acid-added samples; fluorescence spectra of (c) spirit-vinegar-added and (d) acetic-acid-added samples; and mid-IR spectra of (e) spirit-vinegar-added and (f) acetic-acid-added samples vs. authentic apple vinegars for a sample set.

### 5.4.2. Chemometric Analyses

A set of randomly chosen vinegars were adulterated with spirit vinegar and diluted acetic acid (4%) separately. The adulterated set contained both apple-vinegar/spirit-vinegar and apple-vinegar/acetic-acid mixtures, and PLS-DA and OPLS-DA chemometric models were constructed to differentiate the authentic apple vinegars from the mixtures. Separate models were not created for each adulterant since the nature of the adulterant would not be known in a more realistic scenario. Therefore, two classes were created as the authentic and adulterated sets. The whole collected spectral ranges of all the spectroscopic methods were used in the chemometric analyses.

An improved approach was used in deciding on the best classification models (Figure 5.3). Both raw and transformed data, as indicated in Section 5.3.5, were used in generating the PLS-DA and OPLS-DA models. This procedure was repeated three times, and each time a new randomly chosen data set for calibration and validation was used. For each trial, the statistical performance parameters (LV, $R^2_{cal}$, $R^2_{val}$, RMSE, sensitivity, specificity, correct classification rates for calibration and validation) of the models were determined. The models which provided good and robust results for all the trials were designated as our final models. The purpose of this approach was to eliminate the effect of the samples in the model building. Therefore, the chosen robust models had high $R^2$ values for the calibration and validation models, high correct classification rates for classification and validation sets, high sensitivity and specificity values and a low RMSE value regardless of the sample.

Furthermore, the models improved significantly when the samples with a 1% adulteration level were eliminated from the sample set. Since this is a very low level of mixing for the economic gain of fraudsters, the 1% samples were taken out from the sample set, and the models were constructed with the samples with higher adulteration levels. After the removal of the 1%-adulterated samples, the models were built with 107 samples and validated with 52 samples.

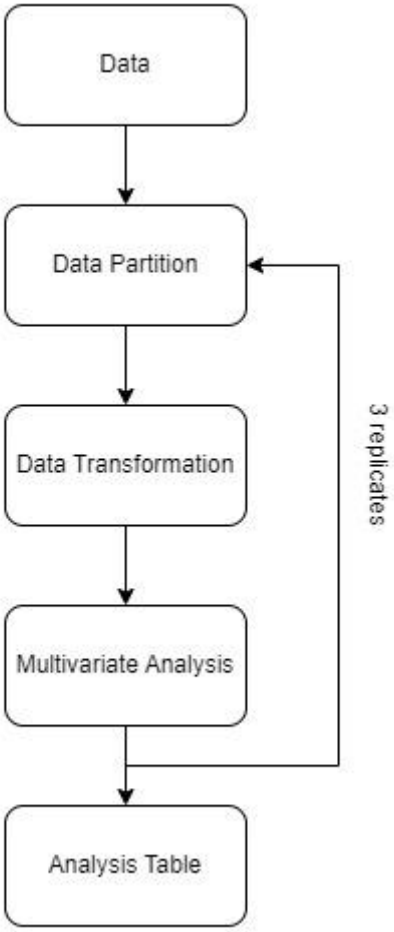


**Figure 5.3.** Flow chart of data analysis.

The only good model, which was built with the fluorescence spectra and had correct classification rates of 90% for calibration, 92% for validation, a sensitivity of 1 and a specificity of 0.92, belonged to the PLS-DA analysis of the MSC-transformed data (Table 5.2). As can be seen from Table 5.2, the sensitivity and specificity values were unacceptable for the second and the third sample sets. It was concluded that this transformation and any other transformations of the fluorescence spectra did not result in any good classification model for the differentiation of the authentic and adulterated vinegars when different calibration and validation sets were used in the second and the third runs. The same type of results was also obtained with the UV–visible spectral data. Although there were models with high correct classification rates for the validation models for the first sample set, similar results were not obtained in the second and the

third runs with different sample sets. For example, the OPLS-DA model after SNV transformation of the data with a correct classification rate of 93% for calibration, a correct classification rate of 92% for validation, a sensitivity of 0.67 and a specificity of 0.94 was the best model (Table 5.2). However, this model with different sample sets did not result in any good specificity value, although these models had high correct classification rates. Therefore, it was concluded that the robustness of classification models had to be decided not only with the correct classification rates but also with the sensitivity and specificity values and that the models had to be checked with different sample sets.

The same type of approach was also used for evaluating the mid-IR data. Six chemometric models constructed with the FTIR data resulted in robust models: the PLS-DA and OPLS-DA models of the raw data, the square-transformed data and the SG-transformed data. Table 5.3 shows the statistical measures related with the performance of these models for three different sets of samples. For each sample set, these models had a high sensitivity and specificity as well as high correct classification rates for calibration and validation. These models had very close performance parameters when they were created with different sample sets. For example, the OPLS-DA model generated with raw data had the same sensitivity value of 1 and specificity values of 0.92, 0.94 and 0.94 for each sample set. Both the PLS-DA and OPLS-DA models produced very similar results in terms of the performances of the models. As an example, the PLS-DA and OPLS-DA models of the SG-treated mid-IR data had the same sensitivity (1), specificity (0.92) and correct classification rate for validation (92%) with the first and the second sample sets (sensitivity: 1, specificity: 0.96, correct classification rate: 96%) (Table 5.3). All six models shown in Table 5.3 were quite satisfactory and could be used successfully in detecting the adulteration of apple vinegar with acetic acid and spirit vinegar. Score plots of the OPLS-DA model of the SG-transformed mid-IR data for three different sample sets are given in Figure 5.4. As can be seen from this figure, the authentic and adulterated samples could be accurately differentiated from each other with respect to the first LV regardless of the sample set. In addition, this study indicates the importance of constructing classification models with different sample sets so that a more robust and accurate model can be obtained. In addition, not only the correct classification rates but also the sensitivity and specificity values have to be considered in evaluating models.

**Table 5.5.** Statistical measures of models generated using UV–visible and fluorescence spectroscopic data with three different data sets.

| Data Transformation | Sample Set | LV | $R^2_{cal}$ | $R^2_{val}$ | RMSE | Sensitivity | Specificity | $CC_{cal}$% | $CC_{val}$% |
|---|---|---|---|---|---|---|---|---|---|
| MSC-transformed fluorescence data with PLS-DA | First sample set | 3 | 0.96 | 0.11 | 0.303 | 1 | 0.92 | 90 | 92 |
| | Second sample set | 7 | 0.97 | 0.87 | 0.119 | 0.2 | 0.91 | 100 | 85 |
| | Third sample set | 4 | 0.96 | 0.43 | 0.243 | NaN | 0.9 | 94 | 90 |
| SNV-transformed UV–visible data with OPLS-DA | First sample set | 1 + 7 | 0.98 | 0.47 | 0.241 | 0.67 | 0.94 | 93 | 92 |
| | Second sample set | 1 + 7 | 0.99 | 0.46 | 0.243 | 0 | 0.9 | 93 | 88 |
| | Third sample set | 1 + 7 | 0.99 | 0.44 | 0.247 | NaN | 0.9 | 93 | 90 |

**Table 5.6.** Statistical measures of models generated using mid-IR data with three different data sets.

| Data Transformation | Sample Set | LV | $R^2_{cal}$ | $R^2_{val}$ | RMSE | Sensitivity | Specificity | $CC_{cal}$% | $CC_{val}$% |
|---|---|---|---|---|---|---|---|---|---|
| **PLS-DA** | | | | | | | | | |
| Raw | 1st sample set | 8 | 0.99 | 0.77 | 0.159 | 1 | 0.92 | 99 | 92 |
| | 2nd sample set | 10 | 0.99 | 0.85 | 0.128 | 1 | 0.94 | 100 | 94 |
| | 3rd sample set | 10 | 0.99 | 0.86 | 0.125 | 1 | 0.94 | 100 | 94 |
| Square | 1st sample set | 10 | 0.99 | 0.85 | 0.13 | 1 | 0.94 | 100 | 94 |
| | 2nd sample set | 10 | 0.99 | 0.84 | 0.135 | 1 | 0.94 | 99 | 94 |
| | 3rd sample set | 10 | 0.99 | 0.83 | 0.139 | 1 | 0.94 | 99 | 94 |
| Savitzky-Golay | 1st sample set | 8 | 0.99 | 0.75 | 0.165 | 1 | 0.92 | 99 | 92 |
| | 2nd sample set | 10 | 0.99 | 0.83 | 0.138 | 1 | 0.96 | 100 | 96 |
| | 3rd sample set | 10 | 0.99 | 0.84 | 0.134 | 1 | 0.94 | 100 | 94 |
| **OPLS-DA** | | | | | | | | | |
| Raw | 1st sample set | 1+8 | 0.99 | 0.84 | 0.133 | 1 | 0.92 | 99 | 92 |
| | 2nd sample set | 1+9 | 0.99 | 0.85 | 0.128 | 1 | 0.94 | 100 | 94 |
| | 3rd sample set | 1+9 | 0.99 | 0.86 | 0.125 | 1 | 0.96 | 100 | 96 |
| Square | 1st sample set | 1+8 | 0.99 | 0.81 | 0.143 | 1 | 0.94 | 100 | 94 |
| | 2nd sample set | 1+8 | 0.99 | 0.78 | 0.157 | 1 | 0.94 | 99 | 94 |
| | 3rd sample set | 1+7 | 0.99 | 0.83 | 0.139 | 1 | 0.96 | 99 | 96 |
| Savitzky-Golay | 1st sample set | 1+8 | 0.99 | 0.82 | 0.140 | 1 | 0.92 | 99 | 92 |
| | 2nd sample set | 1+9 | 0.99 | 0.83 | 0.138 | 1 | 0.96 | 100 | 96 |
| | 3rd sample set | 1+5 | 0.99 | 0.84 | 0.134 | 1 | 0.92 | 95 | 92 |

Most studies about vinegar authentication using spectroscopic techniques have focused on differentiation with respect to the source or type of vinegar [15,16,17,18,19,20,21]. However, studies which investigated the determination of the mixing of different types of adulterants with vinegar also exist in the literature. An electronic nose system was used in detecting the addition of acetic acid and spirit vinegar to apple vinegar in conjunction with the use of PCA and an artificial neural network (ANN), and correct classification rates of 93.3% for acetic acid and 94.7% for synthetic vinegar were determined for the ANN models [35]. Laser diode fluorescence spectroscopy data were evaluated with various intelligent chaotic algorithms to detect the presence of molasses, rice, cider and white wine vinegars in sherry vinegar; as a result, relative errors in predicting the adulterant concentration as low as 1.4% were obtained [22]. One study which investigated the determination of glacial acetic acid in Shanxi aged vinegars used excitation–emission matrix fluorescence spectroscopy data in combination with various chemometric approaches, and a model with a correct classification rate of 84.2%, a sensitivity of 0.83 and a specificity of 0.85 was obtained [20]. Since the adulterated product was a special type and was aged, the larger compositional differences between the authentic and adulterated samples could be the reason for the better success rate in that study compared to our case. In another study, in which the detection of spirit-vinegar- and acetic-acid-adulterated grape vinegars were studied using UV–visible and FTIR spectroscopy, the models created with UV–visible data had a correct classification rate of 95.2%, a sensitivity of 0.857 and a specificity of 0.964, and the FTIR data resulted in a model with 96.7% correct classification rate, a sensitivity of 0.857 and a specificity of 0.982 [24]. The same success was not obtained for apple vinegar adulteration with spirit and acetic acid using UV–visible spectroscopy in the current study. This could be related to the pigment composition of the apple vinegars. The authentic apple vinegars used in this study had a wide phenolic content range, and this was also reflected in the UV–visible spectra of the authentic vinegars (Figure 5.1a) and hence in the models generated using these spectra. In addition, the type of phenolic compounds present in apple and grape vinegars are different. Since UV–visible spectroscopy measurements are based on absorption due to colored compounds, the types and amounts of these compounds could be associated with the difference in the success of UV–visible spectroscopic data in the detection of adulteration of apple and grape vinegars. However, the evaluation of the FTIR spectral data resulted in a very good differentiation of apple vinegar adulteration, and the results of this study are comparable with the results of a

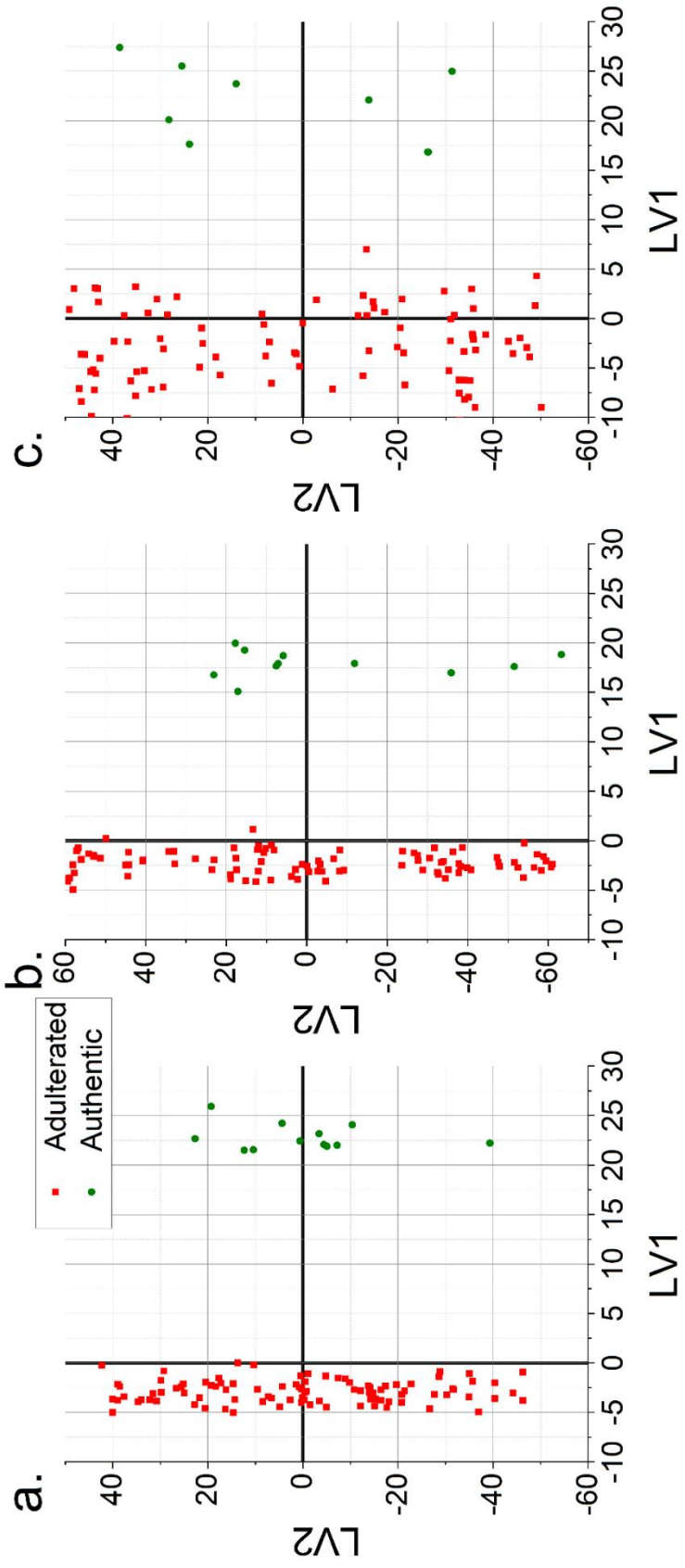


**Figure 5.4.** OPLS-DA score plots (LV1 vs. LV2) of Savitzky–Golay-transformed mid-IR data constructed with three different sample sets (a–c).

previous study done using grape vinegar [24]. While fluorescence and UV–visible spectroscopic measurements are based on the detection of fluorescent and colored compounds, respectively, FTIR spectral data can provide more compositional information of all the organic constituents of analyzed samples, and this could be the reason for the more satisfactory performance of this technique, regardless of the vinegar type.

## 5.5. Conclusions

The mixing of spirit vinegar and acetic acid with apple vinegar was investigated with UV–visible, fluorescence and mid-IR spectroscopy in combination with chemometric tools. Classification models used to separate the authentic and adulterated samples were created with testing models with three different data sets, and it was concluded that this step is important in choosing robust and accurate classification models. The performance of only the mid-IR spectroscopy was considered as successful in determining the presence of spirit vinegar and acetic acid in the apple vinegar, and models were able to determine adulteration with at least a correct classification rate of 92%, a sensitivity of 1 and a specificity of 0.92. Therefore, mid-IR spectroscopy in combination with a chemometric classification system can be used as a rapid analysis technique in determining the adulteration of apple vinegars.

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# CHAPTER 6

# SUMMARY AND CONCLUSIONS

The primary objective of this study was to predict multiple quality and chemical parameters associated with vinegar production and determine its authenticity using spectroscopic profiles analyzed with chemometrics. Within this investigation, diverse quality metrics, phenolic compounds, organic acids, and sugar profiles of vinegars derived from Sultaniye and Alicante grape varieties, produced through submerged and surface fermentation techniques, were estimated using FTIR and UV-Vis spectral data in conjunction with PLS and OPLS regression analyses. The study yielded precise, dependable, and robust prediction models, where $R^2_{cal}$ and $R^2_{val}$ exceeded 0.9 for most quality parameters.

Vinegar, being a commonly adulterated food product, poses a challenging task due to the extensive range of potential adulterants and their distinct characteristics. The adulteration practice of substituting lower-cost alternatives for higher-quality vinegars, as well as fraudulent labeling, are prevalent issues in the vinegar authentication. With the growing demand for premium-quality vinegars, the incidence of adulteration is on the rise. Consequently, there is an urging need for rapid and cost-effective authentication methods to detect low-quality ingredients, estimate product age, and detect instances of false labeling.

Both targeted and non-targeted methods have been employed to detect adulteration in vinegar. The potential of UV–vis and FTIR spectroscopy, coupled with chemometric techniques and artificial neural network (ANN) analysis, was explored for the detection of adulteration in grape vinegars involving the addition of acetic acid and spirit vinegar. The ANN and OPLS-DA models, developed from UV–vis and FTIR spectroscopic data, exhibited high accuracy in classification, sensitivity, and specificity for the adulteration levels above 5%. UV–vis and FTIR spectroscopy in combination with chemometric methods can provide easy detection of grape vinegar adulteration requiring minimal sample preparation.

In this study, effectiveness of three spectroscopic techniques (fluorescence, UV–visible, mid-infrared) in detecting mixtures of acetic acid/apple vinegar and spirit vinegar/apple vinegar within a range of 1% to 50% was also assessed. To identify the most reliable models, an improved classification approach, utilizing multiple calibration and validation sets was employed. Remarkably, only the mid-infrared data yielded robust and precise classification models, achieving a high classification rate of up to 96%, sensitivity of 1, and specificity of up to 0.96 in distinguishing adulterated samples from genuine apple vinegars.

These successful findings underscore the potential of utilizing FTIR and UV-Vis spectral data in tandem with chemometric analysis as cost-effective, safe, and rapid methods for monitoring vinegar fermentation processes. Simultaneous analysis of these parameters could significantly enhance the quality control during fermentation and assist in verifying the product's authenticity. The premise of this study, which posited the effectiveness of the investigated spectroscopic techniques in identifying vinegar adulteration involving spirit vinegar and acetic acid, has been validated by the obtained results. Consequently, UV–vis and FTIR spectroscopy, in conjunction with chemometric methodologies, offer a straightforward means of detecting grape vinegar adulteration with minimal sample preparation. Therefore, mid-IR spectroscopy, when coupled with a chemometric classification system, can serve as a rapid analytical technique for detecting apple vinegar adulteration.

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# APPENDICES

# APPENDIX A

## STANDARD CURVES USED IN THE ANALYSES

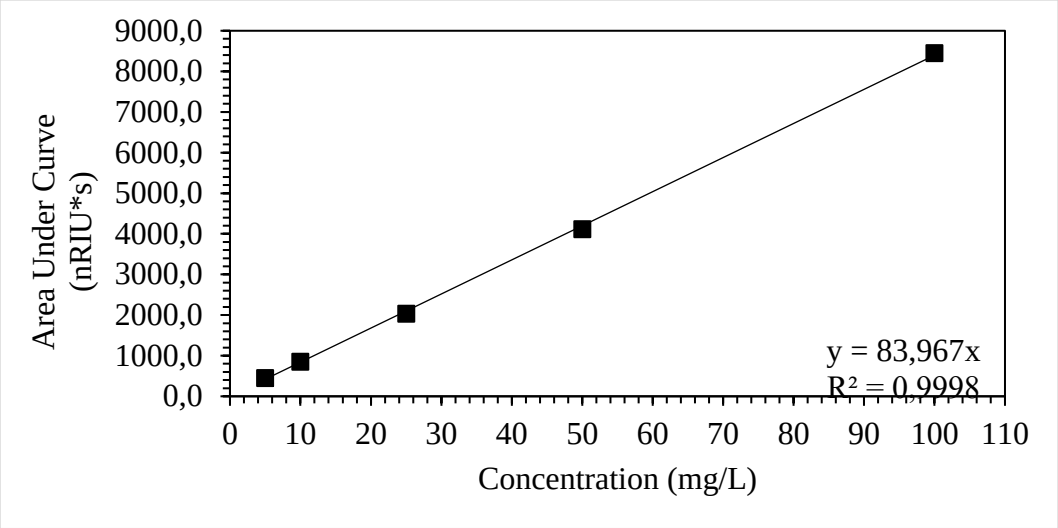


**Figure A.1.** Standard calibration curve for citric acid

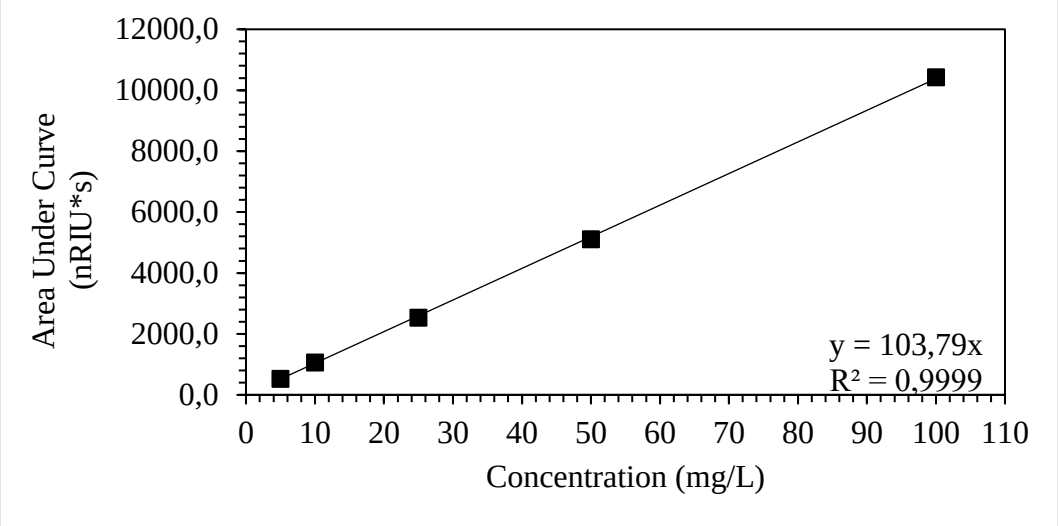


**Figure A.2.** Standard calibration curve for tartaric acid

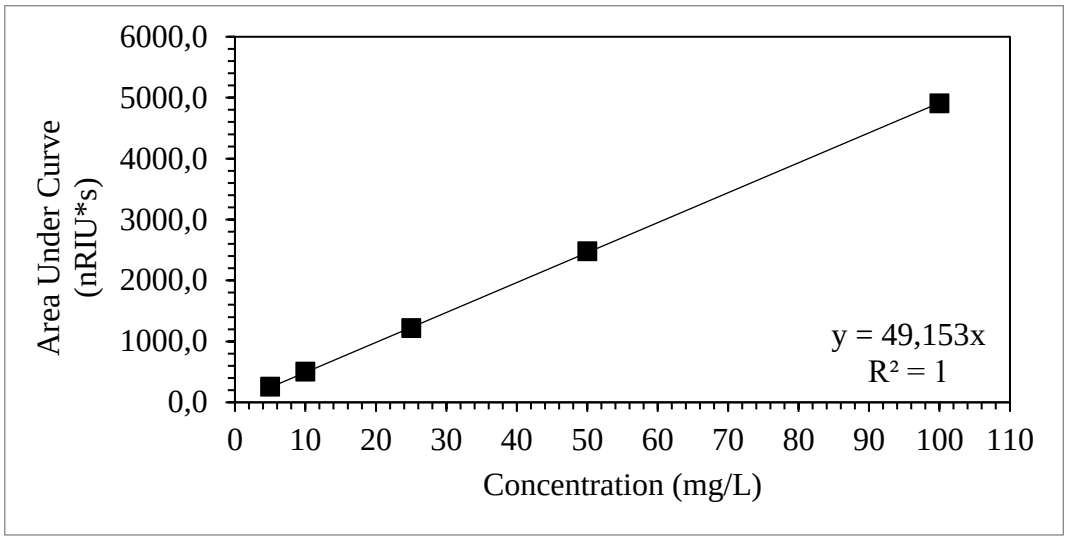


**Figure A.3.** Standard calibration curve for malic acid

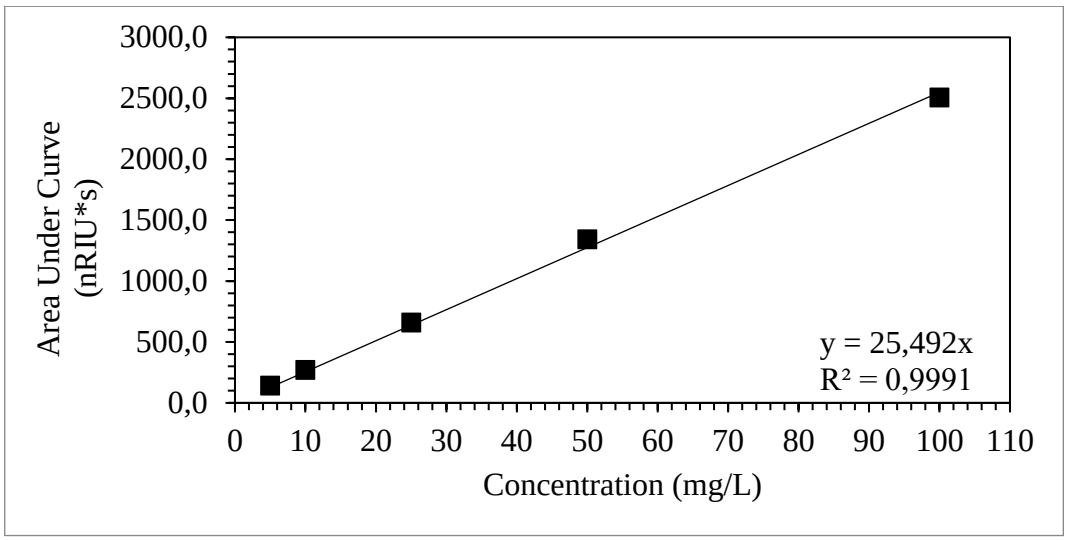


**Figure A.4.** Standard calibration curve for succinic acid

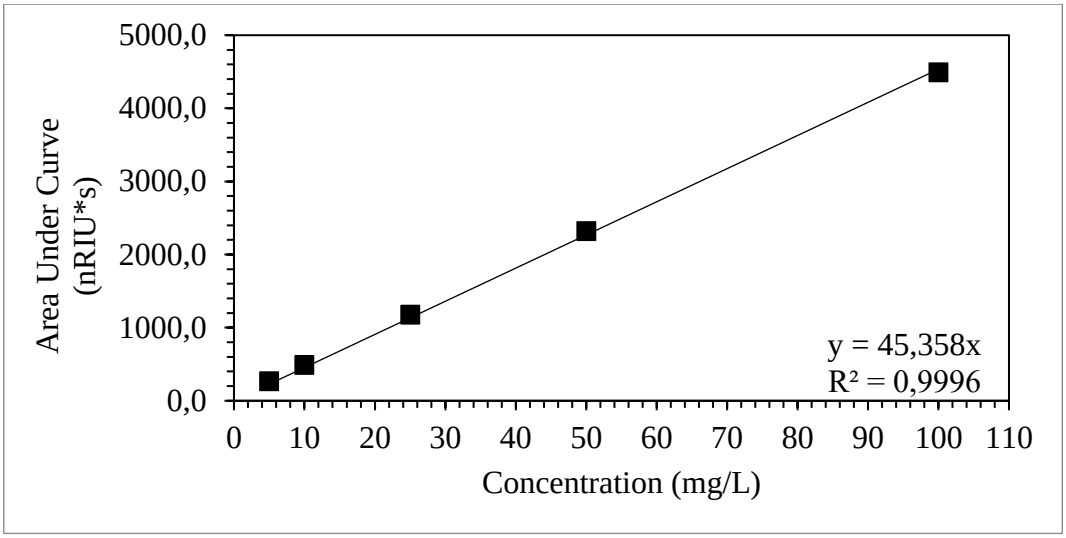


**Figure A.5.** Standard calibration curve for lactic acid

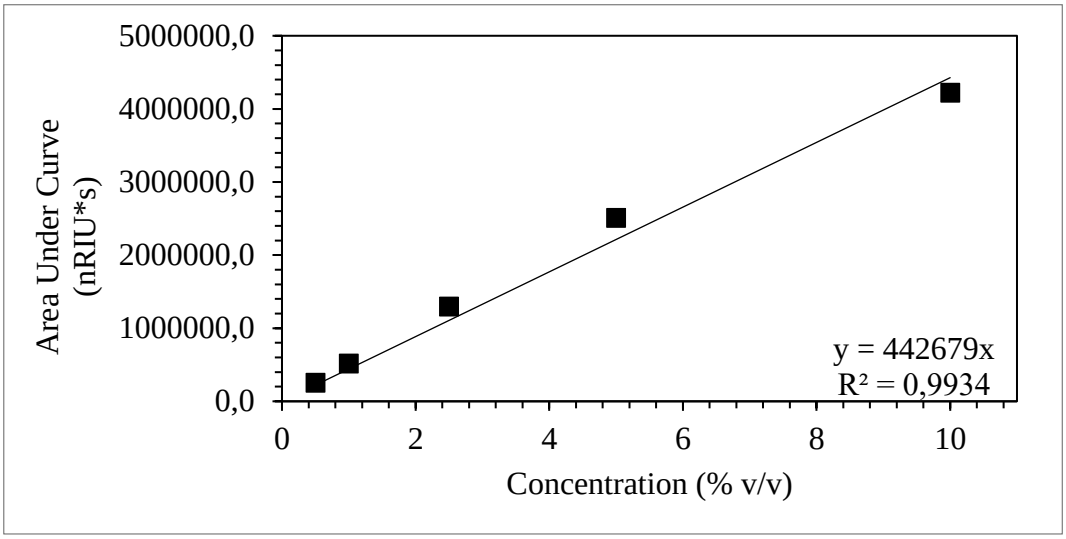


**Figure A.6.** Standard calibration curve for acetic acid

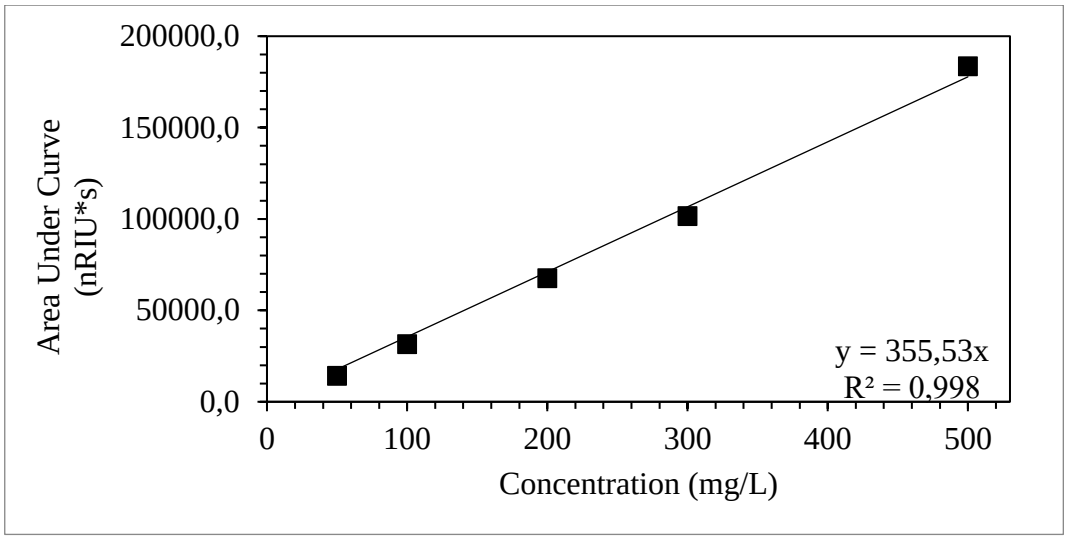


**Figure A.7.** Standard calibration curve for sucrose

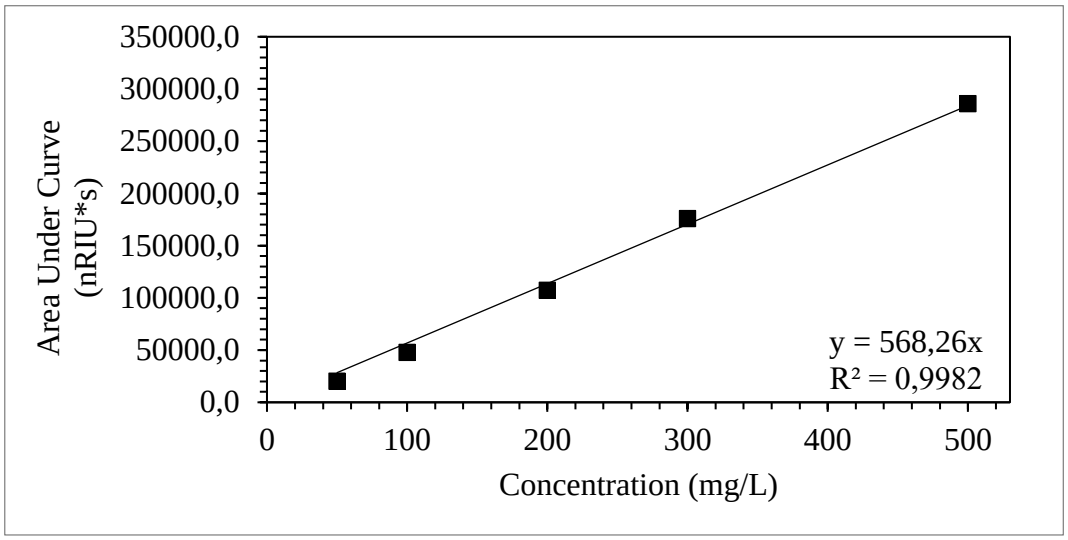


**Figure A.8.** Standard calibration curve for glucose

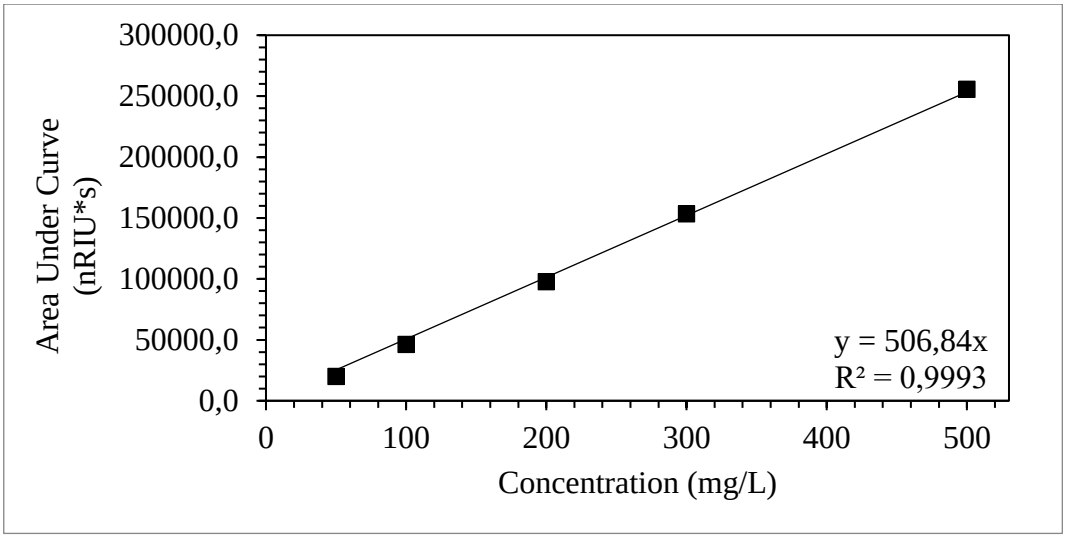


**Figure A.9.** Standard calibration curve for fructose

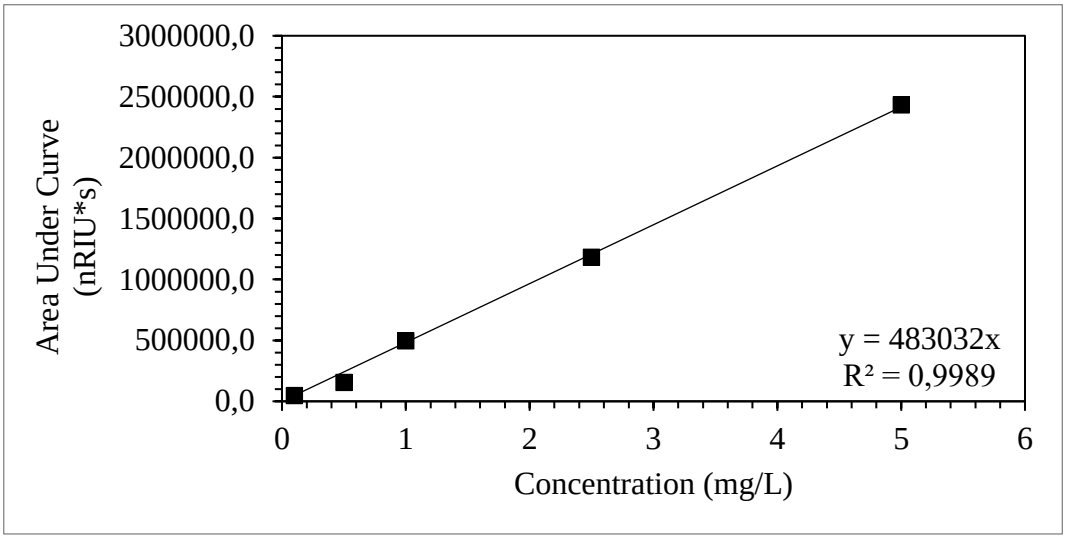


**Figure A.10.** Standard calibration curve for ethanol

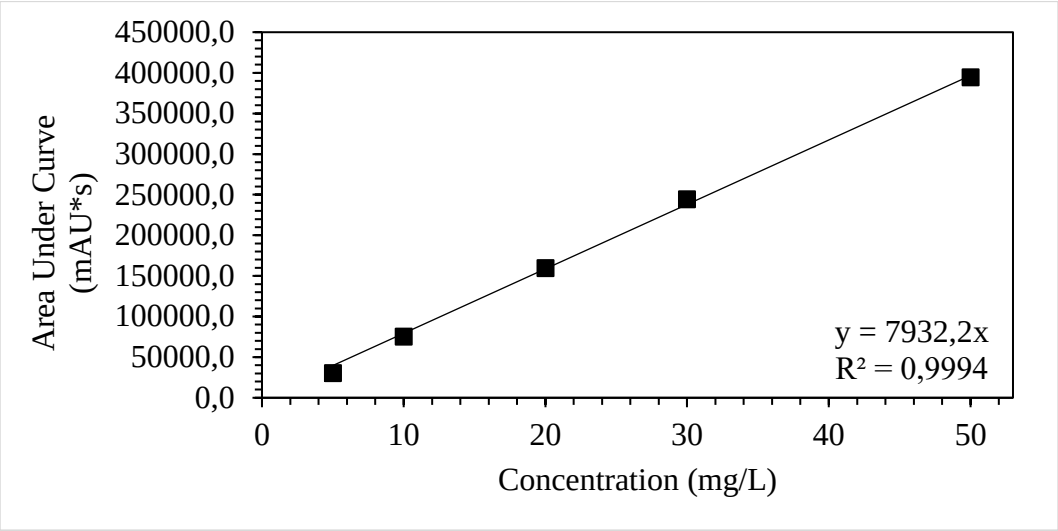


**Figure A.11.** Standard calibration curve for gallic acid

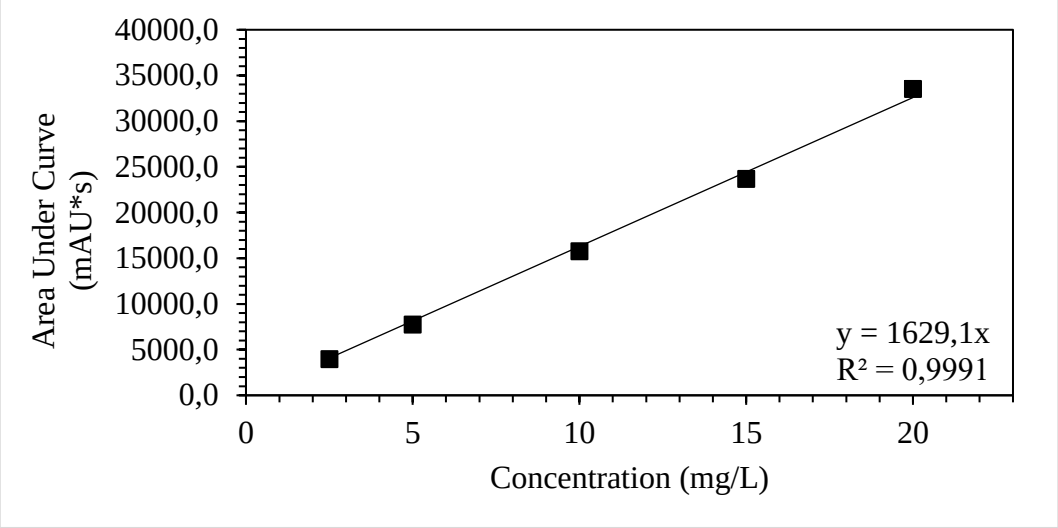


**Figure A.12.** Standard calibration curve for catechin

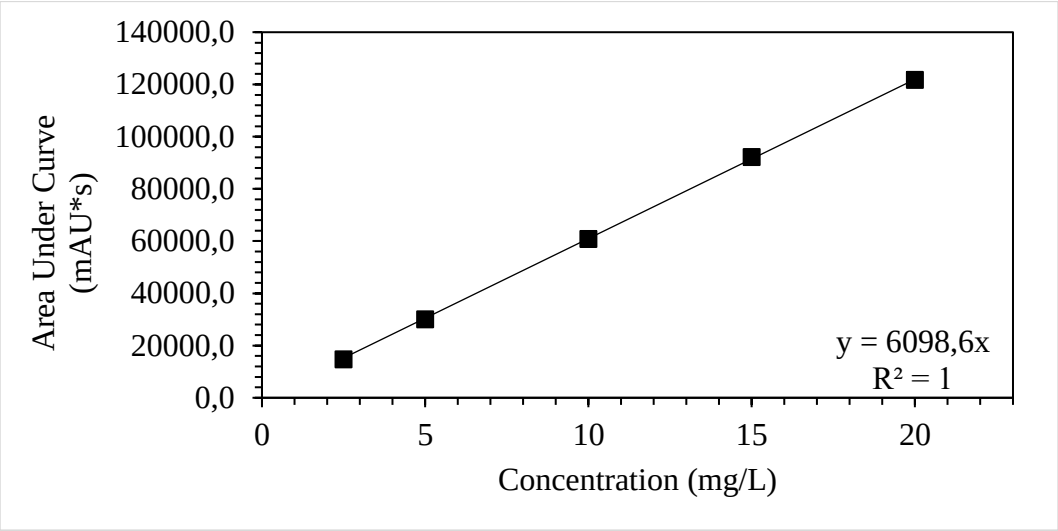


**Figure A.13.** Standard calibration curve for vanillic acid

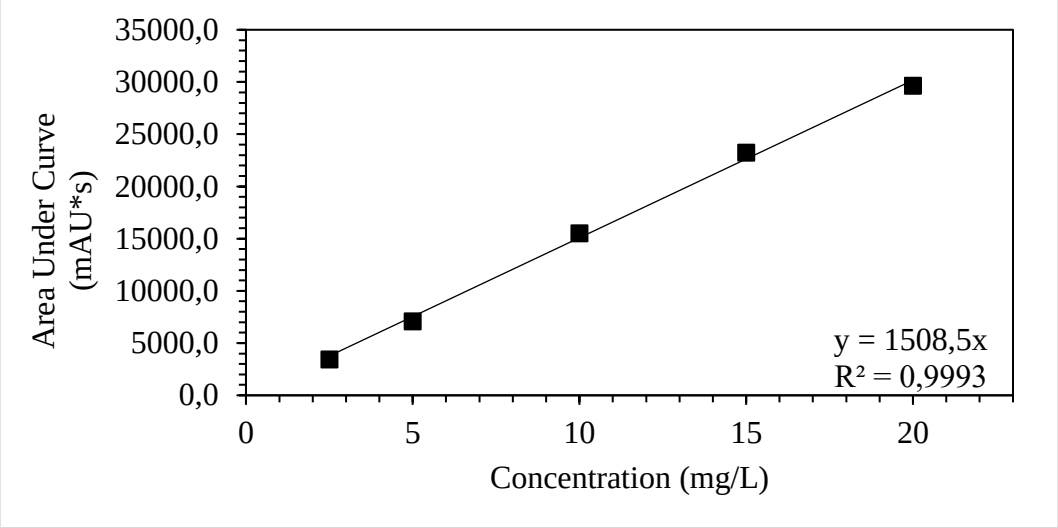


**Figure A.14.** Standard calibration curve for epicatechin

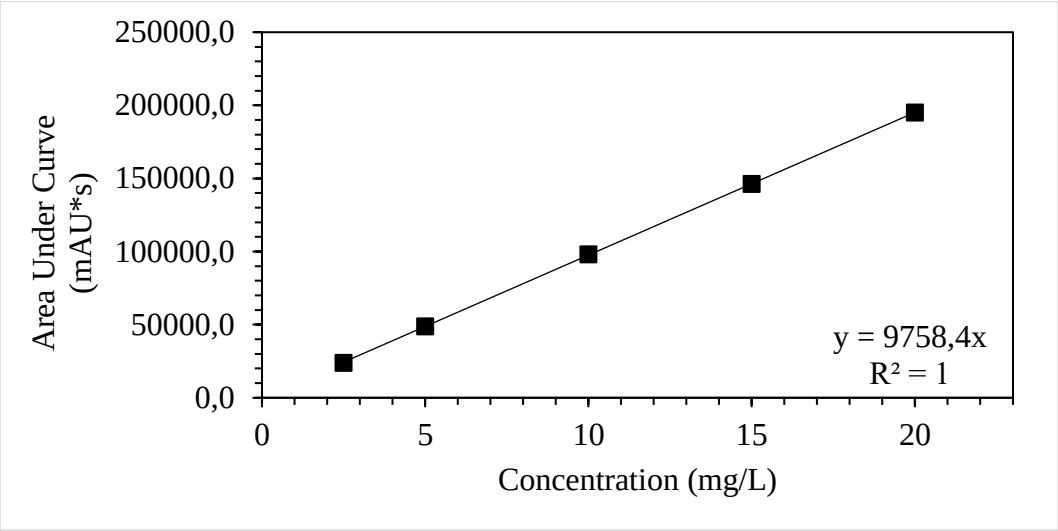


**Figure A.15**. Standard calibration curve for syringic acid

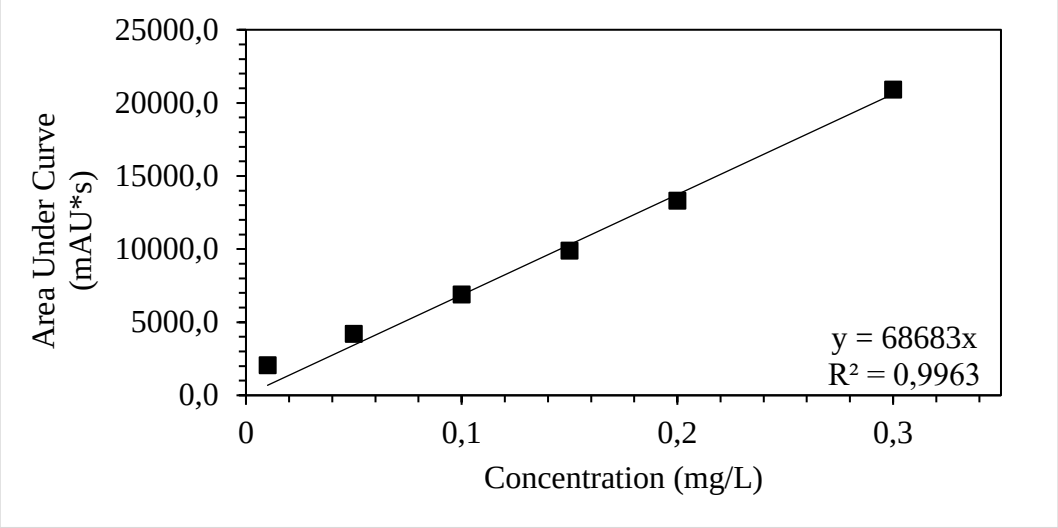


**Figure A.16.** Standard calibration curve for chlorogenic acid

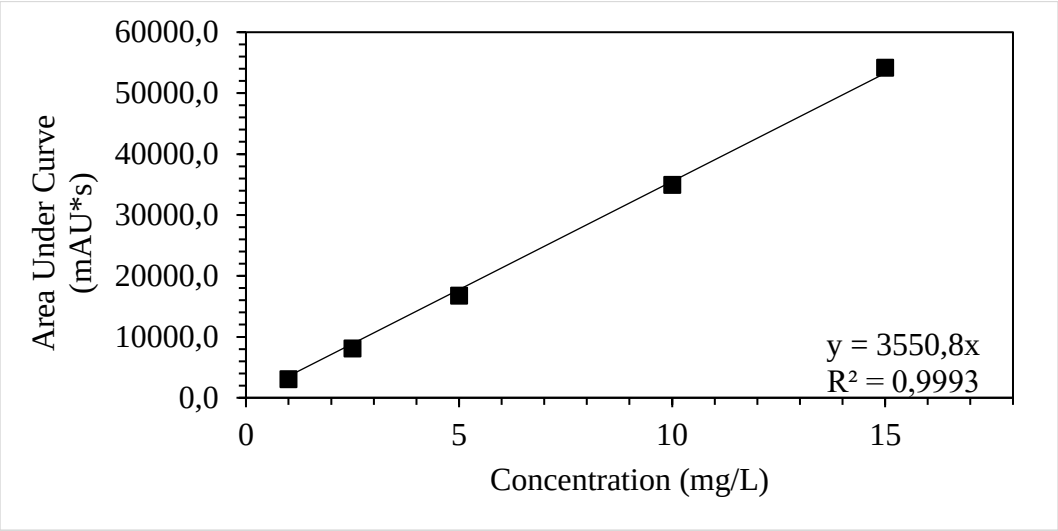


**Figure A.17.** Standard calibration curve for caffeic acid

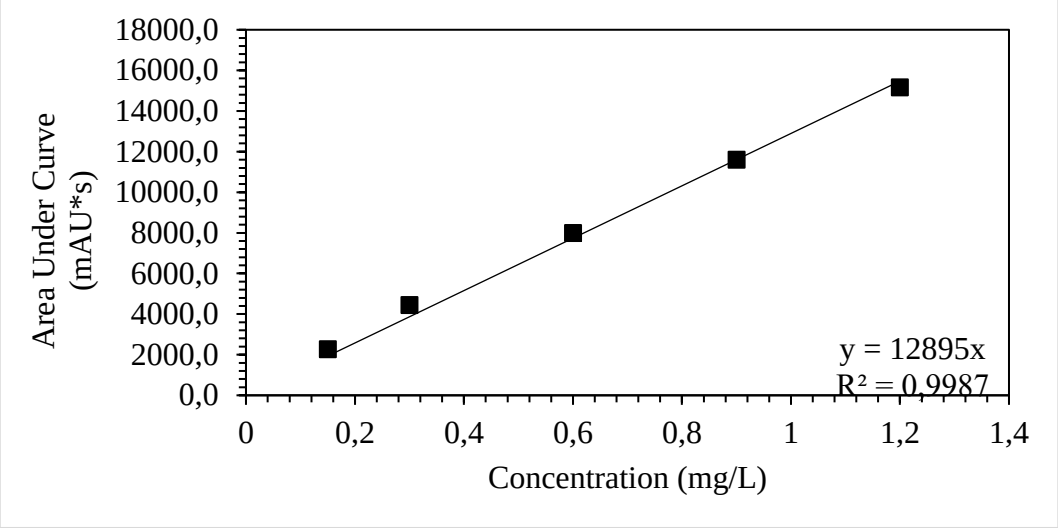


**Figure A.18.** Standard calibration curve for coumaric acid

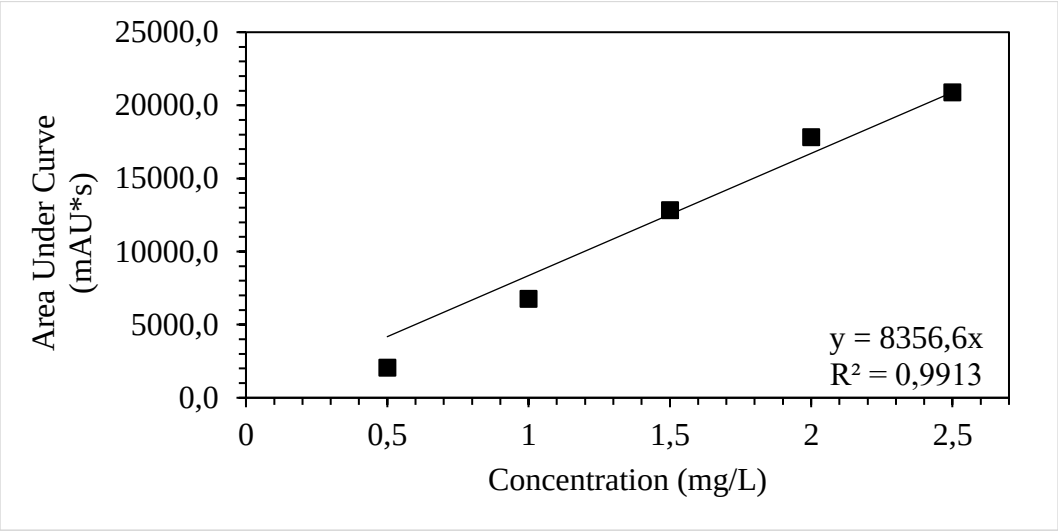


**Figure A.19.** Standard calibration curve for ferulic acid

# APPENDIX B

## MEAN, MINIMUM AND MAXIMUM VALUES OF COMPONENTS OF WINE VINEGAR

| Component | Mean | Minimum | Maximum |
|---|---|---|---|
| Citric Acid (ppm) | 164 | 5 | 2163 |
| Tartaric acid (ppm) | 1101 | 3 | 7610 |
| Malic Acid (ppm) | 746 | 44 | 2845 |
| Succinic Acid (ppm) | 1520 | 202 | 6974 |
| Lactic Acid (ppm) | 3472 | 94 | 18573 |
| Acetic acid (ppm) | 2.55 | 0.01 | 7.14 |
| Gallic Acid (ppm) | 3.30 | 0.18 | 29.60 |
| Catechin (ppm) | 2.75 | 0.09 | 61.32 |
| Vanillic Acid (ppm) | 1.16 | 0.02 | 15.20 |
| Epicatechin (ppm) | 3.63 | 0.02 | 14.89 |
| Syringic Acid (ppm) | 0.22 | 0.00 | 3.44 |
| Caffeic Acid (ppm) | 5.01 | 0.01 | 40.86 |
| Coumaric Acid (ppm) | 1.20 | 0.00 | 9.79 |
| Brix ($^{o}$Brix) | 4.75 | 0.50 | 31.23 |
| Titratable Acidity (% v/v HAc) | 2.73 | 0.32 | 7.94 |
| TPC (mg Gallic Acid Eq/L) | 453.80 | 127.00 | 3016.90 |
| TFC (mg Catechin Acid Eq/L) | 165.00 | 1.60 | 1498.80 |
| Sucrose (ppm) | 258 | 0 | 8354 |
| Glucose (ppm) | 12185 | 0 | 183807 |
| Fructose (ppm) | 18263 | 0 | 186992 |
| Ethanol (% v/v EtOH) | 1.21 | 0.00 | 7.94 |
| pH | 3.55 | 2.78 | 4.44 |

# VITA

## Education:

**2017-2023: Ph.D.** Department of Food Engineering, Izmir Institute of Technology, Izmir, Turkey

Thesis title: Application of Non-targeted Analysis Methods in Adulteration Detection and Prediction of Process Parameters of Vinegars, Supervisor: Prof. Dr. Banu ÖZEN

**2014-2017: M.Sc.** Department of Food Engineering, Middle East Technical University, Ankara, Turkey.

Thesis title: Physiological Traits of *Saccharomyces cerevisiae* Strains Isolated from Traditional Wines in Turkey, Supervisor: Prof. Dr. Candan GÜRAKAN

**2009-2014: B.Sc.** Department of Food Engineering, Middle East Technical University, Ankara, Turkey.

## Publications:


Dikici, S., & **Çavdaroğlu, Ç.** (2023). Development of pro-angiogenic wound dressings from 2-deoxy-D-ribose (2dDR)-loaded decellularized plant leaves. *Journal of Materials Science*, 1-17.

Aykac, B., **Çavdaroğlu, Ç.**, & Ozen, B. (2023). "Authentication of pomegranate juice in binary and ternary mixtures with spectroscopic methods." *Journal of Food Composition and Analysis* 117: 105100.

**Çavdaroğlu, Ç.,** & Ozen, B. (2023). "Applications of UV–visible, fluorescence and mid-infrared spectroscopic methods combined with chemometrics for the authentication of apple vinegar." *Foods* 12(6): 1139.

**Çavdaroğlu, Ç.,** & Ozen, B. (2023). "Authentication of vinegars with targeted and non-targeted methods." *Food Reviews International* 39(1): 41-58.

**Çavdaroğlu, Ç.,** & Ozen, B. (2022). "Detection of vinegar adulteration with spirit vinegar and acetic acid using UV–visible and Fourier transform infrared spectroscopy." *Food Chemistry* 379: 132150.

**Çavdaroğlu, Ç.,** & Ozen, B. (2021). "Prediction of vinegar processing parameters with chemometric modelling of spectroscopic data." *Microchemical Journal* 171: 106886.

Berk, B., **Çavdaroğlu, Ç.,** Grunin, L., Ardelean, I., Kruk, D., Mazi, B. G., & Oztop, M. H. (2022). "Use of magic sandwich echo and fast field cycling NMR relaxometry on honey adulteration with corn syrup." *Journal of the Science of Food and Agriculture* 102(7): 2667-2675.

Soyler, A., Cikrikci, S., **Çavdaroğlu, Ç.**, Bouillaud, D., Farjon, J., Giraudeau, P., & Oztop, M. H. (2021). "Multi-scale benchtop 1H NMR spectroscopy for milk analysis." *LWT* 139: 110557.